Program Nr: 1 from the 1999 ASHG Annual Meeting

Development of a strategy to screen for mutations and drugs affecting gene expression and its application to genomic imprinting. *A.L. Beaudet, T.-F. Tsai, K.-S. Chen, J. Weber, M. Justice.* Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX.

We wished to isolate mutations affecting genomic imprinting related to Prader Willi and Angelman syndrome. We used the mouse *Snrpn* promoter which lies within a CpG island that is methylated and silenced on the maternal chromosome as opposed to unmethylated and expressed on the paternal chromosome. In ES cells, we introduced the agouti cDNA as a knockin gene under the control of the *Snrpn* promoter. In nonagouti (*a/a*) mice, paternal inheritance of the fusion gene confers a tannish abdominal color; the abdomen is black if the fusion gene is absent or inherited maternally. Differential methylation and imprinted expression of the *Snrpn* promoter was preserved and switched normally, and administration of 5-azacytidine altered coat color. Two breeding strategies involving ENU mutagenesis of males were utilized: (1) males homozygous for the fusion gene and *a/a* were mutagenized and bred to *a/a* wild type females to yield 100 percent tannish offspring and (2) mutagenized wild type *a/a* males were bred to females *a/a* and homozygous for the fusion gene to yield 100 percent black offspring, such that mutations affecting genomic imprinting could be detected visually. No mutant animals were isolated in the first >100 offspring from breeding scheme 1 and one presumptive mutation or epigenetic event affecting imprinting and causing an unmethylated maternal allele was found in the first >150 animals from breeding scheme 2. These results demonstrate the feasibility of the general method to isolate regulatory mutations affecting transcription. Possible reporter cassettes include visible markers, easily quantitated secreted proteins, and histological markers. Bicistronic cassettes could be used to help discriminate effects not directly involving the fusion gene. This method is useful for studies of gene regulation and development, for drug screening in vivo or in tissue culture, for genetic improvement of animals, and for various medical applications.

Program Nr: 2 from the 1999 ASHG Annual Meeting

Strong Evidence for Linkage of High Density Lipoprotein Cholesterol (HDL-C) Concentrations to a Genetic Location on Chromosome 9p in Mexican Americans. *R. Arya[1], R. Duggirala[1], L. Almasy[2], M.P. Stern[1], P. O' Connell[3], J. Blangero[2]*. 1) Division of Clinical Epidemiology, Department of Medicine, UTHSCSA, San Antonio, TX; 2) Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX; 3) Department of Pathology, UTHSCSA, San Antonio, TX.

Although the genetic basis of coronary heart disease is not clear, numerous studies have examined the genetic architecture of quantitatively intervening phenotypes such as high density lipoprotein cholesterol (HDL-C). It is well understood that HDL-C has strong genetic determinants. However, little is known about the chromosomal regions harboring the major loci influencing HDL-C levels. Therefore, we employed a multipoint variance component linkage approach to identify loci that exert major influence on variation in HDL-C levels.

This study, a part of the San Antonio Family Diabetes Study, was based on phenotypic data from 415 individuals distributed across 27 low income Mexican American pedigrees. Genotypic data for these individuals were available for more than 400 markers. We conducted multipoint variance component linkage analyses as implemented in the computer package SOLAR.

All genetic analyses included age and gender as covariates. Prior to conducting multipoint analyses, the heritability of HDL-C was determined to be 55.0 ± 9.4%. We found significant evidence for linkage (LOD score = 3.6) of HDL-C levels to a genetic location in between markers D9S925 and D9S1121 on chromosome 9p. This locus explains 47.2 ± 13.0% ($p = 0.0000235$) of the total phenotypic variance in HDL-C. Interestingly, this region on chromosome 9p is not that far from the region on chromosome 9p which we previously reported to be linked to type 2 diabetes. This region has also been reported to be linked to fasting glucose levels in Caucasians. Also, six other chromosomal regions (i.e., chromosomes 2,3,7,8,11, and 20) across the genome exhibited LOD scores ranging from 1.2 to 1.7.

In conclusion, we found significant evidence for linkage of HDL-C levels to a genetic location on chromosome 9p in Mexican Americans, which appears to have major influence on HDL-C variation.

Program Nr: 3 from the 1999 ASHG Annual Meeting

Is direct speech compatible with non-directive genetic counseling? Results of a sociolinguistic investigation. *J. Benkendorf[1], M. Prince[1], C. Gordon[2], M. Rose[3], A. DeFina[2].* 1) Ob/Gyn, Georgetown Univ Med Center, Washington, DC; 2) Linguistics, Georgetown Univ; 3) Linguistics, Stanford Univ, Palo Alto, CA.

To date, genetic counseling research has focused on such aspects of non-directive counseling as the giving or withholding of information, and responses to client requests for advice. We investigated the yet unexplored area of how language choices, the "talk" of genetic counseling, facilitate or hinder the counseling process. Under an IRB-approved protocol with informed consent, we audiotaped 40 prenatal genetic counseling sessions. Transcripts of each session detailing all utterances, overlaps and pauses were prepared for discourse analysis, a sociolinguistic tool that includes studying conversational style, speaker-listener symmetry, directness and other interactional patterns. We found that the genetic counseling process requires flexibility in the use of direct and indirect speech. Analysis of our data demonstrates that: 1) indirect speech, marked by the use of hints, hedges and politeness strategies facilitates rapport and mitigates the tension between a client-centered relationship and a counselor-driven agenda; 2) direct speech, or speaking literally, is the most effective strategy for providing information and education; and 3) confusion exists between the use of direct speech and the intent to provide non-directive counseling, especially when facilitating client decision making. In response to client questions indirect phrases i.e., "some people" or "most people" maintained counselor neutrality; however, this well-intended indirectness, used to preserve client autonomy, obstructed direct explorations of client needs. Explanations (indirect), rather than "yes," "no" answers (direct) were often similar barriers. We will present suggestions, based on our data, for promoting the compatibility of direct speech with a non-directive genetic counseling model. This study provides new insights into how "talk" affects the work of genetic counseling and indicates the need for on-going research and professional development in this area. (This work was supported by the Jane Engelberg Memorial Fellowship, an annual grant from the Engelberg Foundation to the NSGC, Inc.).

Program Nr: 4 from the 1999 ASHG Annual Meeting

Genome wide analysis of DNA replication and replication origins in higher eukaryotes. *J. Herrick[1], P. Stanislawski[1], O. Hyrien[2], A. Bensimon[1].* 1) DNA Biophysics Lab, Pasteur Institute, Paris, 75425 France; 2) Ecole Normale Superieure, Genetique Moleculaire, 46 rue dUlm, 75130 Paris cedex 05, France.

A new method for the study of DNA replication in higher eukaryotes is presented. The method is based on a process called molecular combing and allows for the genome wide analysis of the spatial and temporal organization of replication units and replication origins in a sample of genomic DNA. Molecular combing is a process whereby molecules of DNA are stretched and aligned on a glass surface by the force exerted by a receeding air/water interface. Since the stretching occurs in the immediate vicinity of the meniscus, all molecules are identically stretched in a size and sequence independent manner. The application of fluorescence in situ hybridization to combed DNA results in a high resolution optical mapping that is simple, controled and reproducible. The ability to comb large numbers of genomes on a single coverslip (up to several hundred haploid genomes) allows for a statistically significant number of measurements, which in turn results in a mapping resolution of 1 to 4 kb for any given genetic locus. We have applied the method to the study of DNA replication in the Xenopus laevis in vitro replication system. It was found that 1) origins of replication are activated asynchronously throughout the genome and 2) that origin density increases as DNA replication advances. These results represent the first genome wide study of DNA replication in this species and demonstrate that molecular combing is an attractive tool for genomic studies of DNA replication in higher eukaryotes.

Program Nr: 5 from the 1999 ASHG Annual Meeting

Inversion of the circadian rhythm of melatonin in Smith-Magenis syndrome. *H. De Leersnyder[1], J.-C. von Kleist-Retzow[1], A. Munnich[1], B. Claustrat[2], S. Lyonnet[1], M. Vekemans[1], M.-C. De Blois[1].* 1) Department of Genetics, Hôpital Necker, Paris, France; 2) Nuclear Medecine Centre, Hôpital Neurologique, Lyon, France.

Smith Magenis syndrome (SMS) is a multiple congenital anomaly and mental retardation syndrome caused by an interstitial deletion of chromosome 17p11.2. Common features include characteristic facial appearance, short stature, auto and hetero agressivity, tantrums, mental retardation and severe sleep disturbance. We have studied the sleep behavior of 19 SMS children aged 3 to 17 years, through questionnaires (19/19), sleep consultation (10/19) or hospitalisation (10/19) for EEG recording, melatonin, growth hormone and cortisol work up. Children consistently went to bed and woke up early (mean wake-up time 5:00 am). All have naps and sleep attacks. Polysomnography showed reduced 3-4 NREM sleep, fragmented REM sleep and frequent miniarousals. Cortisol cycle showed normal circadian distribution and no peak of growth hormone during the night. Melatonin, the main pineal gland hormone, is known to be secreted in a circadian rhythm controled by the suprachiasmatic nucleus of the hypothalamus and the sympathetic nervous system. Melatonin normally reaches a peak during the night around 2-4 am (60 to 120 pg/ml), while diurnal plasma melatonin falls below 10 pg/ml. In SMS patients, a complete inversion of melatonin circadian cycle was noted. Plasma melatonin levels were below 6 pg/ml between 1 and 5 am, increased until midday (83-114 pg/ml), and decreased at 8 pm. Urinary melatonin and 6-sulfatoxymelatonin closely paralleled plasma melatonin. The night-day ratio of urinary melatonin excretion was dramatically reduced in SMS (<1 in patients compared to 9.5 in normal children). We also noted that tantrums occurred when melatonin increased and sleep attacks at the peak of melatonin secretion. It is tempting to hypothesize that severe hyperactivity in SMS might be due to a constant struggle against sleepiness. These preliminary findings suggest that SMS patients suffer of an inversion of circadian rhythm of melatonin. This study provides what we believe to be the first biological basis of behavioral genetic disorder and strongly suggests that an aberrant biological clock is involved in SMS.

Program Nr: 6 from the 1999 ASHG Annual Meeting

_In vivo_suppressor mutations correct liver disease in a murine model of Hereditary Tyrosinemia type I. *K. Manning[1], M. Al-Dhalimy[1], M. Finegold[2], M. Grompe[1]*. 1) Department of Molecular & Medical Genetics, Oregon Health Sciences University, Portland, OR; 2) Department of Pathology, Texas Children's Hospital, Houston, TX.

Hereditary tyrosinemia type I (HTI) and alkaptonuria (aku) are disorders of tyrosine catabolism caused by deficiency of fumarylacetoacetate hydrolase (FAH) and homogentisic acid dioxygenase (HGD) respectively. HTI is a severe childhood disease that results in liver and kidney dysfunction and often liver cancer, due to the build-up of fumarylacetoacetate (FAA) and maleylacetoacetate (MAA), the two metabolites upstream of FAH in the pathway. Patients with aku, a much more benign adult disorder, have joint problems resulting from a build-up of HGA, the substrate of HGD, but no liver or kidney damage since FAA and MAA are downstream of HGD in the pathway. Mice doubly mutant in *Hgd*and *Fah*were found to be protected from both the liver and renal damage of HTI. Interestingly, all mice that were deficient in *Fah*, but heterozygous for *Hgd*spontaneously developed clonal nodules of functionally normal hepatocytes that were able to divide and repopulate the whole liver. By analyzing RT-PCR products from corrected livers in *Fah*mutant, *Hgd*heterozygous mice, we determined that this rescue was due to inactivating mutations in the wild-type allele of *Hgd*. This model therefore represents the first example of phenotypic reversion by an *in vivo*suppressor mutation in a mammal. Analysis of the *Hgd* mutations in the corrected nodules showed that FAA and MAA can cause a wide spectrum of alterations. These included small deletions and insertions, base substitutions, and also larger alterations (whole gene deletions or gene conversion) resulting in the loss of the entire wild-type *Hgd*allele.

Program Nr: 7 from the 1999 ASHG Annual Meeting

Do laws restricting the use of genetic information reduce discrimination by health insurers? *M.A. Hall, S.S. Rich.* Public Health Sciences, WFU School of Medicine, Winston-Salem, NC.

Methods: Qualitative, comparative, case-study in seven states, with varying laws restricting health insurers' use of genetic information. Expert, semi-structured interviews were conducted in 1998 with 12 regulators, 35 actuaries and underwriters, 30 insurance agents, 29 genetic counselors, and five patient advocates. In a direct market test, an actual small employer and unhealthy individual contacted 143 insurance agents to inquire about obtaining group and individual health insurance, following a fictitious scenario that included a positive BRCA1 test, a family history of Alzheimer's and juvenile diabetes. Also, 148 application forms were content analyzed. **Findings**: (1) There are almost no well-documented cases of health insurers either asking for or using presymptomatic genetic test results in their underwriting decisions, either before or after these laws, or in states with or without these laws. (2) A person with a serious genetic condition that is presymptomatic faces little or no difficulty obtaining health insurance, and there are few indications that the degree of difficulty varies according to state law. (3) At present, health insurers are not interested in using genetic information of this sort, and they do not consider presymptomatic genetic tests or family history a cost-effective way to predict future diseases. (4) If presymptomatic genetic information were easily available, some health insurers would consider using it in some fashion, if that were legal, and most insurers think this information could be much more relevant in the future. (5) Many health insurers regard it as socially or ethically wrong to use genetic information, and this sense can be traced in part to these laws. (6) These laws have not caused, and are not likely to cause, substantial adverse selection or distabilization of the market. **Conclusion**: It is difficult to justify these laws based on reducing an existing threat of genetic discrimination. However, they might be justified based on their ability to reassure the public or their role in shaping attitudes of social responsibility within the insurance industry.

Program Nr: 8 from the 1999 ASHG Annual Meeting

Evaluating the phenotypic effects of SNP variation: sampling issues. *K.M. Weiss[1], A.G. Clark[1], S.M. Fullerton[1], S.L. Taylor[2], D.A. Nickerson[2], C.F. Sing[3].* 1) Departments of Anthropology and Biology, Pennsylvania State University, University Park, PA; 2) Department of Molecular Biotechnology, University of Washington, Seattle, WA; 3) Department of Human Genetics, University of Michigan Medical School, Ann Arbor, MI.

Current technology makes it possible to identify far more SNP variation in candidate genes than can be practically typed in large-scale epidemiological samples. Choosing the most informative subset of variable sites to genotype for testing etiologic effects poses a challenging problem. Attributes of the SNPs such as heterozygosity, lengths of shared DNA segments, level of linkage disequilibrium, and the partitioning on a cladogram that each site imposes may all be important to consider. These points will be illustrated with complete SNP data for the apolipoprotein E gene (*APOE*) in 884 individuals from Rochester, MN. Different site-choice criteria capture different structural aspects of the sampled gene genealogy, related to the hierarchical history and recurrence status of mutation and recombination. Empirical study of the above criteria shows that maximization of haplotype diversity determined by a subset of variable sites appears to be the single most important criterion for extension to population studies, because it provides the most statistical power for subsequent association tests. However, given the variable history of recombination and recurrent mutation among genes, other factors such as the cladistic structure of present-day haplotypes can be important for identifying phenotypic effects. We describe a strategy to optimize site selection for association testing, genotyping sites in a staged fashion coupled with phenotype analysis, illustrated by analysis of risk factors for cardiovascular disease.

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Program Nr: 9 from the 1999 ASHG Annual Meeting

Phenotypes of CMT1A mice and modifier genes. *M. Fontes[1], J.C. Norrell[2], E. Passage[1], J. Pizant[1], F. Clarac[2], N. Levy[1]*. 1) INSERM U491. Medical Genetics and Development..Facult de Mdecine de la Timone, 27 Bd J.Moulin, 13358 Marseille cedex 5, France; 2) UPR CNRS 90111. Neurobiologie et Mouvement. 31 Ch. J.Aiguier, 13402 Marselle Cedex 20, France.

We have constructed 4 mouse lines by introducing a human YAC containing the human gene PMP22 into the murine oocyte (Huxley et al, 1996; 1998). The conclusions of the phenotypic exploration of these mice clearly demonstrate the following points: - The neuropathic phenotype linked to PMP22 overexpression, appears by a non linear mechanism. - The peripheral neuropathy is not a demyelinating neuropathy, but a dysmyelinating disorder, as the myelination process, occurring at birth, is abnormal. - Schwann cell did not express myelinating markers, like Krox20, demonstrating that they are not performing their correct program of myelinisation. Our transgenic model demonstrates thus that pathologies linked to PMP22 overexpression are in fact pathologies of the differentiation of Schwann cells. One of the major problem in mammalian genetics (and therefore medical genetics) is the variability of inherited phenotypes, probably due to a heterogeneity of the genetic background. This is particularly true for CMT 1A, as aymptomatic as well as severely affected individuals, presenting all the CMT duplication, can be observed in the same family. We have thus used our CMT mice, to detect potential variation in the phenotypic presentation of transgenic animals. For that purpose, we used different behavioural tests, in order to quantitatively estimate the locomotion capacity of different animals from the same line. For that purpose, we used the most severely affected line, C22. We will present data indicating that a phenotypic variability, probably genetic background dependant, exists in this line. Moreover we demonstrate that this variability is not due to differences in the extent of dysmyelinization. We also demonstrate that the endogenous PMP22 region is not a modifier locus. We are thus undertaking backcross to screen the murin genome to map these modifiers loci.

Program Nr: 10 from the 1999 ASHG Annual Meeting

Glial cell line-derived neurotrophic factor gene (*GDNF*) as a candidate type-1 neurofibromatosis (NF1) modifier towards intestinal neuronal dysplasia. *M. Bahuau[1], A. Pelet[2], M. Vidaud[1], D. Lacombe[3], D. Vidaud[1], S. Lyonnet[2]*. 1) Génétique Moléculaire, Faculté de Pharmacie, Université Paris V, Paris, France (mvidaud@teaser.fr); 2) INSERM U-393, Hôpital Necker Enfants-Malades, Paris, France; 3) Génétique Médicale, Hôpital Pellegrin-Enfants, Bordeaux, France.

Neurofibromatosis type 1 (NF1) is a common monogenic disorder primarily affecting neural crest-cell derivatives and resulting in cutaneous manifestations of café-au-lait spots and neurofibromas. The gene mutated in NF1 encodes neurofibromin, a member of the so-called GTPase-activating proteins (GAPs) and an upstream downregulator of the p21Ras/Raf/Map kinase and p21Ras/Ral signaling pathways. Though genetic homogeneity is a hallmark of this condition, phenotypic heterogeneity has been exemplified by a wide spectrum of diversity which seems, at least in part, governed by nonallelic, ‘modifying’, loci. However, until this report, these loci had remained putative. The late observation of a family with NF1 and congenital megacolon status resulting from intestinal neuronal dysplasia (IND B), an intrinsic anomaly of the neural crest cell-derived parasympathetic enteric neurons, provided a unique opportunity to quest for such modifiers. The gene encoding glial cell line-derived neurotrophic factor (GDNF), a natural ligand of the RET tyrosine kinase receptor protein, was a particularly attractive candidate. Indeed, *GDNF* lesions seemed to modulate the phenotypic translation of monogenic/chromosomal factors in a spectrum of neural crest-cell involvement ranging from congenital failure of autonomic control (Ondine’s curse) to congenital aganglionic megacolon (Hirschsprung’s disease, HSCR). Screening *GDNF* for a DNA change in the proposita, a missense mutation (Agr93Trp) was evidenced, which had been previously reported in patients with HSCR or Ondine-HSCR. Consistently, in this family, only siblings with both the paternally-derived *GDNF* lesion and the maternally-inherited *NF1* mutation (2424-2425insCCTTCAC) had megacolon status. Such epistatic interaction between *NF1* and *GDNF* is in line with a functional integration of RET and Ras in a complex subcellular signaling network.

Program Nr: 11 from the 1999 ASHG Annual Meeting

Inhibition of caspase cleavage of huntingtin protects neurons from toxicity and aggregate formation. *C.L. Wellington[1], R. Singaraja[1], L. Ellerby[2], J. Savill[1], S. Roy[3], B. Leavitt[1], E. Cattaneo[4], A. Hackam[1], A. Sharp[5], N. Thornberry[3], D. Bredesen[2], D. Nicholson[3], M.R. Hayden[1]*. 1) CMMT, UBC, Vancouver, Canada; 2) Burnham Institute, La Jolla, CA; 3) Merck Frosst, Montreal, Canada; 4) Institute for Phamacological Sciences, Milan, Italy; 5) Dept. Pysch. Beh. Sci., Johns Hopkins University, Baltimore, MD.

Proteolytic cleavage of huntingtin, the causative protein for Huntington Disease (HD), is hypothesized to be a crucial step in pathogenesis. There is evidence for proteolytic cleavage of huntingtin both in brains of affected persons and in a YAC transgenic mouse model of HD, which suggests that cleavage leads to a truncated fragment that is toxic. Expression of N-terminal huntingtin fragments results in increased toxicity and an increased prevalence of intracellular aggregates. As huntingtin is a known caspase substrate, we tested whether inhibiting huntingtin cleavage by caspases protects neurons from toxicity and aggregate formation. Cell permable caspase inhibitors eliminate huntingtin cleavage and provides protection from an apoptotic stress ($p<0.001$, $n=3$). Additionally, we have determined that caspase-3 cleaves huntingtin at D513 and D552 and that caspase-6 cleaves huntingtin at D589. Site-directed mutations at these sites results in a mutant form of huntingtin that is resistant to cleavage by caspases -3 and -6. Viability and caspase activation assays show that the caspase-resistant huntingtin is no longer toxic ($p>0.05$ compared to vector alone vs $p<0.001$ compared to cleavable huntingtin, $n=3$. Furthermore, aggregate formation is reduced in the presence of caspase resistant huntingtin from 14% to 2.5%($p<0.01$, $n=5$). These results show that caspase cleavage of huntingtin is toxic to cells and is likely to play a crucial role in the pathogenesis of HD. These data strongly support the therapeutic potential of caspase inhibitors in the treatment of HD.

Program Nr: 12 from the 1999 ASHG Annual Meeting

Nuclear distribution, chromatin association and DNA-binding activity of the XNP/ATR-X gene product: functional consequences of ATR-X mutations. *L. Colleaux[1], C. Cardoso[1], Y. Lutz[2], C. Mignon[1], M.G. Mattei[1], J.L. Mandel[2], M. Fontes[1]*. 1) INSERM U491, Faculte de Medecine de la Timone, Marseille, FRANCE; 2) INSERM U184, IGBMC, Parc d'innovation, BP 163, Strasbourg, FRANCE.

Mutations in the XNP/ATR-X gene cause in several X-linked mental retardation syndrome: the ATR-X syndrome, the Juberg-Marsidi syndrome or the Carpenter-Waziri syndrome, suggesting that this gene is involved in brain development and facial morphogenesis. To further understand the function of the XNP protein, which is a new Zinc-finger/Helicase protein belonging to the SNF2/SWI protein family, we have investigated the ability of XNP to interact with chromatin-associated proteins, its the cellular localization and the DNA-binding capacities from normal and mutated forms of the zinc finger domains. We demonstrated, using yeast two-hybrid, that XNP can specifically interact with the chromo-shadow domain of the two human heterochromatin proteins HP1a and HP1b, suggesting that XNP is likely part of a multiprotein complex associated with heterochromatin. Secondly, cellular location of the endogenous XNP protein was studied by indirect immunofluorescence. We showed that the XNP protein is found exclusively in the nucleus, concentrated in several specific nuclear structures and that it localizes to the pericentromeric heterochromatin region of condensed chromosomes. Lastly, using in vitro assays, we demonstrated that the zinc-finger domains of XNP can mediate DNA-binding. Alltogether, our data strongly suggest that the XNP protein is likely part of a transcriptional regulator complex that regulates genes transcription by recruiting or maintaining target genes within transcriptionally repressive heterochromatin structures. Additionally, analysis of previously reported XNP missense mutation revealed either modified pattern of nuclear location or altered DNA-binding capacity, providing the first clues towards understanding the functional significance of these mutations and the biological basis of the pathology .

Program Nr: 13 from the 1999 ASHG Annual Meeting

Myelin deficiencies in both the central and peripheral nervous systems associated with SOX10 transcription factor mutations. *K. Inoue[1], Y. Tanabe[2], J. wilson[3], S. Hirabayashi[4], M. Wegner[5], J.R. Lupski[1]*. 1) Molec & Human Genetics, Baylor College of Med, Houston, TX; 2) Neurology, Chiba Children's Hospital,Chiba, Japan; 3) Neurology, Hospital for Sick Children, London, UK; 4) Neurlogy, Nagano Children's Hospital, Nagano, Japan; 5) Molec Neurobiology, Univ of Hamburg, Hamburg, Germany.

SOX10 is a transcription factor preferentially expressed in the late embryonic glial cell lineage and in mature myelin forming cells of both the central nervous system (CNS) and peripheral nervous system (PNS), as well as in the early neural crest cells. Heterozygous *SOX10*loss-of-function mutations have been reported in patients with Waardenburg-Hirschsprung syndrome (WS4) and its murine model, *Dom*. However, neither WS4 patients nor *Dom*mice have dysmyelinating features, raising the question of how SOX10 acts in the glial lineage *in vivo*. We describe three unique patients presenting with severe leukodystrophy compatible with Pelizaeus-Merzbacher disease and peripheral neuropathy consistent with Charcot-Marie-Tooth disease type 1 in addition to WS4. Novel mutations were identified in their *SOX10*gene. One 12-bp deletion starting from the stop codon does not disrupt the coding region but extends the peptide adding a proline-rich tail adjacent to the transactivation domain. The other two mutations result in the termination at the codon 313 prior to the transactivation domain but leave the DNA binding domain intact. These mutations may act in a dominant-negative manner in contradistinction to the loss-of-function alleles associated with WS4. Our findings indicate that dysfunction of SOX10 may lead to deficiency of myelination in the CNS and PNS as well as hypopigmentation and enteric aganglionosis.

Program Nr: 14 from the 1999 ASHG Annual Meeting

Expansion of a novel CAG repeat in the 5' region of gene encoding a subunit of protein phosphatase 2A is associated with spinocerebellar ataxia type 12 (SCA12). *S.E. Holmes[1], E. O'Hearn[1], M.G. McInnis[1], D.A. Gorelick-Feldman[1], J.J. Kleiderlein[1], C.A. Callahan[1], R.G. Ingersoll-Ashworth[1], M. Sherr[1], A.J. Sumner[2], A.H. Sharp[1], U. Ananth[3], W.K. Seltzer[3], M.A. Boss[3], A-M. Vieria-Saeker[4], N.G. Kwak[1], J.T. Epplen[4], O. Riess[5], C.A. Ross[1], R.L. Margolis[1]*. 1) Johns Hopkins Univ Sch Med, Baltimore, MD; 2) Louisiana State Univ Sch Med, New Orleans, LA; 3) Athena Diagnostics, Inc., Worcester, MA; 4) Ruhr-Univ, Bochum, Germany; 5) Univ Rostock, Rostock, Germany.

We have ascertained a large family with a progressive autosomal dominant disorder with a variable age of onset. The phenotype, also variable, includes tremor, ataxia, cerebellar and basal ganglia signs, and dementia, and has been termed SCA12. An expansion of a CAG trinucleotide repeat in genomic DNA of affected individuals was detected with RED (repeat expansion detection). All known CAG expansions were excluded, including SCA8. A segment of genomic DNA containing the repeat was cloned, and a PCR assay of repeat length was developed. The repeat is located in the 5' region of a regulatory subunit of protein phosphatase 2A. The repeat is 7 to 28 triplets in length in control populations, with a heterozygosity of 60 percent. Affected individuals in the pedigree, but not unaffected family members over the age of 60, have an expanded repeat greater than 65 triplets in length (lod = 3.81, theta = 0). We hypothesize that this repeat expansion is the mutation responsible for SCA12. This work was supported in part by the Huntington's Disease Society of America, NS16375, MH01275, and MH50763.

Program Nr: 15 from the 1999 ASHG Annual Meeting

Missense mutations in the 3-phosphoglycerate dehydrogenase gene are associated with a serine biosynthesis defect and neurological impairment. *I.E.T. van den Berg[1], T.J. de Koning[1], H.E.M. Malingré[1], E.A.C.M. van Beurden[1], M. Brink[1], F.L. Opdam[1], J. Jaeken[2], M. Pineda[3], V.J. Ramaekers[4], B.T. Poll-The[1], R. Berger[1], L.W.J. Klomp[1]*. 1) Metabolic disorders, University Medical Center Utrecht; 2) University Hospital Leuven, Belgium; 3) Hospital Saint Joan de Deu, Barcelona, Spain; 4) Medizinische Einrichtungen der Rwth, Aachen, Germany.

3 Phosphoglycerate dehydrogenase (3PGDH) deficiency is a rare disorder of serine and glycine biosynthesis characterized by severe psychomotor retardation and seizures. This disorder is potentially treatable as the convulsions and biochemical abnormalities can be effectively ameliorated by amino acid supplementation therapy. Human 3PGDH cDNA was cloned and the deduced amino acid sequence revealed marked homology to 3PGDH from a variety of other species. RNA blot analysis revealed abundant expression of 3PGDH mRNA in heart, pancreas and brain, but 3PGDH transcripts were also readily detected in other fetal and adult tissues. To investigate the molecular basis of 3PGDH deficiency, 6 patients from 4 unrelated families were tested for sequence variations in the 3PGDH mRNA. Upon RT-PCR and SSCP analysis using patient fibroblast-derived RNA, three sequence variations were detected, one common polymorphism and two missense mutations. A poorly conserved Val at position 425 was mutated into a Met in one patient. In addition, a well conserved Val at position 490 was mutated to Met in all affected individuals from two Turkish families and one German family. In all these patients, the mutations were also detected in chromosomal DNA and were absent in 100 control chromosomes obtained from healthy individuals with similar ethnic background. Family segregation analysis of Val490Met revealed a pattern consistent with autosomal recessive inheritance. Consistent with these results, 3PGDH enzyme activities in fibroblast extracts of all patients were reduced to approximately 15% of control values. Taken together, this work demonstrates that mutations in 3PGDH are responsible for the observed defect in the synthesis of serine and glycine and underscore the importance of 3PGDH enzyme activity in the development and function of the central nervous system.

Program Nr: 16 from the 1999 ASHG Annual Meeting

Neuronal ceroid lipofuscinosis: A novel gene (CLN8) is mutated in human progressive epilepsy with mental retardation and the motor neuron degeneration mouse model. *S. Ranta[1], Y. Zhang[2], L. Lonka[1], A. Messer[3], S. Mole[4], R. Wheeler[4], J. Sharp[4], A. Hirvasniemi[5], A. de la Chapelle[1], T.C. Gilliam[2], A-E. Lehesjoki[1].* 1) Folkhälsan Institute of Genetics, Helsinki, Finland and Dept. of Medical Genetics, University of Helsinki; 2) Depts. of Psychiatry, Genetics and Development, and Columbia Genome Center, Columbia University, New York, NY; 3) Wadsworth Center, David Axelrod Institute, NY State Dept. of Health, Albany, NY; 4) Dept. of Paediatrics, Royal Free and University College Medical School, University College London, United Kingdom; 5) Dept. of Pediatrics, Kainuu Central Hospital, Finland.

Progressive epilepsy with mental retardation (EPMR), an autosomal recessive disorder characterized by onset of generalized seizures between the age of 5 and 10 years and subsequent progressive mental retardation, was recently recognized as a new subtype (CLN8) of neuronal ceroid lipofuscinosis. NCL comprises a group of progressive encephalopathies characterized by the accumulation of autofluorescent lipopigment in various tissues. A missense mutation (70C>G) cosegregating fully with the disease phenotype in all EPMR patients was identified in a novel putative transmembrane protein with no homology to previously known genes or proteins. It was not found in homozygosity in any of 433 control individuals. We assembled the homologous mouse gene (Cln8) sequence from EST clones. At the nucleotide and polypeptide level the human and mouse genes were 82% and 85% identical, respectively. The human mutation site, arginine 24 was conserved in the mouse gene. We localized the mouse gene to the same region as the motor neuron degeneration mouse (mnd), a naturally occurring model for NCL. In mnd/mnd mice a homozygous 1 bp insertion 267-268insC predicting a frame shift and a truncated protein was identified in the Cln8 gene. These data indicate that defects in these orthologous genes underlie NCL disease phenotypes in human and mouse. The identification of the disease gene underlying the mnd mouse is the first description of the genetic basis of a naturally occuring animal model for NCL.

Program Nr: 17 from the 1999 ASHG Annual Meeting

Mice deficient in b-**sarcoglycan exhibit a severe muscular dystrophy and cardiomyopathy.** *M. Durbeej[1], R. Cohn[1], R.F. Hrstka[2], V. Allamand[1], D.P. Venzke[1], B.L. Davidson[3], R. Williamson[2], K.P. Campbell[1]*. 1) HHMI, Department of Physiology and Biophysics, Neurology, University of Iowa, Iowa City, IA; 2) Department of Obstetrics and Gynecology, University of Iowa, Iowa City, IA; 3) Department of Internal Medicine, University of Iowa, Iowa City, IA.

b-sarcoglycan is a transmembrane protein of skeletal, cardiac and smooth muscle fibers. Loss or reduction of this protein in humans results in limb-girdle muscular dystrophy type 2E, a disease that is genetically and clinically heterogenous. To elucidate the function of b-sarcoglycan, the murine b-sarcoglycan gene was disrupted by homologous recombination. Sgcb-null mice developed a severe muscular dystrophy with an early onset and cardiomyopathy. In addition, Sgcb-null mice displayed irregularities of the vasculature due to the lack of the sarcoglycan complex in vascular smooth muscle. Loss of b-sarcoglycan in skeletal, cardiac and smooth muscle resulted in dissociation of the sarcoglycan and dystroglycan complexes at the muscle membranes, suggesting that the link between the cytoskeleton and extracellular matrix is disrupted in muscle cells. Deficiency of b-sarcoglycan also affected expression of e-sarcoglycan, a recently described a-sarcoglycan homologue, in skeletal muscle and also in cardiac and smooth muscle, suggesting that e-sarcoglycan is associated with b-sarcoglycan in muscle. Finally, by injection of recombinant b-sarcoglycan adenovirus the sarcoglycan complex was restored to the skeletal muscle membrane. In summary, the Sgcb-null mouse is a useful animal model for providing novel insights into the pathogenesis of LGMD2 with cardiomyopathy and smooth muscle dysfunction and also for evaluation of gene therapy strategies.

Program Nr: 18 from the 1999 ASHG Annual Meeting

Loss of the sarcoglycan complex and sarcospan leads to muscular dystrophy in b**-sarcoglycan-deficient mice.** *K. Araishi[1,2], T. Sasaoka[1], M. Imamura[1], S. Noguchi[1,3], H. Hama[1], E. Wakabayashi[1], M. Yoshida[1], T. Hori[2], E. Ozawa[1].*
1) Cell Biol, Natl Inst Neurosci , Natl Ctr Neurology/Psychiatry, Kodaira, Tokyo, Japan; 2) Dept. of Physiol., Faculty of Med., Kyushu Univ, Fukuoka 812-8582, Japan; 3) PRESTO, JST, Saitama 332-0012, Japan.

b-Sarcoglycan, one of the subunits of the sarcoglycan complex, is a transmembranous glycoprotein which associates with dystrophin, and is the molecule responsible for b-sarcoglycanopathy, a Duchenne-like autosomal recessive muscular dystrophy. To develop an animal model of b-sarcoglycanopathy and to clarify the role of b-sarcoglycan in the pathogenesis of the muscle degeneration *in vivo*, we developed b-sarcoglycan-deficient mice by using a gene targeting technique. b-Sarcoglycan-deficient mice (BSG -/- mice) exhibited progressive muscular dystrophy with extensive degeneration and regeneration. The BSG -/- mice also exhibited muscular hypertrophy characteristic of b-sarcoglycanopathy. Immunohistochemical and immunoblot analyses of BSG -/- mice demonstrated that the deficiency of b-sarcoglycan also caused loss of all of the other sarcoglycans as well as of sarcospan in the sarcolemma. On the other hand, laminin-a2, a- and b-dystroglycans and dystrophin were still present in the sarcolemma. However, the dystrophin-dystroglycan complex in BSG -/- mice was unstable compared with that in the wild-type mice. Our data suggest that the loss of the sarcoglycan complex and sarcospan alone is sufficient to cause muscular dystrophy, that b-sarcoglycan is an important protein for the formation of the sarcoglycan complex associated with sarcospan, and that the role of the sarcoglycan complex and sarcospan may be to strengthen the dystrophin axis connecting the basement membrane with the cytoskeleton.

Program Nr: 19 from the 1999 ASHG Annual Meeting

Disruption of the sarcoglycan-sarcospan complex in vascular smooth muscle: A novel mechanism in the pathogenesis of cardiomyopathy and muscular dystrophy. *R. Coral-Vazquez[1], R.D. Cohn[1], S.A. Moore[2], J.A. Hill[3], R.M. Weiss[3], R. Davisson[4], V. Straub[1], R. Barresi[1], D. Bansal[1], R.F. Hrstka[5], R. Williamson[5], K.P. Campbell[1]*. 1) Howard Hughes Medical Institute, Department of Physiology and Biophysics and Department of Neurology, University of Iowa College of Medicine, Iowa City, IA; 2) Department of Pathology, University of Iowa College of Medicine, Iowa City, IA; 3) Department of Internal Medicine, University of Iowa, and the Department of Veterans Affairs, Iowa City, IA; 4) Department of Anatomy and Cell Biology, University of Iowa, Iowa City, IA; 5) Department of Obstetrics and Gynecology, University of Iowa College of Medicine, Iowa City, IA.

Dilated cardiomyopathy is a multifactorial disease that includes both inherited and acquired forms of cardiomyopathy. Inherited cardiomyopathy in humans can be associated with genetic defects occurring in components of the dystrophin-glycoprotein complex (DGC). To investigate possible underlying mechanisms in the development of cardiomyopathy we analyzed genetically engineered mice deficient for either a-sarcoglycan (*Sgca*) or d-sarcoglycan (*Sgcd*), which shows a broader tissue expression including smooth muscle. We find that *Sgca*-null deficient mice for a-sarcoglycan, a protein expressed exclusively in skeletal and cardiac muscle do not display any cardiac abnormalities. In contrast *Sgcd*-null develop severe cardiomyopathy as revealed by morphological and physiological assessment of cardiac muscle. The main detectable histopathological features in skeletal and cardiac muscle are large areas of necrosis similar to alterations seen in tissue infarcts due to ischemic injury. While both animal models display disruption of the sarcoglycan-sarcospan (SG-SSPN) protein complex at skeletal and cardiac membranes, only *Sgcd*-null mice reveal perturbed expression of the SG-SSPN complex in smooth muscle. In vivo perfusion of the microvasculature exhibits numerous areas of microvascular constrictions in *Sgcd*-null mice. Therefore, our data demonstrate that disrupting of the SG-SSPN complex in vascular smooth muscle perturbs vascular function leading to ischemic injury, which induces cardiomyopathy and exacerbates muscular dystrophy.

Program Nr: 20 from the 1999 ASHG Annual Meeting

Autosomal XX sex reversal caused by duplication of SOX9. *B. Huang[1,2], S. Wang[3], Y. Ning[4], A.N. Lamb[5], J. Bartley[1]*. 1) Genzyme Genetics, Orange, CA; 2) University of California Irvine; 3) Harbor-UCLA medical Center, Torrance, CA; 4) Gene-Care Genetics Center and the George-Washington University, Washington, DC; 5) Genzyme Genetics, Santa Fe, NM.

SOX9 is one of the genes that play critical roles in male sexual differentiation. Mutations of SOX9 leading to haploinsufficiency can cause campomelic dysplasia and XY sex reversal. We present here the evidence supporting that SOX9 duplication can cause XX sex reversal.

A new born was referred to genetic evaluation due to ambiguous genitalia. The newborn had imcomplete masculinization with severe penile/scrotal hypospadias. The gonads were palpable. congenital adrenal hyperplasia was not identified as the serum electrolytes, 11-deoxycortisol and 17-hydroxyprogesterone were normal. Cytogenetic analysis revealed a mosaic 46,XX,dup(17)(q23.1q24.3)/46,XX karyotype. The dup(17) was observed in 34%of lymphocytes and 78% of the skin fibroblasts. Fluorescence in situ hybridization (FISH)with a BAC clone containing SOX9 gene demonstrated that the SOX9 gene is duplicated on the rearranged chromosome 17. The presence of SRY was ruled out by FISH with a SRY probe and PCR with SRY-specific primers. Microsatellite analysis with 13 markers on 17q23-24 suggests that the mechanism of the duplication is consistent with maternal mitotic unequal cross-over and defined the boundary of the duplicaiton to be approximately 12 centromorgans(cM) proximal and 4 cM distal to the SOX9.

Since SOX9 plays a critical role in male sexual determination and differentiation,its duplication apparently is the most likely etiology for causing the sex reversal in this case.To our knowledge, this is the first autosomal gene reported to be associated with XX sex reversal. Our study suggests that extra dosage of SOX9 is sufficient to initiate testis differentiation in absence of SRY. In addition, this study warrants thorough investigation of SOX9 function in other SRY negative XX sex reversal individuals, including study cell types other than lymphocytes.

Program Nr: 21 from the 1999 ASHG Annual Meeting

The mutational spectrum of the *Sonic Hedgehog* gene in holoprosencephaly: *SHH* mutations cause a significant proportion of autosomal dominant holoprosencephaly. *J.E. Ming[1], L. Nanni[1], M. Bocian[2], K.L. Jones[3], R.A. Martin[4], M.E.M. Pierpont[4], E. Roessler[5], M. Muenke[1, 5]*. 1) Children's Hospital of Philadelphia, Univ. of Pennsylvania School of Medicine; 2) Univ. CA Irvine; 3) Univ. CA San Diego; 4) St. Christopher's Hospital, Philadelphia, PA; Univ. of Minnesota Hospital; 5) Medical Genetics Branch, NHGRI.

Holoprosencephaly (HPE) is a common developmental anomaly of the human forebrain and midface in which the cerebral hemispheres fail to separate into distinct left and right halves. There is a very wide spectrum of expression of the disease. We have previously reported that haploinsufficiency for *Sonic Hedgehog*(*SHH*) can cause HPE. We have now performed mutational analysis of the complete coding region and intron-exon junctions of the *SHH* gene in 344 affected unrelated individuals. Our analysis detected 13 additional previously unreported individuals with *SHH* mutations, including 2 nonsense mutations, 6 missense mutations, 4 deletions, and 1 insertion. The mutations occur throughout the coding region of the gene. No specific genotype/phenotype correlation is evident based on the type or position of the mutation. In conjunction with our previous studies, we have identified a total of 23 mutations in 344 cases of HPE. They account for 13 familial cases and 10 sporadic cases of HPE. Overall, *SHH* mutations were detected in 10 of 27 families (37%) showing autosomal dominant transmission of the HPE spectrum, based on structural anomalies. *SHH* mutations were detected in probands without a family history of HPE at a lower frequency (3.7%). Interestingly, three of the patients with a *SHH* mutation also had nucleotide changes in another gene that is expressed during forebrain development, raising the possibility that interactions of multiple gene products and/or environmental elements may determine the final phenotypic outcome for a given individual. We speculate that the interplay of these factors may account for the wide variability in the clinical features seen in HPE.

Program Nr: 22 from the 1999 ASHG Annual Meeting

Clinical and molecular findings in Ehlers-Danlos syndrome type VIIA. *L. Hudgins[1], C.M. Cunniff[2], L.M. Drummond-Borg[3], M. Atkinson[1], U. Schwarze[1], P.H. Byers[1].* 1) Univ. of Washington, Seattle, WA; 2) Univ. of Arizona, Tucson, AZ; 3) Texas Dept. of Health, Austin, TX.

Ehlers-Danlos syndrome (EDS) types VIIA and B are dominantly inherited connective tissue disorders characterized by congenital hip dislocation and marked joint laxity resulting from mutations that interfere with splicing of exon 6 in the COL1A1 or COL1A2 genes. We identified 3 new individuals with mutations in COL1A1 and EDS VIIA who increase the range of muations and further define the phenotype. The 3 new patients all had de novo mutations of which two were unique (IVS5-1G->C and IVS5-2A->G) while the third (Exon6-1G->C) accounted for 2 of the 3 previously known cases. IVS5-1G->C resulted in use of a cryptic splice acceptor site in exon 6 that deleted 5 amino acids from the protein (including the protease cleavage site) while the exon 6 mutation led to skipping of the entire exon in a minority of transcripts. Cells were not available from the third patient; the same mutation in COL1A2 leads to use of the internal cryptic site. The IVS5-1 G->C mutation abolished proteolytic conversion of all proa1(I) chains produced by the mutant allele while the exonic mutation affected conversion of a minority of the chains. The alterations in proteolytic processing had dramatic effects on collagen fibril assembly. Like the 3 previously reported cases, the 3 new ones had congenital dislocation of the hips, marked joint laxity, positional deformities of the hands and feet, and soft lax skin. Other dislocations, umbilical hernias, large fontanels, and hypotonia were noted in 4/6 of all patients; blue sclerae and easy bruising were seen in 3/6; and inguinal hernias, micrognathia, and fractures were described in 2/6. Mutations in the genes that encode the proteolytic substrate for procollagen conversion, COL1A1 and COL1A2, result in a less severe phenotype than mutations in the enzyme, procollagen N-proteinase (EDS VIIC). The dramatic finding of umbilical hernia in EDS VIIA and C help distinguish them from EDS VIIB. Biochemical and molecular genetic studies can distinguish these clinical entities, and specific diagnosis is important for prediction of clinical course. (NIH AR21557).

Program Nr: 23 from the 1999 ASHG Annual Meeting

FBN2 mutations identified in congenital contractural arachnodactyly patients with aortic root dilatation. *S.G. Carmical, P. Gupta, D.M. Milewicz, E.A. Putnam.* Internal Med/Medical Genetics, Univ of Texas Medical School, Houston, TX.

Congenital contractural arachnodactyly (CCA or Beals syndrome) is an autosomal dominant disorder phenotypically similar to Marfan syndrome (MFS). CCA results from mutations in the FBN2 gene encoding fibrillin-2, while MFS results from mutations in the FBN1 gene encoding fibrillin-1. Features of CCA include dolicholstenomelia, arachnodactyly, scoliosis, congenital joint contractures and abnormal ear helices, but not aortic root dilatation (ARD). Neonatal MFS (nMFS) patients have many of these features, but also have valvular problems and ARD leading to early death. We have identified five patients with classic CCA with ARD. Using FBN2 intron-based, exon-specific primers for exons 23-33, genomic DNA was amplified and then screened by SSCP analysis. Two novel FBN2 mutations have been identified. The first patient (previously reported) had congenital scoliosis, arachnodactyly and joint contractures, and was initially thought to have nMFS when she developed progressive aortic root dilatation at age 2 years. A FBN2 mutation resulting in the missplicing of exon 31 was detected in the mRNA, and confirmed (G->C at +1 of intron 31) using the exon-specific primers. The second patient was diagnosed with CCA at birth and developed ARD at age 18 months. A FBN2 mutation in exon 28 (C1239R) was identified in the genomic DNA from this patient. We have also identified a putative mutation in FBN2 exon 33 in a third CCA patient who has ARD detected at age 6 years, which we are confirming. The fourth patient at age 12 years had a severely dilated ascending aorta. The fifth patient (30 years old) had mild ARD (3.9 cm) and mitral valve prolapse. This familial case has been linked to FBN2 using a polymorphic marker within the gene. None of the patients have undergone aortic surgery or are known to have dissected their aorta. In summary, ARD is a feature of CCA but has not been demonstrated to result in life-threatening aortic disease.

Program Nr: 24 from the 1999 ASHG Annual Meeting

Molecular and Clinical Analyses of the PIV (polydactyly - imperforate anus vertebral anomalies) Syndrome. *C.E. Killoran[1], M. Abbott[2], V.A. McKusick[3], L.G. Biesecker[1]*. 1) Genetic Disease Research Branc, NHGRI/NIH, Bethesda, MD; 2) Department of Psychiatry, Johns Hopkins University, Baltimore, MD; 3) Center for Medical Genetics, Johns Hopkins University, Baltimore, MD.

The PIV syndrome (OMIM 174100) includes an array of malformations including limb, vertebral column, and ano-rectal anomalies. This disorder was designated as a discrete entity in the OMIM database in 1986. We hypothesized that this syndrome is allelic to Pallister-Hall syndrome (PHS, OMIM 146510), a disorder that includes polydactyly and imperforate anus but is not known to cause vertebral anomalies. In addition to these anomalies, PHS is known to include hypothalamic hamartoma with or without pituitary and endocrine dysfunction, renal anomalies, and pulmonary segmentation anomalies. Truncation mutations in the Gli3 zinc finger transcription factor gene (OMIM 165240) cause PHS. We have performed a molecular evaluation of a patient with PIV and reviewed the existing literature on the condition. To determine if the reported patient has a mutation in Gli3, we sequenced exons comprising the open reading frame of this gene. The PIV patient had a deletion of nucleotides 2188-2207, resulting in a frameshift mutation and predicting a truncated protein product. In addition, subsequent clinical studies have shown that this patient has a hypothalamic hamartoma. Our analysis of the available reported cases of PIV syndrome is hampered by the limited clinical data that are presented in the three papers. However, from these reports it is difficult to determine that PIV syndrome is specific and distinct from the VACTERL association. We conclude that the PIV syndrome designation includes patients who have PHS and that the disorder may not be distinct from the VACTERL association. Patients with this constellation of findings should be evaluated for features of both PHS and VACTERL, with targeted use of molecular studies in patients who may have manifestations of PHS.

Program Nr: 25 from the 1999 ASHG Annual Meeting

Abnormal steroid biogenesis in 7 cases of Antley-Bixler syndrome suggests the possibility of digenic inheritance in some patients with this phenotype. *W. Reardon*[1], *R.M. Winter*[2], *S. Malcolm*[3]. 1) Dept Clin Gen, Ctr Medical Gen, Our Lady's Hosp Sick Children, Dublin, Ireland; 2) Dept. of Clinical Genetics, Institute of Child Health,30 Guilford St., London WC1N 1EH, England; 3) Molecular Genetics Unit, Institute of Child Health, 30 Guilford St., London WC1N 1EH.

The Antley-Bixler phenotype has been thought to be caused by an autosomal recessive gene. However patients with this phenotype have been reported with a new dominant mutation at the FGFR2 locus as well as in the offspring of mothers taking the antifungal agent, Fluconazole, during early pregnancy. In addition to the craniosynostosis and joint ankylosis which are the clinical hallmarks of the condition, several patients, especially females, have been reported who had genital abnormalities. We now report abnormal steroid biogenesis in 7 of 16 patients with an Antley-Bixler phenotype. Additionally, we identify FGFR2 mutations in 7 of these 16 patients, including one patient with abnormal steroidogenesis. Our findings, suggesting that some cases of Antley-Bixler syndrome are the outcome of 2 distinct genetic events, allow a hypothesis to be advanced which unites the different and seemingly contradictory circumstances in which Antley-Bixler syndrome has been recognised.

Program Nr: 26 from the 1999 ASHG Annual Meeting

Cholesterol supplementation for patients with the Smith-Lemli-Opitz Syndrome (SLOS): A review of five years' experience and avenues for future research. *E.R. Elias[1], M. Irons[2], A. Fulton[3], G.S. Tint[4], S. Salen[4]*. 1) Coordinate Care Svc, Children's Hosp, Boston, MA; 2) Div of Metabolism, Children's Hosp, Boston, MA; 3) Dept of Ophthalmology, Children's Hosp, Boston, MA; 4) VA Med Ctr, E. Orange, NJ.

We have been treating SLOS patients with a concentrated suspension of cholesterol in soy bean oil since January of 1994, following our discovery of a defect in cholesterol metabolism as the cause of this disorder. Eighteen patients with SLOS, ranging in age from birth to mid teens, and ranging in pretreatment cholesterol levels from 8-90 mg/dl have been followed by us every 1-3 months for up to five years while on treatment. Even when treatment is begun beyond the newborn period, there are benefits of cholesterol supplementation, and no adverse reactions to therapy have been noted. The benefits include improved growth and nutrition, better tolerance of infection, and improvement in behavior. We have discovered problems in our patients, which were not previously known to occur in SLOS. For example, electroretinogram studies have demonstrated abnormal retinal function in all patients studied, and a severe pigmentary retinopathy has been demonstrated in one older patient. A second problem, photosensitivity, is now being studied in vitro in skin fibroblasts, and in vivo using timed exposure to UV light. We have documented acanthocytosis, and the development of hypersplenism and thrombocytopenia in some patients. Hearing loss has been demonstrated in more than half of the patients studied. Ongoing studies are documenting developmental course, CNS structure and metabolic status on MRI/MRS, and hearing status with ABR. Future plans include further investigation of the retinal, skin, hematologic and hearing problems. Our close follow-up of this patient cohort has allowed us to appreciate the complexity of issues seen in children with severe cholesterol deficiency, and to develop a greater understanding of the natural course of SLOS.

Program Nr: 27 from the 1999 ASHG Annual Meeting

Familial oesophageal atresia is linked to chromosome 2. *H.G. Brunner[1], J. Celli[1], E. van Beusekom[1], R.C.M. Hennekam[2], R. Konig[3], M. Frydman[4], J.W. Innis[5], L. Govaerts[5], M. van Steensel[1], H. van Bokhoven[1]*. 1) Dept Human Genetics, University Hospital Nijmegen, Nijmegen, The Netherlands; 2) Dept Human Genetics, University of Amsterdam, The Netherlands; 3) Dept Human genetics, University of Frankfurt, Germany; 4) Medical Genetics, Chaim Sheba Medical center, Tel Aviv, Israel; 5) Dept human Genetics, University of Michigan, Ann Arbor, USA/Erasmus University Rotterdam, The Netherlands.

Oesophageal atresia is a potentially life-threatening congenital abnormality of which the genetic basis is currently unknown. In order to discover the genetic pathways that are involved in oesophageal atresia, we are studying a rare autosomal dominant disorder with digital abnormalities, microcephaly, short palpebral fissures, learning disabilities and oesophageal/duodenal atresia. This condition is known as Oculodigitoesophagoduodenal syndrome (ODED syndrome, MIM #164280). We have studied 4 pedigrees, including a 3 generation Dutch family with 11 affected members. After exclusion of several chromosomal segments harboring candidate genes, we undertook a genome-wide linkage analysis. Clear evidence for linkage between the syndrome and two markers D2S170 and D2S390 (Zmax = 3.91 at 'q'=0)was detected in three families. The critical region spans about 20 cM in the 2p22.3 region. In the fourth family, a set of five consecutive markers showed inheritance of an apparent null allele. This is consistent with a microdeletion in this area. Together, the linkage analysis and the microdeletion restrict the critical region to a 5cM area in 2p22-p23. Interestingly, linkage analysis in a small family with non-syndromic oesophageal atresia is also consistent with this locus. This is the first genetic locus associated with oesophageal atresia in humans.

Program Nr: 28 from the 1999 ASHG Annual Meeting

Prenatal cortical hyperostosis (Caffey Disease): clinical, radiographic and chondro-osseous findings in 9 new cases. *V. Cormier-Daire[1,2], R. Savarirayan[1,3], W.R. Wilcox[1], R.S. Lachman[1], D.L Rimoin[1].* 1) Medical Genetics-Birth Defects Center, Cedars-Sinai Medical Center, Los Angeles, CA; 2) Department of Genetics, Hopital Necker, Paris, France; 3) Victorian Clinical Genetics Service, Melbourne, Australia.

We present the clinical, radiographic and histologic findings of 9 cases of prenatal cortical hyperostosis (PCH/Caffey disease)from 7 families. The cases were characterized clinically by polyhydramnios from the first trimester, premature delivery, generalized edema at birth, respiratory distress, micrognathia and hepatosplenomegaly. Radiographic hallmarks comprised generalized, symmetric osteosclerosis with marked periosteal cloaking and short, bowed long bones. Chondro-osseous findings comprised markedly abnormal bone with thick cortices, a long hypertophic zone, hypercellularity and fibrosis between trabeculae and enlarged marrow spaces. Four out of the 9 cases died in the first hour of life secondary to respiratory distress and one pregnancy was electively terminated. Of the 4 survivors, one is still a neonate and remains intubated. The three others are aged 9 months, 12 years and 13 years. Their bone abnormalities have markedly improved over time apart from persistent abnormal modelling of the long bones. Two of them have global developmental delay. PCH is probably inherited as an autotosomal recessive trait, given the observations in this series that both sexes are affected (7 boys, 2 girls), sib recurrence (2 cases) and consanguinity (1 case). These data, together with the above clinical and radiographic features, strongly suggest that this prenatal form of infantile cortical hyperostosis is a distinct entity, separate from the classical form of Caffey disease.

Program Nr: 29 from the 1999 ASHG Annual Meeting

A previously unrecognized phenotype characterized by obesity, muscular hypotonia, and ability to speak in patients with Angelman syndrome caused by an imprinting defect. *B. Horsthemke, C. Lich, G. Gillessen-Kaesbach.* Institut fuer Humangenetik, Universitaet Essen, Essen, Germany.

The clinical features of Angelman syndrome (AS) comprise severe mental retardation, postnatal microcephaly, macrostomia and prognathia, absence of speech, ataxia, and happy disposition. Most of the patients have a maternal deletion 15q11-q13, paternal UPD 15, or mutations of the *UBE3A* gene. A few per cent of patients have apparently normal chromosomes of biparental origin, but the maternal chromosome 15 carries a paternal imprint. Such an imprinting defect can result from an imprinting center (IC) mutation or occur spontaneously. We report on seven patients who lack most of the typical AS features, but presented with obesity, muscular hypotonia and mild mental retardation. Based on the clinical findings, the patients were initially suspected of having Prader-Willi syndrome (PWS). Cytogenetic studies and microsatellite analysis demonstrated apparently normal chromosomes 15 of biparental inheritance. DNA methylation analysis of *SNRPN* and *D15S63*, however, revealed an AS pattern, i.e., the maternal band was faint or absent. These results suggest that the patients have an imprinting defect and a previously unrecognized form of AS. Most notably, all of the patients lacked ataxia, and three patients were able to speak and to communicate with others. Ataxia and absence of speech were hitherto regarded as almost mandatory features of AS. Following current diagnostic criteria, an experienced clinician would not have made the diagnosis of AS or PWS in these patients. In contrast to PWS, in which obesity usually starts in early childhood, our patients were already overweight in their first year of life. Our accidental finding of these patients strengthens the notion to perform a methylation test in all children presenting with obesity, muscular hypotonia and mental retardation. Knowing the phenotype of the patients described here may also help to recognize this form of AS more often.

Program Nr: 30 from the 1999 ASHG Annual Meeting

Spastic paraplegia, ataxia, mental retardation (SPAR): a novel genetic disorder . *P. Hedera[1], S. Rainier[1], XP. Zhao[1], J. Trobe[1], J. Wald[1], O.P. Eldevik[2], K. Kluin[1], J.K. Fink[1,3]*. 1) Department of Neurology; 2) Department of Radiology, University of Michigan, Ann Arbor; 3) Geriatric Research Education and Clinical Center, Ann Arbor Veterans Affairs Medical Center.

Inherited cerebellar degeneration (ataxias) and inherited spastic paraplegias are genetically diverse disorders. We identified a family with a unique, dominantly inherited, neurologic disorder in which affected individuals exhibit either spastic paraplegia or a combination of cerebellar ataxia, lower extremity spastic weakness, and variable mental retardation. Pedigree analysis is consistent with a single gene, autosomal dominant disorder. Magnetic resonance imaging showed marked atrophy of the cerebellum and spinal cord. Laboratory analysis showed that the disorder was not due to gene mutations that cause spinocerebellar ataxias (SCA) 1, 2, 3, 6, or 7; not linked to other known loci for autosomal dominant ataxia (SCA 5, 8, or 10); and not linked to known loci for autosomal dominant hereditary spastic paraplegia (HSP) (SPG 3, 4, 6 and 8 on chromosomes 14, 2, 15, and 8, respectively). We designate this novel Spastic Paraplegia, Ataxia, mental Retardation syndrome as SPAR. Inter-generation differences in age-of-symptom onset suggested genetic anticipation, a feature of many disorders due to expanded trinucleotide repeat mutations. Using repeat expansion detection, we detected expanded CAG repeats in three affected subjects (but not in unaffected subjects). We showed that the expanded CAG repeat was not expansion of benign CAG repeats on chromosomes 17 and 18.
Our findings suggest that SPAR is due to an expanded CAG repeat. Extreme phenotypic variablity indicates major regional differences in the distribution of brain and spinal cord involvement among affected family members who share mutations in the same gene. Significant ataxia and mental retardation were present only in later generations. We propose that SPAR is due to a CAG repeat expansion. We speculate that regional vulnerability within the CNS to the effects of the SPAR gene mutation is influenced by the variable size of the CAG repeat (which may be expanded in subsequent generations).

Program Nr: 31 from the 1999 ASHG Annual Meeting

Cloning and sequencing of the breakpoints associated with Recombinant 8 syndrome. *S.L. Graw[1], T. Sample[1,2], J. Bleskan[1], D. Patterson[1]*. 1) Eleanor Roosevelt Inst, Denver, CO; 2) University of Colorado at Denver, Denver, CO.

Recombinant 8 syndrome (Rec8 syndrome, San Luis Valley syndrome) is a chromosomal disorder found in individuals with Hispanic ancestry from the San Luis Valley of southern Colorado and northern New Mexico. Affected individuals have mental retardation, congenital heart defects, and other typical features. The syndrome is due to the presence of a recombinant 8 chromosome, with duplication of 8q22 - 8qter and deletion of 8p23.1 - 8pter. This chromosome is the result of recombination of a parental pericentric inversion 8 chromosome, with breakpoints at 8q22 and 8p23.1. The presence of the inversion 8 chromosome in this population is believed to be the result of a Founder Effect.

In order to understand the molecular basis of the Inversion 8 chromosome and other 8p23.1 rearrangements, we have cloned and sequenced both the 8p23.1 and 8q breakpoints. YAC isolation and mapping of the breakpoint sequences confirms that the Inversion 8 chromosome is the result of a simple inversion event involving only chromosome 8. Sequence analysis indicates that the regions surrounding the breakpoints are repetitive, and we postulate that these repetitive sequences may be associated with the rearrangement event. We have used the sequences surrounding the breakpoints to design a PCR-based assay that allows us to differentiate between the normal, Recombinant 8, and Inversion 8 chromosomes.

Program Nr: 32 from the 1999 ASHG Annual Meeting

Clustering of the constitutional t(11;22) breakpoint on both chromosomes 11 and 22 and narrowing the breakpoint to a <200 kb region on 11q23. *T. Shaikh, M. Budarf, L. Celle, E. Zackai, B. Emanuel.* The Childrens Hospital of Philadelphia. Division of Human Genetics and Molecular Biology, Phila., PA 19104.

The t(11;22) (q23;q11.2) is the only known recurrent, non-Robertsonian, constitutional translocation in humans. Balanced carriers of the constitutional t(11;22) translocation are phenotypically normal but are at risk of having progeny with the Supernumerary der(22) t(11;22) Syndrome as a result of malsegregation of the der(22). We have analyzed t(11;22) balanced carriers from multiple unrelated families by FISH to localize the breakpoints on both chromosomes 11 and 22. We had previously reported twelve cases in which the chromosome 22 breakpoint was localized between markers D22S788 (N41) and ZNF74 on 22q11. We have extended this study to include twenty-three unrelated, balanced carriers. The breakpoint localizes to the same 400 kb interval on 22q11 flanked by markers D22S788 and ZNF74. This region of 22q11 contains DNA sequences that are duplicated in multiple regions on chromosome 22. This duplication has hindered the process of traversing the chromosome 22 t(11;22) breakpoint by chromosome-walking. The chromosome 11 breakpoint region of a single t(11;22) carrier has been previously located distal to D11S1340 and proximal to APOA1 in 11q23.2. We report the isolation of a 185 kb BAC (Bacterial Artificial Chromosome) which spans the t(11;22) breakpoint on 11q23. The genomic DNA insert in the BAC contains both D11S1340 and APOA1. Thirteen of the aforementioned twenty-three carriers were analyzed by FISH with this chromosome 11 BAC as probe. In all thirteen cases signal was detected on the normal 11, the der(11) and the der(22) chromosomes. This suggests that the BAC spans the t(11;22) breakpoint on chromosome 11 in multiple t(11;22) carriers. Interphase FISH with this BAC resulted in two discrete signals suggesting that the genomic DNA contained within the BAC does not contain duplicated sequences. Thus, it appears that in multiple unrelated families the breakpoints on both chromosomes 11 and 22 are clustered. We are currently analyzing the chromosome 11 BAC to further sub-localize the t(11;22) breakpoint and identify a rearranged junction fragment from the t(11;22).

Program Nr: 33 from the 1999 ASHG Annual Meeting

Inter-chromosomal homologous recombination could mediate large *NF1* gene deletions. *C. Lopez-Correa[1], H. Brems[1], C. Lazaro[2], X. Estivill[2], M. Clementi[3], S. Mason[3], P. Marynen[1], E. Legius[1]*. 1) Center for Human Genetics, University Hospital Gasthuisberg, Leuven, Belgium; 2) Medical and Molecular Genetics Center-IRO, Hospital Duran I Reynals, Barcelona, Spain; 3) Servizio di Genetica Medica, Universita di Padova, Italy.

Molecular studies were performed in 21 unrelated patients with sporadic Neurofibromatosis type I carrying a large NF1 gene deletion. A high-resolution physical map, containing 53 PACs, was constructed covering the 3' and 5' flanking regions of the NF1 gene. By FISH experiments, PACs 1026A18, 1135F8, 933H9, 93O20 and 160L21 (3' region) as well as PACs 960O18, 409L16 and 926B9 (5' region) were deleted in 19 patients, showing that the deleted region extends at least 500 kb in the 3' and 300 Kb in the 5' region outside the NF1 gene. Four new polymorphic markers were generated in the flanking regions of the NF1 gene and are included in the deleted region. A total set of twelve intra- and extragenic markers was tested in the patients and available parents. The deletions were of maternal origin in 12 cases and of paternal origin in 2 cases. No evidence of different size in the deletions was observed except for one patient with a deletion of paternal origin, who shows a shorter deletion including only the NF1 gene. FISH experiments demonstrate that the boundaries of the deletions are flanked by duplicated sequences. PACs located at the deletion region crosshybridize on chromosome 19p and PACs located at the 3' and 5' borders of the deletion are duplicated signals on different locations on the long arm of chromosome 17. We used three extragenic markes (D17S841, D17S842 and D17S1880) to perform segregation analysis of grandparental haplotypes in three families with three informative generations. Using these markers we were able to show that a meiotic maternal recombination had occurred at the critical deleted region, suggesting an unequal crossing-over event between the two homologous chromosomes 17 possibly mediated by the duplicon.

Program Nr: 34 from the 1999 ASHG Annual Meeting

Molecular characterization of an alpha-satellite junction. *J.E. Horvath[1], C.L. O'Keefe[1], L. Viggiano[2], S. Schwartz[1], M. Rocchi[2], E.E. Eichler[1]*. 1) Dept of Genetics, Case Western Reserve Univ, Cleveland, OH; 2) Instituto di Genetica, Bari, Italy.

The pericentromeric regions of human chromosomes represent a complex area of our genome filled with duplications and repetitive elements. We are investigating the nature of the pericentromeric regions using chromosome specific BACs to simplify the complex organization. We have identified one BAC from chromosome 16 that is 162 KB in size and contains an alpha-satellite/ non-alpha-satellite junction. Large-scale sequence analysis shows that this BAC shares large contiguous blocks of sequence with BACs from chromosomes 2p11, 10p11 and 22q11. Extended chromatin FISH confirmed that this BAC lies near traditional alpha-satellite DNA and contains at least two duplicated genes that abut monomeric alpha-satellite DNA. The paralogous genes (ALD and a novel cDNA) are 94-96% similar to the ancestral loci which map to Xq28 and 4q24 respectively. We have identified G-C rich repetitive elements that lie at the boundaries of these duplications and may be involved in the duplication process. Ten PCR primer pairs were developed along this chromosome 16 BAC in order to characterize the duplications at the sequence level. Using these pairs against a monochromosomal somatic cell hybrid panel of DNAs, we found an average of 4.7 duplications per PCR primer pair in the genome which were 96.4% similar to each other, suggesting that the duplications have occurred recently in evolutionary time. These duplications were confirmed by FISH analysis using long range PCR products and cosmids as probes on human metaphase chromosomes. Comparative FISH analysis using several of these probes indicated that the genic duplications are specific to greater apes. These data provide the first detailed molecular characterization of an alpha-satellite junction and suggest that such regions of the genome may play a role in genomic instability and human genome evolution.

Program Nr: 35 from the 1999 ASHG Annual Meeting

Cytogenetic, molecular and phenotypic analysis about 80 patients with monosomy 5p. *C. Perfumo*[*1], P. Cerruti Mainardi*[*2], A. Cali[1,2], G. Coucourde[2], G. Pastore[2], S. Cavani[1], F. Zara[1], J. Overhauser[3], F. Dagna Bricarelli[1], M. Pierluigi[1]*. 1) Human Genetic Laboratory, Galliera Hospital, Genoa, Italy; 2) Departement of Pediatrics and Genetics, S. Andrea Hospital, Vercelli, Italy; 3) Thomas Jefferson University, Philadelphia, PA.

Cri du Chat Syndrome (CdCS), one of the more common deletion syndromes, involving the short arm of chromosome 5, is characterized by specific dysmorphisms, cat-like cry, microcephaly and psychomotor retardation. 80 patients from the Italian CdCS Register (192 patients) were analysed to study the phenotype-genotype correlation. Molecular cytogenetic analysis showed 62 patients (77.5%) with a 5p terminal deletion with an interval of breakpoints ranging from p13 (locus D5S743) to p15.2 (locus D5S18); 7 patients (8.75%) with a 5p interstitial deletion; 4 patients (5%) with a de novo translocation involving 5p; 3 patients (3.75%) with a 5/autosome translocation inherited from a parent (2 maternal, 1 paternal); 3 patients (3.75%) with mosaic de novo 5p anomalies involving two rearranged cell lines; 1 patient (1.25%) with a 5p deletion derived from a paternal chromosomal inversion. The origin of the deleted chromosome 5 was paternal in 55 patients (90.2%) and maternal in 6 patients (9.8%). The study of 62 patients with terminal isolated deletions highlighted a progressive severity of psychomotor retardation and clinical manifestations related to the deletion size. The 7 patients with interstitial deletions and 1 with a little terminal deletion confirmed the existence of two critical regions, one for dysmorphisms and mental retardation in p15.2 (CdCCR) and the other for the cat cry in p15.3; one of these patients allowed to narrow distally the cat cry region from D5S13 to D5S731. CdCS phenotype seems to prevail both in mosaic and in translocation cases. The magnitude of risk for carriers of translocation involving 5p was defined by the evaluation of personal and reviewed data from 54 pedigrees. **Work supported by Telethon-Italy and Italian Cri du Chat Children's Association**

*** These authors contributed equally to this work**.

Program Nr: 36 from the 1999 ASHG Annual Meeting

Delineation of the critical region for the 9p deletion and duplication syndromes: Implications for overlapping critical regions. *S. Schwartz[1], C.A. Crowe[2], D. Everman[1], M. Eichenmiller[1], M.A. Micale[3], D.L. Satinover[1], L.A. Christ[1]*. 1) Ctr Human Genetics and Dept of Genetics, Case Western Reserve Univ and Univ Hosp of Cleveland, Cleveland, OH; 2) Dept of Pediatrics, MetroHealth Medical Center, Cleveland, OH; 3) Dept of Pathology, Medical College of Ohio, Toledo, OH.

Over the past several years molecular techniques have enhanced efforts to delineate both deletion and duplication syndromes. These methodologies have assisted in characterizing critical regions for some chromosomal deletion and duplication syndromes, and identifying genes responsible for the syndromes. However, little is still known about many syndromes and even less is known about deletion and duplication syndromes involving the same chromosomal region. In order to better understand the relationship between deletion and duplication syndromes, we have utilized molecular cytogenetic technologies to study both the 9p deletion and duplication syndromes. The 9p deletion syndrome is a well characterized syndrome which features includes: dysmorphic facies (trigonocephaly, midface hypoplasia, and upslanting palpebral fissures), hypotonia, and mental retardation. In contrast, growth and developmental retardation, microbrachycephaly, deep and wide-set eyes with downslanting palpebral fissures, bulbous nose and down-turned corners of the mouth, characterize the 9p duplication syndrome.

We have now studied over 50 patients with deletions and duplications involving 9p. We have utilized molecular techniques (using PCR with microsatellite markers) and molecular cytogenetics techniques (FISH with YACs and BACs) to systematically characterize the deletions and duplications. Results from these studies show that: (1) a critical region of less than 1 Mb in size has been identified as responsible for the 9p deletion syndrome; (2) an overlapping region has also been identified as responsible for the 9p duplication syndrome; (3) a contig of this region has been constructed using YACs and BACs. As both the 9p deletion and duplication syndrome have contrasting phenotypic features, it is intriguing that either the deletion or duplication of a similar chromosomal segment is responsible for the contrasting phenotypes.

Program Nr: 37 from the 1999 ASHG Annual Meeting

Molecular cytogenetic analysis of neocentromere formation in seven inv dup(13q) chromosomes. *P.E. Warburton[1], M.P. Dolled[1], J.O. Van Hemel[2], L.C.P. Govaerts[2], C.Y. Yu[3], D. Warburton[3]*. 1) Dept Human Genetics, Mount Sinai Sch Medicine, New York, NY; 2) Dept. Clinical Genetics, University Hospital Dykzigt, Rotterdam; 3) Dept. Genetics and Development, Columbia University, New York, NY.

Neocentromeres are newly derived centromeres found in previously non-centromeric chromosomal locations on rearranged chromosomes. They permit analysis of centromere formation in the absence of the large amounts of repetitive DNA found at normal centromeres. Seven independent patients have been identified with a neocentromere providing mitotic stability to a supernumerary inv dup(13q). The clinical spectrum of partial tetrasomy 13q overlaps trisomy 13. These rearranged chromosomes are missing the normal 13cen and do not hybridize by FISH with pancentromeric alpha satellite DNA, yet contain active centromeres as shown by immunofluorescence (IF) with antibodies to CENP-C and hMAD2. We are using this unique collection of cell lines to determine whether multiple independent 13q neocentromeres have formed on the same genomic DNA and/or at inversion breakpoints, enabling evaluation of the role of primary sequence or chromosomal rearrangement in neocentromere formation. Molecular analysis using FISH with ordered cosmids from the physical map of 13q combined with IF to centromere proteins permits localization of the neocentromere and inversion breakpoints to genomic clones. In three cases, the breakpoint and the neocentromere colocalize to band 13q32; one of these breakpoints has thus far been mapped to a contig of 3 YACs. A fourth case also has a q32 neocentromere, but the breakpoint is in q21. This 13q32 neocentromere has been mapped slightly distal to the YAC contig containing the13q32 breakpoint already defined. Notably, the q32 sequences on the other arm of this symmetrical chromosome do not form a neocentromere. Another inv dup(q21) and an inv dup(q14) both have neocentromeres in q21. Localization of 4 independent neocentromeres to 13q32 and two to 13q21 suggests that sequences in these regions have a propensity for neocentromere formation. The presence of 4 neocentromeres at or near inversion breakpoints also suggests a possible role of chromosomal rearrangement in neocentromere formation.

Program Nr: 38 from the 1999 ASHG Annual Meeting

Epigenetic effects on centromere activity of dicentric X chromosomes. *A.W. Higgins, H.F. Willard.* Case Western Reserve Univ, Cleveland, OH.

Human dicentric chromosomes can be stabilized by inactivation of one of the centromeres (cens), making a structurally dicentric chromosome functionally monocentric. We previously described a somatic cell hybrid system to create in culture dicentric Xp isochromosomes, i(Xp)s, thus providing a tractable model of patient-derived dicentrics for the study of cen structure and function. By using indirect immunofluoresence with an antibody to cen protein-E, a marker for active cens, we reported three catagories of idic(Xp)s: those with two active cens, those with one active cen and others heterogeneous for cen activity. To further examine the clonal inheritance of cen activity, we derived multiple single-cell subclones from two clones: heterogeneous clone A1, in which some cells have one active cen and others have two active cens, and clone B1, in which all cells have two active cens. The majority of A1 subclones were heterogeneous (6/7), although one subclone showed two active cens in all cells. Subclones of hybrid B1 were largely, but incompletely, consistent with B1 itself; 2/5 had two active cens in all cells; 3/5 had rare cells with only one active cen. These data provide evidence that cen activity can switch from the active to the inactive state, even within a clonal population of cells. To explore a possible epigenetic mechanism of cen inactivation, we treated our clones with trichostatin A, TSA, a histone deacetylase inhibitor shown to affect cen function in yeast. TSA treatment of a clone with one active cen in all cells (>120 cells), resulted in 6/233 cells with two active cens, in contrast to mock-treated cultures that never showed two active cens (0/74 cells). TSA treatment of a different clone with two active cens in all cells (>120 cells) resulted in 5/123 cells with only one active cen, compared with mock-treated cultures that retained two active cens in 96 cells. Thus, both idic(Xp)s with one and two active cens show reversal of cen activity state upon TSA treatment. We conclude that: cen activity in dicentric chromosomes is under epigenetic control; both active and inactive cens can switch their epigenetic states in culture; and histone acetylation may play a role in determining and/or maintaining cen function.

Program Nr: 39 from the 1999 ASHG Annual Meeting

Multicolor banding (MCB) of human chromosomes 1, 5 and 13 based on region specific YAC clones and microdissection libraries. *T. Liehr[1], A. Heller[1], H. Starke[1], J. Lemke[1], J. Wirth[2], G. Senger[3], I. Chudoba[4], M. Rocchi[5], U. Claussen[1]*. 1) Inst. Human Genetics and Anthroplogy, Jena, Germany; 2) Max-Planck-Institute for Molecular Genetics, Berlin-Dahlem, Germany; 3) Practice of Medical Genetics and Gynaecology, Regensburg, Germany; 4) MetaSystems Inc., Altlussheim, Germany; 5) Institute of Genetics, Bari, Italy.

Recently, a new multicolor-banding (MCB) technique based on five different fluorochromes has been developed, allowing the differentiation of chromosome region specific areas at the band and sub-band level (Chudoba et al., Cytogenet. Cell Genet., in press, Liehr et al. 1999, Med. Genetik 11:132). This technique is based on regionspecific libraries producing changing fluorescence intensity ratios along the chromosomes. The latter are used to assign different pseudocolors to specific chromosomal regions. Microdissection libraries or nonchimeric chromosome-specific YAC-clones can be used as such regionspecific libraries. We present 10 clinical cases with congenital or acquired complex chromosomal rearrangements involving the chromosomes 1, 5 and 13. In some of these cases neither conventional GTG-banding nor 24-color-FISH could resolve the complex changes, e.g. an institial deletion of #13 [del(13)(q13q22)], an insertion of a part of #1q (31.2-32) in 1p31.2 or an inverted insertion of a part of #1p (22.2-33) in 1p22.2. Using this new and straight forward MCB technique, complex rearrangements, could be clarified with one single flourescence in situ hybridization (FISH) experiment. Work for the creation of MCB probes for other human chromosomes is in progress. 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: 40 from the 1999 ASHG Annual Meeting

High resolution analysis of chromosomes using new M-FISH strategies and multicolor bar coding. *R. Eils[1], A. Bolzer[2], K. Saracoglu[1], J. Kraus[2], C. Fauth[2], S. Uhrig[2], M.R. Speicher[2].* 1) Interd Ctr Sci Computing, Univ Heidelberg, Heidelberg, Germany; 2) Institute of Anthropology and Human Genetics, Ludwig Maximilians University, Munich, Germany.

Multiplex-FISH (M-FISH) allows the visualization of the 24 different human chromosomes in different colors and is therefore capable of identifying readily both simple and complex chromosomal abnormalities. Probe labeling is done employing the combinatorial labeling strategy which needs a minimum of five fluors. In order to increase resolution we increased the number of fluors for probe labeling from five to seven. This results in a significant reduction of probe complexity because triple combinations of fluors can be avoided which facilitates image analysis and reduces pitfalls caused by the combinatorial labeling. This improvement should be of importance for the detection of subtle aberrations in diagnostic applications. However, chromosome specific painting probes are problematic for the detection of structural intrachromosomal rearrangements. The exact resolution limits for cryptic translocations are difficult to establish. To overcome these limitations we constructed multicolor bar codes consisting of multiple combinatorially labeled YAC clones for the analysis of intrachromosomal rearrangements. In a close collaboration with Drs. Lyndal Kearney and Jill Brown (Oxford, UK) screening for subtelomeric rearrangements is done by the simultaneous hybridization of multiple clones from a "second generation" set of chromosome-specific cosmid, PAC and P1 clones, all confirmed as within 500 kb of their respective chromosome end. For analysis of combinatorially labeled region specific probes we developed a technique for multicolor image analysis originally developed for fully automated multicolor karyotyping. It will be shown that by combining color and local neighborhood information even noise dominated M-FISH images can be analyzed in a fully automated way. Thus, even small intrachromosomal rearrangements can now be analyzed in an automated way. These techniques bear the potential to evolve to a powerful tool for the detection of subtle chromosomal rearrangements difficult to access by other means.

Program Nr: 41 from the 1999 ASHG Annual Meeting

Comparative Genomic Hybridization Arrays: Towards a "telomere chip". *C.M. Lese[1], X. Zhang[2], D. Pinkel[2], P. Bao[3], N. Lermer[3], D. Che[3], J. Shi[3], U. Müller[3], D.H. Ledbetter[1]*. 1) Dept of Human Genetics, Univ of Chicago, Chicago, IL; 2) Cancer Center, Univ of California, San Francisco, San Francisco, CA; 3) Vysis, Inc., Downers Grove, IL.

Comparative genomic hybridization (CGH) is a powerful molecular cytogenetic technology that allows genome-wide analysis of DNA copy number in a single assay. By hybridizing patient genomic DNA to normal metaphase chromosome targets in the presence of normal reference DNA, this technique achieves a genome-wide scanning capability similar to conventional cytogenetic banding analysis. The major advantage of CGH is that extracted genomic DNA is used for analysis instead of metaphase chromosomes; however, it is limited by a resolution of about 10-20 Mb, less than that of conventional cytogenetics, and can only identify gains or losses of genetic material. Modifications to the basic CGH method have been successfully achieved using arrays of cloned DNA sequences as the target DNA, thus increasing the resolution of this technique. Using this methodology, we are developing a telomere chip, containing BAC, PAC or P1 clones unique to every human telomere. Since approximately 5% of idiopathic mental retardation is estimated to be due to cryptic unbalanced telomere rearrangements, this technology will not only allow rapid screening of affected probands to identify telomere abnormalities, but will also afford large studies of various patient populations to assess the overall frequency of such rearrangements. To this end, we have begun to validate the feasibility of a telomere chip by verifying telomeric aberrations previously identified by fluorescence *in situ* hybridization (FISH) using chromosome specific telomere probes. In a blind study, the following four cases were tested with a prototype telomere chip: two 22q telomere deletions, an unbalanced 16p;17p translocation resulting in trisomy for 16p and monosomy for 17p and an unbalanced 7p;16p translocation resulting in trisomy for 16p. Using the CGH array technology, we were able to detect ratios of approximately 2:1 or 3:2 for each of the telomere rearrangements examined, thus demonstrating the potential of this technique for wide-spread telomere screening.

Program Nr: 42 from the 1999 ASHG Annual Meeting

Microarray Analysis Discriminates BRCA2 Mutation-Positive Breast Cancer Biopsies from BRCA1 Mutation-Positive and Sporadic Breast Tumors. *I.A. Hedenfalk[1,2,3], D.J. Duggan[2,3], Y. Chen[2], M. Bittner[2], A. Borg[1], J.M. Trent[2]*. 1) Department of Oncology, Lund University, SE-221 85 Lund, Sweden; 2) Cancer Genetics Branch, National Human Genome Research Institute, NIH, Bethesda, MD 20892; 3) Contributed equally to this work.

Distinct pathologic and genetic alterations distinguish BRCA1 and BRCA2 mutation-positive from sporadic (non-familial) breast cancers. Using cDNA microarrays containing 6500 cDNAs, we have compared BRCA1 and BRCA2 mutation-positive and sporadic biopsies against a reference cell line. Relational analysis of individual genes has identified a small subset of genes which are specifically up or down regulated in the majority of the BRCA2 biopsies. Similarly, we have identified a number of genes specific to BRCA1 tumors. "Clustering" of tumor expression data was performed by several approaches, including placing those genes with the most tightly linearly correlated profiles together by use of a hierarchical clustering dendrogram and clustering the samples on the basis of the linear correlation of the total gene set. Multi-dimensional scaling (MDS) analysis was also used to estimate the approximate degree of correlation between tumors across the total set of genes. Our use of systems-level exploration of the transcriptional regulatory networks of these cancers clearly distinguishes BRCA2 tumors from all other biopsies. Statistical analysis of the MDS result has been performed to identify the genes distinguishing BRCA2 from other tumor biopsies. Examples of genes whose expression is a significant discriminator included cyclin D1, which was consistently up-regulated in the BRCA2 biopsies, while unchanged in BRCA1 tumors. It is known that cyclin D1 overexpression is correlated with estrogen receptor (ER) positivity, and in our sample set all BRCA1s analyzed were ER negative, while all the BRCA2s were ER positive. Cyclin D1 expression had not previously been correlated with BRCA1/2 mutation status. We are currently investigating the biological/pathological significance of the other genes identified as key discriminators of BRCA2 tumors in an attempt to define other interesting characteristics of cellular behavior in this cancer.

Program Nr: 43 from the 1999 ASHG Annual Meeting

Tissue microarrays ("tissue chips") for high-throughput cancer genetics: Linking molecular changes to clinical endpoints. *C. Bucher[1], J. Torhorst[1], L. Bubendorf[1,2], P. Schraml[1], J. Kononen[2], H. Moch[1], M. Mihatsch[1], O. Kallioniemi[2], G. Sauter[1]*. 1) Institute of Pathology, University of Basel, Basel, Switzerland; 2) Cancer Genetics Branch, NHGRI, NIH, Bethesda, MD.

The analysis of candidate prognostic or predictive molecular parameters is traditionally hampered by limited availability of precious tissue resources and the large number of tumors needed to obtain significant results. Our recently developed tissue microarray (tissue chip) technology (Kononen et al., Nat. Med. 4:844-7, 1998) can substantially facilitate such studies. In this technology, samples measuring 0.6 mm in diameter from hundreds of tumors are brought on one slide allowing the simultaneous in situ analysis of all tumors. To investigate whether tissue arrays are applicable for the identification of associations between molecular markers and clinical endpoints, we studied breast cancer, where well-established prognostic markers are available. Alterations in the expression of estrogen (ER) and progesteron receptor (PR) expression as well as p53 were studied.To test, whether the known prognostic associations of ER, PR, and p53 can be reproduced on tissue microarray slides, and to examine the role of tissue heterogeneity, four replicate arrays were constructed containing samples from different regions (one central, 3 peripheric) of 557 breast carcinomas with clinical follow up information. Positive immunostaining for ER was found in 78%, PR in 56%, and p53 in 24% of the tumors, when the data from four replicate arrays were combined. The results obtained on all array sections were significantly associated with prognosis (ER: $p<0.0001$, PR: $p<0.0003$, p53: $p<0.05$). These data suggest that associations between molecular alterations can be detected on tissue microarrays. Tissue chips may drastically accelerate translation of results from basic research into clinical applications by allowing rapid testing of multiple molecular markers from the same clinical material.

Program Nr: 44 from the 1999 ASHG Annual Meeting

Identification of Complex Structural Aberrations in Astrocytomas Using Spectral Karyotyping. *J. Squire[1,3], S. Arab[2,4], M. Zielenska[2], P. Marrano[4], M. Taylor[5], L. Becker[2], J. Rutka[5]*. 1) Oncologic Pathology, Princess Margaret Hospital,; 2) Department of pediatric Laboratory Medicine, Hospital for Sick Children; 3) Department of Medical Biophysics and Laboratory Medicine and Pathology, University of Toronto; 4) Division of Medical Genetics, Hospital for Sick Children; 5) Division of Neurosurgery, Toronto, Ontario, Canada.

ABSTRACT Brain tumors are the most common solid tumor occurring in children and one of the leading causes of death among young children and adults. Understanding the molecular detail of tumor transformation and progression is crucial for developing an effective therapeutic strategy. We have used Spectral Karyotyping (SKY) to complement conventional banding techniques in detecting structural aberrations in human brain tumors by analyzing 6 glioma cell lines and 4 primary brain tumor cells including: one low-grade astrocytoma, two gliomas (pediatric; adult), and one ganglioglioma. In total, in the 6 cell lines 90 structural aberrations including balanced and unbalanced chromosome abnormalities were detected. In the 4 primary patient samples studied a simpler pattern of chromosomal aberration was detected. We report here two novel cytogenetic aberrations including a cryptic t(10;21) translocation detected in one pediatric glioma and in one more advanced adult glioma, double minute chromosomes were recognized as being a novel amplicon derived from 15q. Many more aberrations were detected in the astrocytoma cell lines than in the primary tumor preparations. In addition to the novel findings, this study demonstrates the approach of combining molecular cytogenetic techniques to characterize fully the multiple complex chromosomal rearrangements found in the astrocytoma cell lines and primary brain tumors tested. One of the most important purposes of studying brain tumorigenesis lies in the hope that this will lead to a better treatment and/or prevention of the disease. To date, no studies of the use of SKY or M-FISH in the analysis of astrocytomas have been reported.

Program Nr: 45 from the 1999 ASHG Annual Meeting

The spectrum of germline PATCHED mutations in UK families with naevoid basal cell carcinoma syndrome (Gorlin syndrome). *S.J. Rooker[1], C. Hardy[2], P. Fullwood[2], R. Toftgard[3], P. Kogerman[3], P.A. Farndon[2], F.M. Richards[1]*. 1) Medical & Molecular Genetics Section, Division of Reproductive & Child Health, University of Birmingham, The Medical School, Birmingham B15 2TT, UK; 2) West Midlands Regional Genetics Service, Birmingham Women's Hospital, Edgbaston, Birmingham B15 2TG, UK; 3) Karolinska Institute, Dept. of Biosciences, Novum, S14157 Huddinge, Sweden.

Basal cell carcinoma (BCC) is the most common cancer among caucasians. The naeviod basal cell carcinoma syndrome (NBCCS) is an autosomal dominant disorder characterised by developmental abnormalities and susceptibility to a variety of tumours, in particular to multiple BCCs. Mutations in the patched (PTCH) tumour suppressor gene on chromosome 9q22.3-q31 have been identified in NBCCS and in sporadic BCC. We have completed screening of PTCH exons 2 to 22 in the first cohort of 55 NBCCS families (from 140 under investigation) by PCR/SSCP and biddF. Germline PTCH mutations were identified in 35/55 (64%). 34 different mutations were found; 18 frameshifts, 4 splice-site changes, 12 missense and one in-frame deletion, distributed throughout the gene. In the 20 families with no detectable PTCH mutation, 13 displayed classical NBCCS features of which 9 appear to result from a de novo mutation, with clinical evidence for somatic mosaicism in 2 cases. The remaining 7 cases had equivocal phenotypes. We have extended our mutation analysis in these 20 cases to include the 4kb PTCH promoter region and 5' exons 1b, 1 and 1a by PCR/SSCP. A novel 6bp deletion in a GC rich region at position -20 bp upstream of the ATG start site in exon 1b was detected in one NBCCS family. The proband presented as a new mutation with jaw cysts, falx calcification and typical facial features of Gorlin syndrome and her three children with the deletion all had macrocephaly, hydrocephalus and slight developmental delay. The functional significance of this deletion is under investigation.

Program Nr: 46 from the 1999 ASHG Annual Meeting

Suppressor of fused in basal cell carcinomas and medulloblastomas. *A.E. Bale, H.A. Hess, R. Pagliarini.* Dept Genetics, Yale Univ Sch Medicine, New Haven, CT.

Basal cell carcinoma of the skin (BCC) is the most common human cancer. The nevoid basal cell carcinoma syndrome (NBCCS) is an autosomal dominant condition characterized by predisposition to BCCs and medulloblastomas. The NBCCS gene is the human homolog of Drosophila patched(PTCH), a member of the hedgehog (HH) pathway. PTCH (functioning as a negative regulatory subunit)and smoothened (functioning as a positive subunit) form the receptor for HH. Ninety percent of sporadic BCCs have mutations that switch on the HH pathway, inactivating PTCH or activating smoothened. To determine the genetic basis for the remaining 10 percent of sporadic BCCs we are cloning human homologs of downstream members of the Drosophila HH pathway.

Suppressor of fused (SUFU) is a negative regulator of the Drosophila pathway that interacts with fused and costal 2. No human homologs of the latter two genes are known, but we used homology searches to identify a human EST closely related to SUFU. With library screening and 5'RACE, we isolated a 2 kb cDNA predicted to encode a 450 aa protein 52 percent similar to Drosophila SUFU. Northern blots and RTPCR showed that SUFU was expressed at low levels in 8 normal human tissues including epidermis and cerebellum but was overexpressed in 10 BCCs and 4 medulloblastomas. This expression pattern was identical to PTCH, which is known to exert negative feedback on the HH pathway. One BCC with no mutation in PTCH or smoothened had undetectable levels of SUFU but the highest levels of expression of HH target genes in any tumor, supporting the role of SUFU in negatively regulating the HH pathway. Lack of SUFU expression in this tumor may reflect a mutation of the gene, but none has yet been identified.

We mapped SUFU to chromosome 10q23.3 (Stanford Radiation Hybrid Panel, LOD 6.9), a frequent site of LOH in many tumor types. SUFU, like PTCH, may function as a tumor suppressor.

Program Nr: 47 from the 1999 ASHG Annual Meeting

The interaction between the von Hippel-Lindau gene product (pVHL) and hypoxia-inducible factor 1 is disrupted by disease-causing pVHL mutants. *E.R. Maher[1], S.C. Clifford[1], P.H. Maxwell[2], M.S. Wiesener[2], G.W. Chang[2], P.J. Ratcliffe[2]*. 1) Med & Molecular Genetics, Univ of Birmingham, Birmingham, England; 2) Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, England.

We have demonstrated that pVHL plays a critical role in the regulation of Hypoxia inducible factor-1 (HIF-1), a transcription factor which plays a key role in cellular responses to hypoxia, including regulation of genes involved in angiogenesis. HIFa subunits are constitutively stabilised and HIF-1 is activated in VHL-defective cells, whereas transfection of wild-type (WT) pVHL restores oxygen-dependent HIF-1 instability. pVHL and HIFa subunits are co-immunoprecipitated, and pVHL is present in the hypoxic HIF-1 DNA binding complex, thus defining a key function for pVHL in oxygen regulated gene expression, and suggesting that constitutive HIF-1 activation may underlie the angiogenic phenotype of VHL associated tumours. To investigate the role of pVHL/HIF-1 interaction in VHL-mediated tumourigenesis, we have cloned mutant and wild type pVHLs and analysed the interaction between HIF-1 and the mutant pVHLs by (i) DNA binding (band-shift) assay following addition of in vitro transcribed/translated pVHL mutants to VHL (-/-) nuclear extracts, (ii) HIF-1 hypoxic-induction and anti-pVHL immunoprecipitation studies in stably transfected VHL (-/-) RCC cell lines expressing the cloned mutant pVHL constructs. Of the 14 pVHL mutants analysed, most did not interact with HIF-1 by band-shift assay consistent with the hypothesis that disruption of the pVHL/HIF-1 interaction is important for VHL-mediated tumourigenesis. This data and of the results of co-immunoprecipitation and target gene regulation studies is being analysed to identify correlations with previously identified genotype-phenotype associations in VHL disease.

Program Nr: 48 from the 1999 ASHG Annual Meeting

Cloning and Characterization of the Common Fragile Site FRA16D and Its Role in Cancer. *K.A. Krummel, M. Kawakami, L.R. Roberts, D.I. Smith.* Experimental Pathology, Mayo Clinic, Rochester, MN.

Fragile sites (FS) appear as breaks, or gaps, of metaphase chromosomes. The breaks appear in defined locations throughout the mammalian genome. Two classes of FS are identified: rare and common. Rare fragile sites are inherited in a co-dominant fashion and are inducible in only a small portion of the general population. Common fragile sites (CFSs) are present in virtually all individuals. The study of FSs has now lead to the cloning of seven rare, and three common FSs.

To understand the mechanism of CFS expression and the role they play in human disease, we have identified and assembled a BAC contig of 800 Kb across the second most active CFS, FRA16D. FISH-based analysis, against aphidicolin-treated lymphocytes, demonstrates that these BACs cross FRA16D. In contrast to the other three cloned CFSs, FRA16D appears to be somewhat larger. We have now begun make a high-resolution restriction map of the entire contig. Microsatellite markers that map within this contig are those that show the highest loss in prostate and breast tumors, demonstrating that FRA16D deletions are common in these tumors.

FRA16D at 16q23 is a region demonstrating high LOH in both breast and prostate cancers, and is also involved in multiple myeloma (MM) t(14:16) translocations. Multiple myeloma has frequent translocations in this chromosomal band, and we have now precisely localized four of four previously isolated 16q23 breakpoints within FRA16D. We have already identified two candidate genes in the center of the fragile site and are pursuing these as potentially important in cancer progression. One of these genes was identified from microdissected, low grade, prostatic intraepithelial neoplastic cells, and may be important in progression of this cancer. We will also identify new regions of loss and MM translocations. Once these regions are identified, we will superimpose them on the known genes and MM translocation points to determine regions of interest. We will identify the genes in these regions, and investigate which may play a role in FS expression and cancer progression.

Program Nr: 49 from the 1999 ASHG Annual Meeting

A Mouse Model for Compound Heterozygosity at the Bloom Syndrome Locus. *L.D. McDaniel[1], N. Chester[2], M. Watson[1], N. Okamoto[1], P.B. Sills[1], G. Luo[3], A. Bradley[3], P. Leder[2], R.A. Schultz[1].* 1) McDermott Ctr, Univ Texas SW Med Ctr, Dallas, TX; 2) Dept. Genetics, Harvard Medical School, Boston, MA; 3) Inst. Molecular Genetics, Baylor College Medicine, Houston, TX.

Bloom syndrome (BLM) is a rare autosomal recessive disorder characterized by a predisposition cancer, growth delay, immunodeficiency and chromosome instability including increased sister chromatid exchange (SCE). The disorder is caused by mutations in a gene encoding a protein with DNA helicase function. Human cells compound heterozygous for BLM mutations give rise to normal alleles via somatic "intragenic recombination", an event unique to the BLM gene.

Two distinct Blm mouse models have been previously described. The first, generated by insertion of a neo gene cassette into the Blm locus, yielded embryonic lethality in homozygous mutant animals ($Blm^{1/1}$) which is associated with developmental delay, anemia, and increased SCE frequency in MEFs. The second model yielded homozygous mutants ($Blm^{2/2}$) which are viable, exhibit both chromosome instability and a predisposition to tumors in a variety of tissues, but no growth delay. Given the embryonic lethality unique to the $Blm^{1/1}$ genotype and the lack of growth delay in $Blm^{2/2}$ mice, we have further characterized the nature of the defect in the second model. Data will be presented demonstrating that allele 2 represents a duplication of exon 3 sequences which is expected to disrupt reading frame. While the precise nature of the differences between these two models is still under investigation, we have generated a compound heterozygous Blm mouse model ($Blm^{1/2}$). These animals are viable with all relevant genotypes observed at appropriate mendelian ratios. Initial results indicate that the compound heterozygotes exhibit delayed growth, suggesting this model may better mimic those features characteristic of the human disease. These animals are currently being evaluated for chromosome instability and for tumor frequency and tissue distribution relative to that seen in $Blm^{2/2}$animals. Moreover, this model offers the opportunity to assess potential intragenic recombination in vivo and in established MEF cells representing this genotype.

Program Nr: 50 from the 1999 ASHG Annual Meeting

HPV16 Preferentially Integrates into the Common Fragile Sites in Cervical Cancer. *E.C. Thorland, S. Myers, D. Persing, D.I. Smith.* Experimental Pathology, Mayo Clinic, Rochester, MN.

The development of cervical cancer is highly associated with human papilloma virus (HPV) infection. HPV is typically found in an episomal form in premalignant cervical lesions, but HPV integration into the host cell genome is observed in the majority of malignant cervical tumors. Thus, HPV integration is temporally associated with the acquisition of the malignant phenotype. A relationship between the sites of HPV integration and the positions of the common fragile sites (CFS) has been observed at the cytogenetic level. We were interested in exploring the relationship between CFS and the sites of HPV integration in cervical tumors. We utilized a PCR-based method to amplify the junctions between HPV sequences and the surrounding flanking genomic sequences in cervical tumor specimens. Primers based on the isolated flanking sequences were then used to screen BAC libraries to isolate genomic clones at each integration site. These were then used as FISH probes against aphidicolin-induced cells. We have isolated genomic sequences flanking HPV integration sites from several HPV-positive cervical tumor samples. BAC clones containing these sequences were isolated from three tumors. FISH-based analysis with these BAC clones has demonstrated that all three integrations occurred within CFSs. The integrations occurred at chromosomal locations 1q21.3 (FRA1F), 6p22.2 (FRA6C) and 17q23.1 (FRA17B), thus we have cloned sequences within three previously uncloned CFS. These results demonstrate that CFS are targets of HPV integrations in cervical tumors and that CFS expression may be involved in cervical cancer. It has also been proposed that HPV integration events in cervical tumors may disrupt cellular genes important in tumor progression. Interestingly, the integration site that we have cloned at 1q21.3 disrupts a gene which is expressed in all tissues examined and encodes a novel ubiquitin-specific processing protease. This family of genes is involved in many important biological processes including the control of growth, differentiation, oncogenesis and genome integrity, thus this novel gene is a potential target of mutation in cervical tumors and other tumor types.

Program Nr: 51 from the 1999 ASHG Annual Meeting

Mitochondrial DNA (mtDNA) mutations in papillary thyroid carcinoma and differential mtDNA sequence variants in cases with malignant versus benign thyroid tumors. *J.J. Yeh[1,2,3], K.L. Lunetta[2], P.L.M. Dahia[1,2], C. Eng[1]*. 1) Human Cancer Genetics, Ohio State University, Columbus, OH; 2) Dana-Farber Cancer Institute, Boston, MA; 3) Boston University School of Medicine, Boston, MA.

MtDNA defects have been implicated in several degenerative diseases and aging. Studies of tumors with large accumulations of mitochondria, i.e. oncocytic tumors, have led to hypotheses that defects in oxidative phosphorylation may result in a compensatory increase in mitochondrial replication and/or gene expression. Mutation analysis of mtDNA in thyroid neoplasia, one of the most common sites of oncocytic tumors, has been limited. Due to practical technical limitations, there has yet to be a study that systematically analyzes the coding regions of the mt genome in sporadic adult human thyroid tumors, comparing different thyroid pathologies and population controls. Using the recently developed technique of two-dimensional gene scanning, we have successfully examined 22 cases of thyroid tumors, 5 cases of non-neoplastic thyroid pathology, 30 population controls, 9 fetal thyroid tissues and 9 fetal tissues of non-thyroid origin, either kidney or liver. We have identified 3 different somatic mutations (23%) among 13 sporadic papillary thyroid carcinomas. Among all samples, we found a total of 136 variants representing 51 distinct sequence variations. Between 1 and 6 variants were noted per pathologic sample. Cases with thyroid pathology appeared to have more variants per sample compared to normal controls (2.6 vs 1.4, P=0.05, Kruskall-Wallis). Interestingly, carcinomas (N = 16) and adenomas (N = 6) differed significantly only in their distributions of neutral coding variants (p = 0.02). In fact, there was a greater than five-fold difference in the mean number of neutral coding variants that were identified in the adenomas (0.167) versus carcinomas (0.875). Only 1 of 6 (17%) adenoma samples had a neutral variant in a coding region, compared to 10 of 16 (63%) in the carcinoma samples. These findings suggest first, that somatic mtDNA mutations are involved in thyroid tumorigenesis and second, that the accumulation of certain non-somatic variants may be related to tumor progression in the thyroid.

Program Nr: 52 from the 1999 ASHG Annual Meeting

Dioxin-related gene expression and activity in Seveso. *M.T. Landi[1], J. Grassman[2], S. Masten[2], D. Bell[2], D. Consonni[3], P. Mocarelli[3], G. Lucier[2], L. Needham[4], P.A. Bertazzi[3], N.E. Caporaso[1]*. 1) NCI/NIH, Bethesda, MD; 2) NIEHS, Res. Triangle Park, NC; 3) University of Milan, Italy; 4) CDC, Atlanta, GA.

In 1976 an industrial accident contaminated the area of Seveso, Italy, with the highest levels of 2,3,7,8-tetrachloro-dibenzo(p)-dioxin (TCDD) ever recorded in humans. Twenty years after the accident, 62 healthy subjects from the highly contaminated areas (zones A and B) and 59 subjects from the surrounding area, frequency matched for age, gender, and smoking, were studied. Univariate and multivariate regression analyses were conducted. Elevated plasma TCDD levels were present in subjects from the exposed areas (53.2 ppt in zone A, 11.0 ppt in zone B, and 4.9 ppt outside the area; p = 0.0001). TCDD levels in women were significantly higher compared to men in both the B and surrounding areas (p=0.0003, p=0.007, resp.). CYP1A1 (ile-val polymorphism), GSTM1, and GSTT1 genotypes, CYP1A1 gene expression, and CYP1A1-related enzyme activity (EROD), all potentially involved in the pathway between exposure and carcinogenic effects of dioxin, have been studied in these subjects. CYP1A1 expression appeared associated with CYP1A1 genotype (p=0.052); it was higher in the 6 subjects with the CYP1A1 heterozygous variant genotype, but the small number of subjects do not permit any conclusion. In contrast, CYP1A1 gene expression and activity were not modified by the GSTM1 genotype. Multiple regression revealed that current plasma levels of TCDD were associated with CYP1A1 expression (p=0.053), but not with EROD (p=0.625). However, smoking seemed to affect CYP1A1 expression more than TCDD. EROD was strongly associated with CYP1A1 expression (p=0.002), and also with gender (p<0.001) and GSTT1 genotype (p=0.007). There was a significant interaction between gender and GSTT1 genotype (F=0.56; p=0.028): females with the null GSTT1 genotype had higher EROD levels. In conclusion, 20 years after the accident, exposed subjects, particularly females, have high plasma levels of dioxin. These levels are associated with an increased CYP1A1 gene expression, but not with CYP1A1-related enzyme activity (EROD). CYP1A1 and GSTT1 genotypes and gender may modify these associations.

Program Nr: 53 from the 1999 ASHG Annual Meeting

Triggering The Need to Know: Optimizing the Integration of New Genetics Knowledge into Primary Care Practice. *C.I. Barash[1], A. Kindfield[2], S.J. Hayflick[3].* 1) Genetics, Ethics & Policy Consulting, Boston, MA; 2) Montclair University, NJ; 3) Oregon Health Sciences University, Portland, OR.

Unless a clinician's medical decision-making process triggers suspicion of a genetic factor and facilitates testing of that hypothesis, new genetics knowledge will not be integrated into practice. We present findings from a pilot study of clinical genetics problem-solving designed to identify barriers to the use of genetics knowledge in daily practice. We utilized Think Aloud, a proven tool that relies on a subject's verbalizing their medical decision-making process. Using a clinical genetics problem set, we examined 4 experts and 10 novices. The method demonstrated significant differences. Experts demonstrated a broader range of interpretations of signs and symptoms and of diagnostic hypotheses and a more methodical manner of testing candidate hypotheses. Novices displayed fewer genetics hypotheses and a greater ability to be tripped up by pleiotropy and heuristics. Further, we have found that both experts and novices are tripped up by the following factors: 1)physical findings triggered suspicion of genetic involvement whereas family history did not; 2)heuristics bar appreciation of phenotypic variation and can thwart pursuit of genetic thinking, 3)pleiotropy may interfere with indexing and memory recall; and 4)knowledge deficits deter suspicion and pursuit. Additionally, we documented attitudinal and normative barriers to non-directive service provision. Two unexpected findings emerged. 1)The research method itself may embody a solution as subjects were quick to identify knowledge deficits and inappropriate rationales for decisions. Furthermore, the interactive context motivated the subjects to self-correct. 2)Gender emerged as a factor in suspicion triggering and successful pursuit of possible genetic involvement. Our results explain why education alone has not propelled optimal integration of new genetics knowledge into practice, identify new areas for further research into the problem of non-adoption, and provide vital information that can be applied to both educational initiatives and efforts to improve care delivery.

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Program Nr: 54 from the 1999 ASHG Annual Meeting

The family belief and attitude history: an aid to decision making and coping in genetic counseling. *D.L. Eunpu.* Genetic Counseling Program, Beaver College, Glenside, PA.

Decision making and coping can be difficult for couples who receive genetics services. At precisely the time when decisions that may bear lifelong consequences must be made, a couple's emotional and cognitive resources are often at their most compromised. Helping such couples to identify and connect with their individual and family's values, beliefs and attitudes can provide them with sufficient understanding and confidence to make difficult decisions and cope with unexpected results in healthier ways.

The family belief and attitude history is a straightforward method for guiding couples in this exploration process and can be readily adapted to any genetic counseling indication or setting. In this method, a small set of focused questions guides the couple through the process of exploring their attitudes and beliefs in the context of their extended family's values. The process may be incorporated into the genetic counseling session or given as a homework assignment. Since this method focuses on the family's experiences and attitudes, it has the potential to be highly specific for a given family and therefore more syntonic than other methods. The simplicity and effectiveness of this method is outlined and illustrated with case material for three commonly encountered experiences in clinical genetics: the personal and family experience and meaning of (1) being affected by an inherited condition; (2) infertility and pregnancy loss; and (3) loss in general.

Program Nr: 55 from the 1999 ASHG Annual Meeting

Genetic counseling in Ashkenazi Jewish families with deafness: Connexin 26 gene mutation analysis. *L.D. Bason[1], P. Fortina[2], E. Rappaport[3], C. Ponte[3], M.A. Keller[3,4], E.H. Zackai[1], I. Krantz[1]*. 1) Division of Human Genetics and Molecular Biology, The Children's Hospital of Philadelphia, Philadelphia, PA; 2) Department of Hematology, The Children's Hospital of Philadelphia, Philadelphia, PA; 3) Department of Hematology/Oncology, A.I. duPont Hospital for Children, Wilmington, DE; 4) Department of Pediatrics, Jefferson Medical College, Philadelphia, PA.

Mutations in the Connexin 26 gene (*Cx26*) have been found to be the most common cause of non-syndromic autosomal recessive deafness. Several studies have attempted to estimate the carrier frequency of *Cx26* mutations in the general population. Morell et al. (1998) reported a study which found the carrier frequency of one particular mutation, 167delT, to be 4.03% in the Ashkenazi Jewish population. This particular mutation does not appear to be common in the general population. The overall carrier rate of *Cx26* mutations in the Ashkenazi Jewish population was found to be approximately 1 in 21 which is higher than that expected in the general population. This presents an important genetic counseling issue, as many Ashkenazi Jews marry within this population, and therefore there is an increased chance that two carriers of a *Cx26* mutation will marry. We present the results of *Cx26* mutation analysis in 13 Ashkenazi Jewish families. In 6 families, screening revealed that probands were positive for *Cx26* mutations (4 homozygous 167delT; 1 homozygous 35delG; 1 heterozygous 167delT/35delG). As demonstrated in two families in which only second degree relatives to the probands were affected, the high carrier frequency of *Cx26* mutations may lead to pedigrees which give the erroneous impression of dominant inheritance with reduced penetrance. Connexin 26 mutation analysis in Ashkenazi Jewish families will not only establish an etiology for deafness in a large proportion of individuals, but will also clarify the inheritance pattern in families.

Program Nr: 56 from the 1999 ASHG Annual Meeting

Long-term impact on siblings of growing up with a brother with X-linked severe combined immunodeficiency.

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Severe combined immunodeficiency (SCID) is a rare syndrome (1/50,000 births) of profoundly impaired cellular and humoral immunity. In past generations, individuals with SCID died of infections early in life. In 1968, the first successful bone marrow transplant (BMT) was performed. Prompt diagnosis and BMT currently make survival possible for over 80% of affected males with X-linked SCID (XSCID). This treatment typically requires hospitalization for several months in tertiary medical facilities; thus, the burden on the family is considerable. This study attempted to assess the psychological impact on siblings of growing up in a family with a boy with XSCID. Forty adult siblings from families studied by J.M.P were interviewed in depth by J.H.F., and rating scales for their responses were developed. 51% of siblings reported that information provided to them about XSCID had been poor; 67% believed the mother had been unable to mourn the death of the affected; 52% expressed distress over prolonged maternal absence during the affecteds hospitalization. Poor family communication and unresolved parental mourning were significantly related ($p<.001$), as were poor family communication and survivor guilt ($p=.02$). Families in which there had been a spontaneous mutation, rather than a multigeneration history, were significantly more likely to express distress over maternal separation issues related to the prolonged hospitalization of the affected ($p=.03$), perhaps due to stronger maternal guilt ($p=.02$). Long-lasting difficulties for daughters included a desire to repair the mothers unresolved loss of her own child, as well as an attempt to undo the feeling of being flawed as a carrier, by heightened wishes to bear a healthy son. We recommend: 1) attention to family separation during hospitalizations of XSCID patients to minimize feelings of abandonment in siblings; 2) open communication about XSCID; 3) help for parents with mourning the loss of an affected son, to improve the outlook for healthy emotional development of unaffected siblings.

Program Nr: 57 from the 1999 ASHG Annual Meeting

Psychological impact of genetic counseling on women at high risk of developing breast cancer. *B. Meiser[1], P. Butow[2], A. Barratt[3], K. Tucker[1].* 1) Department of Medical Oncology, Prince of Wales Hospital, Randwick, NSW 2006, Australia; 2) Medical Psychology Unit, University of Sydney, NSW 2006; 3) Department of Public Health and Community Medicine, University of Sydney, NSW 2006.

Aims: Conventional models of genetic counseling will be affected by the increasing availability of genetic testing for breast cancer susceptibility. Given the complexity of the information to be conveyed and its potential impact on decision-making about screening and prophylactic strategies, it appears crucial to understand the effectiveness of genetic counseling in conveying information about breast cancer genetics and breast cancer risk and to assess its efficacy in reducing breast cancer anxiety. Method: This prospective, multicentre study includes only women unaffected by breast cancer who attended a familial cancer clinic for advice about their breast cancer risk. Assessment took place one week before genetic counseling and 12 months after attendance at the clinic, using a self-administered questionnaires with validated measures. Results: Data collection for this three-year study commenced in January 1997. As of July 1999, 140 women have completed the baseline questionnaire and have been followed up 12 months post-counseling. Seventy-seven percent of participants had a family history suggesting a dominantly inherited predisposition to breast cancer, and 23% were at moderately increased risk only. Compared to baseline, 12 months post-counseling there was a statistically significant improvement in breast cancer genetics knowledge. Women without post-school qualifications had significantly lower breast cancer anxiety at the 12 month follow up, compared to women with post-school qualifications, after controlling for differences in baseline breast cancer anxiety ($r = 5.51$, 95% CI 0.80, 10.22, $p = 0.022$). Conclusion: Findings demonstrate that genetic counseling leads to improvements of breast cancer genetics knowledge and that this increase is retained 12 months post-counseling. Women with lower educational levels may especially benefit from specialist genetic counseling, since they experience long-lasting decreases in breast cancer anxiety.

Program Nr: 58 from the 1999 ASHG Annual Meeting

Consultees' attitudes towards preventive strategies for Hereditary Breast Ovarian Cancer risk: Cultural differences between Manchester (UK) Marseilles (F) and Montreal (Ca). *C.M. Julian-Reynier[1], G. Evans[2], L. Bouchard[3], F. Eisinger[1,4], B. Kerr[2], W. Foulkes[5], I. Blancquaert[3], H. Sobol[4].* 1) Unite 379, INSERM, Marseilles, France; 2) St-Mary Hospital, Manchester, UK; 3) Universite du Quebec, Montreal, Canada and Conseil D'Evaluation des Technologies; 4) Inst.Paoli-Calmettes-INSERM E9939, Marseilles, France,; 5) McGill University, Montreal, Canada.

The discovery of BRCA1/2 genes and the development of molecular tests have found applications in clinical practice, despite the fact that uncertainties persist for the management strategies of mutation carriers. Objective : we investigated the social acceptability of these management strategies in three countries to determine whether cultural factors had to be taken into account. Methods : A survey was conducted in Marseilles (N=141), France, Manchester (N=130), UK, and Montreal (N=84), Canada, between 1996-1998. All women attending cancer genetic clinics for the first time completed a self-administered questionnaire before the consultation. Results : The highest acceptability was observed for mammography screening with 86% of the respondents finding this examination acceptable before or at the age of 40 years. Chemoprevention was the second preferred option with 60% of consultees that were in favor of it. Prophylactic oophorectomy would be acceptable for 19% of the women before or at the age of 35, and by 45% of them at 40 years old. Prophylactic mastectomy would be acceptable for 16% of the women by the age of 35 and for 22% at 40 years. After multivariate adjustment, British and Canadians were more in favor of prophylactic surgery than French (OR mastectomy= 2.8; 95% CI=1.3-6.3; OR oophorectomy =2.8 95% CI=1.3-5.6) and British were more in favor of chemoprevention than French and Canadians (OR=4.5; 95% CI=2.4-5.8).The cumulated acceptability of mammograms was higher for French and Canadians than for British (OR=3.1 95% CI=1.7-5.7). Conclusions : Face with the same dilemma and with the same scientific evidence for the preventive management of HBOC risk, patients' cultural backgrounds are likely to be a major determinant of ongoing practices.

Program Nr: 59 from the 1999 ASHG Annual Meeting

Assessing patient satisfaction in a BRCA1/2 testing program. *K.A. Schneider[1], L. Digianni[1], S. Kieffer[2], A. Chittenden[1], K. Shannon[3], A. Patenaude[1], J. Garber[1].* 1) Adult Oncology, Dana Farber Cancer Inst, Boston, MA; 2) Univ of Alberta Hospital, Edmonton, Canada; 3) Mass General Hospital, Boston, MA.

Rating the success of genetic counseling encounters is notoriously difficult. One strategy is to assess rates of patient satisfaction. We assessed satisfaction rates in women participating in a BRCA1/2 research testing program by using the Patient Satisfaction Scale (Shiloh et al, AJMG 1990) and Satisfaction with Decisions Scale (Holmes-Rovner et al, Med Decis Making 1996). Participants met with a genetic counselor and oncologist team for 2 visits. 87/165(52.7%) women completed the measures nine months after receiving BRCA1/2 test results. The Patient Satisfaction Scale was scored 1(none) to 4(high). Mean scores were similarly high in the affective (3.83), procedural (3.68), and instrumental (3.77) subscales. Satisfaction with Decisions Scale was scored 1(strongly disagree) to 5(strongly agree) and the mean score regarding decision to have BRCA1/2 testing was 4.8. 52/87(60%) women had breast/ovarian cancer. Genetic test results included 33/87(38%) positives, 14(16%) true negatives (known mutation in family), and 34(39%) indeterminate negatives (no known familial mutation). 6 women with uncertain variant results were excluded. Over 93% of women with positive or indeterminate negative results were highly satisfied that enough time had been devoted to them by the counseling team while only 71% women with true negative results were highly satisfied with the time allotted ($p<.005$). Unaffected women were also less satisfied than cancer patients with the time devoted to them by the counseling team (83% vs 98% $p<.01$). In addition, unaffected women were less satisfied than cancer patients that they were in good hands (83% vs 96%; $p=.05$) and that the counseling team cared for them as a person (70% vs 88%; $p<.05$). Overall, participants reported high levels of satisfaction with the genetic counseling program and remained satisfied with their decision to have BRCA1/2 testing. Women with true negative results were the least satisfied with the time devoted to them by the counseling team, perhaps because they felt a sense of loss of attention after lifelong cancer-focused health care.

Program Nr: 60 from the 1999 ASHG Annual Meeting

Fear of Genetic Discrimination among Providers of Cancer Genetic Counseling. *E.T. Matloff, K.L. Brierley, H.L. Shappell.* Yale Cancer Center/Yale University School of Medicine, New Haven, Connecticut.

Fear of genetic discrimination is one of the most common deterrents of predictive genetic testing for hereditary cancer syndromes. Concerns about discrimination, privacy, and confidentiality have led to federal and state legislation designed to help protect consumers of genetic services from discrimination by insurance companies. Despite these protective measures, concern about genetic discrimination is still prevalent among patients who present for hereditary cancer testing. This study sought to examine if experienced cancer genetic providers share these fears of genetic discrimination. We surveyed the 296 active members of the National Society of Genetic Counselors Cancer Special Interest Group (NSGC Cancer-SIG) regarding what safeguards they would employ to protect themselves against genetic discrimination. The vast majority of respondents would pursue genetic testing for a BRCA or HNPCC mutation (85% and 91%) if they were at 50% risk to carry such a mutation. However, 68% would not bill genetic testing charges to their health insurance company, and 26% would use an alias due to fear of genetic discrimination. Eighty-two percent of counselors would share the information with their physicians, although many would not want their carrier status documented in their medical records. Almost all (98%) of respondents would share their test results with their family members, while only 30% would discuss their results with colleagues. Fifty-seven percent of counselors would seek professional psychological support to help them cope with the results of their testing. The reasoning behind this decision-making and potential ramifications on the counseling session will be discussed.

Program Nr: 61 from the 1999 ASHG Annual Meeting

Improving lay appraisal of the risks/benefits of genetic carrier testing. *T.L. Spinney, N. Callanan, T. Jennings-Grant, C. Lakon, J.R. Sorenson.* University North Carolina at Chapel Hill, Chapel Hill, NC.

As part of a NHGRI funded study assessing at-risk female relatives' acceptance or rejection of free hemophilia A mutation carrier testing, we are evaluating the impact of a risk/benefit appraisal methodology that requires women to construct their own carrier testing risk/benefit profile prior to the testing decision. Drawing on Behavioral Decision Theory (BDT), including those of Janis and Mann and Petty and Cappioco, we ask women in the treatment arm of the study to identify the consequences for themselves and their significant others of being or not being tested, as well as to identify their and their significant others' values regarding carrier testing. BDT suggests that women who consider these factors will experience more decision stability and satisfaction and less decision regret than those who do not. BDT does not predict the decision that will be made. In contrast to the treatment arm of the study, women in the control arm complete a standard informed consent form, wherein they are provided with expert assessments of potential risks and benefits and are asked to consider these before making a testing decision. Developmental and pilot work (N=23) found the method is useable by women of varying ages/educational levels, can be completed in about 20 minutes, and women can construct personal risk/benefit profiles. To date 76 women have completed a standard consent form and 61 a consent form employing the new risk/benefit appraisal format. Equal percents (77%) of women in both arms of the study decide to have hemophilia A carrier testing. We will report six-month data on the decision satisfaction and regret of women in both arms of the study.

Program Nr: 62 from the 1999 ASHG Annual Meeting

Protection of privacy in published pedigrees versus preserving scientific integrity. *R.L. Bennett[1], M. Horwitz[1], T.D. Bird[1,2]*. 1) Medical Genetics, Univ Washington, Seattle, WA; 2) VA Medical Center, Seattle, WA.

Pedigrees are a foundation of human genetic research. A study by Botkin et al (JAMA 1998 vol 279) showed that 19% of the investigators they queried had published a masked or altered pedigree in the last 5 years. They defined "masking" as changing pedigree symbols in a way that is obvious to the reader (i.e., diamonds to mask gender). "Alteration" was defined as changing a feature of the pedigree in ways that may not be obvious to the reader (i.e., changing gender, deleting unaffected siblings from the pedigree). These practices are designed to protect the privacy and confidentiality of the subject(s). But, at what point do such practices hinder the recognition of salient features of the expression of the disorder under study? How does a researcher decide which information is essential to record on a pedigree? we present examples of pedigrees we have published where potentially key data was excluded, and also pedigrees from the same families that have been published in different pedigree frameworks. Since all pedigrees are potentially identifiable, obtaining informed consent before publication is paramount. We review several questions for investigators to consider before a pedigree is included with a manuscript, that help to balance protection of the subject's privacy with the reporting of unaltered data.

Program Nr: 63 from the 1999 ASHG Annual Meeting

Does HLA play a role in mate choice? *E. Genin[1], C. Ober[2], G. Thomson[3]*. 1) INSERM U155, Paris, France; 2) Department of Human Genetics, University of Chicago, Chicago, USA; 3) Department of Integrative Biology, University of California, Berkeley, USA.

Experiments in mice have suggested that genes from the major histocompatibility complex may influence mating. Female mice generally mate with males different from themselves in the H2 region and the recognition could involve odors. Attempts to generalize these findings in humans are difficult because of confounding factors such as ethnic preferences and population stratification and because of the large HLA repertoire. Focus on ethnically homogeneous populations, such as the Hutterites who have a more limited repertoire of HLA haplotypes, may provide the best situation in which to detect non-random mating. However, such populations are often genetic isolates where there may be inbreeding, or alternatively avoidance of mating with relatives, making it difficult to quantify and account for. We have developed a test for assortative mating at a multiallelic locus that is robust to stratification and inbreeding. The test relies on the availability of genotypic information for the parents of the two spouses. It focuses on families where there is haplotype or allele sharing between the parents of both spouses and follows the segregation of these shared haplotypes from heterozygous parents to their offspring. Data on 16-locus HLA haplotypes (Weitkamp and Ober, 1999, Immunogenetics 49: 491-497) from 511 Hutterite married couples have been investigated with this test. Interestingly, a lack of transmissions of shared haplotypes was found when the parents sharing the HLA haplotype were the mothers of the two spouses (12 shared haplotypes were transmitted and 26 non-transmitted from the mothers to the offspring, $p < 0.02$). These results provide additional evidence of negative assortative mating in this population and suggest a preferential choice for mates with HLA haplotypes that differ from the mother's haplotypes. (Supported in part by NIH grant HD21244).

Program Nr: 64 from the 1999 ASHG Annual Meeting

A data-mining approach to fine-scale gene mapping. *B.S. Weir[1], J.C. Brocklebank[2], P.M. Conneally[5], M.G. Ehm[4], J.R. Gilbert[3], J.H. Goodnight[2], W.A. Hassler[1,2], E.R. Martin[3], D.M. Nielsen[1,4], M.A. Pericak-Vance[3], A.R. Rogala[3], A.D. Roses[4], A.M. Saunders[3], D.E. Schmechel[3], B.D. Slotterbeck[3], J.M. Vance[3], D. Zaykin[1,4].* 1) Dept Statistics, North Carolina State Univ, Raleigh, NC; 2) SAS Institute Inc., Cary, NC; 3) Duke University Medical Center, Durham, NC; 4) Glaxo Wellcome, Research Triangle Park, NC; 5) Dept. of Medicine and Molecular Genetics, Indiana University, Indianapolis, IN.

The growing density of marker maps, such as those emerging for single nucleotide polymorphisms (SNPs), not only increases the ability to undertake fine-scale mapping of human disease genes but also increases the difficulty of analyzing the resulting data. Problems of marker dependencies and multiple testing are becoming of greater concern, and generally suggest the use of haplotype analyses instead of single-marker analyses. An alternative approach that may offer some advantages is that based on computer-intensive data-mining. We have explored this direction by employing Enterprise Miner™ software, developed by SAS Institute Inc., to analyze SNP data in the APOE region (chromosome 19q13.2), in a sample of unrelated Alzheimer Disease (AD) cases and independent controls. Here, affection for AD was treated as the target variable. Our analyses confirmed previous single-marker association results, and also revealed multi-marker patterns associated with the disease. These association patterns are useful for both fine-mapping and for prediction of individuals at risk for diseases such as AD. In addition to applying these techniques to the AD data, we investigated the power and false positive rate by utilizing simulated marker data over larger genetic regions. Enterprise Miner™ provides an excellent framework by which methods such as regression, neural networks and decision trees may be applied to genetic analysis. This can be of substantial benefit in the identification of genetic regions potentially involved in disease etiology.

Program Nr: 65 from the 1999 ASHG Annual Meeting

Genotype-phenotype studies based on the full DNA sequence of the Apo E gene demonstrate the challenge we face in the assignment of function to a particular DNA polymorphism. *C. Sing[1], D. Nickerson[2], S. Taylor[2], A. Clark[3], K. Weiss[3], S. Kardia[1]*. 1) Univ. of Mich., Ann Arbor, MI; 2) Univ. of Wash., Seattle, WA; 3) The Pennsylvania State Univ., University Park, PA.

Variation at 2 sites (3932,4070) in the gene that codes for apolipoprotein (Apo) E determine the e**2**, **3** and **4** alleles which are predictors of variation in plasma cholesterol (C), a major risk factor for heart disease. In a study of 456 females and 398 males, representative of Rochester, MN, we evaluated the influence on measures of lipid metabolism of 11 additional variable sites defined by complete sequencing in 24 members of the sample. After removal of genotypes observed only once, we observed 51 and 52 13-locus genotypes in the resulting samples of females (N=408) and males (N=361), respectively. Nine measures of lipid metabolism were considered-plasma total C, HDL C, triglycerides, and Apos AI, AII, B, CII, CIII and E. The additional sites did not improve the ability to predict variation in plasma Apo E or total C levels in either gender. However, significant variation in HDL C and triglycerides was associated with genotypic variation defined by the 11 sites but not with genotypic variation defined by the 2 sites that code for the e**2**, **3** and **4** alleles. Variation among genotypes defined by many different subsets of the 13 sites explained statistically significant amounts of trait variation equally well. For most traits, genotypes based on 3 or more sites explained the greatest amount of phenotypic variation. Many of these combinations did not include the 3932 and/or 4070 sites. These analyses suggest that different sites in a particular gene may influence variation in different traits in the same metabolic system. Many combinations of sites have the same utility in predicting variation in a particular trait. The complexity of the relationships between genome variation and quantitative trait variation that is implied by our study makes clear the difficulty in assigning functionality to particular sites within a gene. The implications for the selection of sites for the study of a particular trait in a particular context are sobering. This research was supported by NIH HL39107; HL58238, 39 & 40.

Program Nr: 66 from the 1999 ASHG Annual Meeting

Interaction effects of variants of the b**3-adrenergic receptor and peroxisome proliferator-activated receptor**-g**2 on obesity in Mexican Americans.** *W.-C. Hsueh[1], S.A. Cole[1], A.R. Shuldiner[2], B.A. Beamer[3], J. Blangero[1], A.G. Comuzzie[1], J.E. Hixson[1], J.W. MacCluer[1], B.D. Mitchell[1].* 1) Southwest Foundation for Biomedical Research, San Antonio,TX; 2) University of Maryland School of Medicine, Baltimore, MD; 3) Johns Hopkins School of Medicine, Baltimore, MD.

Obesity is a complex phenotype influenced by both genetic and non-genetic factors. Previous studies have reported associations between measures of obesity and the Trp64Arg variant of the b3-adrenergic receptor (b3AR) and the Pro12Ala variant of the peroxisome proliferator-activated receptor-g2 (PPARg2). We therefore evaluated the effects of these two variants on obesity and whether their joint effect (interaction) was significantly greater than their combined independent effects. The sample included 453 subjects from 10 large Mexican American families studied as a part of the population-based San Antonio Family Heart Study.

Mean age was 38.7±16.7 years (range: 16-94), and mean body mass index (BMI) was 28.7±13.8 and 30.4±7.4 kg/m^2 in men and women, respectively. The allele frequencies for the b3AR Arg64 variant and PPARg2 Ala12 variant were 18% and 12%, respectively. Association analyses were conducted using the measured genotype approach and statistical significance was evaluated by likelihood ratio test with adjustment for age, sex and diabetes status. The b3AR Arg64 variant was not significantly associated with obesity, but subjects with the PPARg2 Ala12 variant (n=98) had significantly higher levels of fasting insulin (p=0.03), leptin (p=0.009), and waist circumference (p=0.03) compared to those without. Moreover, there was a significantly positive effect associated with having a variant in both b3AR and PPARg2 (n=32) compared to having the PPARg2 Ala12 variant only (p values for interaction test: 0.04 for BMI, 0.02 for fasting insulin levels and 0.01 for leptin levels). These results suggest that epistatic models with genes that have modest effects individually may be useful in understanding the genetic underpinnings of typical obesity in humans.

Supported by HL45522.

Program Nr: 67 from the 1999 ASHG Annual Meeting

African-American hypertensive subjects have less European ancestry than normotensive controls. *E.J. Parra[1], K.G. Hiester[1], H.L. Norton[1], J.T. Baker[1], C. Bonilla[1], F.O. Gulden[1], R.G. Hutchinson[2], R.E. Ferrell[3], E. Boerwinkle[4], M.D. Shriver[1]*. 1) Dept of Anthropology, Penn State University, University Park, PA; 2) Univ. of Mississippi Medical Center; 3) University of Pittsburgh; 4) University of Texas/Houston.

Populations of African descent living in Europe and the United States have more hypertension than do populations of European descent. It has been hypothesized that there are alleles found at higher frequencies among populations of African descent that increase a persons risk of hypertension. If this is true, we expect to observe differences in the admixture proportions between hypertensives and normotensives from populations of mixed African/European ancestry. We have tested a group of African-American hypertensive cases (HT) and normotensive controls (NT) from Jackson, Mississippi, for differences in proportion of European ancestry (*m*) using a panel of especially selected DNA markers. In the NT group (n = 290) *m* = 19.75% ± 1.1% and in the HT group (n = 258) *m* = 15.21% ± 1.1% . Since obesity is a major risk factor for hypertension and it has also been hypothesized that there may be obesity predisposing alleles at higher frequencies in Africa, we classified the sample by obesity status. Levels of *m* are lower but not significantly so in the obese (BMI >30) subjects in this sample (*m* = 16.7%) than the non-obese (BMI <30) subjects (*m* = 18.3%). When the sample is classified by both hypertension and obesity, it is clear that the level of admixture is related both to hypertensive and obesity status. A logistic regression shows that individual admixture, age, and BMI have significant effects on hypertension. These results clearly indicate that there is population substructure in regards to admixture and hypertension and support the hypothesis of a higher frequency of hypertension risk alleles in the African parental populations. These results also emphasize that care should be exercised when using a case/control study design for both hypertension and obesity studies in African-Americans and the appropriateness of using the admixture linkage disequilibrium mapping approach for mapping obesity and hypertension genes.

Program Nr: 68 from the 1999 ASHG Annual Meeting

An application of the combinatorial partitioning method to identify gene-environment combinations that predict blood pressure levels. *S. Kardia[1], M. Nelson[1], E. Boerwinkle[2], S. Turner[3], C. Sing[1]*. 1) University of Michigan, Ann Arbor, MI; 2) University of Texas, Houston, TX; 3) Mayo Clinic, Rochester, MN.

Advances in genome technology have led to an exponential increase in the ability to identify and measure variation in a large number of candidate genes for common diseases. However, traditional methods used to study genotype-phenotype relationships, such as multiple regression, are limited in their ability to search through the high-dimensional genotype space. By integrating methods from machine learning and pattern recognition into a traditional genetic association method we have developed the Combinatorial Partitioning Method (CPM) to identify high risk subgroups that predict risk factor variation. We present here an application of CPM to identify combinations of genetic and environmental factors that predict variation in blood pressure levels in a sample of 126 women and 91 men, ages 20-50 years, ascertained without regard to health status as part of the Rochester Family Heart Study. Genotype information was available at 9 diallelic loci in 6 candidate genes for hypertension - adducin (*ADD*), angiotensinogen (*AGT*), angiotensin II converting enzyme (*ACE*), lipoprotein lipase (*LPL*), apolipoprotein E (*APO E*), and beta-2-adrenergic receptor (*ADRB2*) - and for three indices of environmental context - smoking status, body mass index, and age. Using CPM, all possible partitions of each pair of factors were evaluated and those with p-values<0.01 were then cross-validated. We found that many combinations of genetic and environmental factors predict variation in blood pressure levels and that most predictive sets show strong non-additivity. For example, the partitions that explained the most variability in systolic BP indicated gene-environment interaction involving *ACE* (I/D) and age in women and between *ADD* (Gly460Trp) and age in men (14% and 10%, respectively). Our results also suggest that heterogeneous etiologies for high blood pressure exist in the population at large that can be characterized by different gene-environment combinations. (Supported by NIH HL39107 and HL51021).

Program Nr: 69 from the 1999 ASHG Annual Meeting

Association between alcohol dependence and 5-locus haplotypes on chromosome 11p15.5 in an American Indian population. *A.W. Bergen, L. Wilhelm, R. Vallejo, S. Kim, D. McKeane, R. Robin, D. Goldman, J.C. Long.* Laboratory of Neurogenetics, NIAAA/NIH, Bethesda, MD.

Long et al. (Am J Med Genet 74:386, 1998) reported results for an autosomal genome scan of alcohol dependence in a Southwestern American Indian tribe. The best evidence for linkage was observed at the D11S1984 STR polymorphism on chromosome 11p15.5 (Lod = 3.1, p = 0.00007). This locus maps closely to two dopaminergic genes, D4 dopamine receptor (DRD4) and tyrosine hydroxylase (TH0). In order to follow-up on this finding, 5 loci in close physical proximity on chromosome 11p15.5 were genotyped in 529 subjects. All subjects belong to the same tribe and 152 were in the original study. Each subject was psychiatrically evaluated for alcohol dependence (340 affected and 189 unaffected). The loci tested were STRs at D11S1984, D11S4893, and TH0 (tyrosine hydroxylase), and two sites in the DRD4 coding sequence. Five locus haplotypes were constructed using an EM algorithm and evaluation of family relationships. Statistically significant linkage disequilibrium was demonstrated between all pairs of loci using likelihood ratio statistics with approximate chi-squared distributions. No evidence could be detected for three-way or higher levels of disequilibrium. Strong association between alcohol dependence and 5-locus haplotype ($P < 0.001$) was demonstrated using a multiway contingency table analog. A variety of restrictions on the full 5-locus haplotype model were subsequently evaluated in order to localize the association to haplotypes involving fewer loci. The best restricted-model associated alcohol dependence with 3-locus haplotypes constructed from D11S4893 and the two DRD4 polymorphisms ($P<0.03$). Interestingly, 2-locus haplotypes constructed for the DRD4 polymorphisms were not directly associated with alcohol dependence. Statistical significance for the DRD4 locus was achieved only in the presence of D11S4893 and/or TH0. These findings suggest either the presence of interactions between loci in close proximity on chromosome 11p15.5, or that there is a limit to the resolution of linkage disequilibrium mapping because of high stochastic variance introduced by genetic drift.

Program Nr: 70 from the 1999 ASHG Annual Meeting

Evidence of linkage and association between a novel TGF-b1 gene polymorphism and hip bone density in female twins: use of the S-TDT and multivariate linkage analysis. *T.D. Spector[1], H. Snieder[1], H. Molloy[2], M. Chiano[2], F. Gibson[2], A.J. MacGregor[1], R.W. Keen[1].* 1) Twin & Genetic Epidemiology Research Unit, St Thomas Hospital, London, UK England; 2) Gemini Research Ltd, Cambridge, UK.

Twin and family studies have demonstrated a strong genetic component to bone mineral density (BMD). Transforming growth factor b1 (TGF-b1) is an important regulatory cytokine in bone, and is therefore a potential candidate gene for the determination of BMD. A novel T®C polymorphism in intron 5 of the the TGF-b1 gene has been previously associated with hip BMD. The study's aim was to assess the evidence of linkage disequilibrium using an analysis with data from both the femoral neck (FN) and total hip (HP) sites which are closely correlated.

Full clinical data was available on 1,758 dizygotic (DZ) female twins (mean age 47.5 ± 11.3 yrs). BMD was measured at the two hip sites using DXA (Hologic QDR-2000). Association analysis and the sib-transmission disequilibrium test (S-TDT) were performed using structural equation modelling with the statistical programme Mx. IBD probabilities were estimated using MAPMAKER/SIBS with bivariate analysis also using Mx.

Compared to the "TT" genotype, the "CC" genotype was associated with a significant 5% reduction in FN BMD (c2=7.95, p=0.02), and a non-significant 3% decrease in HP BMD (c2=3.01). The S-TDT provided evidence of linkage disequibrium at only the FN (c2=7.41, p=0.02). Single point linkage analysis demonstrated significant linkage between the intron 5 polymorphism and FN BMD (Dc2=4.67, p=0.03), although stronger evidence for linkage was provided with bivariate analysis using FN and HP BMD in the same model, Dc2=5.05, p=0.02.

This study has demonstrated evidence of linkage disequilibrium through the use of the S-TDT between a novel polymorphism in the TGF-b1 gene and hip BMD. The study highlights the strength of multivariate linkage analysis in DZ twins to identify loci having pleiotropic effects on complex traits.

Program Nr: 71 from the 1999 ASHG Annual Meeting

Genetic Restriction of HIV-1 infection and AIDS progression by promoter alleles of Interleukin 10. *H.D. Shin[1], C. Winkler[1], J.C. Stephens[2], J. Bream[3], H. Young[3], J.J. Goedert[4], T.R. O'Brien[4], D. Vlahov[5], S. Buchbinder[6], J. Giorgi[7], C. Rinaldo[8], S. Donfield[9], A. Willoughby[10], S.J. O'Brien[2], M.W. Smith[1]*. 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) Laboratory of Experimental Immunology, National Cancer Institute, Frederick, MD 21702-1201; 4) Viral Epidemiology Branch, National Cancer Institute, Rockville, MD 20852; 5) Johns Hopkins School of Hygiene and Public Heath for AIDS Link to the Intravenous Experience, Baltimore, MD 21205; 6) San Francisco Department of Public Health, San Francisco, CA; 7) Department of Medicine/CIC, UCLA School of Medicine, Los Angeles, CA; 8) Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; 9) Rho, Inc., Chapel Hill, NC; 10) Adolescent & Maternal AIDS Branch, NICHD/NIH, Bethesda, MD.

Interleukin-10 (IL10) is a powerful TH-2 cell cytokine produced by lymphoid cells that limits HIV-1 replication in vivo, ostensibly by inhibiting macrophage/monocyte replication and secretion of inflammatory cytokines (IL1, TNF , IL6, IL8 and IL12). A genetic epidemiological scan of patients enrolled in AIDS cohorts for candidate gene-linked short tandem repeat (STR) polymorphisms revealed significant genotype associations for HIV-1 infection and progression to AIDS with markers adjacent to and tracking (by linkage disequilibrium) common single nucleotide polymorphic (SNP) variants in the IL10 promoter region. Individuals carrying the IL10-5'-592A (IL10-5'A) promoter allele were at increased risk for HIV-1 infection, and once infected they progressed to AIDS more rapidly than homozygotes for the alternative IL10-5'-592 C/C (IL10-+/+) genotype, particularly in the later stages of HIV-1 infectionAlternative IL10 promoter alleles are functionally distinct in relative IL10 production, in retention of an ETS transcription factor recognition sequence, and in binding to specific putative nuclear transcription factors suggesting a potential mechanism for IL10 allele influence on AIDS outcomes. The inhibitory influence of IL10 on HIV-1 infection and AIDS induction translates directly to genotype specific survival revealed by quantitatively distinct IL10 promoter alleles. 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: 72 from the 1999 ASHG Annual Meeting

Segregation analysis of complex diseases with variable age of onset : comparison of different methods by a simulation study and application to French families of prostate cancer. *L. Briollais[1,2], A. Valéri[3,4], E. Drelon[3], F. Tores[1], P. Berthon[3], P. Mangin[3,4], G. Fournier[3,4], O. Cussenot[3,4,5], F. Demenais[1]*. 1) INSERM U358, Hôpital Saint-Louis, Paris; 2) Registres des cancers, Martinique; 3) CeRePP/JE2153, Université Paris VII, Hôpital Saint-Louis, Paris; 4) Services d'urologie des CHU de Brest (A.V. and G.F.), Hôpital Saint-louis (O.C.) et CHU de Nancy (P.M.); 5) Institut Universitaire de France, Paris, France.

Few models of segregation analysis were developed to account for variable age of onset. The unified model (UM) can only take into account age at examination. In the Regressive Hazard Model (RHM), Abel and Bonney (1990) have incorporated survival analysis concepts into the regressive logistic model of Bonney, but interpretation of familial dependence parameters is difficult. We have proposed to extend an other formulation of the regressive approach, the Regressive Threshold Model (RTM) (Demenais 1991) to account for variable age of onset. This new formulation is based on an underlying liability to the disease, individuals affected at age k have a liability comprised between two time-dependent thresholds $[T_{k+1},T_k[$, individuals affected (unaffected) by age at examination k have a liability higher (lower) than the midpoint of this interval. The age-of-onset distribution is thus a genotype-dependent step-function. As compared to RHM, familial dependences can be expressed by odds-ratios specific to relatives' age of onset. The performances of three models (UM, RHM, RTM) were compared in analysing familial data generated with variable age of onset and a sample of 691 French families of prostate cancer. All analysis models were robust with respect to false conclusion of a major gene, the best results were obtained under RTM. The power to detect the major gene was higher under RHM than RTM, but the fit of the age-dependent penetrance with respect to the true value, was better under RTM. Segregation analyses of prostate cancer confirmed these results, all models concluded to the transmission of a rare dominant gene but with different penetrance estimations. These results assessed the ability of this new RTM approach to analyse complex diseases with variable age of onset.

Program Nr: 73 from the 1999 ASHG Annual Meeting

Testing for gene-environment interaction using affected sib-pairs. *F.Z. Sun[1], W.D. Flanders[1], Q.H. Yang[2], H.Y. Zhao[3], M.J. Khoury[2]*. 1) Emory Univ, Atlanta, GA; 2) Centers for Disease Control and Prevention, Atlanta, GA; 3) Yale Univ, New Haven, CT.

Testing for gene-environment interaction is an important problem in genetic epidemiology. Several designs are available to test for gene-environment interaction such as case-control studies, case-only studies, family based case-control studies and family studies. Families with affected sib-pairs are routinely collected to find the genetic bases of complex diseases. Here we compare several methods for detecting gene-environment interaction using affected sib-pairs. We considered the following tests: (1) comparing the fraction of affected sib-pairs sharing 0 alleles identical by descent (IBD) when the environmental factor is present with the fraction of sib-pairs sharing 0 alleles IBD when the environmental factor is absent T1; (2) comparing the average allele sharing IBD when the environmental factor is present with the average allele sharing IBD when the environmental factor is absent, T2; (3) regressing the average allele sharing probability with respect to the number of sibs having the environment, T3. We compare the tests under two models. In the first model, the environmental factor is independently distributed among individuals. We show that generally T3 is the most powerful. In the second model, the environmental factor is the same within a family. T3 and T2 are the same and are generally more powerful than T1. We also show that even for the most powerful test, the sample size needed to have a reasonable power to detect the gene-environment interaction is usually too large to be realistic. Thus alternative methods should be used to detect gene-environment interactions.

Program Nr: 74 from the 1999 ASHG Annual Meeting

Searching for Parent-of-Origin Effect in Complex Disorders. *F. Hagighi*[1], *S.E. Hodge*[2,3,4]. 1) Genetics and Development, Columbia University, New York, NY; 2) Psychiatry,Columbia University, New York, NY; 3) New York State Psychiatric Institute, New York, NY; 4) Division of Biostatistics, Columbia University, School of Public Health, New York, NY.

Parent-of-origin effect refers to the differential expression of disease in the offspring based on the sex of the transmitting parent. We use parent-of-origin effect in a broad sense to include such known underlying biological phenomena as genomic imprinting, trinucleotide expansion, and mitochondrial inheritance. Our primary goal is to identify and further quantify the magnitude of the gender-based parental effect on the penetrance of disease in the offspring. This would be of interest in studies of complex disorders with unknown etiologies, such as panic disorder, where there is an apparent sex-based difference in the risk of developing the disease. We have developed a likelihood-based approach to test for the presence of parent-of-origin effect in nuclear families. The likelihood calculation handles all possible parental mating types and variable sibship sizes. The likelihood is parameterized to include maternal and paternal transmission penetrances, as well as sex-dependent penetrances. We have also incorporated a general ascertainment model in our likelihood formulation, which allows for separate male and female ascertainment probabilities. We demonstrate the likelihood derivation and assess its utility under different inheritance and ascertainment models. We have written a simulation program to simulate data to test for: (1) difference in the estimated maternal versus paternal transmitting penetrances, (2) difference in the estimated female versus male penetrances, and (3) effect of the differential male and female ascertainment probabilities on the estimation of maternal and paternal penetrances and sex-based penetrances. The simulation and the likelihood calculation programs are available to the research community.

Program Nr: 75 from the 1999 ASHG Annual Meeting

A lipid-lowering gene exists on chromosome 13q. *H. Knoblauch[1], B. Mueller-Myhsok[2], A. Busjahn[1], L. Ben Avi[3], S. Baehring[1], H. Baron[1], S. Heath[4], H.D. Faulhaber[1], S. Shpitzen[3], A. Aydin[1], A. Reshef[3], R. Uhlmann[1], M. Rosenthal[1], O. Eliav[3], D. Schurr[1], A. Lowe[5], Y. Friedlander[3], H. Schuster[1], F.C. Luft[1], E. Leitersdorf[3]*. 1) Franz Volhard Clinic and Max Delbrueck Center for Molecular Medicine, Berlin, Germany; 2) Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany; 3) Center for Research, Prevention and Treatment of Atherosclerosis, and Department of Social Medicine, Hadassah University Hospital and School of Public Health, Jerusalem, Israel; 4) Laboratory of Statistical Genetics, Rockefeller University, New York; 5) Applied Biosystems Inc., Foster City, CA.

A lipid-lowering gene has been postulated from familial hypercholesterolemia (FH) families having heterozygous persons with normal LDL levels and homozygous individuals with LDL levels similar to heterozygous FH persons. We studied such an FH family which also had non-FH members with lower than normal LDL levels. We performed linkage analyses and identified a locus at 13q defined by markers D13S154 and D13S158. FASTLINK and GENEHUNTER yielded LOD scores >4, while an affected sibpair analysis yielding a peak multipoint LOD of 4.82, corresponding to a p value of 1.26x10-6. A multipoint quantitative-trait (QTL) linkage analysis with MLBQTL verified this locus as a QTL for LDL levels. To test the relevance of this QTL in an independent normal population, we studied monozygotic (MZ) and dizygotic (DZ) twin subjects. An MZ-DZ comparison confirmed genetic variance on lipid concentrations. We then performed an IBD linkage analysis on the DZ twins and their parents with markers at the 13q locus. We found strong evidence for linkage at this locus with LDL ($p<0.0002$), HDL ($p<0.004$), total cholesterol ($p<0.0002$), and body mass index ($p<0.0001$). These data provide support for the existence of a new gene influencing lipid concentrations in normal man.

Program Nr: 76 from the 1999 ASHG Annual Meeting

Evidence of linkage of familial hypoalphalipoproteinemia to chromosome 11q23. *E.N. Kort[1], D.G. Ballinger[2], S. Hunt[3], A. Thomas[3], V. Abkevich[3], B.R. Bowen[4], K. Harshman[2], A. Bandley[2], B. Campbell[2], K. Bulka[2], B. Wardell[2], M. McDermott[2], J. Fraser[2], T. Thorne[2], P. Hopkins[3], A. Gutin[2], M. Samuels[2], R. Williams[3], M. Skolnick[2]*. 1) Genetic Research, Intermountain Health Care, Salt Lake City, UT; 2) Myriad Genetics, Inc., Salt Lake City, UT; 3) Cardiovascular Genetics, University of Utah, Salt Lake City, UT; 4) Novartis Institute for Biomedical Research, Summit, NJ.

Coronary heart disease (CHD) accounts for half of the 1 million deaths annually ascribed to cardiovascular disease and for almost all of the 1.5 million acute myocardial infarctions. Within families having early and apparently heritable CHD, dyslipidemias have a much higher prevalence than in the general population; 20-30% of early familial CHD has been ascribed to primary hypoalphalipoproteinemia (low HDL). This study assesses the evidence for linkage of low HDL to chromosomal region 11q23 in 112 large Utah pedigrees ascertained with closely related clusters of early CHD and expanded on the basis of dyslipidemia. Linkage analysis was performed using 22 STRP markers in a 55 cM region of chromosome 11. Two-point analysis based on a general phenotype model yielded a LOD of 2.9. Multipoint analyses were performed with Mcsim, a monte-carlo, markov chain linkage analysis program. The general model yielded a multipoint LOD of 1.6. Model optimization was performed using a two-point heterogeneity LOD statistic (HTLOD) in which the multipoint haplotype results from Mcsim analysis make each marker fully informative. Several phenotype submodels, with different numbers of liability classes based on HDL levels, were analyzed. The most parsimonious model gave a HTLOD of 4.8, defining a 10 cM region. Simulations were performed to allow interpretation of this MOD-type statistic, yielding a p value of 2 x 10-4. This low HDL linkage defines a region that is clearly distinct from the ApoAI/CIII/AIV gene cluster approximately 20 cM proximal, and represents a novel localization for this phenotype.

Program Nr: 77 from the 1999 ASHG Annual Meeting

A Genetic Locus Near 1p32 Causes Autosomal Dominant Hypercholesterolemia in an Extended Utah Pedigree.

S.C. Hunt[1], P. Hopkins[1], T. Thorne[2], K. Bulka[2], B. Wardell[2], M. McDermott[2], A. Bandley[2], J. Fraser[2], M. Skolnick[2], R. Williams[1], M. Samuels[2]. 1) Dept Cardiovascular Genetics, Univ Utah, Salt Lake City, UT; 2) Myriad Genetics, Inc., Salt Lake City, UT.

In a genomic search for genes predisposing to familial combined hyperlipidemia/familial hypercholesterolemia, employing 585 autosomal markers, Utah kindred 1173 showed significant genetic linkage to a locus located near chromosome 1p32. The multipoint LOD score for this 5-generation kindred is 6.8 using a high penetrance dominant transmission model with a hypercholesterolemia phenotype. 17 out of 18 affected individuals share a common haplotype, and the haplotype is penetrant in 17 out of 18 carriers. Affected haplotype carriers span an age range from 4-67 years old. Three affected haplotype carriers have early onset coronary heart disease. Specific recombinant individuals in the pedigree restrict the region of linkage to an approximately 14 cM interval between polymorphic markers D1S2130 and D1S405. This region overlaps with a region recently identified by Varret et al., named FH3.

Program Nr: 78 from the 1999 ASHG Annual Meeting

Evidence of a gene on chromosome 3p for familial hypobetalipoproteinemia. *R.J. Neuman[1], B. Yuan[1], S.H. Duan[1], P.Y. Kwok[1], N.L. Saccone[1], J.L. Weber[2], J.S. Wu[1], K-Y. Liu[1], G. Schonfeld[1]*. 1) Washington Univ Med Ctr, St Louis, MO; 2) Ctr for Medical Genetics, Marshfield Medical Research Foundation, Marshfield, WI.

Familial hypobetalipoproteinemia (FHBL) is a disorder of lipid metabolism characterized by extremely low levels of apolipoprotein B (apoB) and total cholesterol. In a small minority of FHBL kindreds mutations have been identified in the apoB gene on chromosome 2 (2p23-24) which cosegregate with the FHBL phenotype. We have identified a 3-generation FHBL kindred (38 genotyped members) that is not linked to the apoB gene. To locate other chromosomal regions containing genes for low apoB levels, a genome wide search was conducted using 384 polymorphic markers spaced 10 cM apart on average (Weber Laboratory). Initially, individuals with apoB levels < 5th percentile for their age and sex were considered affected (n=10). Two young individuals in the 3rd generation with borderline apoB levels were considered unknown. Under a quasi-autosomal dominant mode of transmission the MLINK program gave two-point LOD scores > 1 on chromosomes 2, 3, 13, 19, 20, and 22. Additional markers were then typed in the region of these signals. MLINK LOD scores on chromosome 3 increased to 3.35 (t=0.0, penetrance=0.75) in the region of D3S2407. GENEHUNTER confirmed this finding with an NPL score of 7.5 (p=0.0004).

We conducted quantitative analyses on apoB levels (rather than the dichotomous phenotype) corrected for age and sex using variance component methods from LOKI and SOLAR. Results indicated strong evidence for linkage of FHBL in the same chromosomal region on 3p as the previous analyses. LOKI, fitting multiple chromosomes simultaneously, placed over 20 more QTLs than expected by chance into this area and SOLAR produced a two-point LOD score of 3.3. Linkage to a 2 cM region near D19S916 on chromosome 19 was also indicated by both analyses although not as strong as the chromosome 3 finding. These data indicate a heretofore unidentified locus may be responsible for FHBL in this kindred, thus demonstrating the genetic heterogeneity of FHBL.

Program Nr: 79 from the 1999 ASHG Annual Meeting

Fine scale mapping confirms the linkage of a region on Chromosome 2 with essential hypertension in Chinese. *H. Wang[1], M. Xiong[2], S. Chu[1,2], L. Jin[2,3,4], G. Wang[1], W. Yuan[1], S. Mao[1], W. Zhang[1], H. Zhou[1], Y. Zhan[1], S. Dong[1], G. Zhao[1], W. Huang[3], D. Zhu[1]*. 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 at Shanghai, Shanghai, China; 4) Institute of Genetics, Fudan University, Shanghai, China.

In a previous genome-wide scanning study performed in 79 Chinese families, we have identified three microsatellite markers (D2S168, D2S151, and D2S142) that show suggestive linkage to genes susceptible to essential hypertension (EH), with D2S151 and D2S142 in one candidate region and D2S168 in the other. To replicate the results of genome-wide screening, we extended the linkage analysis to 240 Chinese families containing 1080 individuals and 920 affected sib-pairs. Nine highly polymorphic microsatellite markers around the two aforementioned regions were typed. The average gap between these markers is about 5 cM. The multipoint parametric and non-parametric linkage (NPL) test using GENEHUNTER confirmed a linkage of EH to the region defined by D2S142 and D2S151. The LOD score peaks are at 147 cM (1.574, p=0.0035) and 159.5 cM (1.67, p=0.0028) and the NPL Z score peaks are at 141 cM (1.348, p=0.0887) and 161.3 cM (1.92, p=0.028). This observation was further supported by a transmission-disequilibrium test of the two markers in this region: D2S2196 (P=0.01) and D2S2370 (P=0.001). However, the linkage with D2S168 could not be replicated in the extended study. Our observations in the aforementioned positive region replicated finding of Krushkal et al. (1999) in Rochester family study where they showed a positive linkage of EH at D2S1334 (145cM) with p-value of 0.0353 in Caucasians. These data strongly suggest the region containing D2S142 and D2S151 may harbor genes responsible for the development of essential hypertension in Chinese population.

Program Nr: 80 from the 1999 ASHG Annual Meeting

Genome Scan in Dutch Dyslipidemic Families Reveals Novel Blood Pressure Loci. *H. Allayee[1], T.W.A. de Bruin[2], R.M. Cantor[1], B.E. Aouizerat[1], A.J. Lusis[1], J.I. Rotter[3].* 1) Department of Human Genetics, UCLA, Los Angeles, CA; 2) Department of Medicine, Academic Hospital, Maastricht, the Netherlands; 3) Division of Medical Genetics, Steven Spielberg Research Center, Los Angeles, CA.

Genes contributing to the common forms of essential hypertension and blood pressure (BP) variation are largely unknown. This may result from the underlying extensive genetic heterogeneity of this disorder. One approach to reduce such heterogeneity is to conduct gene finding efforts in families ascertained for common metabolic syndromes with associated hypertension. Familial combined hyperlipidemia (FCHL), a common lipid disorder characterized by hypercholesterolemia and/or hypertriglyceridemia with a frequency of 1% in Western populations, and 10% in survivors of early onset coronary disease, is a metabolic syndrome associated with insulin resistance, central obesity and an increased frequency of hypertension. Insulin resistance, defined by fasting insulin and more direct measures, is both associated with hypertension in cross-sectional studies and predictive in prospective studies. Furthermore, insulin resistance is also accompanied by increased free fatty acid (FFA) and increased apolipoprotein B secretion. In the present study, we analyzed a 10cM genome-wide scan in 18 Dutch FCHL pedigrees (N=240) to search for genes contributing to BP in this metabolic syndrome. A multipoint genome scan of systolic (S)BP and diastolic (D)BP identified a region on chromosome 4 exhibiting a LOD score of 3.9 with SBP (peak marker D4S2366). Interestingly, FFA levels mapped to this region, with a LOD score of 2.4. In addition, evidence for linkage of DBP to the lipoprotein lipase gene locus on chromosome 8p (LOD=1.8, peak marker D8S136) was observed. This supports previously reported findings for BP in a different population. The a-adducin gene, a gene involved in renal sodium handling, resides within the chromosome 4 peak and has been associated with elevated blood pressure in both human populations and in animal models of hypertension. This genome-wide scan of BP has identified two loci which may contribute to hypertension associated with this insulin resistant dyslipidemic syndrome.

Program Nr: 81 from the 1999 ASHG Annual Meeting

Whole-genome linkage disequilibrium mapping of the genes underlying blood pressure variation in an isolated Chinese population. *J. Xiao[1], Y. Zhao[1,2], M. Xiong[3], W. Huang[2,4], W. Zhang[1,2], X. Liu[1,2], W. Li[3], F. Hu[1,5], H. Wu[1], D. Lu[1], J. Tan[1], Z. Chen[2,4], E. Boewrinkle[3], L. Jin[1,2,3]*. 1) Inst Genetics, Fudan Univ, Shanghai, China; 2) National Human Genome Center at Shanghai, Shanghai, China; 3) Human Genetics Center, University of Texas Houston, Houston, TX; 4) Center of Molecular Medicine, Ruijin Hospital, Shanghai Second Medical University, Shanghai, China; 5) Department of Cardiovascular Diseases, Suzhou College of Medicine, Suzhou, China.

Essential hypertension is a common and important risk factor for cardiovascular diseases in industrialized nations. We used a recently developed and powerful statistical method, linkage disequilibrium based regression (LDR), to conduct a whole-genome linkage disequilibrium mapping of the genes involved in the inter-individual variations of blood pressure (BP) in an isolated Chinese population living in Xishan Island, using the microsatellite markers consisting ABI Mapping Set Version 2. We identified 61 and 31 candidate regions that show significant linkage at $p < 0.05$ for systolic BP variation and diastolic BP variation in that population, respectively, without correction for multiple tests. Among them, 41 candidate regions are supported by at least two neighboring markers and 5 regions showed positive linkage to both systolic BP and diastolic BP. Interestingly, 22 of the regions identified in this study overlap with the candidate regions identified by TDT in a Caucasian population in the Rochester Heart Study. In both studies, blood pressure was treated as a quantitative trait. We therefore concluded that (1) a large number of genes are involved in the determination of BP, and (2) a large proportion of the genes involved in the determination of BP are shared between different ethnic groups.

Program Nr: 82 from the 1999 ASHG Annual Meeting

A second locus for inherited venous malformations maps to chromosome 1p. *J.T. Calvert[1], T.J. Riney[1], M.C. Speer[2], N.C. Nevin[3], S.A. Simpson[4], D.A. Marchuk[1]*. 1) Genetics, Duke University Medical Center, Durham, NC; 2) Medicine (Medical Genetics), Duke University Medical Center, Durham, NC; 3) Northern Ireland Regional Genetics Centre, Belfast, Northern Ireland; 4) Department of Medical Genetics, Aberdeen Royal Hospitals, Aberdeen, Scotland.

Venous malformations are a common abnormality of the vasculature and may occur sporadically or as an autosomal dominant form. One familial form of venous malformations has previously been linked to chromosome 9p. Mutations in the gene TIE2, an endothelial specific receptor tyrosine kinase, have been identified in four different families. We have identified two new families which show autosomal dominant inheritance of venous malformations. These two families were used in a whole genome linkage scan at a 10 cM resolution. We established linkage to chromosome 1p with a combined maximum lod score of 5.07 in these two families thereby demonstrating locus heterogeneity for inherited venous malformations. The 18 cM linkage interval determined by recombination within affected individuals is defined by markers D1S435 and D1S221. Investigation of candidate genes within the interval should provide new insights into lesion formation in inherited venous malformations.

Program Nr: 83 from the 1999 ASHG Annual Meeting

A gene for inherited cutaneous venous anomalies ("glomangiomas") localizes to chromosome 1p21-22. *M.S. Vikkula[1], L.M. Boon[1], P. Brouillard[1], A. Irrthum[1], L. Karttunen[2], M.L. Warman[3], R. Rudolph[4], B.R. Olsen[2], J.B. Mulliken[5].* 1) Laboratory of Human Molecular Genetics, Christian de Duve Institute & Université catholique de Louvain, Brussels, Belgium; 2) Department of Cell Biology, Harvard Medical Boston, MA; 3) Department of Genetics, Case Western Reserve University School of Medicine, Cleveland, OH; 4) Division of Plastic and Reconstructive Surgery, Scripps Clinic and Research Foundation, La Jolla, CA; 5) Division of Plastic Surgery, Childrens Hospital, Boston, MA.

Venous malformations (VMs) are congenital abnormalities of veins. Although VMs usually occur sporadically, we have been able to identify families with autosomal dominant inheritance. Using linkage analysis we have established that in some families VMs cosegregate with marker alleles on chromosome 9p21 (Boon et al., Hum. Mol. Gen.,9; 1583-87, 1994); the mutation causes ligand-independent activation of an endothelial cell specific receptor tyrosine kinase, TIE-2 (Vikkula et al., Cell, 87; 1181-90, 1996). Now we show that VMs with glomus cells (known as glomangiomas), inherited as an autosomal dominant trait in five families, are not linked to 9p21, but instead link to a new locus on 1p21-p22, called VMGLOM (LOD score 12.70 with theta = 0,00), defined by AFMA205XD5 and D1S2775. Penetrance is close to 100%, but with variable expressivity for the number and size, time of occurrence, and painfulness of lesions. In contrast to the individuals with a TIE-2 mutation, members of the families showing linkage to VMGLOM did not exhibit any mucosal lesions. The linked locus is 4-6 cM (or 4-6 Mbp) based on current linkage and physical maps. We exclude three known positional candidate genes, DR1 (depressor of transcription 1), TGFBR3 (TGFb receptor 3) and TFA (tissue factor). We hypothesize that cutaneous venous anomalies (glomangiomas) are caused by mutations in a novel gene that may act to regulate angiogenesis, in concert with the TIE-2 signaling pathway.

Program Nr: 84 from the 1999 ASHG Annual Meeting

A progressive cardiac conduction defect maps to 3p21.1q. *J.J SCHOTT[1], F. Kyndt[1], V. Probst[2], H. Le Marec[2]*. 1) Cardiovascular Genetics, INSERM CJF96-01, 1 rue Gaston Veil, Nantes, France; 2) Cardiovascular department, CHU Nantes, France.ø.

Progressive cardiac conduction defect (PCCD), also called Lev's or Lenegre's disease is the most common cardiac conduction disturbance with progressive alteration of conduction through the His-Purkinje system leading to complete AV-block. It was considered as a progressive degenerative and aging cardiac disease affecting the conduction system of the heart. We have identified a four generations kindred (53 members accepted to participate in the study) with autosomal dominant transmission of a typical PCCD. Standard 12 leads ECG and signal-averaged ECG (SAECG) were performed in all members. Patients were defined as affected when right (RBBB) or left bundle branch block (LBBB) or widening of QRS complexes > 120msec with axial deviation were present, without other cause of cardiac disease. 14 patients were obviously affected (mean SAECG QRS duration =135±7 msec), none of them had structural heart disease. Cardiac conduction defect were : RBBB (n=5), LBBB (n=2), left anterior (n=2) or posterior hemiblock (n=1) and long PR interval (>210 msec) (n=9), 4 patients received pacemaker implantation because of syncope or complete AV-block. Clinical follow-up has shown that PCCD increases with age. 9 patients younger than 40 had abnormal QRS duration (mean SAECG QRS duration =1063 msec), their genetic status was considered unknown. In the 26 remaining patients mean SAECG QRS duration was 892 msec. Linkage analysis allowed us to exclude the previously described locus on chromosome 19q22.3 and loci for other hereditary heart disease with associated conduction defects. Marker flanking the SCN5A locus (D3S1260) was strongly linked to the disease (LOD score 6.05 at 0% of recombination). Identification of 2 recombinations event allowed us to refine the candidate interval including the SCN5A gene between D3S2338 and D3S3559. SCN5A gene is a strong candidate for PCCD. Identification of the genetic defect will allow to genotype patients with unknown status and should give new insight in the physiopathology of heart blocks.

Program Nr: 85 from the 1999 ASHG Annual Meeting

Mapping of a first locus for autosomal dominant myxomatous mitral valve prolapse to chromosome 16p11.2-p12.1. *S. Disse-Nicodeme[1], E. Abergel[2], A. Berrebi[3], A.-M. Houot[1], J.-Y. Le Heuzey[2], B. Diebold[2], L. Guize[2], A. Carpentier[3], P. Corvol[1,4], X. Jeunemaitre[1,4]*. 1) INSERM U36, College de France, Paris, France; 2) Departement de Cardiologie Medicale, Hopital Broussais, Paris, France; 3) Service de Chirurgie Cardiovasculaire, Hopital Broussais, Paris, France; 4) Laboratoire de Biologie Moleculaire et Centre d'Investigation Clinique, Hopital Broussais, Paris, France.

Myxomatous mitral valve prolapse (MMVP), also called Barlows disease, is a common cardiac abnormality and affects up to 5% of the population. It is characterized by excess of tissue resulting in billowing of the mitral leaflets which may be complicated by prolapse, with typical histological findings such as myxomatous degeneration, degradation of collagen and elastin. Previous reports have proposed an autosomal dominant inheritance of the trait with age- and sex-dependent expression. We performed a systematic echocardiographic screening of the first-degree relatives of 17 patients who underwent mitral valve repair, and identified four pedigrees showing such inheritance. Genome-wide linkage analysis of the most informative pedigree (24 individuals, 3 generations) gave significant linkage for markers mapping to chromosome 16p, with a two-point lod score maximum for D16S3068 (Zmax=3.30 at q=0). Linkage to D16S3068 was confirmed in a second family (Zmax=2.02 at q=0), but excluded for the two remaining families, demonstrating the genetic heterogeneity of the disease. Multipoint linkage analysis performed on the two linked families, using nine additional markers with two different statistical models, gave a maximum multipoint lod score of 5.45 and 5.68 for D16S3133, according to the model used. Haplotype analysis defined a 5-cM minimal MMVP-1 locus between D16S3068 (16p11.2) and D16S420 (16p12.1). The identification of this locus represents a first step towards a new molecular classification of mitral valve prolapse.

Program Nr: 86 from the 1999 ASHG Annual Meeting

The creation of mouse models for mitochondrial DNA (mtDNA) disease. *D.C. Wallace[1], S.E. Levy[1], J.E. Sligh[1,2]*. 1) Center for Molecular Medicine, Emory Univ School of Medicine, Atlanta, GA; 2) Department of Dermatology, Emory Univ School of Medicine, Atlanta, GA.

A wide spectrum of degenerative diseases have now been associated with mtDNA mutations. However, the complexity of cytoplasmic genetics and of mitochondrial biochemistry has made it difficult to investigate the pathophysiology of these diseases or to develop effective therapies. Consequently, well defined mouse models for mtDNA disease are essential. We now report an effective procedure for the introduction of mtDNA mutations into the female mouse germline. A cultured mouse cell line carrying mouse NZB mtDNAs was created by generating a r^0(mtDNA-deficient) LM(TK^-) cell line and fusing it with synaptosomes from NZB mouse brain. Synaptosome cybrids were selected in HAT medium without uridine or pyruvate. The [LM(TK^-) /NZB mtDNA] cells were then enucleated and fused to female 129/Sv embryonic stem (ES) cells, which had been cured of their mitochondria by treatment with the mitochondrial poison rhodamine-6G (R6G). NZB mtDNAs differ from C3H [LM(TK^-)] and 129/Sv mtDNAs by about 108 nucleotides. ES cells, virtually homoplasmic for the NZB mtDNAs, were then injected into blastocysts and chimeric female mice recovered. Mating of these females resulted in transmission of the NZB mtDNAs to a founder female, which in turn transmitted the NZB mtDNAs to multiple male and female offspring. All of the second generation mice proved to be heteroplasmic for the NZB and 129/Sv mtDNAs. Using the same strategy, mouse 501-1 cells carrying chloramphenicol resistance (CAP^R) mutant mtDNAs were enucleated and fused to R6G-treated female 129/Sv ES cells. ES cells containing the CAPR mtDNAs were recovered and injected into blastocysts. Highly chimeric females were obtained and found to harbor CAP^R mtDNAs in their tissues. These animals are now being mated to determine if the CAP^R mtDNA will be maternally transmitted. With these methods, mtDNA variants from either whole animals or cultured cells can now be transferred into the mouse germline at will.

Program Nr: 87 from the 1999 ASHG Annual Meeting

Dedifferentiation of vascular smooth muscle cells initiates vascular disease in an animal model of Marfan syndrome. *N. Biery[1], T. Bunton[2], D. Keene[3], L. Sakai[3], B. Gayraud[4], F. Ramirez[4], H. Dietz[1]*. 1) Inst of Gen Med,HHMI,Johns Hopkins Univ,Baltimore,MD; 2) Dept Pathol,Johns Hopkins Univ, Baltimore,MD; 3) Shriners Hosp,Portland,OR; 4) Mt Sinai Sch Med,NY,NY.

Marfan syndrome (MFS) is associated with early death due to aortic aneurysm. The condition is caused by mutations in the gene (FBN1) encoding fibrillin-1, a major constituent of extracellular microfibrils. Prior observations suggested that a deficiency of microfibrils causes failure of elastic fiber assembly during late fetal development. Our phenotypic characterization of mice homozygous for a targeted hypomorphic allele (R1) of Fbn1 revealed an unanticipated pathogenetic sequence for aneurysm. Ultrastructural analysis revealed the formation of elastic lamellae in -/- mice that were abnormally smooth, manifesting the loss of cell attachments that are normally mediated by fibrillin-1. Adjacent vascular smooth muscle cells (VSMC) adopted a dedifferentiated phenotype characterized by the synthesis of fetal markers including tropoelastin mRNA. VSMC processes were found adjacent to zones of apparent degradation of elastic lamellae. Intense elastolysis correlated with immunohistochemical detection of matrix metalloproteinase (MMP)-9 in the aortic media. Early vascular lesions showed focal regions of calcification that were often associated with breaks in elastic lamellae. Once the internal and external elastic lamellae were breached, VSMC adopted a migratory and proliferative phenotype characteristic of dedifferentiated cells, leading to intimal hyperplasia. Recruitment of inflammatory cells into the vessel wall late in pathogenesis was associated with complete loss of architecture and aueurysm formation. These data suggest that VSMC dedifferentiation initiates a nonproductive attempt to remodel a mature elastic matrix that culminates in the vascular manifestations of MFS. In retrospect, we find all of these pathologic changes in archived specimens from affected patients. This refined understanding of pathogenesis will facilitate development of therapeutic strategies. We are currently determining whether a transgene that overexpresses a potent tissue inhibitor of MMPs can modulate disease phenotype.

Program Nr: 88 from the 1999 ASHG Annual Meeting

Math1 & atonal: Functionally conserved genes essential for balance, hearing & proprioception. *N.A. Bermingham[1,2], N. Ben-Arie[1,2], B.A. Hassan[1,2], H.J. Bellen[1,2], A. Lysakowski[3], H.Y. Zoghbi[1,2].* 1) Howard Hughes Medical Institute; 2) Baylor College of Medicine, Houston Tx; 3) University of Illinois.

Compare a fly and mammal: superficially there are no obvious similarities; for example a fly has antennae for the detection of sound while mammals have ears. However, a closer look at their DNA and gene expression revealed similarities between Drosophila and mice. In this study we present data illustrating functional conservation between the basic helix-loop-helix transcription factor ato and one of its closest homologs mouse atonal homolog 1 (Math1). ato is required for the development of the eye and chordotonal organs, the sense organs of stretch, proprioception and hearing. Loss of ato results in loss of chordotonal organs, located in the body wall, leg joints and antennae. To define Math1 expression pattern during development and evaluate its in vivo role, we replaced the Math1 open reading frame with the b-galactosidse reporter gene. Xgal staining of heterozygous embryos revealed expression in the dorsal spinal cord, external germinal layer (EGL) of the cerebellum, brainstem, inner ear hair cells, Merkel cells of the skin, joints and gut. Math1 null mice die immediately after birth due to respiratory failure, they lack the EGL, have no inner ear hair cells and have significant reduction of lacZ staining in the Merkel cells. Inner ear hair cells and Merkel cells are mechanoreceptors that mediate hearing, balance and pressure sensation respectively. The loss of hair cells occur as early as E13.5 in sensory epithelia, demonstrating that Math1 is essential for their genesis. To further study the functional conservation between Math1 and ato, we overexpressed Math1 in ato mutant flies which led to partial rescue of the ato phenotype. These data demonstrate that Math1 is essential for granule neurons and inner ear hair cells. They also underscore the functional conservation between Math1 and ato. It is fascinating that mechanoreceptors in flies and mammals require similar molecules for their genesis and development. Lastly, the finding that Math1 is essential for hair cell genesis is potentially of therapeutic benefit for age-related deafness and balance disorders.*Math1 ato*.

Program Nr: 89 from the 1999 ASHG Annual Meeting

Spatial separation and differential demethylation of parental genomes in mouse preimplantation embryos. *T.H. Haaf[1], W.G. Mayer[1], A. Smith[1], A. Niveleau[2], R. Fundele[1]*. 1) Max-Planck-Institute of Molecular Genetics., Berlin, Germany; 2) CNRS, Université J. Fourier de Grenoble, La Tronche France.

An enormous body of data from classical genetics, nuclear transplantation experiments and human imprinting disorders, suggests that normal mammalian development and adult phenotype require the participation of both a maternal and a paternal genome. The one-cell embryo is formed from two very different sets of chromatin: the highly compact, genetically totally inert sperm DNA, and the undermethylated maternal egg chromatin. These differential gametic marks are largely reset during embryonic development into their functional forms. Until now the general belief was that the paternal and the maternal chromosome complements were mixed together after breakdown of the pronuclear envelopes during progression through the first metaphase, to form the zygotic genome. We present the first comprehensive analysis of the higher-order nuclear organization of parental chromatin during early mammalian development. Mouse eggs fertilized with BrdU-labeled sperm and detection of BrdU in preimplantation embryos of the next generation, and differential heterochromatin staining in mouse (*Mus musculus* x *M. spretus*) interspecific embryos demonstrate topological separation of parental genomes after nuclear envelope breakdown. Each chromatin set occupies approximately one half of the nuclear volume. Separation of chromatin according to parental origin is preserved up to the four-cell embryo stage and then gradually disappears. This spatial separation is associated with differential epigenetic reprogramming. Anti-5-methylcytosine staining shows that the paternal genome is largely and actively demethylated within six to eight hours after fertilization. Similar genome-wide demethylation of the female genome occurs by a replication-dependent mechanism around the second and third cleavage stages. Separate nuclear compartments at the time during preimplantation development, when extreme degrees of differential methylation occur, may be associated with chromatin remodeling to program the appropriate patterns of developmental gene expression.

Program Nr: 90 from the 1999 ASHG Annual Meeting

Clarifying the Role of Gamma Crystallin in Congenital CCL Cataract. *E. Heon[1,2], M.K. Priston[1], G.D. Billingsley[1], N.H. Lubsen[3], F.L. Munier[4].* 1) Eye Research Institute of Canada; 2) University of Toronto, Dept of Ophthalmology, Research Institute The Hospital for Sick Children, Toronto, Ontario, Canada; 3) Department of Biochemistry, University of Nijmegen, The Netherlands; 4) Ocular Genetics Unit, Division of Medical Genetics, CHUV, Hôpital Ophtalmique Jules Gonin, Lausanne, Switzerland.

Cataracts constitute the leading cause of blindness worldwide and the mechanisms of lens opacification remain unclear. The Coppock-like cataract is an autosomal dominant cataract characterized by dust-like opacification of the fetal nucleus with frequent involvement of the zonular lens. It had been associated with sequence changes of the crystallin gamma E pseudogene at 2q33-35. Further studies were done to elucidate the true role of gamma crystallin (CRYG) in the formation of Coppock-like cataracts. Direct cycle sequencing of Gamma C, D, and E were performed in the original family affected with Coppock-like cataract and linked to chromosome 2q33-35. Mutational analysis of CRYGE demonstrated that the proposed Coppock-like cataract sequence changes were polymorphic, present in 23% of the control population. Sequencing of CRYGD failed to document any disease causing mutation. A novel missense mutation identified in a highly conserved segment of exon 2 of gamma C 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. This is the first evidence that CRYGC plays a role in human cataract formation. There is currently no evidence that upregulation of the pseudogene CRYGE causes cataracts. The identification of cataract-related genes is critical to the better understanding of the biology of cataract formation and progression.

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Program Nr: 91 from the 1999 ASHG Annual Meeting

Analysis of TIGR protein structure supports *homoallelic complementation* to account for autosomal dominant heterozygote-specific inheritance of glaucoma. *S. Moisan[1], M.A. Rodrigue[1], D. Vermeeren[1], T.D. Nguyen[2], J.R. Polansky[2], J. Morissette[1], V. Raymond[1].* 1) Molecular Endocrinology, CHUL Research Ctr, Quebec, PQ, Canada; 2) Ophthalmology, UCSF, CA.

We reported that 4 homozygotes carrying the autosomal dominant (AD) K423E mutation within the trabecular meshwork-induced glucocorticoid response (*TIGR*) gene, also known as myocilin, did not manifest primary open-angle glaucoma. $TIGR^{K423E}$ may be thus the 1st mutation that causes an AD heterozygote-specific phenotype in humans. To account for this particular mode of inheritance, we envisaged *homoallelic complementation*. According to this model, similar to metabolic interference, $TIGR^{K423E}$ would act as a dominant negative mutation resulting in defective heteromultimers but functional homomultimers. To test for this model, we investigated TIGR protein structure. COS-7 cells were transfected with vectors encoding various *TIGR* cDNA constructs. Newly synthesized proteins were analyzed by immunoblotting. Under non-reducing conditions, $TIGR^{\text{wild-type(wt)}}$ was detected as 2 major complexes composed of TIGR polypeptides migrating at approximately 120 and 240 kDa. Under reducing conditions, both complexes were resolved into 2 lower MW forms migrating at about 57 and 63 kDa. Site-directed mutagenesis and glycosidase treatments demonstrated that the 63 kDa isoform was N-glycosylated at Asn-57. Co-transfection of $TIGR^{K423E}$ cDNA with its wt counterpart generated 2 high MW complexes identical to those observed with the wt construct alone. On the other hand, co-transfection of the wt cDNA with a $TIGR^{Q368Stop}$ recombinant, that yielded a truncated polypeptide and was associated with glaucoma, generated 1 additional intermediate complex formed by wt/Q368Stop heterodimers at about 100 kDa. Deletion and cross-linking studies revealed that TIGR leucine zipper was essential for dimerization. Our results showing that $TIGR^{wt}$ and $TIGR^{K423E}$ polypeptides formed homomultimers are consistent with homoallelic complementation to account for the unaffected mutant homozygotes. These data also suggest that heterodimer formation between a normal and a mutated TIGR polypeptide may be a critical aspect of TIGR disease-causing mutations.

Program Nr: 92 from the 1999 ASHG Annual Meeting

Retinoschisin, the X-linked Retinoschisis protein, is a secreted photoreceptor protein. *D. Trump[1], C. Grayson[1], S.N.M. Reid[2], J. Sowden[3], J.A. Ellis[1], D.B. Farber[2], J.R.W. Yates[1]*. 1) Medical Genetics, Cambridge Institute for Medical Research,Cambridge University Cambridge, England; 2) Jules Stein Institute, UCLA School of Medicine, Los Angeles, CA, USA; 3) Developmental Biology and Neural Development Unit, Institute of Child Health, University College London, UK.

X-linked retinoschisis (RS) leads to visual deterioration in affected males due to splitting in the inner layers of the retina. The causative gene RS1 was recently identified and numerous inactivating mutations have been found in RS families. RS1 encodes a 224 amino acid protein retinoschisin. Our recent studies indicate that retinoschisin is a secreted photoreceptor protein. We first investigated the expression pattern of RS1 in the human and mouse retina using in situ hybridisation with full length RS1 cDNA digoxygenin-labelled riboprobes and found that RS1 is expressed only in the photoreceptors and not in other layers of the retina. We also generated a polyclonal antibody against a 14 amino acid peptide from a unique region within retinoschisin. This detects a 28-30 KDa protein in reduced retinal samples, but multimers sized 48 KDa and above under non-reducing conditions and is the first anti-retinoschisin antibody to be characterised. Immunohistochemistry studies indicate retinoschisin is present both in the photoreceptors and the inner nuclear layer of the retina suggesting the protein is secreted from photoreceptors and functions within the inner retinal layers. Furthermore, differentiated Weri cells (a retinoblastoma cell line) were found to express RS1. Retinoschisin was present in the medium confirming that it had been secreted. Therefore, our results indicate that X-linked retinoschisis is caused by a secreted photoreceptor protein. This is the first example of a secreted photoreceptor protein causing a retinal dystrophy.

Program Nr: 93 from the 1999 ASHG Annual Meeting

A new locus for autosomal recessive Congenital Microphthalmia maps to 14q24.3 and caused by a homozygous mutation in the CHX10 gene. *E.F. Percin[1,2], K. Arici[2], D.J. Horsford[3], A. Kocak-Altintas[4], A.N. Akarsu[5], R.R. McInnes[3], M. Sarfarazi[1]*. 1) Molecular Ophthalmic Genetics; Univ. Connecticut Health Center, Farmington; CT; 2) Medical Biology & Ophthalmology, Cumhuriyet Univ. Turkey; 3) Devel Biol, Hosp for Sick Children, Toronto; 4) Ophthalology Dept, Ankara Hospital; 5) DNA/Cell Bank, Hacettepe Univ., Turkey.

Microphthalmia is a clinically heterogeneous group of developmental eye disorders that usually present itself as an isolated entity but occasionally several affected subjects clustered within the same family. Small eye, high hyperopia and high eye/lens volume ratio typically characterize isolated microphthalmia. Congenital cataract and coloboma are also commonly associated with this condition. We have recently screened 9 multiply affected families with congenital microphthalmia for possible linkage to 14q and several other regions on chromosomes 18 (RX) and 20 (EYA2). None of these families showed any apparent linkage to RX, EYA2 or a previously reported locus on 14q32. However, 3 smaller families showed homozygosity for a region on 14q21-q22 that contains the OTX2 gene (see Sarfarazi et al). Herein, we report the mapping of a new autosomal recessive microphthalmia locus to the 14q24.3 region in another three-generation consanguineous Turkish family. The affected members of this family have bilateral microphthalmia, congenital cataract, coloboma of iris and scleral thickening. The optic nerve is within the normal thickness with no apparent aplasia as confirmed by computerized tomography. Hapolotype analysis of 18 DNA markers from 14q24.3 positioned the disease locus within a 6.3 cM that is flanked by 2 DNA markers of D14S77 and D14S61. A region of homozygosity was identified for a total of 5 DNA markers, including a CA-repeat that is located within the 2nd intron of the CHX10 gene. Radiation hybrid mapping of the CHX10 positioned this gene 1.71 cR below D14S71. Cloning and sequence analysis of this gene identified a homozygous mutation (R200Q) in this family (see Horsford et al). Support: TUBITAK, NATO-B2.

Program Nr: 94 from the 1999 ASHG Annual Meeting

Mutations in a human homolog of Drosophila crumbs cause retinitis pigmentosa with preserved para-arteriolar retinal pigment epithelium (RP12). *A. den Hollander[1], J. ten Brink[2], Y. de Kok[1], S. van Soest[2], I. van den Born[2], M. van Driel[1], D. van de Pol[1], U. Kellner[3], C. Hoyng[4], A. Westerveld[2], H. Brunner[1], E. Bleeker-Wagemakers[2], A. Deutman[4], J. Heckenlively[5], F. Cremers[1], A. Bergen[2].* 1) Dept. of Human Genetics, University Hospital Nijmegen, Nijmegen, the Netherlands; 2) The Netherlands Ophthalmic Research Institute, Amsterdam, the Netherlands; 3) Dept. of Ophthalmology, University Clinic Benjamin Franklin, Free University Berlin, Berlin, Germany; 4) Dept. of Ophthalmology, University Hospital Nijmegen, Nijmegen, the Netherlands; 5) Dept. of Ophthalmology, University of California, Los Angeles, CA.

Retinitis pigmentosa (RP) comprises a clinically and genetically heterogeneous group of diseases that afflicts approximately 1.5 million people worldwide. Affected individuals suffer from a progressive degeneration of the photoreceptors, eventually resulting in severe visual impairment. To isolate candidate genes for chorioretinal diseases, we cloned cDNAs specifically or preferentially expressed in the human retina and the retinal pigment epithelium (RPE) through a novel suppression subtractive hybridization method. One of these cDNAs (RET3C11) mapped to chromosome 1q31-q32.1, a region harboring a gene involved in a severe form of autosomal recessive RP which is characterized by a typical preservation of the para-arteriolar RPE (RP12). The full length cDNA encodes an extracellular protein with 19 EGF-like domains, 3 laminin A G-like domains and a C-type lectin domain. It is homologous to Drosophila crumbs protein, and denoted CRB1 (crumbs homolog 1). In seven unrelated RP patients with preserved para-arteriolar RPE, we identified a homozygous AluY insertion disrupting the open reading frame, two homozygous missense mutations, and seven compound heterozygous mutations in the CRB1 gene. Its similarity to crumbs suggests a role in cell-cell interaction and possibly in the maintenance of cell polarity in the retina. The distinct RPE abnormalities observed in RP12 patients and the unknown function of CRB1 in the retina, suggest that CRB1 mutations trigger a novel mechanism of photoreceptor degeneration.

Program Nr: 95 from the 1999 ASHG Annual Meeting

The 4N cell cycle delay in Fanconi anemia cells is caused by blocked DNA replication not a G2/M checkpoint.

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Fanconi anemia (FA) is an autosomal recessive disease characterized by progressive bone marrow failure and cancer susceptibility. Cells from FA patients are sensitive to DNA crosslinks, but the molecular defect remains elusive. In order to gain a better understanding of the defect underlying this disease, we investigated the cellular response and cell cycle kinetics of normal and FA cells to DNA interstrand crosslinks (XLs). Primary fibroblasts from FA patients group A and C, their retrovirally corrected controls, and wild-type controls were exposed to different doses of psoralen-UVA light. Cells were synchronized, and the XLs were introduced at specific times in the cell cycle. Cell viability, DNA content, and chromosome breakage were analyzed. In contrast to previous reports in the literature, we found that high doses of XLs produced an irreversible cell cycle arrest in S phase, not in G2/M. At lower doses, the DNA content of arrested cells was almost 4N and cells eventually reentered the cell cycle. Interestingly, XLs introduced specifically during G2 did not inhibit or delay the subsequent mitosis, but resulted in an arrest in the following S-phase. Chromosome breakage was absent, even at the highest doses of XLs. In contrast, mitosis was completely blocked when XLs were introduced during S phase. XL-induced chromsome breakage was only observed if cells had gone through S-phase first. These results imply that DNA replication is absolutely required to elicit a cellular response to DNA interstrand crosslinks. Indeed, cells treated and maintained in G1 were more resistant to psoralen-UVA than cycling cells. Comparison of FANCA and FANCC cells to their isogenic controls confirmed that FA cells were specifically sensitive to DNA interstrand XLs, and not monoadducts. The S-phase arrest was induced at similar doses of XLs in FA cells and controls, but the kinetics of recovery were much slower in FA cells. Thus, we hypothesize that FA proteins play a crucial role in the resolution of XL-induced replication arrest. A model for the cellular recognition of XLs will be presented.

Program Nr: 96 from the 1999 ASHG Annual Meeting

Positional cloning of the Fanconi Anemia complementation group D (*FANCD*) gene. *C.D. Timmers, J.A. Hejna, C. Reifsteck, S.B. Olson, R.E. Moses, M.J. Thayer, M. Grompe.* Department of Molecular and Medical Genetics, Oregon Health Sciences University, Portland, OR.

Fanconi Anemia (FA) is an autosomal recessive disease characterized by progressive bone marrow failure, multiple congenital anomalies and a predisposition to cancer. FA cells are hypersensitive to interstrand DNA cross-linking agents. At least 8 separate complementation groups exist and 3 out of 8 genes have previously been cloned by cDNA complementation strategies. We have previously reported the mapping of the rare FA group D gene (*FANCD*) to chromosome 3p26 using microcell mediated chromosome transfer. Deletion analysis of > 300 microcell hybrids of *FANCD*$^-$ cells containing an additional human chromosome 3, narrowed the gene location to a 200 kb interval. Three candidate cDNAs in the critical region were extensively analyzed. One candidate gene had 2 sequence alterations (1 paternal, 1 maternal) in the *FANCD*$^-$ patient cell line, PD20. These changes were not present in 600 control chromosomes. The paternal allele carries a R1236H missense mutation in exon 37. The maternal allele contains a nt372A->G base pair change in the -2 position of the splice donor site between exons 5 and 6. 49 independent cDNA clones carrying this change all contained varying amounts of sequence from intron 5. In contrast, all paternally derived cDNAs and multiple wild-type control cDNAs, were properly spliced. We therefore consider the nt372A->G change to be a splice mutation and the candidate cDNA to be the *FANCD* gene. The *FANCD* gene encodes a 1472 amino acid protein with no identifiable motif, except for a single putative transmembrane domain. The mRNA is ubiquitously expressed at very low levels. The gene has 43 exons, the first being non-coding. Database searches revealed a high degree of conservation with a plant (Arabidopsis thaliana) protein, but no other homologies. Interestingly, overexpression of FANCD using conventional mammalian expression vectors causes rapid cell death consequently preventing functional complementation.

Program Nr: 97 from the 1999 ASHG Annual Meeting

Cellular resistance to cross-linkers requires direct interaction between the Fanconi anemia proteins FANCA and FANCG in the nucleus. *F.A.E. Kruyt, F. Abou-Zahr, H. Mok, H. Youssoufian.* Dept. Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Fanconi anemia (FA) is an autosomal recessive disease characterized by bone marrow failure, developmental defects, and cancer predisposition. Hypersensitivity to crosslinkers, a hallmark of FA cells, suggests that the FA disease gene products (FANCA, FANCC and FANCG) are involved in cellular defense mechanisms. We recently demonstrated that the FANCA and FANCG proteins interact directly with each other (Waisfisz et al. PNAS, in press). Here we have determined these interaction domains more precisely and investigated the effect of complex formation on cross-linker sensitivity. Deletion mapping of FANCA reveals a novel protein interaction domain involving the residues 18-29 in the amino terminus, and alanine mutagenesis in this region reveals that at least two arginines and a leucine residue are important for binding to FANCG. Reciprocal mapping experiments show that two non-contiguous regions in FANCG between amino acids 400-475 and 585-622 contribute to binding to FANCA. The latter domain greatly enhances the binding affinity. Immunofluorescence studies show a nearly identical expression pattern for the two proteins in the nuclear and cytoplasmic compartments of Hela cells. Moreover, in co-transfection experiments, FANCG mimics the expression pattern of nuclear- or cytoplasmic-targeted FANCA generated by fusions to heterologous nuclear-import and -export signals, respectively. Thus, the subcellular distribution of FANCG correlates closely with that of FANCA. FANCA-FANCG interactions are functionally important as removal of the interaction domain on FANCA disrupts the ability of wild type FANCA to correct the sensitivity of FA group A cells to mitomycin C. Conversely, stable expression of FANCA mutants with an intact FANCG-interaction domain induces hypersensitivity to mitomycin C in HeLa cells, most likely through a dominant-negative mechanism. Our findings demonstrate that FANCA-FANCG complex formation is necessary for cellular resistance to crosslinkers.

Program Nr: 98 from the 1999 ASHG Annual Meeting

Identification, characterisation and expression of the SEDL gene for X-linked spondyloepiphyseal dysplasia tarda (SEDL). *J. Gecz[1,2], A.K. Gedeon[1], S. Chiveralls[1], T. Cox[3], S. Heuertz[4], M. Le Merrer[4], G.E. Tiller[5], J.C. Mulley[1,3]*. 1) Department of Cytogenetics and Molecular Genetics, Centre for Medical Genetics, Womens and Childrens Hospital, North Adelaide, Australia; 2) Department of Paediatrics, Adelaide University, Adelaide, Australia; 3) Department of Genetics, Adelaide University, Adelaide, Australia; 4) Institute Necker, INSERM U-393, Paris, France; 5) Division of Medical Genetics, Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, TN, USA.

Spondyloepiphyseal dysplasia tarda (SEDL) is a rare, genetically heterogeneous osteochondrodysplasia of which both autosomal and X-linked forms have been described. We refined the location of the SEDL gene locus at Xp22.2 to an interval of less than 170 kb by critical recombination events at DXS16 and AFMa124wc1 in two Australian families. Three previously characterised and one new candidate gene were detected in the region. The latter in which novel mutations were detected in ten unrelated SEDL families was identified as SEDL. The SEDL gene is transcribed as a 2.8 kb transcript composed of 6 exons and shows multitissue expression and alternative splicing pattern, icluding cartilage. It has a pseudogene on chromosome 19 (SEDLP) which is also transcribed, but as a truncated 0.75 kb transcript. Although there are six nucleotide differences between the ORF of the SEDL gene and the SEDLP pseudogene, both putative protein products would be 100% identical. Southern blot analysis of SEDL indicate presence of other pseudogene(s) and/or copies elsewhere in the genome. The SEDL gene encodes a 140 amino acid protein with a putative role in ER-to-Golgi vesicular transport. Subcellular localisation studies with both FLAG epitope tagged and GFP constructs would support this hypothesis. Identification and characterisation of this gene enables diagnosis of carriers in affected families and extends the understanding of the molecular pathways involved in skeletal development.

Program Nr: 99 from the 1999 ASHG Annual Meeting

Truncating mutations in the SEDL gene at Xp22.2 cause spondyloepiphyseal dysplasia tarda (SEDL). *G.E. Tiller[1], A.K. Gedeon[2], V.L. Hannig[1], D. Dozier[1], B.G. Kousseff[3], W.G. Cole[4], J.L. Haines[1], M.E. Garcia[1], J.C. Mulley[2,5], J. Gecz[2,5]*. 1) Vanderbilt University School of Medicine, Nashville, TN; 2) Women's and Children's Hospital, North Adelaide, SA, Australia; 3) University of South Florida, Tampa, FL; 4) Hospital for Sick Children, Toronto, Canada; 5) University of Adelaide, Adelaide, SA, Australia.

Spondyloepiphyseal dysplasia tarda (SEDL) is a genetically heterogeneous disorder characterised by mild-moderate short stature and early-onset osteoarthritis. Both autosomal and X-linked forms have been described. We have recently identified the locus for the X-linked recessive form, which maps to Xp22.2 [Gedeon AK et al., Nature Genetics, in press]. Recombination events in two Australian SEDL families narrowed the critical region to the 170kb interval between DXS16 and AFMa124wc1. Within this region, a gene was identified which generates a 2.8kb transcript encoding a 140 amino acid protein. Northern analysis and RT-PCR demonstrated expression of SEDL in multiple tissues, including cartilage and osteoblasts. Three unique dinucleotide deletions were identified among three Australian pedigrees. We now report characterization of unique mutations among three North American SEDL pedigrees and one New Zealand pedigree, including a splice donor site mutation 3' to exon 3, two frameshift mutations in exon 5, and a putative splice acceptor site mutation 5' to exon 5. All mutations are predicted to significantly truncate the expressed protein. Homologues of the SEDL gene include a pseudogene on chromosome 19, as well as expressed genes in mouse, rat, Drosophila, C. elegans, and yeast. The latter homologue, p20, has a putative role in vesicular transport from endoplasmic reticulum to Golgi. These data suggest involvement of a previously unappreciated intracellular pathway in cartilage homeostasis.

Program Nr: 100 from the 1999 ASHG Annual Meeting

Overexpression of a novel protein containing 40 consecutive glutamines results in extranuclear aggregates. *J.K. Cooper, H. Bruce, M.F. Peters, F.C. Nucifora, Jr., D.A. Gorelick-Feldman, J.J. Kleiderlein, C.A. Ross, R.L. Margolis.*
Div. of Neurobiology, Dept. of Psychiatry, Johns Hopkins Univ Sch of Med, Baltimore.

In an effort to identify candidate genes for neurological and psychiatric disorders, we have recently cloned a series of cDNAs encoding long stretches of polyglutamine. One of these, initially termed CAGH44, is a novel cDNA that encodes 40 consecutive glutamines and maps to 6q14-15. We have now cloned a complete open reading frame for this cDNA, performed preliminary sequence and expression analyses, and overexpressed the protein in several cell lines. The open reading frame encodes 431 aa, with at least one region of alternative splicing. The predicted protein is hydrophilic and contains a near perfect C2H2 zinc finger motif. Preliminary Northern analysis suggests a low level of expression in multiple brain regions. Overexpression of cDNAs containing the complete open reading frame in COS7, HEK293, or N2a cell lines resulted in moderately large cytoplasmic aggregates of the protein. Overexpression of a construct containing 79 consecutive glutamines resulted in very large extranuclear aggregates. Understanding the functional and structural characteristics of this protein may help elucidate some of the pathogenic properties of the expanded glutamine repeats that lead to disease. The work was support in part by the HDF, HS16375, MH01275, and MH50763.

Program Nr: 101 from the 1999 ASHG Annual Meeting

Color bar-coding the BRCA1 gene: a usefull strategy for the detection of large rearrangements. *S. Gad[1], A. Aurias[2], N. Puget[3], A. Mairal[2], C. Schurra[4], M. Montagna[5], S. Mazoyer[3], A. Bensimon[4], D. Stoppa-Lyonnet[1]*. 1) Oncology Genetics, Institut Curie, Paris, France; 2) INSERM U509, Institut Curie, Paris, France; 3) International Agency for Research on Cancer, Lyon, France; 4) Biophysique de l'ADN, Institut Pasteur, Paris, France; 5) Department of Oncology and Surgical Sciences, University of Padova, Italy.

Besides small sized and point mutations of the BRCA1 gene, large rearrangements may be at the origin of breast-ovarian cancer predisposition. Ten deletions have been reported so far, ranging in size from 510 pb to 23.8 kb, scattered over the gene, and detected by Southern-blot. A duplication has also been identified by the analysis of lymphocyte transcripts. These rearrangements are due to unequal recombination between Alu sequences. To detect large alterations, which are expected to have a certain part in the BRCA1 mutation spectrum, it seemed us important to have a panoramic view of the entire gene, including coding and non coding sequences, and flanking regions. We have therefore developped a multi-color hybridization strategy applied to the Dynamic Molecular Combing, a technic which allows reproductive measures of DNA fragments thanks to a constant stretching rate of 2 kb per micron, developped by A Bensimon. The BRCA1 region was visualized by using a full-four-color bar-code leading to split up the region of about 120 kb in ten fragments. This approach was first tested on two control DNAs. On a second step, three rearrangements recently described were analysed. A 23.8 kb deletion was directly visualized at the microscope screening as one of the probe had disappeared, giving therefore qualitative informations. Thanks to the CartographiX software, quantitative informations were obtained, leading to an estimation of the deletion size and of its standard deviation. A 6 kb duplication was then visualized quantitatively. Finally, a 3 kb deletion could be suspected, giving us therefore an estimation of the method resolution. This approach, which can be broaden to the study of other genes, is promising as it is expected to detect, besides deletions, duplications, inversions, and insertions which are probably underreported so far.

Program Nr: 102 from the 1999 ASHG Annual Meeting

Functional consequences of mutations in zinc-binding sites of the BRCA1 RING finger. *J.E. Meza[1,2], P. Brzovic[3], R. Klevit[3], M.-C. King[1,2].* 1) Div Medical Genetics, Univ Washington, Seattle, WA; 2) Department of Genetics, Univ Washington, Seattle WA; 3) Department of Biochemistry, Univ Washington, Seattle, WA.

The BRCA1 RING finger comprises two zinc-binding sites with conserved residues at Cys24, Cys27, Cys44, and Cys 47 at Site I and Cys 39, His41, Cys61, and Cys 64 at Site II. The RING finger domain enables BRCA1 to homodimerize and, preferentially, to heterodimerize with BARD1. Naturally occurring missense mutations occur in patients at BRCA1 Site II residues Cys 61, Cys 64 and Cys 39, but only one Site I missense (Cys24Arg) has been observed in one family. Why are missense mutations in the RING finger observed only at these residues in patients? Do certain conserved residues contribute more than others to the stability of the BRCA1 RING finger domain? We constructed independent mutations of two Site I conserved residues and the four conserved residues comprising Site II. The various Site II mutant constructs were all capable of heterodimerizing with BARD1, while the Site I mutant constructs were not. Site I missense mutations may be deleterious as heterozygotes and hence rarely observed in vivo, despite the relatively high frequency of protein-truncating mutations in this region. Site I mutations perturb the structure of the RING finger domain to a greater extent than do Site II mutations. Substitutions at positions Cys 61 and Cys 64, both in Site II, render the BRCA1 RING finger more susceptible to proteolysis than do mutations at other Site II conserved residues. Missense mutations at Cys39 or His 41 may be less severe and hence less frequently observed in cancer patients than mutations at the other Site II residues. Interestingly, the BARD1 component of the heterocomplex is equally resistant to proteolysis in the wildtype and Site II mutant heterocomplexes. These results demonstrate that Site II mutations of the BRCA1 RING finger domain have a localized effect, and that certain conserved residues contribute more than others to the stability of the BRCA1 component of the heterodimer.

Program Nr: 103 from the 1999 ASHG Annual Meeting

Genetic Studies of Ovarian Cancer in Jewish Women. *R. Moslehi[1,2], B. Karlan[3], D. Fishman[4], A. Fields[5], J. Brunet[1], J.M. Friedman[2], S. Narod[1]*. 1) Cntr for Research in Women's Health, Women's College Hosp, Toronto, ON, Canada; 2) Dept of Medical Genetics, Univ of British Columbia, Vancouver, BC, Canada; 3) Dept of Obstetrics & Gynecology, Cedars Sinai Med Cntr, Los Angeles, CA; 4) Dept of Obstetrics & Gynecology, Northwestern Univ, Chicago, IL; 5) Obstetrics, Gynecology & Women's Health, Albert Einstein College of Med, Bronx, NY.

We ascertained 238 Ashkenazi Jewish women with ovarian cancer, unselected for age or family history, from 11 hospitals in North America and Israel. We also interviewed 386 Jewish controls. Detailed medical and family histories were obtained on all patients and controls. Cases were tested for the three common *BRCA1* (185delAG and 5382insC) and *BRCA2* (6174delT) mutations in this population. We reviewed pathology and surgical reports on the ovarian cancers in 210 probands. 78 (37.1%) were *BRCA1* or *BRCA2* mutation carriers. Mutation carriers had a significantly higher frequency of serous tumours than the non-carriers (91.0% versus 75.8%, p=.01). 71 of 171 (41.5%) patients with serous tumours carried one of the three mutations. 6 of 12 (50.0%) patients with endometrioid tumours were mutation carriers. 1 of 7 (14.3%) patients with clear cell tumours carried a mutation. None of the patients with mucinous (n=8), transitional cell (n=2), borderline (n=8) or sex cord tumours (n=2) were carriers. This indicates that genetic testing for *BRCA1* and *BRCA2* mutations can be limited to patients with serous, endometrioid or clear cell ovarian tumours.

The cumulative incidence of cancer was calculated for all 1371 first-degree relatives of the cases and 2213 first-degree relatives of the controls. The relatives of the ovarian cancer cases experienced a greater risk of any cancer to age 65 compared to the controls (19.4% versus 11.5%, p=.0001). Excluding cancers of the breast and ovary, the cumulative incidence of cancer in the female first-degree relatives to age 65 was 11.4% versus 7.0% in the relatives of the controls (p=.035). The male relatives of the *BRCA2* carriers were at a significantly higher risk for cancer before age 65 than the male relatives of the *BRCA1* carriers (21.4% versus 4.4%; p=.01).

Program Nr: 104 from the 1999 ASHG Annual Meeting

Relevance of germline mutations in CDKN2A gene in breast cancer families with associated history of melanoma. *A. Ganguly, L. Godmilow, S. Palmer, T. Ganguly.* Dept Genetics, Univ Pennsylvania, Philadelphia, PA.

Pedigree analysis of a large cohort of breast cancer families indicated the presence of a significant number of families with coexistence of breast cancer and melanoma. The incidence of two cancers is often in the same individual and suggests a common genetic predisposition for both diseases. We have studied a small subset of 24 such individuals for germline mutations in BRCA genes as well as in CDKN2A, the gene known to predispose to melanoma. Six individuals carried mutations in the BRCA1(3 frameshift) or BRCA2 (1 nonsense, 2 frameshift) genes and 6 individuals carried mutations in the CDKN2A gene(1 frameshift, 6 missense). Three individuals carried mutations in both BRCA1/2 and CDKN2A genes. In all three individuals, the onset of melanoma was earlier than breast cancer. In addition, there were four individuals with breast cancer and/or first degree relative with melanoma that were BRCA1/2 mutation negative but CDKN2A mutation positive. The overall incidence of CDKN2A mutations (29%) in this subgroup of predominantly breast cancer families is comparable to that observed in familial melanoma patients. It is known that the products of BRCA1/2 and CDKN2A genes are tumor suppressors involved in two separate pathways that inhibit cell growth. These results suggest the two different genes possibly interact and specifically modify the role of each other in the process of breast cancer pathogenesis. Since the fraction of breast cancer families that can be accounted for by mutations in BRCA1/2 genes is only 30%, it is important to consider mutations in additional cancer susceptibility loci in these families. Therefore the mutations in CDKN2A gene might be relevant in explaining the molecular basis in a larger fraction of breast cancer families.

Program Nr: 105 from the 1999 ASHG Annual Meeting

High frequency of sequence variants in women of African descent undergoing *BRCA1* or *BRCA2* testing. *M. Robson[1], C. Duteau-Buck[1], H. Valdimirsdottir[2], J. Guevara[1], R. Baum[1], J. Hull[1], D. McDermott[1], M. Pinto[1], L. Scheuer[1], K. Offit[1]*. 1) Dept Human Genetics, Mem Sloan-Kettering Cancer Ctr, New York, NY; 2) Mt. Sinai School of Medicine, New York, NY.

Few studies have described the results of genetic testing for inherited breast and ovarian cancer susceptibility in individuals of African descent. Families in the present study were offered genetic testing after direct referral to a cancer risk counseling clinic or after recruitment from a screening population. Following pre-test counseling and informed consent, 57 individuals from 49 families underwent *BRCA1* and/or *BRCA2* testing. Of the 53 individuals from 49 families who underwent *BRCA1* coding sequence analysis, 5 individuals (9.4%) from 5 families (10.2%) were heterozygous for presumably deleterious *BRCA1* mutations. An additional 21 *BRCA1* sequence variants of uncertain significance were detected in 16 individuals (30.2%) from 16 families (32.7%). *BRCA2* sequence analysis was performed on 33 individuals from 28 families (4 individuals tested only for previously identified mutations). Of the 29 individuals from 28 families undergoing complete *BRCA2* coding sequence analysis, 2 (6.9%) were found to carry presumably deleterious mutations. An additional 17 individuals (58.6%) from 16 families (57.1%) were found to carry a total of 28 *BRCA2* sequence variants of uncertain significance. Of 29 individuals (28 families) undergoing both *BRCA1* and *BRCA2* analysis, 21 persons (72.4%) from 20 families (71.4%) had at least one sequence variation of uncertain significance. More than one variant was noted in 17 individuals from 16 families. Several variants (3 *BRCA1* , 5 *BRCA2*) were observed in more than 1 family. The high prevalence of genetic variants of uncertain significance must be taken into account when providing counseling regarding *BRCA* testing to individuals of African descent.

Program Nr: 106 from the 1999 ASHG Annual Meeting

Frequency of breast cancer attributable to BRCA1 and BRCA2 in a population-based series of Caucasian and African-American women. *H. Mu[1], B. Newman[2], C. Rousseau[1], S. Payne[1], K. Gordon[1], D. Gold[1], C.-K.J. Tse[2], R.C. Millikan[2], M.-C. King[1]*. 1) Departments of Medicine and Genetics, University of Washington, Seattle, WA; 2) UNC Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC.

To determine the public health impact of inherited mutation in BRCA1 or BRCA2 on breast cancer, both genes were fully screened in a population-based series of sequentially diagnosed Caucasian and African-American breast cancer patients from North Carolina. New results for BRCA2 and recently discovered large genomic alterations complete our previously published results on BRCA1 in the same cohort (Newman et al., JAMA 279: 915-921, 1998). BRCA2 was analyzed for germline variants in the coding sequence, splicing junctions and neighboring intronic regions using multiplex single-strand conformation analysis, heteroduplex analysis, and DNA sequencing of 203 cases. In addition, 373 cases were screened for the large genomic mutations. After adjustment for sampling probabilities, the weighted prevalences (%) of breast cancer attributable to BRCA1 or BRCA2 were 7.4 (95% CI, 1.3-12.1) for Caucasian women, 1.0 (95% CI, 0-3.0) for African-American women, and 6.1 (95% CI, 1.2-9.8) overall. Among probands with at least three affected relatives, 13% carried a BRCA1 or BRCA2 mutation. Among breast cancer probands with any relative with ovarian cancer, 22% carried a mutation. Among American breast cancer patients generally, those from these high risk families can most benefit from full genotyping of BRCA1 and BRCA2.

Program Nr: 107 from the 1999 ASHG Annual Meeting

Prevalence of BRCA1 in a hospital-based population of Dutch breast cancer patients. *H. Papelard[1,2], I. Stec[3], G.H. De Bock[1], R. Van Eijk[3], T.P.M. Vliet-Vlieland[2], C.J. Cornelisse[4], R.A.E.M. Tollenaar[2], P. Devilee[3,4].* 1) Department of Medical Decision Making, Leiden University Medical Center, Leiden, The Netherlands; 2) Department of Surgery, Leiden University Medical Center; 3) Department of Human and Clinical Genetics, Leiden University Medical Center; 4) Department of Pathology, Leiden University Medical Center.

Testing for breast cancer predisposition is still controversial. It is hampered among others by incomplete knowledge about the frequency of disease related mutations in patients not selected on the basis of family history status. We investigated the prevalence of disease related BRCA1 mutations in a series of 642 consecutively collected Dutch breast cancer patients. All patients had primary invasive breast cancer, were surgically treated at the Leiden University Medical Center between 1984 and 1996, and had provided a blood sample for research purposes. They were tested for germline mutations in the BRCA1 gene using Detection of Small Deletions and Insertions (DSDI). Data on family history and bilaterality of the cases were obtained retrospectively. Ten protein truncating mutations were detected and one in frame deletion with an unknown relation to disease risk. Four patients carried the Dutch founder deletion of 510 bp encompassing exon 22. The prevalence of BRCA1 was estimated to be 2.0% among the general breast cancer population in the Netherlands, and 9.5% and 6.4% among cases diagnosed under 40 or under 50, respectively. All mutation carriers were under 50 years of age at diagnosis of their first breast cancer, 5 did not have any relative with breast cancer. The proportions of bilateral breast cancer among carriers and non-carriers did not differ significantly. These data indicate that in the general Dutch breast cancer population the great majority of BRCA1 mutations will be found among women under 50 years of age. Futhermore, they suggest that a substantial proportion of carriers will be missed when family history of breast cancer is used as the sole selection criteria for mutation screening.

Program Nr: 108 from the 1999 ASHG Annual Meeting

A single nucleotide polymorphism in the 5'UTR of *RAD51* is associated with the risk of breast cancer among *BRCA1/2* mutation carriers. *W. Wang[1], M.A. Tucker[2], M.M. Doody[3], R.E. Tarone[4], J.P. Struewing[1]*. 1) Laboratory of Population Genetics; 2) Genetic Epidemiology Branch; 3) Radiation Epidemiology Branch; 4) Biostatistics Branch, Division of Cancer Epidemiology and Genetics, NCI, Bethesda, MD.

The average risk of breast cancer among *BRCA1/2* mutation carriers may be 50% or lower, suggesting that genetic or environmental modifiers of risk may exist. The hRAD51 protein co-localizes with both the BRCA1 and BRCA2 proteins and all three may be involved in repairing DNA damage. To investigate whether *RAD51* is a modifier of *BRCA1/2*, we searched for polymorphisms by re-sequencing cDNA from 11 breast cancer patients. No coding polymorphisms were identified, but we observed two single nucleotide polymorphisms (snp's) in the 5' untranslated region (5'UTR), designated snp135g/c and 172g/t. We then compared the breast cancer incidence curves for subjects with different *RAD51* snp genotypes, using cox proportional hazards models, adjusted for correlated observations. Two populations of female *BRCA1/2* mutation carriers were studied: 86 were from the Washington Ashkenazi Study, a community-based study, and 77 were from 19 NCI breast/ovarian cancer-prone families with identified mutations. Thirteen of 51 breast cancer cases carried the "c" allele at snp135g/c, while nine of 112 women without breast cancer carried this allele. This represents an odds of disease of 3.6 (95% CL 1.9, 6.9) for carriers of the "c" allele, a highly statistically significant difference (P<.00001, Wald test). The association was consistent between study populations and for both BRCA1 and BRCA2 mutation carriers. The "c" allele was present in approximately 5% of Jewish controls and 16% of non-Jews. The snp135g/c was not significantly associated with breast cancer risk among women who did not carry BRCA1/2 mutations, and there was no association with risk for snp172g/t in either group. The impact of the snp135g/c on hRAD51 protein is being investigated and if confirmed, would lend further support for the epidemiologic evidence of *RAD51* being an important modifier locus for breast cancer risk.

Program Nr: 109 from the 1999 ASHG Annual Meeting

BRCA1 in sporadic breast cancer: genomic loss and protein expression in primary tumors. *S.R. Payne[1], R. Gonzalez-Hernandez[2], M.-C. King[1,3].* 1) Dept of Genetics, University of Washington, Seattle, WA; 2) Dept of Pathology, University of Washington, Seattle, WA; 3) Dept of Medicine (Division of Medical Genetics), University of Washington, Seattle, WA.

In inherited breast cancer, BRCA1 acts as a classic tumor suppressor in that both normal copies of *BRCA1* are lost, one in the germline and one somatically. In sporadic tumors, large somatic deletions including *BRCA1*, as reflected by loss of heterozygosity (LOH), occur frequently, while somatic point mutations are rare. One argument for the role of BRCA1 in sporadic breast cancer has been the high rate of allelic loss observed for the *BRCA1* locus. Sporadic invasive breast tumors were evaluated for protein expression and genomic loss at *BRCA1*. Protein expression was evaluated by immunohistochemistry using the N-terminal monoclonal antibody MS110. Genomic loss was evaluated by LOH using multiple markers within and flanking *BRCA1*, by Long PCR (7-12kb fragments) of the entire *BRCA1* genomic region and by Southern analysis. BRCA1 protein expression was significantly decreased in 72% (49/68) of invasive ductal carcinomas and correlated significantly with tumor grade, as previously observed (Wilson et al, 1999 Nat Genet 21:236, Lee et al, 1999 Histopath 34;106). No large genomic rearrangements of somatic origin were detected, although one germline rearrangement was detected by Southern analysis. Although allelic loss at all intragenic markers is not associated with BRCA1 protein reduction, allelic loss at the marker nearest the transcription start site (D17S1323) is associated with reduction of BRCA1 protein. This observation might reflect specific targeting of either the *BRCA1* promoter or *BRCA1* exon 11 for loss. The function of BRCA1 that is involved in maintenance of genetic stability throught the G2-M checkpoint has been shown to require exon 11 (Xu et al, 1999 Mol Cell 3:389). A high proportion (57%, 8/14) of the tumors that retained both *BRCA1* alleles showed loss of the BRCA1 protein. In this subset of tumors other mechanisms may be responsible for *BRCA1* inactivation.

Program Nr: 110 from the 1999 ASHG Annual Meeting

Somatic alterations of chromosome 8p in sporadic and BRCA2 999del5 linked breast cancer. *S. Ingvarsson, B.I. Sigbjornsdottir, G. Ragnarsson, B.A. Agnarsson, C. Huiping, R.B. Barkardottir, V. Egilsson.* Department of Pathology, University Hospital of Iceland, IS-101 Reykjavik, Iceland.

We have previously analysed the difference of chromosome alterations in tumours of individuals carrying a BRCA2 mutation and sporadic tumours [Ingvarsson S etal Cancer Res 58:4421, 1998; Tirkkonen M etal Cancer Res 57:1222, 1997]. Furthermore, we have shown altered expression of p53 and Fhit and frequent LOH (loss of heterozygosity) at the corresponding gene loci in BRCA2 linked breast cancer [Bergthorsson JT et al Eur J Cancer 34:142, 1998; Eiriksdottir G etal Oncogene 16:21, 1998; Ingvarsson S etal Cancer Res 59:2682, 1999]. Frequent alterations of the short arm of chromosome 8 are observed in a variety of tumour types, including breast cancer, suggesting the presence of one or more tumour suppressor genes in this region. Therefore, we used 11 microsatellite markers to analyse LOH at chromosome 8p in 151 sporadic breast tumours and 50 tumours from individuals carrying the BRCA2 999del5 mutation. Fifty percent of sporadic tumours compared to 78% of BRCA2 linked tumours exhibit LOH at one or more markers, showing that chromosome 8p alterations in breast tumours from BRCA2 999del5 carriers are more pronounced than in sporadic breast tumours, possibly due to misrepaired double-strand breaks. The pattern of LOH is different in the two groups, whereas a higher proportion of BRCA2 tumours have LOH at a large region of chromosome 8p. In the total patient material, LOH of 8p is associated with LOH at other chromosome regions e.g. 1p, 3p, 6q, 7q, 9p, 11p, 13q, 17p and 20q, but no association is found between LOH at 8p and LOH at chromosome regions 11q, 16q, 17q and 18q. Furthermore, an association is detected between LOH at 8p and positive node status, large tumour size, aneuploidy and high S-phase fraction. Breast cancer patients with LOH at chromosome 8p have a worse prognosis than patients without this defect. Multivariate analysis suggests that LOH at 8p is an independent prognostic factor. We conclude that chromosome 8p carries a tumour suppressor gene or genes, which loss results in growth advantage of breast tumour cells, especially in carriers of the BRCA2 999del5 mutation.

Program Nr: 111 from the 1999 ASHG Annual Meeting

Prophylactic surgery in BRCA1/2 mutation carriers: predictive factors and follow-up. *H. Meijers-Heijboer[1], L. Verhoog[2], C. Brekelmans[2], A. van Geel[2], C. Seynaeve[2], A. van den Ouweland[1], D. Majoor-Krakauer[1], P. Devilee[3], J. Klijn[2]*. 1) Dept Clinical Genetics, Erasmus University Medical Center Rotterdam, Netherlands; 2) Dept Internal Oncology, Erasmus University Medical Center Rotterdam; 3) Leiden University Medical Center, Netherlands.

Background Germline mutations in the BRCA1 and BRCA2 genes highly predispose to breast and ovarian cancer. In families with known BRCA1 or BRCA2 alterations, identification of mutation carriers is of clinical relevance in view of the options of surveillance or prevention. Methods In the Rotterdam Family Cancer Clinic we assessed the utilisation of presymptomatic DNA-testing and prophylactic surgery within 53 consecutive families with a known BRCA1/2 mutation including 682 unaffected subjects with a 50% risk (275 women and 271 men) or with a 25% risk (136 women) for carrying a mutation. Also the short-term clinical outcome of surgical prevention was evaluated. Results Overall, presymptomatic DNA-testing was requested by 38%(257/682) of the subjects. Significant predictive factors for DNA-testing were the genetic risk for a mutation (50% vs 25% = 57% vs 29%), gender (women vs men = 57% vs 22%), age in women (< 50 years vs > 50 years = 66% vs 38%) and parenthood both in women (yes vs no = 65% vs 46%) and in men (yes vs no = 28% vs 9%). Of the unaffected women with an identified mutation and eligible for prophylactic surgery, 51% (35/68) opted for bilateral mastectomy and 64% (29/45) for oophorectomy. Parenthood was a major predictive factor for prophylactic mastectomy. The decision period for presymptomatic DNA testing and for prophylactic surgery was less than 1 year in the majority of 50% risk carriers and unaffected mutation carriers respectively. After prophylactic mastectomy no invasive breast cancer was found in an extended series of 76 BRCA1/2 mutation carriers during a median follow-up of 27 months (expected without intervention 6 cases; p=0.028). Conclusion Especially young women with children opt for both DNA-testing and prophylactic mastectomy. Increasingly, prophylactic surgery is based on identification of a breast cancer gene mutation rather than on risk estimations derived from the family history.

Program Nr: 112 from the 1999 ASHG Annual Meeting

Osteogenesis imperfecta (OI): pregnancy characteristics, mode of delivery, and neonatal outcome. *E.Y. Cheng, R. Cubert, S. Mack, M.G. Pepin, P.H. Byers.* University of Washington, Seattle, WA.

It is often recommended that a fetus known to have a non-lethal form of OI be delivered by cesarean section. To determine if the mode of delivery influenced the rate of fracture or survival, we analyzed data concerning 296 pregnancies in which the diagnosis of OI was confirmed either prior to or after delivery. From a database of 1016 individuals in whom OI was confirmed by biochemical studies of cultured fibroblasts or chorionic villus mesenchymal cells, from 1987 to 1994, we identified 547 that involved children or pregnancies. Of 547, obstetric information was available for 296 that included electively terminated pregnancies or early miscarriages in 129 instances and 167 affected live-born individuals for whom delivery information was available. There were 29 pregnancies with OI type I, 50 with OI type II, 5 with OI type II/III, 14 with OI type III, 37 with OI type III/IV, and 32 with OI type IV. The obstetrical records were reviewed for prenatal detection, method of delivery, presence of prenatal and/or postnatal fractures, and length of survival. The diagnosis of OI was suspected prenatally as a result of ultrasound studies in 39 (23%). The rate of breech presentation at term (44/120, 37% of infants delivered at term) was significantly higher than expected (3-4%). The rate of cesarean delivery was 54% (90/167). Among those delivered by cesarean section the diagnosis of OI was known prior to delivery in 14 (16%). Cesarean delivery affected neither the fracture rate per infant nor the number of infants with fractures among those with the non-lethal OI phenotypes. Furthermore, cesarean delivery did not prolong the survival of infants with the lethal form of OI. Although knowledge of a diagnosis of OI may have played a role in the mode of delivery, it was not the major determinant of cesarean delivery. Instead, breech presentation (39/90 delivered by cesarean section) appeared to a better predictor of non-vaginal delivery. These data suggest that prenatal studies to identify the type of OI, for determination of mode of delivery alone, may be of limited value because the major reason to alter mode of delivery is related to obstetric issues.

Program Nr: 113 from the 1999 ASHG Annual Meeting

Steroid sulphatase deficiency: genotype and phenotype in cases ascertained by maternal serum screening. *J.R.W. Yates[1,2], R. McMahon[3], W. Gelson[3], L.R. Willatt[3], P.R. Raggatt[4], C. Carr[4], D.A. Aitken[5], S.F. Goodburn[2]*. 1) Dept of Medical Genetics, Cambridge University, Cambridge, UK; 2) Dept of Medical Genetics, Addenbrooke's Hospital, Cambridge, UK; 3) Molecular Genetics and Cytogenetics Laboratories, Addenbrooke's Hospital, Cambridge, UK; 4) Dept of Clinical Biochemistry, Addenbrooke's Hospital, Cambridge, UK; 5) Institute of Medical Genetics, Yorkhill, Glasgow, UK.

Cases of steroid sulphatase (STS) deficiency were ascertained from 87,967 consecutive pregnancies undergoing maternal serum screening for Down syndrome. Investigation of viable pregnancies with very low unconjugated oestriol (uE3) levels identified 25 cases of STS deficiency, a prevalence of 1 in 3500. In all but one case, the diagnosis was established by assay of STS activity, demonstration of an STS gene deletion or postnatal confirmation of X linked ichthyosis (XLI) in the baby (in the remaining case STS deficiency had been proven in a previous pregnancy). 11/25 (40%) cases had a family history of XLI. One pregnancy was terminated following ultrasound diagnosis of severe cleft lip and palate. Two pregnancies suffered intrauterine deaths at 41 and 42 weeks gestation. Deletions of the STS gene were present in 18/19 (95%) of the cases tested. Typing of markers DXS996, DXS1139, 5'STS, 3'STS, 22S1 and DXS278 identified two patients with unusually large deletions. In one case deleted for 3'STS to DXS996 inclusive, the baby showed marked developmental delay. At 23 months of age locomotor development was at the 15 month level and other skills at around 9 months. A maternal uncle with the same deletion had learning disability. In the other case, deleted for DXS278 to DXS996 inclusive, the baby had intrauterine growth retardation, bilateral talipes, hypotonia and delayed motor development. At 2 years 10 months his Developmental Quotient (Griffith's Scale) excluding locomotor skills was 82 (normal range 80 - 120). These data (1) support previous evidence for an association between STS deficiency and increased risk of intrauterine death (2) suggest that contiguous gene deletions with STS deficiency and learning disability may be commoner than previously thought.

Program Nr: 114 from the 1999 ASHG Annual Meeting

Multicenter study of fetal cell recovery from maternal circulation and analysis. *D. Lewis.* for the NIFTY group [D. Lewis, F. Bischoff and J.L. Simpson (Houston); S. Elias (Chicago); D. Bianchi, K. Klinger, K. Dukes, L. Sullivan (Boston); M. Evans (Detroit); W. Holzgreve and S. Hahn (Basel); L. Jackson and R. Wapner (Philadelphia); F. de la Cruz and H. Shifrin (Bethesda)].

The NIFTY (**N**ational **I**nstitutes of Health **F**etal Cell S**T**ud**Y**) study is designed to assess the feasibility and accuracy of using fetal cells in maternal blood as a screening test for cytogenetic aneuploidies. The purpose of this report is to highlight the variables that impact upon Y-chromosome detection. METHODS-Six centers have collaborated for five years developing protocols, sharing expertise and methodologies including fluorescent activated cell sorting (FACS) or magnetic activated cell sorting (MACS) using either the mini-MACS or Immunicon system. Although the ultimate goal is aneuploidy screening, Y-chromosome detection by FISH was used initially as a surrogate for sensitivity. Blood from 2072 women in the first or second trimester was processed by differing methods. RESULTS-Several factors were associated with detection of Y-containing cells: cell separation technique, gestational age, and time of processing. Because these variables are dependent, univariate comparison of methodologies is not possible. Selection for Y-positive fetal cells in confirmed male pregnancies was better using either mini-MACS or Immunicon than FACS. However, mini-MACS showed a far higher false positive rate (26%) than FACS (7%) or Immunicon (5%) in female pregnancies. In addition, specimens prepared using the Immunicon method were of a younger gestational age (12.7wks vs 15.2wks for both mini-MACS and FACS) and were processed faster (5.3hrs vs 22.9 and 22.4hrs, respectively). CONCLUSION-Although the NIFTY study group has made great strides in isolating fetal cells from the maternal circulation, univariate comparisons among centers are not yet meaningful because confounding variables exist. Thus, definitive conclusions as to the best laboratory methodologies are not yet possible. NIFTY centers are now sharing specimens to assess confounding variables and to optimize protocols for the detection of fetal aneuploidy.

Program Nr: 115 from the 1999 ASHG Annual Meeting

Fetal cell microchimerism: FISH detection of male cells in females with autoimmune disease. *K.L. Johnson[1], D.K. Zhen[1], B. Srivatsa[1], T. McAlindon[2], J.L. Nelson[3], D.W. Bianchi[1].* 1) Division of Genetics, Dept of Pediatrics, Tufts Univ School of Medicine, Boston, MA; 2) Dept of Medicine, Boston Univ Sch of Med, Boston, MA; 3) Fred Hutchinson Cancer Research Institute, Seattle, WA.

Background: It has been hypothesized that fetal cell microchimerism, resulting from the persistence of fetal cells post partum, plays a role in the higher incidence of autoimmune disease that occurs in women after their childbearing years (Arth Rheum 1996;39:191). This hypothesis has been tested in studies that have detected male DNA in peripheral blood and skin from women with scleroderma. We undertook this study to determine the extent of fetal cell microchimerism in autopsy specimens from women with autoimmune disease that had a history of having had males.

Methods: We performed FISH using X and Y chromosome specific probes on paraffin-embedded tissue obtained from autopsy specimens from 4 women with systemic scleroderma (SSc) and 1 woman with systemic lupus erythematosus (SLE). The number of nuclei bearing X and Y signals was counted. The morphology and location of the male cells was also recorded.

Results: Male cells were seen in the spleen, lung, and skin tissue of the women affected with SSc, with concentration of male cells in the spleen. In the patient with SLE, male cells were seen in the lung, skin and colon.

Conclusions: Although a causal relationship remains to be elucidated, these data suggest that microchimerism may play a role in the pathogenesis of a variety of autoimmune diseases through the migration of histocompatible fetal cells to maternal lymphopoietic organs and subsequent development of a graft-versus-host response. Further efforts will include analysis of additional organs and confirmation of fetal origin of the cells using micromanipulation and DNA polymorphisms.

Program Nr: 116 from the 1999 ASHG Annual Meeting

Female Carriers of X-Linked Lethal Traits Show an Increased Frequency of Spontaneous Abortion and a Gender Bias among Liveborn Children. *M. Lanasa[1], W.A. Hogge[2], C. Kubik[3], T. Prosen[2], E. Hoffman[4].* 1) Dept Biochem & Mol Gen, Univ Pittsburgh Sch Medicine, Pittsburgh, PA; 2) Genetics and OB/GYN, Magee-Womens Hospital, Pittsburgh, PA; 3) Reproductive Endocrinology, Magee-Womens Hospital, Pittsburgh, PA; 4) Center for Genetic Medicine, Children's National Medical Center, Washington DC.

Recurrent spontaneous abortion (RSA) is a highly prevalent disorder; however, an underlying etiology is not determined for nearly half of RSA patients. Female carriers of X-linked lethal traits should show an increased rate of spontaneous abortion, due to the loss of hemizygous male pregnancies receiving the defective X. Moreover, such females might be identified via the molecular phenotype of skewed X chromosome inactivation, as the skewing represents secondary selection against cells with the defective X active. To support of this hypothesis, we have undertaken a case control study comparing the frequency of highly skewed X chromosome inactivation among women with RSA to population based controls. Here, we report findings in an additional 19 women, and compare gender bias in offspring. We have previously reported 7/48 of women with RSA to show skewed X inactivation (AJHG, July 1999). Using our original definition of skewed X chromosome inactivation as the preferential expression of one allele in ³90% of peripheral leukocytes, we have found 9/67 women with RSA show this extent of skewing; 1/67 controls show this level of skewed X chromosome inactivation ($p<0.02$, Fisher's Exact Test). Moreover, if we define skewed X inactivation as ³89%, then 11/67 cases and 2/67 controls show this extent of non-random X inactivation ($p<0.02$). This model also predicts a gender bias among the liveborn offspring of females with skewed X inactivation. When combined, the 11 women with RSA and skewed X inactivation (³89%) have had 13 successful pregnancies, with 9 female and only 4 male children. This observation approximates the expected 2:1 female to male ratio, and approaches statistical significance ($p=0.08$, binomial distribution; H_0 = female to male ratio equal to 1:1).

Program Nr: 117 from the 1999 ASHG Annual Meeting

Imprinting status and mutation analysis of the *HPEG3* gene on chromosome 19q13.4 in familial hydatidiform mole. *I.B. Van den Veyver[1], R. Slim[2]*. 1) Depts. of Obstetrics and Gynecology and of Molecular and Human Genetics, Baylor Col Medicine, Houston, TX; 2) Dept. of Biochemistry, American University of Beirut, Lebanon.

We previously mapped a locus for autosomal recessive recurrent molar pregnancy with biparental genomic contribution to a 3.5-5 Mb region on chromosome 19q13.4 between D19S924 and D19S890. This region is syntenic to proximal mouse chromosome 7 and contains a cluster of zinc finger genes. Two genes (*peg3* and *zim1*) in the mouse syntenic region are known to be imprinted. *peg3* is maternally imprinted and is differentially expressed in androgenetic mouse embryos. Partial cDNA sequence and abundant expression in ovary and placenta of its human homologue (*HPEG3*) has been reported. We therefore characterized the full length *HPEG3* by sequence analysis and cDNA library screening and searched for mutations in the affected women of two unrelated families with familial hydatidiform moles. *HPEG3* contains seven coding exons and three 5' untranslated exons spanning 30.4 Kb of genomic DNA. Its largest open reading frame is 4764 bp encoding a 1588 amino acid zinc finger protein. Sequence analysis strongly predicts two promoters in a CpG rich region in the first intron. Direct sequencing of all coding exons and flanking introns did not reveal any mutations. We found expressed polymorphisms in exons 2 and 7 that were used to study allele-specific expression of *HPEG3*. Using RT-PCR and restriction enzyme digestion on informative lymphoblast cell lines, we detected preferential expression of the paternal allele. Southern analysis of genomic DNA with methylation sensitive restriction enzymes revealed methylation sensitive sites in the CpG island. These results indicate that *HPEG3* is imprinted. We will continue imprinting analysis of this and other genes in 19q13.4 in a variety of tissues to determine if *HPEG3* is part of a novel cluster of imprinted genes in humans. We will investigate whether a mutation in an imprinting control region in 19q13.4 or an autosomal recessive mutation in a non-imprinted gene causes the defect in the affected women and whether these genes play a role in the pathogenesis of hydatidiform moles.

Program Nr: 118 from the 1999 ASHG Annual Meeting

Identification and characterization of the candidate genes involved into proliferation and/or improper migration of primordial germ cells in gcd mutant mice. *A.I. Agoulnik[1], C.E. Bishop[1,2]*. 1) Dept OB/GYN, Baylor Col Medicine, Houston, TX; 2) Dept. Human and Molecular Genetics, Baylor Col Medicine, Houston, TX.

In human populations infertility is a common problem affecting 10-15% of all individuals. Defects in migration and/or proliferation of the primordial germ cells during embryonic development can lead to both Sertoli Cell Only syndrome in males and Premature Ovarian Failure in females. Identification of the genes that play a role in the population of the genital ridge is a key to the better understanding of these abnormalities. An autosomal mouse transgene insertional mutation gcd (germ cell deficient) has been proposed as a model for these diseases. Males and females homozygous for gcd exhibit sterility due to a reduced number of primordial germ cells reaching the genital ridge by 11.5 days post coitum. Using a transgene DNA as bait we have cloned the genomic fragments adjacent to the insertion site and, the corresponding genomic region from normal DNA. We mapped the mutation to a genetic interval of less than 1cM using interspecific backcross analysis. It was established that the transgene insertion caused a deletion in genomic DNA. A BAC contig of the critical region has been constructed. A single 200 kb BAC was found, which covers the deleted gcd interval. Using genomic DNA sample sequencing approach in combination with BLAST analysis of the available human genomic sequence information of the homologous region in the human chromosome (2p15-p16) and ESTs, we constructed a gene map of the mouse gcd region. Two candidate genes within critical area for gcd that lack the 3exons in the mutants have been identified. One of the genes is a member of a conserved family of Serine/Threonine kinases. The other gene has no homology in Genbank. An expression pattern of the genes, analysed by Northern blots and in situ hybridization , indicates their involvement in germ cell proliferation.

Program Nr: 119 from the 1999 ASHG Annual Meeting

The role of CHX10 in eye development: human *CHX10* mutations in microphthalmia and identification of *Mitf* as a Chx10 downstream target. *D.J. Horsford[1], L. Ploder[1], E.F. Percin[2], J. Yu[1], A. Duncan[3], D. Cox[4], E. Traboulsi[5], M. Sarfarazi[2], R.R. McInnes[1]*. 1) Devel Biol, Hosp for Sick Children, Toronto; 2) U Conn Health Centre; 3) Montreal Childrens Hosp; 4) U Alberta; 5) Cleveland Clinic.

The retinal homeodomain (HD) protein Chx10 is required for murine eye development, since *Chx10*$^{-/-}$ mice are microphthalmic. Mapped human microphthalmic loci have no known associated genes. To determine whether *CHX10* mutations cause human eye defects, we cloned and mapped *CHX10* to 14q24.3 and screened 371 patients with ocular defects, including 127 with microphthalmia. In a Turkish microphthalmic kindred (Percin *et al* ASHG'99) and in a sporadic case, we found homozygous R200Q and R200P mutations, respectively; these were absent from >800 control (110 Turkish) alleles. R200, which contacts the PO_4 backbone, is highly conserved (R in 342/344 HDs). To prove these alleles were nulls, the CHX10 DNA binding site was identified, and in EMSA studies, neither mutant bound the 8bp CHX10 target we defined, in contrast to control CHX10. To ascertain how loss of CHX10 function causes microphthalmia, we compared the expression of Chx10 candidate target genes in the retinas of control vs *Chx10*$^{-/-}$ mice. Unexpectedly, we found that the *Mitf* transcription factor is ectopically expressed (E10.5 to P0) in neuroretina of *Chx10*$^{-/-}$ mice, whereas *Mitf* expression in the *Chx10*$^{-/-}$ RPE was normal. To determine whether *Mitf* is required for *Chx10*$^{-/-}$ microphthalmia, we generated *Mitf*^{mi}*/Chx10*$^{-/-}$ double mutants, which had dramatic rescue of the *Chx10*$^{-/-}$ phenotype: eye size and differentiation at P0 is largely normalized. We conclude that 1) *CHX10* null alleles cause isolated human microphthalmia, 2) the 8bp CHX10 DNA binding sequence is unique vs. other HD proteins, 3) Chx10 represses *Mitf* expression, and *Mitf* is the first regulatory gene identified downstream of Chx10, 4) the near-identical phenotypes of mouse and human *Chx10* mutants indicate that Chx10 function is orthologous in these species. Since the role of Mitf in the RPE is to downregulate RPE cell proliferation, we propose that a major role of Chx10 in normal eye development is to repress *Mitf* in the neuroretina, allowing retinal progenitor proliferation.

Program Nr: 120 from the 1999 ASHG Annual Meeting

***Eya1* is required for cell survival and tissue interactions during ear and kidney formation.** *R.L. Maas[1], H. Peters[1], S. Heaney[1], J. Adams[2], M.C. Brown[2], P-X. Xu[1].* 1) Genetics Division, Dept. Medicine, Brigham & Women's Hospital and Harvard Medical School, Boston, MA; 2) Dept. Otology and Laryngology, Harvard Medical School and Eaton-Peabody Laboratory, Massachusetts Eye and Ear Infirmary, Boston, MA.

Haploinsufficiency for the human *Eyes Absent 1* gene, *EYA1*, results in Branchio-Oto-Renal (BOR) syndrome, but how *EYA1* functions in kidney and ear formation is unknown. To address this question, we inactivated the *Eya1* gene in mice. While *Eya1* heterozygotes exhibit a conductive hearing loss similar to BOR syndrome, *Eya1* homozygotes completely lack ears and kidneys due to defective inductive tissue interactions and apoptotic regression of the organ primordia. Inner ear development in *Eya1* homozygotes arrests at the otic vesicle stage and all components of the inner ear and specific cranial sensory ganglia fail to form. In the kidney, *Eya1* homozygosity results in an absence of ureteric bud outgrowth and a subsequent failure of metanephric induction. Expression of glial-derived neurotrophic factor (GDNF), which is required to direct ureteric bud outgrowth via activation of the c-ret receptor tyrosine kinase (RTK), is not detected in *Eya1* homozygous metanephric mesenchyme. In both *Eya1* mutant ear and kidney development, similar to a genetic pathway elucidated in *Drosophila* organogenesis, *Pax* gene expression is preserved, while expression of *Six* genes, homologs of the *Drosophila* gene *sine oculis*, is never activated. These results indicate that *Eya1* controls critical early inductive signaling events involved in ear and kidney formation and integrate *Eya1* into the genetic regulatory cascade controlling kidney formation upstream of GDNF. In addition, our results suggest that an evolutionarily conserved *Pax-Eya-Six* regulatory hierarchy is utilized in mammalian ear and kidney development.

Program Nr: 121 from the 1999 ASHG Annual Meeting

Homeobox genes in development of the pituitary gland and other organs. *S.A. Camper, H. Suh, K.R. Douglas, L.J. Cushman, M.L. Brinkmeier, I. Nasonkin, P.J. Gage.* Dept Human Gen, 4301 MSRB III, Univ Michigan Medical Sch, Ann Arbor, MI.

Several homeobox genes are critical for pituitary gland development (Trends in Genetics 24, 284,1998). The correlation between the phenotypes of mouse mutants and human genetic diseases affecting pituitary function is excellent. Loss of *POU1F1* (*Pit1*) and *PROP1* function results in combined pituitary hormone deficiency (MIM 173110, 601538) and disruption of *HESX1* causes septo-optic dysplasia (MIM 601802). Pitx2 is a homeodomain transcription factor that is expressed prior to *Hesx1*, *Prop1* and *Pit1* in pituitary ontogeny. We assessed the requirement for *Pitx2* by generating hypomorphic (neo) and null (-) alleles in mice. Neo/+ and +/- mice are viable and exhibit abnormalities that mimic Rieger syndrome (RGS), a disorder affecting eyes and teeth caused by haploinsufficiency of PITX2 or mutations at other loci (MIM 180500, 601499). The RGS phenotype can include heart, umbilicus and somatic growth defects. Both the neo and null alleles of *Pitx2* cause embryonic lethality. The -/- fetuses exhibit ectopia cardis and omphalocele. The consistent heart defects in neo/neo fetuses reveal the requirement for a threshold level of Pitx2 and suggest RGS patients should be screened for heart defects. Pituitary gland development is arrested in -/- fetuses; therefore the growth insufficiency of Rieger patients could result directly from PITX2 effects in the pituitary. The -/- fetuses have eye defects, implying that the high frequency glaucoma in Rieger patients may result from structural eye defects. Interestingly, Pitx2 deficiency causes isomerization of the lungs but not other organs, revising its postulated role in left-right axis determination. This allelic series establishes that *Pitx2* is required for the development of multiple organs in a dosage-sensitive manner. We have also generated a *Pitx2* allele that can be deleted by conditional or tissue-specific expression of cre transgenes. We are using these *Pitx2* alleles and mouse mutations in *Pit1* and *PROP1* to analyze gene interactions, identify target genes, and further explore the function of *Pitx2*.

Program Nr: 122 from the 1999 ASHG Annual Meeting

Understanding developmental compensatory mechanisms using the activins as a model system. *C.W. Brown[1], D.E. Houston-Hawkins[1], M.M. Matzuk[2]*. 1) Department of Molecular and Human Genetics; 2) Departments of Pathology,Cell Biology, Molecular and Human Genetics; Baylor College of Medicine, Houston, TX.

The TGF-b superfamily has been classified on the basis of the substantial protein sequence similarity among its members. As in other protein superfamilies, this phenomenon raises questions as to whether these proteins can function interchangeably, particularly during early events in embryogenesis. Activin bA (ActbA) homozygous null mutant mice demonstrate disruption of whisker, palate, and tooth development, leading to neonatal lethality, indicating that this TGF-b superfamily member is essential for normal development. We have now targeted the ActbA gene with a construct that encodes the closely related activin bB (ActbB) mature region (functional domain); however, the construct preserves all sequences which direct the transcription and subsequent processing from the ActbA locus. The hybrid ActbA/ActbB knock in allele has been designated, $actbA^{bB\text{-}KI}$. Homozygous mutant mice were generated with two copies of the activin knock-in allele ($actbA^{bB\text{-}KI}/actbA^{bB\text{-}KI}$). These mice were viable and fertile but were significantly smaller than both their wild-type (+/+) and heterozygous ($actbA^{bB\text{-}KI}/+$) littermates. Tooth and whisker development were normal. Interestingly, hemizygous mice ($actbA^{bB\text{-}KI}/-$) had a more severe phenotype that included profound postnatal growth delay, delayed hair and whisker growth, and abnormal growth of the external genitalia. In contrast to homozygous mice, which survived for more than one year, the hemizygous mice died between 3 and 4 weeks of age. These experiments have provided novel information about the biological functions of the activins. They have also enhanced our understanding of compensatory mechanisms that occur during development by offering insight into the relative importance of expression patterns versus specific ligand-receptor interactions. Finally, the knock-in mice have confirmed that gene dosage is an important modulator of the phenotypes that are associated with hypomorphic alleles.

Program Nr: 123 from the 1999 ASHG Annual Meeting

Enzyme replacement therapy in mucopolysaccharidosis I: One year followup of ten patients. *E.D. Kakkis[1], J. Muenzer[2], G. Tiller[3], L. Waber[4], J. Belmont[5], M. Passage[1], B. Izykowski[1], J. Phillips[1], I. Walot[1], R. Doroshow[1], R. Hoft[1], K.T. Yu[1], S. Okazaki[1], D. Lewis[1], R. Lachman[1], E.F. Neufeld[1]*. 1) Harbor-UCLA Medical Center and UCLA; 2) University of North Carolina at Chapel Hill; 3) Vanderbilt University; 4) University of Texas Southwestern Medical Center; 5) Baylor College of Medicine.

Recombinant human alpha-L-iduronidase has been administered to 10 patients with MPS I on a weekly basis for over one year. The patients ranged in age from 5 to 22 years and cover the spectrum of severity. Initial evaluations performed at 6, 12, and 26 weeks demonstrated a rapid reduction in hepatosplenomegaly and urinary GAG excretion. Clinical assessments also demonstrated improvement in joint mobility. Endurance/fatigue, pain, and severe headaches improved by patient report. Follow-up evaluations at 52 weeks show that liver size is normal in 8/10 patients and urinary GAG remains low. Range of motion continues to improve in knee, elbow, or shoulder joints in some patients. In the 6 prepubertal patients height increased by an average of 4.9 % and weight increased by an average of 13% during the 52 week period. New York Heart Association scores improved by at least one class in 10/10 patients. Pulmonary function testing in one patient showed 230% improvement in FEV1 and FVC. Adverse events include urticaria in 5 patients, recurring in 4 patients, and ameliorated or resolved in 2 of 4. Complement activation occurred in 4 patients resolved by 26 weeks and did not recur at 52 weeks. Antibodies to iduronidase were observed in 4 patients but the titers are declining.

Program Nr: 124 from the 1999 ASHG Annual Meeting

Direct enzyme replacement with recombinant human iduronidase in a murine model for MPS I. *L.A. Clarke[1], C.S. Russell[1], T. Matlock[1], S. Gibson[1], T. Pourbahrami[1], D. Brooks[2], J.J. Hopwood[2].* 1) Dept Medical Genetics, BC Children's Hosp, Vancouver, BC, Canada; 2) Lysosomal Diseases Research Unit, Women's and Children's Hospital, North Adelaide, South Australia.

It is hoped that exogenous adminitration of purified iduronidase will provide an important therapeutic to alleviate many of the symptoms of MPS I. Studies of enzyme replacement in the canine model for MPS I have shown marked response in some tissues but not in others. The small number of animals used for these studies and the limiting supply of enzyme available have limited the conclusions form these important studies. We have previously reported the generation of a murine model for MPS I and now report on the use of this model for the testing and evaluation of direct enzyme replacement. Three dosing regimes have been used which included; 1) 10,000 units/kg/weekly (unit = 1 nmole/min) from 4 weeks to 25 wks, 2) 10,000 units/kg/weekly from day one to 25 weeks, 3) 4000 units/kg/weekly from 4 weeks to 25 weeks. Biochemical evaluation of tissues showed higher than normal specific activity of iduronidase in all tissues tested except the brain, where no appreciable activity was noted. In all dosing regimes there was disappearance of storage vacuoles from the liver, spleen, kidney and heart. The morphology of the growth plate and cortical bone was improved in all mice but a normal morphology was not restored. The CNS showed no appreciable alteration of pathology. All animals produced antibodies to iduronidase but no obvious hypersensitivity reactions were noted.

Program Nr: 125 from the 1999 ASHG Annual Meeting

Functional correction of old *mdx*mouse muscle using a gutted adenoviral vector expressing human dystrophin. *C. DelloRusso[1], J. Scott[2], D.J. Hartigan-O'Connor[4], A. Robinson[2], S.V. Brooks[1,3], J.S. Chamberlain[2,4]*. 1) Departments of Physiology; 2) Human Genetics; 3) Biomedical Engineering; 4) Cellular and Molecular Biology Graduate Program, University of Michigan, Ann Arbor, MI.

Duchenne muscular dystrophy (DMD), a degenerative lethal muscle disorder that affects one in 3500 newborn males, is caused by mutations in the dystrophin gene. Adenoviral vectors are promising tools that may be used to deliver the dystrophin gene to affected muscle to alleviate the symptoms of DMD. A concern with gene transfer is that old muscles may not be transduceable by adenovirus, limiting the age of patients treatable by gene therapy. We show adenoviral infection of dystrophic *mdx*mouse muscle aged 6 weeks to 24 months, however a five-six fold drop in infectivity was observed by 24 months. Another concern with gene transfer is that first generation adenoviral vectors elicit a potent immune response that prevents long term transgene expression. Therefore, we constructed a “gutted” adenoviral vector that contains a full-length human dystrophin cDNA driven by a muscle specific promoter. This viral construct contains only the viral sequences necessary in *cis*for replication and packaging of viral particles, and lacks any reporter gene. Injection of this virus into six week old *mdx*mouse muscle produced high levels of full-length human dystrophin. These dystrophin-positive fibers excluded the vital dye Evans Blue, demonstrating improved muscle sarcolemma integrity in the presence of human dystrophin. For analysis of restored muscle function, we showed that a novel eccentric contraction protocol demonstrates marked differences in the force deficit after stretch when *mdx*muscle is compared with wild type muscle. Eleven month old *mdx*mice were injected with the human dystrophin virus and analyzed with this assay after five days. Injected muscles showed a highly significant decrease in the force deficit compared with the contralateral uninjected muscle. In contrast, there was no significant difference between uninjected and sham injected muscles. Collectively, these data demonstrate a functional correction in old *mdx*mouse muscle by delivery of full-length human dystrophin in a gutted adenoviral vector.

Program Nr: 126 from the 1999 ASHG Annual Meeting

Functional Rescue of the Tibialis Anterior and Treatment of Large Muscle Groups in a Model for Limb Girdle Muscular Dystrophy Using a rAAV Vector. *D. Dressman[1,2], X. Xiao[2], J. Li[2], Y.P. Tsao[2], E.P. Hoffman[1,2], J.F. Watchko[3]*. 1) Center for Genetic Medicine, Children's National Medical Center, Washington, DC; 2) University of Pittsburgh, School of Medicine, Department of Molecular Genetics and Biochemistry, Pittsburgh PA; 3) Magee Women's Hospital, Department of Pediatrics, Pittsburgh PA.

A subset of the limb girdle muscular dystrophies (LGMD), the recessively inherited sarcoglycanopaties are caused by mutations in one of the four muscle expressed sarcoglycan genes (a-, b-, g-, or d-). Gene delivery using a recombinant adeno-associated virus (rAAV) has recently generated considerable interest for the biochemical rescue of muscle in muscular dystrophy. We previously reported the first successful delivery of a sarcoglycan gene by rAAV using the d-sarcoglycan deficient hamster (Bio14.6) model. (Li et. al. Gene Therapy 6:74-82, 1999) Here we show data proving feasibility of gene delivery to rescue entire muscles. Intramuscular injection of d-sarcoglycan into the dystrophic hamster's tibialis anterior (TA) muscle led to a greater than 97% recovery in muscle strength, both in specific twitch and titanic force. Normal muscle weight was also restored. There was no evidence of inflammation or other toxic side effects of the treatment. Expression appeared permanent, consistent with the ability of AAV to stably integrate into the host's genome. To extend these results to the intramuscular injection of larger (e.g. human) muscles, we describe the design and used of a 10 needle injection manifold for the efficient and reproducible injections of virus the entire hamster quadriceps (~1cm^2). The successful treatment of large muscle groups, and the biochemical and physiological recovery of the d-sarcoglycan deficient muscle demonstrates the feasibility of gene therapy in human LGMD patients with rAAV vectors.

Program Nr: 127 from the 1999 ASHG Annual Meeting

Isolation of muscle-derived stem cells with the potential of differentiating into muscle and hematopoietic lineages.

E. Gussoni[1], Y. Soneoka[2,3], C.D. Strickland[1], E.A. Buzney[1], M.K. Khan[4], A.F. Flint[1,2], R.C. Mulligan[2,3], L.M. Kunkel[1,2]. 1) Genetics, Children's Hosp, Boston, MA; 2) Howard Hughes Medical Institute, Children's Hospital, Boston, MA; 3) Department of Genetics, Harvard Medical School, Boston, MA; 4) Department of Surgical Research, Children's Hospital, Boston, MA.

Muscle precursor cells (myoblasts) are thought to be stem cells of skeletal muscle capable of repairing damaged or injured myofibers (Mauro, 1961; Bischoff, 1994; Grounds, 1990). Myoblast transplantation has been proposed as a cell-based therapy for several disorders of muscle, including Duchenne muscular dystrophy (DMD). Studies in the dystrophin-deficient mdx mouse (Karpati, 1989; Partridge, 1989) and in human clinical trials (Law, 1990; Mendell, 1995; Morandi, 1995; Gussoni, 1992; Huard, 1992; Karpati, 1993; Neumeyer, 1998) have demonstrated that myoblast transplantation is a safe but inefficient technique. Recent findings have suggested that myoblasts may consist of a heterogeneous population of mature cells mixed with a small percentage of early stage muscle progenitor cells that are more efficient at regenerating damaged muscle (Qu, 1998; Beauchamp, 1999). By adapting a technique previously used to isolate pluripotent hematopoietic cells (Goodell, 1996), we have isolated a population of stem cells from normal male mouse skeletal muscle and introduced them into lethally irradiated mdx females via the circulation. Donor male muscle stem cells can be detected by the presence of the Y-chromosome in recipient skeletal muscle, where they are responsible for the systemic delivery of the dystrophin gene and expression of dystrophin protein in up to 9% of the myofibers. Surprisingly, donor muscle stem cells can also be found in the bone marrow and spleen as differentiated hematopoietic cells. These data suggest that stem cells isolated from adult skeletal muscle have the ability to differentiate into a variety of mesodermal tissues under the influence of the local environment, and should constitute a valuable resource for future therapies of muscle disorders.

Program Nr: 128 from the 1999 ASHG Annual Meeting

Analysis of 200 patients undergoing Bone Marrow Transplant shows allelic disequilibrium between drug related toxicity and a common exonic polymorphism in the CPSI gene and correlates with disruption of urea cycle intermediates. *M.L. Summar[1], N. Scott[1], E. Cummings[1], H. Hutcheson[1], S. Dawling[3], B. Christman[2].* 1) Dept Pediatrics/Div Genetics, Vanderbilt Univ Medical Ctr, Nashville, TN; 2) Dept. Medicine, Vanderbilt Univ Medical Ctr,Nashville, TN; 3) Dept. Pathology, Vanderbilt Univ Mecical Ctr, Nashville, TN.

Bone marrow transplantation (BMT)is increasingly being used as primary therapy for malignancies. Hepatic venocclusive disease (HVOD), a clinical syndrome of hyperbilirubinemia, hepatomegaly,and fluid retention early after BMT, is one of the major dose limiting toxicities after BMT, afflecting 20% of the patients in our study. HVOD has a mortality rate of 50%. Coordinated metabolism of intermediates through the urea cycle allows for efficient formation of arginine, the substrate required for generation of nitric oxide (NO), an evanescent molecule that antagonizes platelet activation and promotes vasodilation. We previously reported a tentative link between a common carbamyl phosphate synthetase I (T1405N, het = .43) and HVOD in 40 BMT patients. Expanding our data set to 200 patients has added further evidence that indivuduals homozygous for T1405 form of CPSI have a very low incidence of HVOD (1 individual of 39 HVOD patients). Using Hardy Weinberg as a predictor of expected allele frequencies and Chi Square analysis, we demonstrated a high degree of disequilibrium between T1405N and HVOD ($p < 0.001$). Analysis of the urea cycle intermediates arginine and citrulline in all BMT patients shows a 50% decrease in citrulline from pre-transplant levels and an increase in arginine levels. Patients who developed HVOD had lower pre-treatment citrulline and arginine levels and experienced a greater drop in citrulline and no increase in arginine. This data suggests that there is a chemotherapy related decrease in urea cycle function which is exacerbated by a pre-existing genetic compromise of CPSI function. This combination appears to result in susceptibility to HVOD. The T1405N polymorphism can be easily screened for and we are testing therapeutic modalities to circumvent the decrease in arginine.

Program Nr: 129 from the 1999 ASHG Annual Meeting

Microglial Activation in Sandhoff Disease and Suppression After Bone Marrow Transplantation. *R. Wada[1], F. Norflus[1], C.J. Tifft[1,2], R.L. Proia[1].* 1) Genetics of Development and Disease Branch, NIDDK, NIH, Bethesda, MD; 2) Department of Medical Genetics, Children's Natl Medical Center, Washington DC.

In glycosphingolipid (GSL) storage diseases, such as Tay-Sachs disease, Sandhoff disease and some forms of Gaucher disease, an inherited lysosomal enzyme deficiency results in the accumulation of GSLs in the central nervous system (CNS). The GSL accretion leads to neuronal dysfunction and apoptosis through pathogenic mechanisms that are only poorly understood. We have studied a Sandhoff disease mouse as a prototype for the GSL storage diseases to uncover mechanisms of neurodegeneration. At 4 months old, when neurologic manifestations were extreme, apoptotic neuronal cell death was prominent in thalamic nuclei, brainstem and spinal cord. Global gene expression analysis using cDNA microarrays containing approximately 8,000 sequences revealed an up-regulation of macrophage/microglial genes in the CNS of 4 month old Sandhoff disease mice. Immunohistological analysis confirmed that CNS regions exhibiting prominent apoptotic neuronal death were populated by expanded numbers of macrophages/microglia. These macrophages/microglia intensely expressed markers of activation. In brain samples from a human case of Sandhoff disease, macrophage/microglial expansion and activation was confirmed. Bone marrow transplantation (BMT) of Sandhoff disease mice ameliorated neurologic manifestations and apoptotic neuronal death without demonstrable reduction in CNS storage of GSLs. We have found that BMT suppressed macrophage/microglial expansion and activation. These findings implicate the activation of the macrophage/microglial system in the neurodegeneration of Sandhoff disease and possibly other GSL storage diseases. They also suggest that the therapeutic effect of bone marrow transplantation is, in part, the result of the suppression of macrophage/microglial activation.

Program Nr: 130 from the 1999 ASHG Annual Meeting

Lysosomal acid lipase gene targeted mice: Long-term physiologic outcomes. *H. Du[1], J. Mishra[1], M. Heur[1], M. Duanmu[1], D. Witte[2], G.A. Grabowski[1].* 1) Div Hum Genetics, Children's Hosp Medical Ctr, Cincinnati, OH; 2) Div Pediatric Pathology, Children's Hosp Medical Ctr., Cincinnati, OH.

Lysosomal acid lipase (LAL) is essential for the hydrolysis of triglycerides (TG) and cholesteryl esters (CE) in lysosomes. It's deficiency produces two human phenotypes: Wolman disease (WD) and cholesteryl ester storage disease (CESD). To understand this pathophysiology and physiologic role of LAL, a mouse model, null for LAL mRNA and protein, was created by gene targeting. The lal-/- mice are normal at birth, develop to adulthood, and are fertile. The lifespan is foreshortened (~8 mos avg.). The lal-/- mice show age dependent progressive hepatosplenomegaly. The percentage of liver weight relative to body weight in lal-/- mice was 8.2% at 1.5 mos, 22.4% at 5 mos, and 35.1% at 8 mos. (lal +/+ = 5-6%). The total liver cholesterol (free and esterified) in lal-/- is 15-fold > lal +/+ at 1.5 mos. This increased from 19 and 43-fold at 5- and 8-mos., respectively. The total liver TG in lal-/- mice increased from 5-fold to 9-10 fold relative to lal+/+ in these same age groups. Electron microscopic analyses of liver showed the major lipid storage in the lysosomal compartment. Immunohistochemical staining with antibodies against albumin (hepatocyte), desmin (Ito cells) and CD68 (monocyte differentiated macrophages), showed the lipid storage in Kupffer cells. The progressive accumulation of TG and CE in lal-/- mice with also was observed in macrophages of the spleen and small intestine. Progressive rapid loss of white and brown adipose tissue was observed. By age of 6- to 8-mos., the lal-/- mice are completely lacking in white and brown adipose tissue. To evaluate the overall effects of LAL null, the mRNAs from livers of 4-month lal-/- and lal+/+ mice were compared. The cDNA arrays showed 34 mRNAs up- and 15 mRNAs down- regulated >5-fold, and 75 mRNAs up- and 40 mRNAs down- regulated between 5 and 2.5-fold increased in lal-/- vs lal +/+ livers. This mouse model provides evidence for the critical roles of LAL in cellular cholesterol and fatty acid metabolism including adipocyte differentiation and fat mobilization.

Program Nr: 131 from the 1999 ASHG Annual Meeting

LYSOSOMAL NEURAMINIDASE DEFICIENCY IN MICE: a model for sialidosis. *N. DE GEEST, L. MANN, J. DE SOUSA-HITZLER, C. HAHN, R. ROTTIER, A. D'AZZO.* GENETICS, St Jude Child Res Hosp , Memphis, TN.

Lysosomal neuraminidase (neur) initiates the degradation of sialoglycoconjugates in lysosomes. The enzyme is related to two lysosomal storage disorders in children: sialidosis is directly caused by structural mutations in the neur gene, while galactosialidosis is associated with a combined deficiency in both neuraminidase and acid beta-galactosidase, secondary to a defect in the protective protein/cathepsin A. We have generated a neur deficient line as a murine model for sialidosis. A 10 kb genomic fragment encompassing the 4 kb neur gene was used to construct the targeting vector for homologous recombination in embryonic stem cells. Disruption of the neur locus was achieved by the insertion of a lacZ-neo-cassette within the first exon. Germline transmission of the targeted allele led to the generation of knock-out mice. Null mutants were obtained with a frequency of ~21 %, indicating the absence of pre- or neonatal death. Newborn mice were visibly smaller and weighed 25 % less than their heterozygous and wild-type littermates. Neuraminidase activity, measured in tissues from (-/-) animals, confirmed the inactivation of the neur gene. Urine samples from 1 month old (-/-) mice showed abnormal excretion of high molecular weigth oligosaccharides, which is diagnostic of the disease. Tissue sections from 1 and 2 month old (-/-) mice stained with X-Gal demonstrated high expression of the beta-galactosidase reporter gene in the proximal tubuli of the kidney, and to a lesser extent in the Kupffer cells of the liver, the red pulp of the spleen, and vascular endothelial cells in the brain. The occurrence of phenotypic changes in the different organs was monitored using tissue sections stained with H/E and Periodic Acid Shiff (PAS). In line with the expression pattern of beta-Gal, extensive vacuolation was observed in the kidney where the epithelial cells of the proximal tubuli appear filled with swollen lysosomes. In conclusion, mice completely devoid of neur present early in life with characteristic features of the human disorder sialidosis and represent an ideal tool to study the function of lysosomal neuraminidase in vivo.

Program Nr: 132 from the 1999 ASHG Annual Meeting

A novel anion transporter gene is mutated in sialic acid storage diseases. *F.W. Verheijen[1], E. Verbeek[1], N. Aula[2], C.E.M.T. Beerens[1], A.C. Havelaar[1], M. Joosse[1], L. Peltonen[2], P. Aula[3], H. Galjaard[1], P.J. van der Spek[4], G.M.S. Mancini[1]*. 1) Department of Clinical Genetics, Erasmus University, Rotterdam, The Netherlands; 2) Department of Human Molecular Genetics, NPHI, Helsinki, Finland; 3) Department of Medical Genetics, University of Helsinki, Finland; 4) Akzo-Nobel/N.V.Organon, Oss, The Netherlands.

Sialic acid storage diseases (SASD) are recessively inherited lysosomal storage disorders characterized by an excessive tissue storage and excretion of sialic acid. The clinical form frequent in northern Finland (Salla disease), produces from early infancy on a slowly progressive neurodegeneration associated with disturbed myelination in the central nervous system. The infantile type of the disease (ISSD) entails generalized storage with visceromegaly, dysostosis, fetal hydrops and outcome within the first years. Salla disease and ISSD are allelic disorders mapping to chromosome 6q14-q15. Biochemical studies of lysosomal sialic acid transport earlier demonstrated that a H^+/anionic sugar symporter mechanism is impaired in patient lysosomal membranes. Functional characterization of the purified lysosomal carrier from rat liver pointed to a group of candidate transporter gene families, among which ACS (anion-cation symporters) family members. Here we report the discovery of a novel gene, *AST*, belonging to the ACS family of cation/anion symporters, encoding for a 495 amino acid protein with a predicted transport function, sialin. A recurrent homozygote mutation (R39C) of *AST* was observed in Finnish Salla disease patients, while six ISSD patients showed six different mutations in the *AST* gene, which predictively disrupt sialin function. These data demonstrate that mutations in *AST* are the primary cause of lysosomal SASD.

Program Nr: 133 from the 1999 ASHG Annual Meeting

Dietary Folic Acid Rescues Neural Tube Defects in *Crooked tail (Cd)*. *M.L. Carter, S. Ulrich, M.E. Ross.* Laboratory of Molecular Neurobiology and Development, Dept Neurology, UMHC, Univ Minnesota, Minneapolis, MN.

In human populations, the vitamin folic acid (FA) can reduce the recurrence rate of neural tube defects (NTDs) by as much as 50-70%, though the mechanism of this rescue is unknown. We have identified a new genetic mouse model, *Crooked tail* (*Cd*), in which NTDs are sensitive to dietary FA supplementation. In this strain, heterozygotes display a kinked tail while homozygotes exhibit a high incidence of the rostral NTD exencephaly; *Cd/Cd* individuals able to close the neural tube demonstrate subtle cortical malformation. We have placed the *Cd* locus to a 0.2 cM interval on the GCD genetic map, and identified tightly linked markers (LOD=65.3) which permit genotyping of embryos prior to phenotypic expression; physical mapping in a BAC contig is underway. In this study, heterozygous mating pairs were maintained on diets containing varying levels of FA, and genotype-phenotype correlations were examined in pups and in embryos. The risk of NTDs was reduced by up to 55% (x^2=31.11, p<0.005); this rescue was dose-dependent and did not require previous FA deficiency. As seen in humans, female *Cd/Cd* embryos were more prone to exhibit exencephaly; they were also more responsive to FA rescue (x^2=96.8, p<0.005). A shift was seen in the phenotypic expression of *Cd*: at lower levels of FA, early lethality was the most common outcome, but as FA levels increased, the most common phenotype shifted first to exencephaly, and then to neural tube closure. Thus, the *Cd* strain offers the opportunity to identify the biochemical, genetic and gender-specific factors which contribute to FA responsive NTDs.

Program Nr: 134 from the 1999 ASHG Annual Meeting

A resource of characterized bacterial artificial chromosome clones. *V.G. Cheung, M. Morley, H.L. Dalrymple, S. Narasimhan, J. Watts, A. Bruzel.* Pediatrics, Univ Pennsylvania, Philadelphia, PA.

Despite the success of the genome project, it is still difficult for investigators to obtain from a single source mapped bacterial artificial chromosome (BAC) clones that cover entire chromosomes. Our laboratory is establishing a set of resources that includes a BAC clone repository and a publicly available database, GenMapDB (http://genomics.med.upenn.edu/vcheung). This resource is designed to facilitate physical mapping, construction of genomic microarrays and high - resolution mapping of chromosomal mutations. For example, the clones can be used as starting material for constructing contigs of genomic regions of interest, as templates for building microarrays or as probes for fluorescent *in situ* hybridization.

Currently in the repository, there are about 1,000 BAC clones that span twelve human chromosomes (chromosome 13 to 22, X and Y) at 1-2 Mb resolution. When completed, it will contain clones that cover the entire human genome at 1-Mb resolution. All the clones are part of the Roswell Park Cancer Institute human BAC library. They are anchored to sequence tagged sites (STS) markers. The clones are mapped by a combination of filter-hybridization and STS-content PCR. The clones are characterized using pulse-field gel electrophoresis and *Hind*III fingerprinting.

GenMapDB is a freely available database that contains information about the mapped BAC clones. The database is designed to allow users to search for clones mapping to a region of a chromosome or an entire chromosome. Hyperlinks to other genome databases are set up to provide additional information on the STS-markers that are used as anchors for our mapped BAC clones.

In this presentation, we will describe data on the clones that are in the repository and demonstrate the database, GenMapDB.

Program Nr: 135 from the 1999 ASHG Annual Meeting

Large-scale sequencing of the Human Genome. *W. Barbazuk, L. Hillier, M.A. Marra, J.D. McPherson, R.K. Wilson, R.H. Waterston.* Genome Sequencing Center ,Washington Univ Sch Med, St Louis, MO.

The goal of the Human Genome Project is to represent the human genome in its entirety with high quality, highly redundant DNA sequence by 2003. In order to maximize data access by the research community, the HGP has embarked on a scheme to produce a working draft of the genome by the spring of 2000 (Science 19 March 1999, p. 1822). This working draft should contain 90% of the genome represented by 5 fold sequence coverage. To minimize duplication of effort, and provide added value to the sequence data, we are striving to map all clones prior to their selection for sequencing. Our strategy for mapping is focused on developing a database of BAC clone fingerprints obtained from HINDIII restriction digest patterns. This collection of fingerprints, currently estimated to represent 5X coverage of the genome, is being derived systematically from the RPCI-11 human genomic BAC library. Overlapping clones are identified and assembled into contigs. Contigs are anchored and ordered by hybridization with probes designed from markers with known chromosomal origin. Extensive use is also being made of the database of BAC end sequences available from TIGR(http://www.tigr.org/tigr_home/index.html). STSs designed from available BAC endsequence are screened against mapping panels (Washington University) and the TNG radiation hybrid panel (David Cox, Stanford University) to anchor BAC clones; and frequent searches of all publicly available mapped sequence against the BAC end sequence database are conducted by Greg Schuler at the NCBI. A comprehensive discussion of these mapping methodologies and progress will be discussed. HGP progress and data can be accessed at http://www.ncbi.nlm.nih.gov/genome/guide/ and a summary of these mapping data can be found at exit http://genome.wustl.edu/gsc/cgi-bin/ace/ctc_choices/ctc.ace.

Program Nr: 136 from the 1999 ASHG Annual Meeting

RefSeq and LocusLink: NCBI's new curated resources for human genes. *K. Pruitt, D. Maglott, H. Sicotte, K. Katz, G. Schuler.* NCBI, NIH, Bethesda, MD.

Public sequence archives include data that are redundant, incomplete, outdated or even contradictory. Thus there is a need for a stable, non-redundant, and curated integration of sequence data and associated biological information. NCBI is meeting this need by providing two new interdependent resources: RefSeq and LocusLink.

The RefSeq project currently provides reference sequences for human mRNAs and their protein products. RefSeq records, generated in collaboration with external experts, provide a foundation for the functional annotation of the human genome as well as a stable reference point for gene expression, mutation, and polymorphism studies. Furthermore, they are used within NCBI to anchor UniGene clusters, to identify sequence variants in dbSNP that fall in genes, and to support annotation of genomic contig sequence data generated by the Human Genome Project.

Much of the annotation added to a RefSeq record is derived from LocusLink. LocusLink provides a stable public LocusID associated with sequence accessions, official nomenclature, alternate descriptors, summary descriptions, sequence data, and other resources. A flexible search interface supports queries on any text in the record.

ReqSeq records are generated by a process consisting of (1)establishing an accurate gene-to-sequence association; (2) identifying the longest sequence that most closely matches that seed sequence; and (3) creating a provisional record from the seed sequence by incorporating current nomenclature, map locations, citations, a MIM number, and the LocusID. The provisional RefSeq records are reviewed by biologists who review the initial gene-to-sequence association, add information including a summary of gene function, and, more importantly, correct, re-annotate, or extend the RefSeq sequence using data available in other GenBank records. Both provisional and reviewed RefSeq records are made publicly available via the NCBI Entrez retrieval system, nucleotide and protein non-redundant BLAST databases, FTP, and the LocusLink web site (http://www.ncbi.nlm.nih.gov/LocusLink).

Program Nr: 137 from the 1999 ASHG Annual Meeting

Gene-specific SNP discovery from EST databases. *J. Yu, Z. Yang, M. Kibukawa, W. Hughes, D. Passey, M.V. Olson, P. Green, G.K.-S. Wong.* The Human Genome Center, Dept of Medicine, University of Washington, Seattle, WA.

We will report on the experimental validation of a set of single-nucleotide-polymorphisms (SNPs) that were originally identified by a computer analyis of 538,601 human ESTs from the WUSTL/Merck EST project. A searchable database, indexed by both map position and sequence content, has been constructed. A subset of 500-1000 candidate SNPs, ranked by biological significance, and representing the intersection of the most promising SNP candidates and the identifiable EST contigs (i.e., by a Blast search against GenBank), have been re-sequenced in an ethnically-diverse panel of up to 24 individuals. Issue considered include: (1) the quality of original EST reads, (2) the design of genomic PCR primers from EST sequence, (3) the fidelity of the reverse transcriptase, (4) any potential confusion over paralogous genes, and (5) the failure to replicate real SNPs with low allele frequencies.

Program Nr: 138 from the 1999 ASHG Annual Meeting

SNP allele-frequency determination in pooled DNA samples by kinetic PCR. *R. Higuchi[1], S. Germer[1], M.J. Holland[2]*. 1) Human Genetics Dept., Roche Molecular Systems, Alameda, CA; 2) Dept. of Biological Chemistry, School of Medicine, UC Davis, Davis, CA.

We have developed an accurate yet inexpensive and high-throughput method for determining the allele frequency of bi-allelic polymorphisms in pools of DNA samples. The assay combines kinetic (real-time quantitative) PCR with allele-specific amplification and requires no post-PCR processing. In essence, the relative amounts of each allele in a sample are quantified. This is done by dividing equal aliquots of the pooled DNA between two separate PCR reactions, each of which contains a primer pair specific to one or the other allelic SNP variant. For pools with equal amounts of the two alleles, the two amplifications should reach a detectable level of fluorescence at the same cycle number. For pools that contain unequal ratios of the two alleles, the difference in cycle number between the two amplification reactions can be used to calculate the relative allele amounts. We demonstrate the accuracy and reliability of the assay on known relative mixtures of DNAs homozygous for each allele from a SNP from 20:1 to 1:20, and on pools of both human and mouse DNAs over 9 different SNPs altogether. In most cases, measurement variance contributes considerably less than sampling error to the overall uncertainty in estimating population allele frequencies. We believe that by providing a means for SNP genotyping thousands of samples simultaneously, inexpensively and reproducibly, this method is a powerful strategy for detecting meaningful polymorphic differences in association studies and genome wide linkage disequilibrium scans.

Program Nr: 139 from the 1999 ASHG Annual Meeting

Large-scale detection and genotyping of mouse single-nucleotide polymorphisms. *K. Lindblad[1], N. Patil[2], E. Winchester[1], D. Wang[1], E. Robinson[1], M.J. Daly[1], J. Hirschhorn[1], P. Sklar[1], N. Shah[2], J. Warrington[2], T.J. Hudson[1,3], E. Lander[1].* 1) Whitehead Institute/MIT Center for Genome Research, Cambridge, MA; 2) Affymetrix Inc, Santa Clara, CA; 3) Dept of Medicine and Human Genetics, Montreal General Hospital Research Institute, McGill University, Canada.

A single nucleotide polymorphism (SNP) is a position in the genome where two alternate bases each occur at an appreciable frequency in a population. SNPs are abundant in mammalian genomes and amenable to automated genotyping. SNPs are especially well suited to genotyping of mouse crosses: While such bi-allelic markers are less informative than microsatellites in humans, they are completely informative in a cross of inbred mouse strains. In order to generate a set of useful SNPs for the mouse genome, we performed a screen of 3717 mouse sequence tagged sites (STSs) in a DNA panel of 8 inbred strains using GeneChip probe arrays. Altogether, 2848 SNPs were found in 1755 STSs (325 ESTs and 1428 random sequences). Not surprisingly, the majority of the SNPs detected were variations between M. m.castaneus (CAST/Ei) and the seven lab strains screened. The mean SNP frequency was 1/202 bp between M. m. castaneus and lab strains. Among lab strains a mean pairwise SNP frequency of 1/1029 bp was found. Sixty-eight percent of the STSs containing SNPs had previously been assigned map positions. A map was constructed yielding a mean inter-marker distance of 2.6 cM as an average for M. m. castaneus versus lab strains. The pair-wise mean inter-marker distance among lab strains was 7.5 cM. To test the usefulness of these SNPs, 100 well-spaced SNPs between A/J and C57BL/6J were chosen and a genotyping panel developed. The SNPs were assayed by PCR amplification in two pools of 50 loci, followed by genotyping by single-base extension (SBE) in 6 pools of 16-17 loci and detection in 6 lanes on an ABI 377. By loading multiple times on a gel, a cross of 48 mice can be genotyped for 100 markers on two gels. This effort is a first step towards a high-density SNP map of the mouse, as well as an efficient system for genotyping SNPs, that will be useful in positional cloning of single gene traits as well as for dissection of complex traits.

Program Nr: 140 from the 1999 ASHG Annual Meeting

The mouse brain transcriptome by Serial Analysis Of Gene Expression (SAGE): Differences in gene expression between P30 brains of the Ts65Dn mouse model of Down syndrome and normals and between males and females.

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Gene discovery and expression studies in mouse brains will enable the quantification and monitoring the expression of thousands of genes in space and time and help to analyse how these expression patterns vary in events such as changes in behavioral state, onset of disease and response to drugs. The segmental trisomy 16 mouse, Ts65Dn, is a model of Down syndrome, the most common genetic cause of mental retardation. The SAGE was used to analyse differences in the transcriptome of Ts65Dn males and normals, and normal female and male brains. We have collected a total of 153,000 SAGE tags from whole P30 mouse brains; 51,000 from normal males, 51,000 from Ts65Dn males and 51,000 from normal females. A total of 45,000 unique transcripts were observed in the combined sample, near the plateau of novel transcript discovery. Only 32% of those tags showed a match to the mouse UniGene database, and therefore 68% of the mouse transcripts are yet to be identified. In differential gene expression comparisons, analyses of ~102,000 tags, 51,000 from both the Ts65Dn and normal males revealed that the vast majority of transcripts are expressed at similar levels. 355 transcripts showed differences ($P<0.05$), 72 at 7-fold or more. Of these, 17 were from known genes, 45 undescribed and 10 identical to mouse ESTs. In comparisons of 51,000 tags from normal males versus equal number from females, again the majority of transcripts were expressed at similar levels. 421 transcripts showed differences ($P<0.05$), 92 at 7-fold or more. Of these, 16 were from known genes, 53 undescribed and 23 identical to mouse ESTs. Confirmation of the SAGE results is now being done by hybridization of microarrays containing the differentially expressed genes.

Program Nr: 141 from the 1999 ASHG Annual Meeting

Genome-wide expression analysis identifies novel genes participating in cardiac hypertrophy. *C.J. Friddle[1], J. Bristow[1,2], T. Koga[1], E.M. Rubin[1]*. 1) Genome Sciences, Lawrence Berkeley National Lab, Berkeley, CA; 2) Pediatrics, UCSF, San Francisco, CA.

Cardiac hypertrophy is a significant risk factor for cardiac failure, affecting 15% of the adult population, including 50% of those with hypertension. Single gene disorders account for a small fraction of these cases.

Studies have identified several genes that play important roles in the onset and progression of cardiac hypertrophy. These studies have primarily focussed on genes known to function in the heart. To identify novel mediators of hypertrophy, without prior assumptions regarding individual genes, we have taken a genome-wide approach using cDNA microarrays to study 2 pharmacological models in mice. This system allows us to precisely turn on and off the hypertrophic stimulus and assess gene expression at each stage of the process. Isoproterenol (ISO) was given continuously and mice were sacrificed at 4 times during the first day and then daily for 7 days. Similar timepoints were obtained for Angiotensin II (AII). Both drugs induced substantial increases in heart size. Total RNA was labeled from both ISO and control left ventricles and hybridized to a microarray. We arrayed 670 ESTs from murine heart libraries, 200 cDNAs previously implicated in hypertrophy, and 900 transcription factor cDNAs.

Genes with significant changes in expression (>2 SD) on 3 or more arrays were catalogued. Given our data set, we expect 5 clones to meet these criteria by chance, but 51 clones actually changed. Consistent with the known effects of ISO, 16 genes involved in oxidative energy metabolism were identified. We also identified 3 genes known to be altered by ISO. The remaining genes included 14 anonymous ESTs, 6 transcription factors, 5 receptors, 3 matrix elements and 3 sarcomeric ESTs. We performed similar analyses with AII and identified 11 genes that respond to both ISO and AII. To test whether these genes are directly involved in the response to ISO and AII, we are over expressing 5 genes in mice. This study illustrates a genome-wide approach to the identification of novel genes that play a role in disease processes *in vivo*.

Program Nr: 142 from the 1999 ASHG Annual Meeting

Genomic Studies of Human Erythropoiesis. *J.L. Miller, A.N. Gubin, J.M. Njoroge, T. Lee, L.B. Mitchell.* Lab Chem Biol, NIDDK/NIH, Bethesda, MD.

We have begun a genomic-scale analysis of transcriptional events involved in human erythropoiesis. Populations of primary human erythroid cells at defined stages during their terminal differentiation in suspension culture were isolated as a source of mRNA. Expression libraries from those cells were produced to provide a transcriptional phenotype of the cell populations. For the more differentiated cell populations, we developed a novel strategy to selectively subtract globin cDNA and thereby augment the identification of nonglobin genes. To date, we have sequenced 4468 clones (2329 independent clones) and created an internet-linked database of erythroid Expressed Sequence Tags (EST). Approximately 85% of the erythroid EST share significant homology with full-length mRNA transcripts or EST identified in other libraries. Among the full-length transcripts are several dozen transcription factors, 17 cell surface receptors, and 5 secreted proteins. The remaining 15% of the erythroid EST (385 independent clones) were classified as novel erythroid EST based on a lack of sequence homology with 2 million mRNA sequences currently deposited in public databases. Despite a lack of homology with expressed sequences, the genomic loci of 19 novel erythroid EST were identified by homology searches of human genome databases. Two of the novel erythroid EST unexpectedly map to regions within the beta-globin locus but outside the globin coding regions. Another novel erythroid EST maps to a genomic locus previously linked with fetal cell globin production (Xp22.2-22.3). In addition to electronic comparisons, we have constructed high density arrays of erythroid EST (E-chips) to prioritize the study of individual transcripts. The E-chips are produced by PCR amplification of the erythroid EST followed by robotic spotting of the PCR products on glass slides. Messenger RNA from erythroid cells or other tissues is then fluorescently labeled using a single round of reverse transcription and hybridized to the E-chip. Preliminary data from hybridizations with bone marrow and erythroleukemia cells suggest the arrays will provide a useful differentiation assay to rapidly screen the relative expression levels of genes transcribed in erythroid cells.

Program Nr: 143 from the 1999 ASHG Annual Meeting

Identification and comparison of housekeeping/maintenance genes in normal adult and fetal tissue. *J.A. Warrington, A. Nair, M. Mahadevappa.* Affymetrix Inc., Santa Clara, CA 95051.

Gene expression levels of about 7100 genes were measured in seven different human adult tissues and four different fetal tissues using high-density oligonucleotide arrays to identify genes involved in cellular maintenance. The adult tissues share a set of 700 transcripts. The fetal tissues share a set of 591 transcripts. 242 of the transcripts are detected at the same level of abundance in all of the adult tissues and 339 are detected at the same level in the fetal tissues. These transcripts can be considered maintenance or housekeeping genes. Results of a comparison of fetal and adult maintenance genes will be reported. Because our goal was to identify genes that are involved in maintaining cellular function in normal individuals we minimized the effect of individual variation by screening mRNA pooled from many individuals. This information is useful for establishing average normal expression levels and will be useful as a reference in a related study in which we are measuring gene expression in 30 different tissues across a number of individuals to better understand normal expression variation (www.geneindex.org). Additionally, we will report transcripts uniquely expressed in each of the seven adult tissues and each of the four fetal tissues.

Program Nr: 144 from the 1999 ASHG Annual Meeting

Genome-scale translational research with tissue and cellular microarrays: Identifying and prioritization of targets for diagnostics and therapeutics. *J. Kononen[1], D. Rohwer-Nutter[1], S. Leighton[3], T. Pohida[3], J. Kakareka[3], G. Sauter[2], O.-P. Kallioniemi[1]*. 1) Lab of Cancer Genetics, Nhgri/NIH, Bethesda, MD; 2) Institute for Pathology, University of Basel, Switzerland; 3) Dept. of Engineering, NIH.

As the number of novel genes and molecules increases, and high-throughout parallel analysis of many genes at a time (such as the cDNA microarray technology) has become available, there is an urgent need to develop methods for increasing the number of specimens that can be analyzed in an experiment. This is important to assess the diagnostic, prognostic or therapeutic significance of newly-discovered genes and molecules. We developed the tissue microarray technology (Kononen et al., Nat. Med. 4:844-7, 1998) to facilitate translational research by allowing up to 1000 tissue specimens to be analyzed in a single experiment. Based on recent technology improvements, it is now possible to construct many replicate arrays, each of which can be sectioned 300 times. For example, from a series of 1000 tumors (each 15 x 15 mms), almost 100 000 replicate tissue microarray sections can be be genarated. This provides a unique approach to utilize archival tissue materials for genome-scale molecular analyses, especially in a collaborative setting, where a number of investigators/centers analyze the same arrayed tissue material at different sites. The data and resulting images of hybridizations and stainings are deposited in a common database, which allows correlative molecular profiling of cancer. We have developed a molecular profling database based on FISH, mRNA ISH and immunohistochemical experiments on 500 breast cancer specimens with clinical follow-up information. Furthermore, we have proven that established prognostic markers in breast cancer (such as ER, PR, HER-2, and p53), can be reliably analyzed on tissue microarrays, and discovered new prognostic indicators for breast cancer (such as DNA amplification at 17q23, p=0.002). Finally, we have developed techniques for arraying cell lines. This allows generation of arrays from cytological, hematological and other clinical specimens for detailed molecular analyses.

Program Nr: 145 from the 1999 ASHG Annual Meeting

Identification and Characterization of Differentially Expressed Genes in Nonobese Diabetic (NOD) Mice by cDNA Microarray. *Q.G. Ruan, S. Eckenrode, Q. Fang, J.X. She.* Department of Pathology,Immunology and Laboratory Medicine,University of Florida, Gainesville, FL 32610.

Type I or insulin-dependent diabetes mellitus (IDDM) is a polygenic autoimmune disease that affects 0.3% of the world's population. It is anticipated that the expression levels of certain genes in patients are different from normal controls and that other genes may change their expression levels along with the autoimmune progression. To identify these differentially expressed genes, we have analyzed 18,342 cDNA clones on high density cDNA microarrays in a diabetic mouse model. Total RNA was extracted from spleens of the nonobese diabetic (NOD) mouse, three control strains (B6, CBA and Balb/C) and an autoimmune strain (MRL/mpj) at 10 weeks of age. Hybridizations were repeated in multiple experiments and reproducible results were obtained. Gene-specific semiquantitative RT-PCR was also used to verify the differential expression for genes identified using cDNA microarrays. Comparison between B6 and NOD revealed 41 genes that exhibit at least 4-fold expression differences. Of these 41 genes, 15 are known genes or have homology to known genes; 26 are unrelated to any other sequence in the databases. Thirty-five of the 41 genes are consistent in all three control strains (B6, CBA and Balb/c), while the MRL/mpj mice, an autoimmune lupus model, have similar expression levels as in NOD for 30 of the 35 genes. These results suggest that 30 differentially expressed genes are related to autoimmunity and 5 genes are diabetes-specific. We have also compared gene expression between non-diabetic NOD (10 week old) and diabetic NOD mice. Six genes were up-regulated in diabetic NOD mice compared to non-diabetic NOD and other controls at 10 weeks of age. These genes are secondary changes due to the autoimmune process. Studies of primary and secondary differences should help us understand the molecular immunopathogenesis of IDDM and other autoimmune disease and identify novel molecular markers for disease prediction.

Program Nr: 146 from the 1999 ASHG Annual Meeting

Isolation of an FMRP-associated mRNP and the identification of nucleolin and the fragile X-related proteins as components of the complex. *S. Ceman, V. Brown, S.T. Warren.* Howard Hughes Medical Institute and Departments of Biochemistry, Pediatrics and Genetics, Emory University School of Medicine, Atlanta, GA.

The loss of FMR1 expression due to trinucleotide repeat expansion leads to fragile X syndrome, a cause of mental retardation. The encoded protein, FMRP, is a member of a gene family that contains the fragile X-related proteins, FXR1P and FXR2P. FMRP has been shown to be a nucleocytoplasmic shuttling protein that selectively binds a subset of mRNAs, forms mRNP complexes, and associates with translating ribosomes. In order to identify proteins that interact with FMRP, we developed a cellular system expressing N-terminal Flag-FMR1 cDNA. We attempted to express this transgene in a number of cultured cell lines and although we were able to isolate numerous drug-resistant colonies of P19, J1 and COS cells, none expressed Flag-FMRP. In contrast, approximately 40% of the transfected murine L-M(TK-) clones expressed Flag-FMRP. Interestingly, L-M(TK-) cells also have the lowest levels of endogenous FMRP, thus, overexpression of FMRP may be toxic to cells. Here we describe a cell culture system from which we could isolate epitope-tagged FMRP along with RNA and at least six other proteins. We identify two of these proteins as the FXR1 and FXR2 proteins using specific antisera and identify a third protein as nucleolin using mass spectrometry. The presence of nucleolin is confirmed by both reactivity with a specific antiserum as well as reverse co-immunoprecipitation where anti-nucleolin antiserum immunoprecipitates endogenous FMRP from both cultured cells and mouse brain. The identification of nucleolin, a known componenent of other mRNPs, adds a new dimension to the analysis of FMRP function, and the approach described should also allow the identification of the remaining unknown proteins of this FMRP-associated mRNP as well as the bound RNA.

Program Nr: 147 from the 1999 ASHG Annual Meeting

Mutation of the E6-AP Ubiquitin Ligase Reduces Nuclear Inclusion Frequency While Accelerating Polyglutamine-Induced Pathology in *SCA1* Transgenic Mice. *C.J. Cummings[1], E. Reinstein[4], Y-H. Jiang[1], A. Ciechanover[4], H.T. Orr[3], A.L. Beaudet[1], H.Y. Zoghbi[1,2].* 1) Pediatrics and Human Molecular Genetics, Baylor College of Medicine, Houston, TX; 2) Howard Hughes Medical Institute, Houston, TX; 3) University of Minnesota, Minneapolis, MN; 4) Technion-Israel Institute of Technology, Haifa, Israel.

Spinocerebellar ataxia type 1 (SCA1) is a neurodegenerative disorder caused by an expansion of a polyglutamine tract in ataxin-1. In affected neurons of SCA1 patients and transgenic mice, mutant ataxin-1 aggregates in a single ubiquitin-positive nuclear inclusion (NI) that alters the distribution of the proteasome. The presence of neuronal ubiquitin-positive deposits is a common trait for many neurodegenerative disorders, yet how these deposits form and what role they play in pathogenesis is not yet clear. The ubiquitin-proteasome pathway (UPP) is essential to control the levels and activities of normal and misfolded proteins. In this study, we examined the role of the UPP in SCA1 pathogenesis. We show that while wild-type ataxin-1 [2Q] and mutant [92Q] are polyubiquitinated equally well in cell free extract, the mutant form is three fold less sensitive to degradation. Furthermore, degradation of ataxin-1 is inhibited *in vivo* by the specific proteasome inhibitor, *clasto*-Lactacystin b-lactone, and this inhibition also leads to an increase in the frequency and size of nuclear aggregates in transfected cells. These results suggest the expanded glutamine tract affects proteasomal degradation of mutant ataxin-1 and not its ubiquitination. To further investigate the role of the UPP *in vivo*, we bred the *SCA1* transgenic mice to mice lacking expression of *Ube3a* (E6-AP ubiquitin-protein ligase) in Purkinje cells. Purkinje cells from the double mutant animals have a significant reduction in the frequency of NI. Despite the reduced frequency of NI, the SCA1 specific Purkinje cells morphological changes in the *SCA1/Ube3a* mice are remarkably worse than those of *SCA1* mice. These results implicate the UPP in SCA1 pathogenesis and demonstrate unequivocally that the presence of NIs does not correlate with polyglutamine-induced neurodegeneration.

Program Nr: 148 from the 1999 ASHG Annual Meeting

An inducible PC12 cell model of Huntington's disease; insights into pathogenesis. *L.M. Thompson[1], J.S. Steffan[1], A. Kazantsev[2], B.A. Apostal[1], D. Greenwald[1], S. Raffioni[1], Y.-Z. Zhu[1], E. Preisinger[2], D. Housman[2].* 1) Dept Biological Chemistry, Univ California, Irvine, Irvine, CA; 2) Center for Cancer Research, MIT, Cambridge, MA.

Huntington's disease is an autosomal dominant, neurodegenerative disorder caused by a CAG/polyglutamine repeat expansion within the coding region of huntingtin. It is one of eight such disorders characterized by progressive degeneration of specific neurons. We have generated an ecdysone inducible PC12 cell model containing exon 1 of huntingtin with normal and expanded range polyglutamine repeats. We find that the expression of huntingtin is tightly controlled with high levels of induction and that expanded repeat exon 1 localizes to membrane fractions upon cellular fractionation experiments. Aggregates are observed in a percentage of cells expressing expanded repeat exon 1, either as cytosolic and perinuclear aggregates or as nuclear inclusions. Aggregates are also observed within the neurite extensions. PC12 cell lines expressing either normal range or expanded repeat protein are able to differentiate normally upon addition of NGF. A peptide inhibitor of ubiquitination, Leu-Ala was found to inhibit neurite outgrowth and cause cell death of differentiated cells, but not of proliferating cells, suggesting a dependence for maintenance of neuronal differentiation upon ubiquitination. In our system, we find no evidence of classic apoptosis, using a variety of experimental conditions. We do, however, find that transcription of specific genes is repressed by the expression of huntingtin; this repression is most pronounced when mediated by the expanded repeat containing protein. In addition, the interaction of a protein involved in apoptosis with huntingtin will be described.

Program Nr: 149 from the 1999 ASHG Annual Meeting

Analysis of polyglutamine-mediated cellular dysfunction in *Caenorhabditis elegans*. *P.W. Faber, A.C. Hart.* Cancer Ctr, Massachusetts General Hospital and Dept. of Pathology, Harvard Medical School, Boston, MA.

Huntington's Disease (HD) is one of 8 dominant human neurodegenerative disorders caused by expansion of an unstable CAG-trinucleotide repeat encoding polyglutamine (polyQ). How expanded polyQ stretches cause disease is largely unknown. We generated a *C. elegans* (nematode with a 10 day lifespan) model for polyQ-mediated cellular dysfunction. N-terminal fragments (aa 1-171) of huntingtin containing polyQ stretches of either 2, 23 or 95 and 150 residues were expressed in the ASH sensory neuron. Degeneration and death of these neurons was analyzed.

Expression of Htn-Q150, but not Htn-Q2, Htn-Q23 or Htn-Q95, causes age dependent ASH degeneration, but no ASH cell death, in 8-day-old animals. Despite the absence of cell death this degeneration requires *ced-3* caspase function, suggesting that Htn-Q150 activates the apoptotic cell death pathway. Htn-Q150 coexpression with subthreshold levels of a toxic (but non-apoptotic!) transgene, an OSM-10::GFP fusion protein, causes enhanced degeneration and cell death in both 3- and 8-day-old animals. This cell death depends on *ced-3* function, supporting the notion that Htn-Q150 activates the apoptotic cell death pathway.

Animals that express just Htn-Q150 are being used in a genetic screen for recessive enhancers of Htn-Q150 mediated ASH degeneration. We seek mutations that induce >30% Htn-Q150 mediated ASH degeneration in 3-day-old animals (unenhanced <1% degeneration). So far, one mutation, *rt13*, has been identified out of 13,000 mutagenized animals. 79% of *rt13* ASH neurons show degeneration at 3 days in the presence of Htn-Q150 expression. In the absence of Htn-Q150 this number is 1%. Marking the ASH with a non-toxic GFP marker revealed that *rt13* actually induces Htn-Q150 mediated cell death. Preliminary results indicate that this cell death requires *ced-3* function. Importantly, neither expression of Htn-Q2 nor the toxic OSM-10::GFP fusion protein induces ASH degeneration nor cell death in 3-day *rt13* animals. *rt13* has been mapped to a small chromosomal interval. Identification of the molecular nature of *rt13* will provide important insights in polyQ pathogenesis.

Program Nr: 150 from the 1999 ASHG Annual Meeting

Transgenic mice carrying the human DM region: a model for CTG repeat intergenerational and somatic instability Analysis of the CTG repeat amplification influence in transgenic mice. *C. Junien[1,3], H. Seznec[1], A.-S. Lia[1], O. Agbulut[2], C. Duros[1], C. Fouquet[1], H. Radvanyi[3], G. Gourdon[1]*. 1) Inserm UR383, Hopital Necker Enfants Malades, Paris Cedex 15, France; 2) CNRS Faculte de medecine Pitie-Salpetriere, Paris, France; 3) Service Central de Biochimie, Hopital Ambroise Pare, Boulogne, France.

The molecular basis of myotonic dystrophy (DM) is a CTG expansion located in the 3' untranslated region of the DM protein kinase gene (DMPK) but the mechanisms involved in the pathophysiology are not fully understood. Dramatic instability with very large intergenerational increases and high levels of somatic mosaicism is observed in patients. We have generated 3 types of transgenic mice containing 45 kb of the human genomic DM region with the three genes DMWD, DMPK, DMAHP and either 20, 55 or 320 CTG repeat. Analysis of the CTG repeat length in the 3 models (DM20, DM55 or DM320) and over more than 5 generations showed a striking similarity with DM: 1) intergenerational instability of the CTG repeat shows a bias towards expansion, a threshold between 20 and 55 CTG and is size-, sex-, and age-dependent; 2) somatic instability revealed by PCR and SP-PCR on different tissues is seen only in the DM55 and DM320 mice and is size- and age-dependent with a bias towards expansion and no correlation with replication and transcription. These results suggest that large genomic DNA and human chromatin environment are required. Different efficiencies in DNA repair mechanisms during replication and/or in non dividing cells are very likely to be involved. To define precisely which gene is affected by the CTG expansion and what is the size-threshold for the CTG repeat to affect one particular gene, we analysed the three transgenes expression in the three models by RPA. The tissue-specific expression was similar to that in humans. Various phenotypic analyses or tests have been performed on the DM320 mice in muscle, eye, heart, and for behaviour and insulin resistance. Histological anomalies are detected in muscle. This model is the first one to faithfully reproduce the features of CTG triplet repeat instability observed in human DM patients.

Program Nr: 151 from the 1999 ASHG Annual Meeting

NF1 microdeletions are mediated by homologous recombination between duplicons. *M.O. Dorschner, M.A. Weaver, V.P. Sybert, K.G. Stephens.* Department of Medicine, University of Washington School of Medicine, Seattle, WA.

Neurofibromatosis type 1 patients with a submicroscopic deletion spanning the NF1 gene are remarkable for an early age at onset of cutaneous neurofibromas, suggesting the co-deletion of a novel locus that potentiates neurofibromagenesis. Construction of a 3.5 Mb BAC/PAC contig at chromosome 17q11.2 and analysis of somatic cell hybrids from microdeletion patients showed that 14 of 17 cases had deletions of 1.5 Mb in length. The deletions encompassed the entire 350 kb NF1 gene, 3 additional genes, 1 pseudogene, and 13 ESTs. The critical region harboring the putative locus that exacerbates neurofibroma development was narrowed to 1 Mb by the identification of a smaller deletion in one of the 3 remaining patients. Of the 14 cases with 1.5 Mb deletions, both the proximal and distal breakpoints mapped within chromosomal regions of high homology, termed NF1 duplicons. These duplicons, with an estimated length of 15-100 kb, harbor at least 4 ESTs and an expressed SH3GL pseudogene. Therefore, homologous recombination between duplicons either in cis or in trans on sister chromatids is a predominant mechanism of NF1 microdeletion. Refined breakpoint mapping will facilitate identifying sequences within the duplicons that are susceptible to chromosome breakage and recombination. The number of NF1 duplicons in the genome is unknown, however, we have identified a third one at chromosome 17q24. These data suggest that NF1 duplicons may also play a role in germline or somatic rearrangements other than those resulting in neurofibromatosis type 1.

Program Nr: 152 from the 1999 ASHG Annual Meeting

The SCA8 transcript is an antisense RNA to a brain-specific transcript encoding a novel actin-binding protein (KLHL1). *M.D. Koob, M.L. Moseley, K.A. Benzow, C.M. Johnson, J.P. Nemes, L.P.W. Ranum, J.W. Day.* Institute of Human Genetics, Univ Minnesota, Minneapolis, MN.

We recently cloned a CTG expansion mutation that causes spinocerebellar ataxia type 8 (SCA8) and demonstrated that the SCA8 expansion is transcribed in the CTG-orientation, as is the case for myotonic dystrophy, and not in the CAG-orientation, as is seen with the other SCAs. No extended open reading frames are present in any of the SCA8 splice variants isolated. During the isolation of the SCA8 transcript we unexpectedly identified a mRNA containing a long open reading frame transcribed in an orientation opposite to that of the SCA8 transcript, suggesting that the SCA8 transcript is an endogenous antisense RNA. The sense transcript encodes a 748 aa protein that is highly homologous to the Drosophila KELCH gene and has been given the name Kelch -like 1 (KLHL1). We have isolated the mouse homologue of the KLHL1 transcript and have found that both the predicted open reading frame and promoter region are highly conserved between mouse and human. KLHL1 is predicted from sequence analysis to have the POZ/BTB protein:protein interaction domain present in kelch and in a number of zinc finger proteins, and to also have the six kelch motif repeats that are thought to constitute the actin-binding domain of kelch. The domain organization of KLHL1 is highly similar to that of the kelch-related, nerve-specific human gene NRPB (ENC-1). NRPB, which was identified as a specific molecular marker of neural induction in vertebrates, has been shown to participate in neuronal process formation and is believed to be a nuclear matrix protein. Northern and dot-blot analyses indicate that the KLHL1 sense transcript is primarily expressed in specific regions of the brain, including the cerebellum, frontal lobe, and subthalamic nucleus. We are currently performing in situ hybridization to more precisely determine the timing and localization of transcription. Experiments to elucidate the normal function of the KLHL1 protein and to determine the interactions between the KLHL1 sense and SCA8 antisense transcripts are underway. (See also abstract by MA Janzen et al.).

Program Nr: 153 from the 1999 ASHG Annual Meeting

Characterization of a transgenic model for SBMA. *D.E. Merry[1], J. Woods[1], J. Walcott[1,2], L. Bish[1], K.H. Fischbeck[3], A. Abel[3]*. 1) Biochemistry and Molec. Pharm., Thomas Jefferson Univ., Phila., PA; 2) Program in Pharmacology, Univ. of Pennsylvania Sch. of Med., Phila., PA; 3) Neurogenetics Branch, NIH, Bethesda, MD.

Spinal and bulbar muscular atrophy (SBMA) is a degenerative motor neuron disease caused by expansion of a polyglutamine tract within the androgen receptor (AR). Pathology studies of SBMA patients reveal intranuclear inclusions that contain N-terminal epitopes of the AR. We previously created truncated AR constructs with highly expanded CAG repeats and showed that the mutant AR is toxic, forms protein aggregates, and is abnormally processed. We have created transgenic mice with a highly expanded (112 CAG), truncated AR, using the prion protein (PrP) and the neurofilament light chain (NF-L) promoters to direct expression. Eight founders obtained with the PrP promoter show phenotypes ranging from mild, with abnormal hindlimb flexion, to severe, with gait abnormalities, tremor, seizures, and early death (PrP-AR112-10). Analysis of line PrP-AR112-10 revealed neuronal intranuclear inclusions. These were shown to contain ubiquitin, Hsp70, HDJ-2/HSDJ, the 20S core proteasome, and the 19S regulatory proteasome cap. Analysis of muscle pathology revealed no evidence for neurogenic atrophy in this line, suggesting that the gait abnormalities in these mice likely reflect central neuronal dysfunction. Immunostaining of other PrP founder animals with a similar phenotype, but of later onset, did not reveal nuclear inclusions, suggesting that inclusions are not required for the neuronal toxicity in this model. In addition to PrP transgenic mice, two lines obtained with the NF-L promoter show neurologic phenotypes; one exhibits abnormal hindlimb flexion, while the other displays severe hindlimb gait abnormalities and hindlimb and perineal muscle weakness. The finding of hindlimb gait abnormalities in the absence of other neurological impairment, in transgenic mice created with the NF-L promoter, indicates that expression patterns play a major role in determining phenotype in this model system. These mice represent a useful model for understanding the molecular pathogenesis of SBMA.

Program Nr: 154 from the 1999 ASHG Annual Meeting

Transgenic mice harboring a full-length human DRPLA gene with highly expanded CAG repeats exhibit severe disease phenotype. *T. Sato[1], M. Yamada[2], M. Oyake[1], K. Nakao[3], K. Nakamura[3], M. Katsuki[3], H. Takahashi[2], S. Tsuji[1]*. 1) Dept. of Neurology; 2) Pathology, Brain Research Inst., Niigata Univ., Niigata; 3) Center for Experimental Medicine, Inst. of Medical Science, Univ. of Tokyo, Japan.

To create animal models for Dentatorubral-pallidoluysian atrophy (DRPLA), we have previously generated transgenic mice harboring a single copy of a full-length human mutant DRPLA gene with 76 CAG repeats (HMG **8**:99-106, 1999). Although these mice showed no obvious phenotypes, we found one mouse among thousands of hemizygous mice which bred transgenic offsprings exhibiting behavioral abnormalities. About half of the transgenic offsprings showed the phenotype and contained highly expanded CAG repeats, indicating that the mouse was mosaic with respect to the size of CAG repeats of the transgene. With intensive breeding of the mouse, we obtained transgenic mice harboring a full-length mutant DRPLA gene with highly expanded CAG repeats and analyzed the phenotype in detail. Using litter-sprit technique, behavioral analysis and neuropathological studies were performed every 2 weeks from 4 to 14 weeks.The mosaic mouse carried the mutant DRPLA genes with ~129 and 76 CAG repeats which had no interruptions. The hemizygous transgenic offsprings with ~129 CAG repeats began to reveal myoclonic movement at 3 weeks. Ataxic phenomenon and myoclonic movement rapidly progressed, and epilepsy was observed around 12 weeks. Neuropathological examinations revealed massive accumulation of ubiquitinated neuronal intranuclear inclusions in selected neurons such as dentate nucleus and cerebral cortex after 9 weeks. The brain weight, prior to body weight, decreased progressively from 6 weeks, and finally all the hemizygous mice died by 16 weeks. As a result of CAG repeat instability, we obtained the transgenic mice with highly expand CAG repeats. The behavioral abnormalities and the neuropathological findings of the mice were quite similar to those of human DRPLA patients with early onset. Since the transgenic mice contain a mutant DRPLA gene driven by its own promoter, the mice are a good model for exploring the molecular mechanisms of neuronal loss and its regional selectivity commonly underlying CAG repeat diseases.

Program Nr: 155 from the 1999 ASHG Annual Meeting

FMR1 YAC transgenic mice: rescue of the FMR1 knockout mouse and behavior of the CGG trinucleotide repeat. *A.M. Peier, K.L McIlwain, R. Paylor, D.L. Nelson.* Molecular & Human Genetics, Baylor College of Medicine, Houston, TX.

Fragile X syndrome, the most common cause of inherited mental retardation, is the result of unstable expansions of a CGG trinucleotide repeat in the 5' untranslated region of the FMR1 gene. Fmr1 knockout mice exhibit similar, although less severe, characteristics of the human phenotype such as learning impairment, hyperactivity and macroorchidism. Although recent data supports a role in RNA metabolism, the function of FMRP remains to be elucidated. We have adopted a YAC transgenic approach in order to address questions pertaining to the stability of the CGG trinucleotide repeat and the developmental timing requirements for FMR1. Using homologous recombination in yeast we have introduced a number of alterations into the human FMR1 locus on a YAC including a premutation sized repeat. YAC transgenic mice have been generated that carry the human FMR1 gene containing various sized CGG repeats. A premutation sized allele (CGG90) has exhibited moderate instability to date in both paternal and maternal transmissions. RT-PCR analysis using human specific primers demonstrates that these mice express the YAC transgene. Several transgenic lines were subsequently bred to the Fmr1 knockout mouse. Western analysis and immunohistochemistry demonstrates that the protein produced from the human FMR1 gene is appropriately expressed. An observed reduction in testicular weights in knockout mice carrying the YAC transgene compared to non transgenic knockout littermates indicates rescue of the macroorchidism phenotype. Mice were examined for behavioral phenotypes using a battery of assays including open-field, rotarod, startle habituation, and conditioned fear. In the open-field, Fmr1 knockout mice were hyperactive compared to the knockout mice carrying the human FMR1 gene and wildtype mice. In addition, Fmr1 knockout mice displayed more anxiety-related responses in the open-field when compared with knockout mice carrying the YAC and with wildtype mice. These findings suggest that the YAC carrying the human FMR1 gene may have the capacity to ameliorate the behavioral consequences of Fmr1 deficiency in mice.

Program Nr: 156 from the 1999 ASHG Annual Meeting

Linkage of a Gene for Kartagener Syndrome to Chromosome 15q. *M.P. Witt[1], D.F. Wyszynski[4], Y-F. Wang[4], A. Miller-Chisholm[4], J. Pawlik[2], M.H. Khoshnevisan[4], C. Sun[4], S. Wang[4], Y-J. Zhang[4], E. Rutkiewica[1], J. Zebrak[2], A. Kapelerova[3], S.R. Diehl[4].* 1) Inst Human Genetics, Polish Academy of Sciences, Poznan, Poland; 2) Bronchology and Cystic Fibrosis Clinic, Inst of Tuberculosis and Lung Diseases, Rabka, Poland; 3) 2nd Clinic of Pediatrics, University Hospital, Bratoslava, Slovakia; 4) National Institute of Dental and Craniofacial Research, Craniofacial Epidemiology and Genetics Branch, NIH, Bethesda, MD, USA.

Kartagener syndrome (KS), a subtype of primary ciliary dyskinesia (PCD), is a genetic disorder characterized by situs inversus, bronchiectasis and the craniofacial symptom chronic sinusitis. Its caused by ciliary immotility/dysmotility due to ultrastructural defects of cilia. Most often its transmitted as an autosomal recessive trait, although genetic heterogeneity is postulated. We conducted linkage studies to search for PCD disease genes in 51 KS families. We genotyped 65 microsatellite markers on chromosome 15 and obtained a maximal pairwise LOD score of 4.23 providing support of linkage with marker D155154. Nonparametric sibpair analyses also support this. With results from multipoint linkage analyses, we've localized a KS gene within a 3 cM region between 77cM and 80cM from the telomere of 15p. Evidence of an association with marker alleles is also suggested. By contrast, both pairwise and multipoint LOD scores calculated for 19 PCD families without situs inversus (CDO, ciliary dysfunction only families) were negative, showing no linkage to this region. Future studies will search the whole genome for more genes. To clone the KS gene, we've been searching genomic databases for dynein, kinesin and forkhead genes that map to 15q to identify candidates for mutation analyses in our families.

Program Nr: 157 from the 1999 ASHG Annual Meeting

Identification of a locus for primary ciliary dyskinesia (PCD; Kartagener syndrome) on chromosome 19. *H.M. Mitchison[1], A.J. Walne[1], S.L. Spiden[1], H. Blau[2], H. Mussaffi-Georgy[2], H. Simpson[3], M. El Fehaid[4], M. Cheehab[4], M. Al-Dabbagh[4], H.D. Hammum[4], R.M. Gardiner[1], E.M.K. Chung[1], M. Meeks[1].* 1) Department of Paediatrics, Royal Free and University College Medical School, University College London, UK; 2) Schneider Children's Medical Center of Israel, Petach Tikva, Israel; 3) School of Medical Sciences, University Sains Malaysia, Kelantan, Malaysia; 4) Riyadh Al Kharj Hospital Programme, Saudi Arabia.

Primary ciliary dyskinesia (PCD) is an autosomal recessive disorder (incidence 1: 20,000) characterised by sinusitis, pulmonary infections, bronchiectasis and subfertility. Approximately 50% of patients have defects of laterality, usually *situs inversus*(Kartagener syndrome). The clinical phenotype is caused by dysmotility of the cilia associated with a variety of abnormalities of ciliary structure. Six families of Arabic origin were ascertained. Parents are first cousins in five of the families and the resource includes 37 individuals with 13 affected children, four of whom have *situs inversus.* A ciliary ultrastructural defect of absent outer dynein arms was documented. A genome-wide linkage search using homozygosity mapping was undertaken. A region of excess homozygosity was identified on chromosome 19q13.4-qter below marker *D19S418*, spanning a region of 13 cM. GENEHUNTER analysis gave a maximum multipoint lod score of 4.73 with an alpha of 0.7. Haplotype analysis is consistent with linkage in four of the families. This is the first definitive report of linkage for a PCD locus and work is ongoing to refine the critical region in order to identify potential candidate genes. Chromosome 19q was one of five regions suggestive of linkage in a genome wide search undertaken in 33 nuclear PCD pedigrees (J-L Blouin et al. Am J Hum Genet 63: A282, 1998).

Program Nr: 158 from the 1999 ASHG Annual Meeting

A gene for laterality defects maps to 3p14.2-3p12. *S.H. Morelli[1], L.T. Pagotto[1], B.S. Reid[3], H. Ruttenberg[1], H.J. Yost[1,2], M. Bamshad[1,4].* 1) Department of Pediatrics, University of Utah, Salt Lake City, UT; 2) Huntsman Cancer Institute, University of Utah, Salt Lake City, UT; 3) Department of Pediatric Radiology, Primary Childrens Medical Center, Salt Lake City, UT; 4) Shriners Hospitals for Children, Salt Lake City, UT.

Disturbances of left-right asymmetry (i.e. laterality defects) such as asplenia and polysplenia are observed in approximately 1/10,000 livebirths. Laterality defects are often accompanied by severe congenital heart defects. Genes causing laterality defects could underlie more common congenital heart defects. Few genes have been identified causing laterality defects in humans. Using extensive pedigree linking, we have ascertained large, multiplex families in Utah in which laterality defects segregate in complex patterns. Strict diagnostic criteria were developed, and all participants underwent comprehensive imaging of the thoracic and abdominal cavities. A single family with multiple affected individuals in 3 generations demonstrated laterality defects segregating in an autosomal dominant pattern. This family was used to perform a genome-wide screen for genes causing laterality defects. A maximal two-point lod score of 3.05 (at q = 0.0) was obtained with marker D3S1261. Fine mapping has defined an interval of linkage between D3S2329 and D3S1663, corresponding to a location 3p14.2-3p12. This region comprises a genetic distance of 27 cM, and evaluation for possible candidate genes is underway.

Program Nr: 159 from the 1999 ASHG Annual Meeting

Hydrolethalus Syndrome Maps to a Highly Restricted Region on Chromosome 11q23-25. *I. Visapaa[1], R. Salonen[2], P. Paavola[1], L. Peltonen[1,3].* 1) Department of Human Molecular Genetics, National Public Health Institute and Department of Medical Genetics, University of Helsinki, Finland; 2) Prenatal Genetics, Department of Obstetrics and Gynecology, Helsinki University Central Hospital, Finland; 3) Department of Human Genetics, UCLA, Los Angeles, USA.

Hydrolethalus syndrome (MIM 236680) is a multiple midline malformation syndrome leading to stillbirth or death during the first day of life. It is characterized by hydrocephaly with absent midline structures of the brain, micrognathia, polydactyly, anomalous nose, eyes and ears, cleft lip or palate, keyhole shaped defect of the occipital bone, club feet and abnormalities of heart, lungs and genitalia. This recessive syndrome occurs almost exclusively in Finland, where the incidence is 1:20 000, and only a few cases have been described elsewhere in the world. The molecular pathogenesis of hydrolethalus syndrome is totally unknown. We assigned the hydrolethalus syndrome locus to 11q23-25 using genome scan with DNA samples of only 15 affected individuals. Consequent linkage analysis in eight families with denser marker set on chromosome 11 confirmed the assignment of the hydrolethalus syndrome locus to a 8.5 cM region between markers D11S4144 and D11S1351. The locus was further restricted to a 0.5 - 1 cM interval between markers D11S933 and D11S934 by linkage disequilibrium and ancestral haplotype analysis. The maximum LOD score obtained in multipoint linkage disequilibrium analysis was 21.1 at the marker D11S4158. The results suggest locus and allelic homogeneity of hydrolethalus syndrome in Finland and form the basis for the identification of the causative gene of this developmental disturbance.

Program Nr: 160 from the 1999 ASHG Annual Meeting

An association approach using eight affected individuals from Tasmania, Australia maps a locus for keratoconus.

J.M Fullerton[1], P. Paprocki[2], S. Foote[3], D. Mackie[1], R. Williamson[1], S. Forrest[1]. 1) Murdoch Institute, Royal Children's Hospital, Melbourne, Victoria, Australia; 2) Liley and Paprocki Optometrists, Burnie, Tasmania, Australia; 3) Walter and Eliza Hall Institute, Melbourne, Victoria, Australia.

Founder populations, such as Iceland and Finland, have long been used to identify novel candidate genes for single gene disorders. This study explores the island state of Tasmania, Australia as a founder population, where a minimalistic approach was used to map the locus for keratoconus. Keratoconus is a progressive degeneration of the central cornea, which is present at a five fold increased incidence in the population of the North-West coast of Tasmania. Familial keratoconus accounts for 10% of cases world wide, usually as an autosomal dominant trait. We collected mouthwash samples from six apparently unrelated individuals and one sib pair with keratoconus from Burnie, a town of 25000 people on the North-West coast. A genome wide search was conducted on all autosomes, using 343 polymorphic dinucleotide markers at 10cM intervals across the genome. An association approach was used, where markers were assessed for common allele sharing in all eight individuals on at least one chromosome. The genome-wide search revealed four markers on three chromosomes (11q, 15q, 18p and 18q) where the maximum allele sharing score of 7/7+1 was observed. This approach eliminated 98% of the markers from the genome set from further analysis. The four 7/7+1 loci were assessed by allele sharing simulations using control allele frequencies, and analysis of additional markers adjacent to each 7/7+1 marker. Initial investigation of the 18p region found a high proportion of allele sharing over a 5cM interval in a total of 24 Tasmanian patients of unknown genetic relationship. Collection of parental DNA from eight patients revealed a founder haplotype spanning a minimum of 2.7Mb. This region is currently under investigation for candidate genes. We propose that Tasmania is, at least in part, a founder population, and can be used to identify genes for monogenic disorders.

Program Nr: 161 from the 1999 ASHG Annual Meeting

Identification of a putative locus for keratoconus on Chromosome 21. *L.X. Zu[1], H.Y. Yang[3], Y.P. Wang[3], K.P. Figueroa[1], J. Rotter[3], S.M. Pulst[1], Y.S. Rabinowitz[2].* 1) Dept Neurology, Cedars-Sinai Medical Center, Los Angeles,CA; 2) Cornea-Genetic Eye Institute, Medical Genetics Birth Defects Center,Cedars-Sinai Medical Center, Los Angeles,CA; 3) Division of Medical Genetics,Cedars-Sinai Medical Center ,Los Angeles,CA.

Keratoconus is a corneal dystrophy with an incidence of 1 in 2,000. It is a major cause of visual disability worldwide and a leading cause for cornea transplantation in Western developed countries. Recent segregation studies have suggested that keratoconus has a genetic component in its expression, and both autosomal dominant and recessive modes of inheritance have been observed. Since the incidence of keratoconus is greatly increased in Down syndrome, we examined linkage of chromosome 21 markers in an extended pedigree with keratoconus. We determined the presence of keratoconus in ten individuals in three generations using standardized videokeratography. Seven of them had clinical signs. Assuming autosomal dominant inheritance and a penetrance of 0.6 the highest multipoint lod score of 2.4 was obtained for marker D21S1437. Non-parametric linkage (NPL) scores were estimated using the GENEHUNTER program, and a maximum NPL score of 12.9 (p=.0.0017) was obtained for the same marker D21S1437 which is approximately 10 cM distal to the centromere of Chromosome 21. Haplotype analysis defined the region of linkage to a 5.4 cM region flanked by markers D21S1905 And D21S1409 which covers approximately 4 MB of physical distance. While it is likely that keratoconus is a complex trait caused by multiple genes, identifying one gene will greatly facilitate the subsequent identification of other modifying alleles.

Program Nr: 162 from the 1999 ASHG Annual Meeting

Homozygosity mapping of a locus for autosomal recessive external ophthalmoplegia to chromosome 17p12-p13.1.

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External ophthalmoplegia (EO) is a clinically and genetically heterogeneous condition characterized by weakness of ocular muscles, ptosis and involvement of peripheral skeletal muscles. The disorder usually results from mitochondrial deletions and several nuclear genes for autosomal dominant progressive EO (PEO) have been mapped to chromosomes 10, 3 and 4. Here we report on a large Muslim kindred originating from a highly inbred community with EO diagnosed in 6 individuals. In some of the patients, ophthalmoplegia was not isolated and, in particular, weakness of the shoulder girdle was also present. Laboratory investigations did not show any mitochondrial defect, including a muscle biopsy in the most severely affected patients. Since the parents of the EO patients were not affected, we hypothesized that EO behaved as an autosomal recessive trait in this family, and performed a genome-wide search for regions of homozygosity. Among 400 markers tested, we found homozygosity in all affected patients for 8 cM interval mapping to chromosome 17p12-p13.1. The maximum lod score was 4.33 for markers AFMa219ye9 (D17S1803) and AFMa070WD1. Two independent recombination events that flanked the region of homozygosity were observed at loci D17S1812 and D17S947. Three candidate genes are mapping in this region, one of them (COX10) being already excluded by direct DNA sequencing. We hope that the identification of a gene for autosomal recessive EO might also shed some light on the other variants, especially as none of the autosomal dominant loci for PEO has been ascribed to chromosome 17 so far.

Program Nr: 163 from the 1999 ASHG Annual Meeting

Evidence for a susceptibility locus for phonological coding dyslexia on chromosome 6q13-q16.2. *T.L. Petryshen[1], B.J. Kaplan[2], L.L. Field[1]*. 1) Dept. of Medical Genetics, Univ. of Calgary, Calgary, Alberta, Canada; 2) Alberta Children's Hospital and Dept. of Paediatrics, Univ. of Calgary, Calgary, Alberta, Canada.

Phonological coding dyslexia (PCD) is a common, genetically-complex disorder characterized by difficulty processing the phonetic (sound) units of language despite normal intelligence. We studied 96 Canadian kindreds having at least two members diagnosed as affected with PCD by psychometric testing for linkage between a *qualitative* PCD phenotype (affected, unaffected, uncertain) and chromosome 6 microsatellite markers. Using parametric analyses, highly suggestive evidence for linkage was detected at 4 markers spanning 12cM on chromosome 6q13-q16.2 (LOD scores 2.3-2.6 at markers D6S254, D6S965, D6S286, D6S251 under a high penetrance 0, .8, 1 model). Nonparametric SIBPAL analyses of 357 sibpairs derived from one nuclear family per kindred were consistent with linkage (lowest p value = 0.054 at D6S254). Results were even more interesting analyzing a subsample of 228 sibpairs under 18 years old, for whom the psychometric tests may be more definitive (p value = 0.016 at D6S254). As other groups have employed *quantitative* measures of reading disability in their linkage studies, we also investigated linkage to quantitative measures of phonological coding and spelling in our families using SIBPAL. In both the 357 all-age sibpairs and the 228 under 18 sibpairs, convincing evidence for a locus influencing reading disability was found in the 6q13-q16.2 region, supporting the qualitative PCD results. The most significant results were with the spelling trait, which involves phonologic and orthographic skills (all-age sibpairs lowest p value = 0.00076 at D6S254, and p values < 0.05 at 6 other markers; under 18 sibpairs lowest p value = 0.0011 at D6S286, and p values < 0.05 at 5 other markers). The results were also significant for the phonological coding trait (all-age sibpairs p values < 0.04 at two markers; under 18 sibpairs p value < 0.03 at one marker). In conclusion, 1) we have identified a susceptibility locus for dyslexia on chromosome 6q13-q16.2 in a sample of 96 Canadian families, and 2) this locus was detectable using both qualitative and quantitative methods of analysis.

Program Nr: 164 from the 1999 ASHG Annual Meeting

A novel syndrome of episodic muscle weakness maps to Xp22.3. *M.F. Buckley[1], M.M. Ryan[2], P.J. Taylor[1], J.A. Donald[3], R.A Ouvrier[2], G. Morgan[4], G. Danta[5], K.N. North[2].* 1) Molecular & Cytogenetics Unit, Prince of Wales Hospital, Sydney, New South Wales, Australia; 2) Neurogenetics Research Unit, The New Childrens Hospital, Sydney, New South Wales, Australia; 3) Dept of Biological Sciences, Macquarie University, Sydney, New South Wales, Australia; 4) Dept of Medical Genetics, Sydney Children's Hospital, Sydney, New South Wales, Australia; 5) Dept of Neurology, The Canberra Hospital, Canberra, ACT, Australia.

We describe a family with a novel disorder characterised by episodic muscular weakness, vacuolar myopathy and X-linked inheritance.

Eight males in three generations demonstrate the characteristic features of the disorder. Episodes of severe muscle weakness are typically precipitated by febrile illness or anaesthetics, affect the facial and extra-ocular musculature as well as the trunk and limbs and resolve spontaneously over weeks to months. Younger members of the family are normal between episodes but during relapses there is generalised weakness, ptosis and fluctuations in strength. In some cases fatiguability can be demonstrated. The proband has chronic weakness and fatiguability that is partially responsive to anticholinesterases. The clinical phenotype has features suggestive both of the familial myasthenic syndromes and of ion-channel disorders such as the periodic paralyses. Electron microscopy demonstrated a vacuolar myopathy with dilated sarcotubular systems and proliferation of the sarcoplasmic reticulum.

We have localised the gene for the condition to chromosome Xp22.3 with a maximum two-point Lod score of 4.52 (Theta = 0.0) between OACA2 and DXS9985. Centromeric and telomeric recombinations limit the region of peak LOD score to that between DXS7103 and DXS1224. The physical distance between the two flanking markers is approximately 2.8Mb. Candidate genes within the region include:*CLCN4, APXL, TBL1, TCH, GAPL.* The gene symbol EMWX (episodic muscle weakness X-linked) has been approved for this condition.

Program Nr: 165 from the 1999 ASHG Annual Meeting

The locus for one form of bipolar affective disorder (BPAD) in a large Turkish family with autosomal dominant inheritance maps on chromosome 20p11-q13. *U. Radhakrishna[1], S. Senol[2], H. Herken[4], K. Gucuyener[3], C. Gehrig[1], A.N Akarsu[5], S.E Antonarakis[1]*. 1) Medical Genetics, University of Geneva Medical School & Hospital, Geneva, Switzerland; 2) Dept. of Psychiatry; 3) Pediatric Neurology, Gazi University; 4) Dept. of Psychiatry, Gaziantep University; 5) (TUBITAK) DNA/Cell Bank and Gene Research Laboratory, Hacettepe University, Ankara, Turkey.

Bipolar affective disorder (BPAD), or manic-depressive illness, is a common complex, polygenic disorder characterized by recurrent cyclic episodes of mania and depression. The lifetime prevalence of BPAD is 0.5%-1.5%. Family, twin, and adoption studies strongly implicated a genetic predisposition/susceptibility in the etiology of BPAD. In some rare families, BPAD is inherited as autosomal dominant condition. We studied a large Turkish BPAD pedigree with autosomal dominant inheritance that consists of 80 people with 13 affected individuals (9 females and 4 males). The phenotypes in the members of this family were determined using DSM-IV criteria. The age of onset ranged from 15-40 years with an average of 25 years. The phenotypes included recurrent manic and major depressive episodes; there was usually full remission with lithium treatment. DNA samples were collected from 35 individuals after informed consent. A genomewide genotyping and linkage analysis using over 225 highly informative polymorphic microsatellite markers showed strong evidence for the BPAD locus in this pedigree on chromosome 20p11-q13. The highest 2-point LOD score of 4.04 at theta=0 was obtained with four markers. Haplotype analysis enabled the mapping of the BPAD locus in this family between markers D20S186 and D20S171, in a region of approximately 61 cM. No other genomic region resulted in a positive lod score. The contribution of this locus to the BPAD of the general population is unknown. The sequence analysis and a complete list of the genes in the critical region will provide a wealth of candidates for mutation analyses. The study of additional families linked to the chromosome 20 will narrow down the critical genomic region and facilitate positional cloning of this BPAD gene.

Program Nr: 166 from the 1999 ASHG Annual Meeting

The gene for autosomal recessive Robinow syndrome is located in 9q. *H. Van Bokhoven[1], E. van Beusekom[1], J. Celli[1], H. Kayserili[2], H.G. Brunner[1]*. 1) Dept Human Genetics 417, University Hospital Nijmegen, Nijmegen, The Netherlands; 2) Division of Medical Genetics, University of Istanbul, Istanbul, Turkey.

Robinow syndrome has hypertelorism, short stature, mesomelic shortening of the forearms and forelegs and hypoplastic genitalia as its most characteristic features. Both autosomal dominant and autosomal recessive forms exist. The genetic basis for Robinow syndrome is not yet known, but the phenotype of the autosomal recessive form suggests that the mutation may affect a gene that is involved in vertebral segmentation. We have used homozygosity mapping to localise the gene for autosomal recessive Robinow syndrome in five families of Turkish descent. Our results indicate that the Robinow syndrome gene is located in 9q21-q23 (Zmax = 5.72 at 'Q'=0). A Detailed analysis with markers spanning the 14cM critical region between D9S257 and D9S176 shows haplotype sharing between two of the families, which reduces the critical interval to 11cM between D9S1796 and D9S176. No obvious ancestral haplotype was present in this sample of five families. The results suggest that multiple mutant alleles at a single locus in 9q are involved in the high frequency of Robinow syndrome in Turkey.Q.

Program Nr: 167 from the 1999 ASHG Annual Meeting

Frataxin is an iron-storage protein. *G. Isaya, J. Adamec, F. Rusnak, W.G. Owen, S. Naylor, L.M. Benson.* Pediatric & Adolescent Medicine and Biochemistry & Molecular Biology, Mayo Clinic and Foundation, Rochester, MN.

Friedreich's ataxia (FRDA) is an autosomal recessive neurodegenerative disease caused by a deficiency of frataxin, a nuclear-encoded mitochondrial protein. Studies in yeast have shown that frataxin is required for mitochondrial iron efflux, suggesting that FRDA results from oxidative damage secondary to mitochondrial iron accumulation. The function of frataxin, however, is not yet known. In this study, the mature form of the yeast frataxin homologue (mYfh1p) was expressed in bacterial cells and purified to homogeneity. Isolated recombinant mYfh1p is a soluble monomer with a molecular mass of 13,783 Da and contains £0.4 atoms of iron per subunit. The frataxin monomer shows no significant tendency to associate in the absence of added iron. Aerobic addition of ferrous ammonium sulfate to mYfh1p, however, results in assembly of a regular spherical complex with an external diameter of 176±10 angstroms and a molecular mass of ~1,1 MDa. The frataxin multimer consists of ~60 subunits and can sequester up to 69±2 iron atoms per subunit into a polynuclear ferric oxohydroxide mineral core. These data indicate that frataxin is an iron storage protein and similar to cytoplasmic ferritin plays a role in iron detoxification and iron reserve in the mitochondrion. This work is supported by grant AG15709 from the National Institute on Aging.

Program Nr: 168 from the 1999 ASHG Annual Meeting

Redesignation of the SJL mouse (SJL-dysf) as a model for human dysferlin-deficient muscle disorders. *R.E. Bittner[1], L.V.B. Anderson[2], E. Burkhardt[3], R. Bashir[2], E. Vafiadaki[2], S. Ivanova[1], I. Maerk[1], H. Hoeger[4], M. Jung[3], M. Storch[1], H. Lassmann[1], J.A. Moss[2], K. Davison[2], R. Harrison[2], K.M.D. Bushby[2], A. Reis[3,5]*. 1) Neuromuscular Research Dept., Inst. of Anatomy and Inst. of Brain Research, Univ. Vienna, Vienna, Austria; 2) Neurobiology Dept. and School of Biochemistry and Genetics, University Medical School, Newcastle Upon Tyne, UK; 3) Mikrosatellitenzentrum, Max-Delbrueck-Centre, Berlin, Germany; 4) Research Institute of Laboratory Animal Breeding, Himberg, Austria; 5) Inst. of Human Genetics, Charite, Humboldt University, Berlin, Germany.

The SJL-mouse strain has been inbred for almost half a century and has subsequently been found to be highly susceptible to numerous experimentally induced autoimmune diseases. It is widely used as a model for experimental autoimmune encephalitis (EAE) and inflammatory muscle disease. Additionally, several observations indicated that the skeletal muscle in SJL mice had several unusual characteristics: these included an increased regenerative capacity, and the spontaneous occurrence of what has been designated an inflammatory myopathy, accompanied by loss of strength. We have now recognized that this muscular weakness is due to an autosomal recessive form of muscular dystrophy and have mapped this Sjl locus to mouse chromosome 6, a region syntenic to human chromosome 2p13. We have also shown that the cDNA of the dysferlin gene, mutations in which cause limb girdle muscular dystrophy 2B (LGMD2B) or Miyoshi myopathy (MM) in humans, is disrupted in the SJL mouse by a 171 bp deletion. Defective dysferlin expression in the mouse strain is reflected by a clinical phenotype as well as muscle pathology consistent with muscular dystrophy. Therefore, the SJL mouse is the naturally occurring animal model for dysferlin-deficient muscle disorders in humans, which may become instrumental in testing therapeutic strategies in LGMD2B/MM. We therefore propose the redesignation of this strain as SJLdysf/dysf. Moreover, our findings will lead to a reassessment of experimental results obtained from the SJL mouse during the last decades.

Program Nr: 169 from the 1999 ASHG Annual Meeting

Embryonic recovery is the mechanism for reduced penetrance of congenital heart defects in mice deficient for the DiGeorge syndrome region. *E.A. Lindsay, D. Su, A. Baldini.* Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX.

A heterozygous deletion within the chromosome band 22q11 (*del22q11*) is the basis for the most common deletion syndrome in humans, which includes DiGeorge and velocardiofacial syndromes. *Del22q11* is associated with cardiovascular abnormalities and although the heart phenotype is common, it is characterized by reduced penetrance and variable expressivity. We have generated a mouse model of the *del22q11* - associated heart phenotype (see abstract from Baldini et al.) and the resultant mice (*Df1*/+) have the same types of heart defects as those seen in *del22q11* patients. Here we show that as in humans, the penetrance of the heart phenotype is reduced, as only 25-30% of mutant embryos are affected at term (E18.5). We show that the primary embryological defect underlying the cardiovascular abnormalities in *Df1*/+ mice is in the development of the 4th aortic arch arteries. The defect varies from reduction in size of the arteries to their complete absence, and the defect can be either unilateral or bilateral. The abnormality is unlikely to be caused by defective migration of cardiac neural crest, because only the 4th arch arteries are affected, and the 3rd and 6th arch arteries develop apparently normally. Surprisingly, we found that the abnormal 4th arch artery phenotype is fully penetrant in E10.5 mutant embryos, but that by E11.5 only 50% of mutant embryos are affected. This reduction in penetrance of the heart phenotype as embryogenesis progresses is not explained by loss of mutant embryos. We conclude that a substantial fraction of *Df1*/+ embryos recovers from the primary embryological lesion, so that only ~25% have permanent anatomical defects at term. Hence, the deletion affects the development of the 4th arch arteries in all embryos, but the intervention of a recovery mechanism, considerably reduces the penetrance of heart defects at term. This is an unexpected mechanism for reduced penetrance of a haploinsufficiency phenotype. The understanding of the genetic and molecular basis of the recovery mechanism may open a way to pharmacological intervention which will enhance the recovery and prevent heart defects.

Program Nr: 170 from the 1999 ASHG Annual Meeting

Haploinsufficiency of Genes from the DiGeorge Syndrome Region Causes Heart Defects in Mice. *A. Baldini, A. Botta, V. Jurecic, S. Carattini-Rivera, Y-C. Cheah, A. Bradley, E.A. Lindsay.* Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX.

DiGeorge Syndrome is associated with heterozygous deletions of 22q11 (*del22q11*). *Del22q11* is also an important cause of cardiovascular defects. Many candidate genes have been proposed for this deletion syndrome, but the haploinsufficient genes are unknown. To study the pathogenesis of this syndrome we are generating mouse models of *del22q11*. This approach is feasible because the deleted region is evolutionary conserved and because the mouse is an excellent model for human structural heart defects. Using chromosome engineering, we have generated mice carrying chromosome deletions of the region homologous to the *del22q11* region. The phenotype caused by one of these deletions, *Df1*, which spans ~1 Mb of the region, was analyzed in detail. Heterozygously deleted (*Df1*/+) mice had cardiovascular abnormalities of the same type as those associated with *del22q11*, suggesting that *Df1* and *del22q11* interfere with the same developmental pathway. Defects included aortic arch patterning abnormalities, ventricular septal defects and defects of cardiac outflow tract alignment. Using this model we characterized the primary embryological defect and determined that this is unlikely to be caused by a neural crest migration defect (see abstract from Lindsay et al.). To understand whether the *Df1*/+ phenotype is due to haploinsufficiency of deleted genes or to an effect of the rearrangement on neighboring genes, we generated a mouse line carrying a duplication (*Dp1*) complementary to *Df1*. If the *Df1*/+ phenotype were due to haploinsufficiency of deleted genes, Dp1 should rescue it. Crosses between *Dp1*/+ and *Df1*/+ mice generated *Df1/Dp1* mice which had no heart defects. Hence, the *Df1*/+ phenotype is a true haploinsufficiency phenotype due to deletion of genes located within *Df1*. The *Df1*/+ mouse model reveals the pathogenic basis of the most clinically dramatic aspect of DiGeorge syndrome and related phenotypes and uncovers a novel mechanism leading to aortic arch abnormalities. These mutants are also the first example of a mouse model of a human deletion syndrome generated by chromosome engineering.

Program Nr: 171 from the 1999 ASHG Annual Meeting

Effect of human TWIST mutations in Saethre-Chotzen syndrome on TWIST protein dimerization. *M.E. Cohen[1], W.A. Paznekas[1], M.K. Francis[2], E.W. Jabs[1]*. 1) Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD; 2) Lankenau Medical Research Center, Wynnewood, PA.

Craniosynostosis, premature fusion of calvarial sutures, is a common malformation. Saethre-Chotzen syndrome (SCS) is a human autosomal dominant, craniosynostosis syndrome with phenotypic variability including facial asymmetry, ptosis, downslanting eyes, hypertelorism, dysmorphic ears, brachydactyly, syndactyly, and broad great toes. In SCS, mutations in TWIST, a basic helix-loop-helix transcription factor (bHLH), have been identified. More than 40 different TWIST mutations (nonsense, missense, insertion) and chromosomal deletions outside or including the coding region are known and are presumed to cause loss of function. In other organisms, twist protein is normally activated to bind E-box (CANNTG) containing DNA target(s) by dimerization to itself or other bHLH proteins. To determine dimerization partners for the human TWIST protein, we used an *in vitro* assay. Dimer complexes were isolated by affinity purification and analyzed by SDS-PAGE. GST-TWIST protein bound to glutathione sepharose was allowed to bind to ^{35}S-bHLH proteins. The labeled proteins were generated by *in vitro* transcription and translation of plasmids coding for bHLH proteins. We found that TWIST homodimerizes and heterodimerizes to E2-2, E2-5, and E12 which are known to bind to E-boxes in enhancers of human light and heavy chain Ig genes. To determine the dimerization ability of mutant TWIST found in SCS patients, we used the above assay with eight ^{35}S-TWIST mutants created by site-directed mutagenesis. We found loss of dimerization only for truncating mutations (E126X in Helix I and Q161X in Helix II regions), while others retained dimerization competence (R118H, 352del3 R118Q in the basic DNA binding region; R132P, P139S, D141Y, 433del23 K145G in the helix-loop-helix region) under various salt conditions (0.1 to 1.0 M NaCl). Our data suggest there are alternative mechanisms by which mutations may cause loss of TWIST function, e.g., affecting DNA binding or interaction with regulatory proteins. These alterations caused by different TWIST mutations may play a role in the variability of the SCS phenotype.

Program Nr: 172 from the 1999 ASHG Annual Meeting

A defective gene associated with atherosclerosis: Tangier disease is caused by mutations in the ATP binding cassette transporter 1 (*ABC1*). *S. Rust[1], M. Rosier[2], H. Funke[1], J. Real[3], Z. Amoura[4], J.-C. Piette[4], J.-F. Deleuze[2], H.B. Brewer[5], N. Duverger[2], P. Denéfle[2], G. Assmann[1]*. 1) Molecular Genetics, Inst. f. Arteriosclerosis Res., Muenster, NRW, Germany; 2) Core Genomics & Cardiovascular Departments, Rhône-Poulenc Rorer, 91006 Evry, France; 3) Universidad Departamento de Medicina, Hospital Clinico Universitario, Avda V Blasco Ibanez 17, 46010 Valencia, Spain; 4) Service de Médicine Interne, Hopital Pitié-Salpétrière, Paris, France; 5) National Institutes of Health, National Heart Lung and Blood Institute, Bethesda, Maryland, USA.

Tangier disease was first discovered nearly 40 years ago by Donald Fredrickson and colleagues in two sibs living on Tangier Island in Chesapeake Bay, Virginia, USA. This is an autosomal codominant condition characterized in the homozygous state by the absence of HDL cholesterol from plasma, hepatosplenomegaly, peripheral neuropathy, and, frequently, premature coronary artery disease (CAD). In heterozygotes, HDL cholesterol levels are about half-normal. Impaired cholesterol efflux from macrophages leads to the presence of foam cells throughout the body which may explain the increased risk of coronary heart disease in some Tangier families. We here report that we have refined our previous linkage of the Tangier disease gene to a 1 cM region between markers *D9S271* and *D9S1866* on chromosome 9q31 in which we found the human ATP cassette binding transporter 1 (*ABC1*) gene. Moreover, a change in *ABC1* expression level upon cholesterol loading of phorbol ester-treated THP1 macrophages was found, substantiating *ABC1*s role in cholesterol efflux. The full length cDNA was cloned and the gene was sequenced in several unrelated families including the original case from Tangier Island. Six different gene disrupting mutations (deletions/insertions, stop codons) were discovered demonstrating that defects in *ABC1*, a member of the ABC transporter superfamily, are the cause of Tangier disease. The involvement of ABC transporters in cholesterol efflux may open new avenues for anti-atherosclerotic therapies.

Program Nr: 173 from the 1999 ASHG Annual Meeting

Mutations in transportin (ABC1) in Tangier disease and familial HDL deficiency. *A.R. Brooks-Wilson[1], M. Marcil[1], S.M. Clee[2], L-H. Zhang[1], K. Roomp[1], M.J. van Dam[3], L. Yu[4], C. Brewer[5], J.A. Collins[1], H.O.F. Molhuizen[3], B.F.F. Ouellette[2], C.W. Sensen[1], D. Martindale[5], J. Frohlich[2], K. Morgan[5], B. Koop[5], S. Pimstone[1,2], J.J.P. Kastelein[3], J. Genest, Jr.[4], M.R. Hayden[2]*. 1) Xenon Bioresearch, Vancouver, Canada; 2) Centre for Molecular Medicine and Therapeutics, University of British Columbia, Canada; 3) Academic Medical Centre, Amsterdam; 4) Clinical Research Institut of Montreal, Canada; 5) Network of Centres of Excellence, Canada.

Coronary artery disease (CAD) is the major cause of death in Western industrialized countries. Low HDL-cholesterol (HDL-C) is the best discriminator between CAD patients and controls. Tangier disease (TD) is a rare autosomal recessive disorder characterized by very low HDL levels and cholesterol ester accumulation in different tissues. After confirming the previously reported 9q31 linkage of TD (Lod=6.37 at q=0cM for D9S1832), we undertook a genetic approach to identify the TD gene. Concurrently, we established linkage of a more common autosomal dominant condition, Familial HDL Deficiency (FHA) to the same region (Lod=9.67 at q=0cM for D9S277). FHA patients have low HDL-C levels without other TD features. Though distinct clinically and in mode of inheritance, TD and some FHA families share a deficiency of cellular cholesterol efflux. Genetic mapping localized the TD gene distal to D9S127. Recombination events in four efflux-defective FHA families mapped FHA to a 1.1cM region between D9S277 and D9S1866. In light of the similarities between TD and FHA and the overlapping regions to which they mapped, we considered the pooled TD and FHA genetic data as pointing to a smallest region of overlap between D9S127 and D9S1866. We physically mapped the ATP binding cassette 1 (ABC1) gene to this exact interval. ABC1 is a member of a gene family responsible for transport of substances across cell membranes. Mutations in ABC1 implicate it in both TD and FHA, showing that these disorders are allelic. Transportin is a key gatekeeper in cholesterol efflux and a limiting factor in reverse cholesterol transport. Its role will be crucial in the development of new treatments for cardiovascular disease.

Program Nr: 174 from the 1999 ASHG Annual Meeting

A gene related to *Chlamydomonas reinhardtii* dynein IC78 is mutated in human primary ciliary dyskinesia. *G. Pennarun[1], E. Escudier[2], C. Chapelin[1], AM. Bridoux[1], V. Cacheux[1], G. Roger[3], A. Clement[4], M. Goossens[1], S. Amselem[1], B. Duriez[1]*. 1) INSERM U 468, Hopital Henri Mondor, Creteil, France; 2) Service d'Histologie-Embryologie, CHU Pitie-Salpetriere, Paris INSERM U492, Creteil, France; 3) Service d'Oto-Rhino-Laryngologie, Hopital Armand-Trousseau, Paris, France; 4) Service de Pneumologie Pediatrique, Hopital Armand-Trousseau,Paris France.

Primary ciliary dyskinesia (PCD) is a group of heterogeneous inherited disorders of unknown origin, characterized by axonemal abnormalities of respiratory cilia and sperm tails, leading to bronchiectasis and sinusitis, sometimes associated with *situs inversus* (Kartagener's syndrome) and male sterility. The main ciliary defect found in PCD is an absence of dynein arms affecting almost all cilia. Dynein arms are complex axonemal structures bound to peripheral microtubules; their key role in the sliding movements between adjacent microtubules is required for ciliary and flagellar beating. Strikingly, several immotile strains of *Chlamydomonas*, a biflagelled unicellular alga, present axonemal ultrastructural defects, which are reminiscent of those reported in PCD patients. In particular, the flagellar ultrastructural phenotype of *Chlamydomonas* mutants lacking the dynein intermediate chain IC78 is similar to the axonemal ultrastructural abnormality observed in several PCD patients (i.e. absence of outer dynein arms); we therefore designed a strategy, based on evolutionary conservation of both axonemal ultrastructure and genes encoding axonemal proteins to isolate a human sequence related to IC78. This led to the cloning of a novel human gene, *DNAI1*, mapped to chromosome 9. Two loss-of-function mutations of *DNAI1* have been identified in a PCD patient with a lack of outer dynein arms. In addition, the use of intragenic *DNAI1* polymorphisms allowed us to demonstrate the existence of a genetic heterogeneity in this condition. These data, which reveal the critical role of *DNAI1*, the first gene involved in PCD, in the proper development of human axonemal structures, open up new ways for identifying additional genes involved in PCD and related developmental defects.

Program Nr: 175 from the 1999 ASHG Annual Meeting

Mutations in the human homolog of the murine *dl* gene cause autosomal recessive and dominant hypohidrotic ectodermal dysplasia. *J. Zonana[1], D.J. Headon[2], A.W. Monreal[1], S.L. Street[1], P.A. Overbeek[2], B.M. Ferguson[1]*. 1) Dept of Molecular and Medical Genetics L-103, Oregon Health Sciences Univ, Portland, OR; 2) Depts of Cell Biology and Molecular and Medical Genetics, Baylor College of Medicine, Houston, TX.

X-linked hypohidrotic ectodermal dysplasia results in abnormal morphogenesis of teeth, hair and eccrine sweat glands. The gene (*ED1*) responsible for the disorder has been identified, as has the analogous X-linked gene (*Ta*) in the mouse. Autosomal recessive disorders, phenotypically indistinguishable from the X-linked forms, exist in humans and at two loci (*cr, dl*) in the mouse. Dominant disorders, possibly allelic to the recessive loci, are also seen in both species. Recently, a candidate gene has been identified from the *dl* locus. Utilizing the murine cDNA and its sequence, we have isolated and characterized its human homolog. Mutations in the candidate gene produce both recessive loss of function, as well as likely dominant negative affects. We identified mutations in five families with autosomal recessive inheritance and in two with dominant inheritance. The disorder does not map to the candidate gene locus in all recessive families, implying the existence of at least one additional human autosomal locus. The putative protein is predicted to have a single transmembrane domain, and shows partial similarity to two separate domains of the tumor necrosis factor receptor family (TNFR). One possibility is that the protein functions as a multimeric receptor. Of interest, ectodysplasin-A, the product of the ED1 locus, has significant similarity to the TNF ligand family. Additional work will be required to test if the two proteins function as ligand and receptor in a common developmental pathway.

Program Nr: 176 from the 1999 ASHG Annual Meeting

Analysis of genetic and functional thresholds in ND5 gene mutation carrying cells reveals a tight regulation of gene expression in mouse mitochondria. *Y. Bai, R.M. Shakeley, G. Attardi.* Biology, Caltech, Pasadena, CA.

A mouse cell variant carrying in heteroplasmic form a nonsense mutation in the mitochondrial DNA (mtDNA)-encoded ND5 subunit of the respiratory NADH dehydrogenase has been isolated and characterized. The derivation from this mutant of a large number of cell lines containing between 4 and 100% of the normal number of wild-type ND5 genes has allowed an analysis of the genetic and functional thresholds operating in mouse mitochondria. In wild-type cells, ~40% of the ND5 mRNA level was in excess over that required for ND5 subunit synthesis. However, in heteroplasmic cells, the functional mRNA level decreased in proportion to the number of wild-type ND5 genes over a 25-fold range, pointing to the lack of any compensatory increase in rate of transcription and/or stability of mRNA. Most strikingly, the highest ND5 synthesis rate was just sufficient to support the maximum NADH dehydrogenase-dependent respiration rate, with no upregulation of translation occurring with decreasing wild-type mRNA levels. These results indicate that, despite the large excess of genetic potential of the mammalian mitochondrial genome, respiration is tightly regulated by ND5 gene expression.

Program Nr: 177 from the 1999 ASHG Annual Meeting

Nephrogenic diabetes insipidus: a new endoplasmic reticulum storage disease? *R.S. Wildin, D.E. Cogdell, B.J. Filanoski.* Molecular & Medical Genetics, Oregon Health Sciences University, Portland, OR.

Ninety percent of congenital nephrogenic diabetes insipidus (NDI) is caused by mutations in the X-linked AVPR2 gene encoding the V2 vasopressin receptor (V2R), a G protein-coupled receptor activated by arginine vasopressin (AVP). To follow up on prior work showing lack of function of V2R expressed in cultured cells from NDI alleles, we measured the cell surface AVP-binding of V2R expressed from 16 missense, one nonsense, and one frame shift alleles derived from NDI patients. Three (L81F, R113W, N321Y) had detectable binding with < 10% normal receptor number and mild decreases in affinity (k_d = 19.7, 4.5 and 6.9 nM, resp., vs. 2.6 nM for wild type [w.t.]) correlating with signal transduction only at high AVP concentrations. The remaining mutant alleles (L53R, N55D, P95L, G122R, D136-frame shift, S167L, R181C+V215M, Y205C, L219P, M272K, W293X, S315R, N317K, G12E+C319R, W323R) expressed receptors with no AVP binding, though a few signalled at high doses of AVP.

The possibility of poor surface expression and intracellular retention of mutant V2Rs was assessed by confocal microscopy of transfected cells with immunofluorescent staining. V2R distribution was like wild type in L53R, Y205C and W293X, while the others showed marked concentration of fluorescence in a novel dome-shaped arc surrounding a nucleic acid-containing structure adjacent to the nucleus. Co-staining for protein disulfide isomerase, an ER protein, identified the latter structure as endoplasmic reticulum. The second class of mutants also showed reduction or elimination of granular, reticular, and "surface" staining observed with w.t. V2R. This suggests that many mutant V2Rs may be retained in the ER due to improper folding, thereby preventing transport to the Golgi and cell surface. Thus, X-linked NDI may belong to the growing class of Endoplasmic Reticulum Storage Diseases (ERSD), genetic diseases caused by mutations in multiple transmembrane domain proteins, like CFTR, Rhodopsin, and AQP2, that are prone to critical misfolding.

Program Nr: 178 from the 1999 ASHG Annual Meeting

Paternal origin of FGFR2 mutations in sporadic cases of Crouzon and Pfeiffer syndromes. *R.L. Glaser[1], W. Jiang[1], S. Boyadjiev[1], D. Johnson[2,3], S. Walsh[2], M. Oldridge[2], S.A. Wall[3], A.O.M. Wilkie[2,3], E.W. Jabs[1]*. 1) Inst for Genetic Med, Johns Hopkins Univ Sch Med, Baltimore, MD; 2) Inst of Mol Med, John Radcliffe Hosp, Oxford, UK; 3) Oxford Craniofacial Unit, Radcliffe Infirmary, Oxford, UK.

The first association between older paternal age and Crouzon (CZ) and Pfeiffer (PF) syndromes was described by Jones et al in 1975. These syndromes are autosomal dominant craniosynostotic disorders which are due to fibroblast growth factor receptor 2 (FGFR2) mutations. Here we molecularly prove the origin of different mutations to be paternal for 19/19 informative cases ($p=2x10^{-6}$ when compared to the probability being equal for either parent). Polymorphisms used to determine the parental origin of the FGFR2 mutations were either previously identified by Moloney et al (1996) or identified by sequencing 4871 bp between introns 7 and 10, spanning exons IIIa to IIIc, and performing heteroduplex analysis. We identified a 2 bp insertion (TATA/TA) in the intron upstream of and a C/T polymorphism in the intron downstream of exon IIIc. Using the amplification refractory mutation system (ARMS), we screened 39 families, 19 (10 CZ, 9 PF) of which were informative for either the 2 previously identified polymorphisms or the TATA/TA polymorphism. The C/T polymorphism was uninformative. 11 different mutations including splice site and missense mutations not in the context of a CpG dinucleotide were found by enzyme digest or allele specific oligonucleotide hybridization. Advanced paternal age was noted for the fathers of CZ and PF patients (35.11 ± 8.39 yrs, $p<0.05$, compared to controls). Our data extends previous clinical evidence based solely on statistical analyses as well as reports of advanced paternal age associated with paternal origin of only 1 to 2 recurrent mutations causing Apert syndrome (FGFR2) or achondroplasia (FGFR3). Our results suggest that older men have accumulated or are more susceptible to germline mutations. While the exact mechanism behind the age effect has yet to be shown, various hypotheses suggest that replication or repair processes involved in spermatogenesis may increase the rate of mutation in older men.

Program Nr: 179 from the 1999 ASHG Annual Meeting

Microdeletions: An important cause of craniosynostosis. *E.H. Zackai, D.M. McDonald-McGinn, K.W. Gripp, R. Kirschner, S. Bartlett, L. Whitaker, B.S. Emanuel, C.A. Stolle.* The Children's Hospital of Philadelphia.

While major progress in the identification of molecular causes of craniosynostosis has been made, there remain a sizable number of patients whose etiology is unknown. We have now identified chromosomal microdeletions 22q11.2 and 7p21.2 as an important cause of craniosynostosis in our patient population. Two patients, 1 with bicoronal and 1 with unicoronal, who were negative for point mutations in *FGFR1*, *2*, *3*, and *TWIST*, were also noted to have clinical findings consistent with a chromosome 22q11.2 deletion. *FISH* utilizing the commercially available probe, N25, confirmed the diagnosis in both patients. Literature review revealed craniosynostosis in 5/550 patients with a 22q11.2 deletion (Ryan et al., 1997). This is comparable to the experience with our 22q11.2 deletion population (2/250) (McDonald-McGinn et al., 1999) and is much higher than the general population incidence of craniosynostosis (1/2500). Three patients with bicoronal craniosynostosis and physical findings consistent with Saethre-Chotzen syndrome were negative for mutations in the *TWIST* gene, as well as point mutations in *FGFR1*, *2*, and *3*. Following a report by Johnson et al. (1998) describing patients with Saethre-Chotzen due to a microdeletion of 7p21.2, we reanalyzed these 3 patients by Southern blot analysis of EcoR1 digested genomic DNA hybridized to 32P- labeled probes specific for the *TWIST* gene (in order to detect partial or complete deletions) and the beta globin gene (as an internal standard for DNA loading). All 3 patients were found to have deletions of the *TWIST* gene on 7p21.2. This was confirmed by *FISH* using a cosmid probe spanning the *TWIST* gene. Of note, all had learning disabilities, as was seen in the majority of Johnson's microdeletion patients. This makes the microdeletion of *TWIST* an important consideration in patients with Saethre-Chotzen phenotype (3/10 Johnson et al.; 3/11 this study). Our prior experience with mutational analysis in 82 patients with craniosynostosis, including both familial and isolated cases, yielded only a 56% detection rate. Therefore, we believe that microdeletion testing is an important adjunct in identifying the etiology of craniosynostosis.

Program Nr: 180 from the 1999 ASHG Annual Meeting

Prader-Willi syndrome: Phenotypic differences observed in an objective study of deletion and disomy. *J.E. Allanson[1], C. Clericuzio[2], S.B. Cassidy[3]*. 1) Dept Genetics, Children's Hosp Eastern Ont, Ottawa, ON, Canada; 2) University of New Mexico, Albuquerque, NM; 3) Case Western Reserve University, Cleveland, OH.

When a syndrome has several etiologic mechanisms genetic background may influence phenotype. In Prader-Willi syndrome (PWS), deletion of the paternal copy of chromosome 15q11-13 is found in 70% of individuals, while maternal uniparental disomy (UPD) is seen in most of the remainder. A previous retrospective study documented a higher likelihood of an atypical face, which was often rounder with a broader forehead and nasal bridge, in those with UPD. The current prospective objective study was undertaken to expand on this subjective observational assessment. We have had the opportunity to examine 109 individuals with Prader-Willi syndrome, 57 females and 52 males. 44 have a deletion; 18 have UPD ; in the remainder a clinical diagnosis was made by an experienced physician . A series of anthropometric measurements was obtained on each subject (by JEA)using published methods. For each dimension, age- and sex-matched normal standards were available. The raw data were converted to Z scores to control for age and sex differences. Pattern profiles were compiled for each age and sex. Our results show that individuals with PWS due to maternal UPD have a longer face; a more protuberant nose with a narrower base; a smaller mouth and a broader or more prominent mandible. Both etiologic groups have dolichocephaly with reduced craniofacial widths (excluding the lower jaw) and short palpebral fissures. These findings which complement the prior subjective study may explain the delay in diagnosis of PWS caused by maternal UPD which has been reported, particularly in females.

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Program Nr: 181 from the 1999 ASHG Annual Meeting

A Natural History of Cleidocranial Dysplasia. *S.C. Cooper[1], B. Lee[2], G. Zhou[2], C. Flaitz[1], J. Hecht[1]*. 1) University of Texas, Houston, TX; 2) Baylor College of Medicine, Houston, TX.

Cleidocranial dysplasia (CCD) is an autosomal dominant skeletal dysplasia. Affected individuals have hypoplastic/aplastic clavicles and multiple dental abnormalities. Mutational analysis of the causative CBFA1 gene has recently become available. This study sought to systematically delineate the natural history of CCD and establish genotype/phenotype correlation. This study ascertained 90 affected individuals and 56 first and second-degree relative controls from the United States, Canada, Europe, and Australia. Significant skeletal findings include genu valgum in 27%, scoliosis in 18%, and pes planus in 57%. Recurrent sinus infections and other upper airway breathing complications were observed significantly more often in CCD individuals. Hearing loss was reported by 39% of affected individuals. In addition, from birth throughout life, individuals with CCD are more likely to have recurrent ear infections. Dental abnormalities included cysts in 12%, supernumerary teeth in 70%, and underbite malocclusion in 66%. Although 23% of CCD individuals reported learning disabilities, there were no differences in delay in school and the highest degree earned between affected and control individuals. The primary Cesarean-section rate among affected women was 69%. Finally, affected individuals were more likely to have surgery (51%) and other health complications (37%) than controls. However, no clear patterns emerged in terms of the types of surgeries required or the types of health complications found in individuals with CCD. Of the 19 individuals who underwent mutational analysis, mutations were found in the runt domain that both affected DNA binding and did not affect DNA binding. There was also a mutation found in the PST domain that affects target gene transactivation. These findings suggest children with CCD should be monitored closely for orthopedic complications, upper airway breathing complications, and ear infections. In addition, formal hearing evaluations should be performed starting in early childhood. Finally, pregnant women with CCD should be monitored closely for cephalopelvic disproportion, which may necessitate Cesarean-section delivery.

Program Nr: 182 from the 1999 ASHG Annual Meeting

Evaluation of the validity of the published clinical diagnostic criteria for Prader-Willi Syndrome. *M. Gunay-Aygun, S. Schwartz, S. Heeger, S.B. Cassidy.* Dept Genetics, Ctr Human Gen, Case Western Reserve Univ, Cleveland, OH.

Prader-Willi syndrome(PWS) is a complex multisystem disorder whose clinical diagnosis may be difficult and whose genetic basis is heterogeneous. Approximately 70 % of PWS is caused by deletion of the paternally derived 15q11-q13(DEL), 28 % by maternal uniparental disomy of chromosome 15(UPD) and less than 2 % by an abnormality in the imprinting process. Although molecular tests for these defects are currently available, clinical diagnostic criteria continue to have critical importance especially for selection of appropriate patients for molecular testing. We, therefore, did a retrospective review of all our patients with molecular confirmation of PWS to assess the validity of the previously published clinical diagnostic criteria (Holm et al., Pediatrics, 1993; 91:398-402).

Charts of the 90 patients(68 DEL, 21 UPD) with molecularly confirmed PWS were reviewed. One patient had abnormal methylation with biparental inheritance presumably due to an imprinting defect. Age range was 5 months to 60 years (median: 14.5 yrs.)(DEL;median:14 yrs., range 5 mo-60 yrs., UPD; median: 18 yrs., range 5 yrs-42 yrs).

The sensitivities of major criteria varied between 97.75 %(developmental delay/mental retardation) and 81.71 %(characteristic facial features), and of minor criteria varied between 93.06 %(speech and articulation defects) and 36.76 %(sleep disturbance or sleep apnea). Interestingly, the sensitivities of 5 of the minor criteria were higher than the sensitivity of characteristic facial features which is a major criterion. 10 out of 90 patients (11 %) with molecular diagnosis did not meet the clinical diagnostic criteria (all with DEL). However, bias of referral may have influenced our results. Though this study is retrospective and less than 100 % of patients were assessed for each criterion, these results suggest that revision of the published clinical diagnostic criteria for PWS may improve the clinician's ability to select appropriate patients for molecular testing. We will propose such criteria.

Program Nr: 183 from the 1999 ASHG Annual Meeting

Clinical screening criteria for adults with 22q Deletion Syndrome. *A.S. Bassett[1], E. Chow[1], L. Scutt[1], H. Dorman[1], R. Weksberg[2]*. 1) Psychiatry, U of Toronto, CAMH- Queen Street Division, Toronto, ON, Canada; 2) Medical Genetics, Hospital for Sick Children, Toronto, ON, Canada.

22q Deletion Syndrome (22qDS), including velocardiofacial and DiGeorge syndromes, is associated with 22q11 deletions, but is under-recognized especially in adults. The prior probability of having 22qDS is about 2% in schizophrenia (SZ) and 15% in tetralogy of Fallot (TOF). Objective: To better identify adults with 22qDS we developed screening criteria and compared features of subjects with and without a 22q11 deletion. Methods: 41 adult subjects (n=32 SZ, n=9 TOF; 23 male, 18 female; median age 27 years) meeting screening criteria (2 or more of 6 features: learning disorder (LD), hypernasal speech/history of palate anomaly, suggestive facial appearance, history of other (non-cardiac) congenital anomaly, hypocalcemia or athymia) had standard FISH testing (probe N25, Vysor) for 22q11 deletions. Results: 21 subjects who met screening criteria (44% of SZ and 78% of TOF subjects) had a 22q11 deletion. Deleted subjects had more screening criteria features than non-deleted subjects ($p<.001$) but the modal number of screening criteria met was 3 for both groups; the range was 2-5 for deleted and 2-4 for non-deleted subjects. The proportion of subjects meeting individual criteria, and most common features (facial, LD, and palate), did not differ between deleted and non-deleted subjects. 29% of adult deleted subjects did not have "typical" facies. Conclusions: Applying screening criteria can increase the prior probability of detecting 22qDS in at-risk populations and may help clinicians recognize adults at risk for 22qDS.

Program Nr: 184 from the 1999 ASHG Annual Meeting

The 22q11.2 deletion: cast a wide fishing net! *D.M. McDonald-McGinn, A. Laufer-Cahana, D.A. Driscoll, B.S. Emanuel, E.H. Zackai.* The Children's Hospital of Philadelphia, Philadelphia, PA.

A 22q11.2 deletion has been identified in most patients with DiGeorge syndrome, VCFS, and CTAF and occasionally in Opitz G/BBB and Cayler cardiofacial syndrome. In addition, deletion studies are becoming standard for some defects such as conotruncal cardiac anomalies and velopharyngeal incompetence. However, we've found a sizable group of previously undiagnosed individuals identified only following the diagnosis in a relative. Here we report this first unselected cohort and make a plea for careful attention to the history and physical upon presentation for care. Sixteen adults (6M;10F;20-52yrs) were identified following the detection of the deletion in their child, making the familial incidence in our population of 305 patients 6%. Only 4 parents had major findings including cleft palate(2), hypocalcemic seizures, & schizophrenia. Some had minor anomalies such as hernia & scoliosis. The majority had mild dysmorphia. Educational history in 15 revealed: associates degree(1), regular ed(9); LD(3); special ed(2). Occupational history in 14 revealed: maintenance, chef, farmer, security, milkman, & homemaker(9). Six had an additional affected child prior to their diagnosis and 2 had an additional affected child following their diagnosis. Three of these 8 children had no visceral findings; 5 had major anomalies including CHD(3), vascular ring/VPI(1), & cleft palate(1). Deletion sizing demonstrated the same large 3Mb deletion in most families. Three families had smaller deletions. No phenotype-genotype correlations could be made. In addition, 4 males were diagnosed only following the identification of a deletion in their co-twins. Thus, analysis of this series of 28 unselected patients, many with mild manifestations, emphasizes the importance of broadening the index of suspicion in order to provide appropriate recurrence risk counseling, cognitive remediation, & medical management. In addition, this data serves to highlight the importance of deletion testing in all parents of affected probands. Further, it underscores the lack of familial concordance and the lack of phenotype-genotype correlations in this quite common and often complex contiguous gene deletion.

Program Nr: 185 from the 1999 ASHG Annual Meeting

The Revised Diagnostic Criteria of Marfan Syndrome: A Clinical Analysis. *B.G. Kousseff, J.W. Jennings, J.D. Ranells.* Dept Pediatrics, Med Genetics, Univ South Florida, Tampa, FL.

In 1996 the diagnostic criteria for Marfan syndrome (MFS) were revised (De Paepe et al., 1996). Both major and minor criteria were redefined, more rigorous requirements for relatives of unequivocally affected individuals were made, and molecular genotype as a major criterion was included. In light of this, we performed a retrospective phenotypic analysis on 104 individuals evaluated for MFS between 1-2-82 and 12-31-97. They were part of the 32,715 probands/families evaluated during the period. The object of the analysis was to select individuals who met the revised criteria for mutation screening and molecular analysis of FBN1. This was done with the intent to minimize false positive diagnoses. Of the104 individuals, 60 (58%) were diagnosed clinically as having MFS. However, as per the revised criteria only 33/104 (32%) had the condition; the remaining 27 individuals met only some of the criteria. In 6 individuals from 2 families, autosomal dominant ectopia lentis appeared to be the diagnosis and in two the MASS phenotype was more likely than MFS. In the remaining 19, 5 had a first-degree relative meeting the revised criteria for MFS. All of them were younger than 5 years. An additional 5 were younger than 8 years of age at their most recent evaluation. Together with 9 older individuals, they had a negative family history; 9/14 met the old MFS criteria. Since the revised criteria include looking for protrusio acetabulae, dural ectasia, cornea plana and myopia based on measured axial length of the ocular globe and they were not part of the evaluations, it was expected that a few MFS patients, diagnosed the old way, may not meet the revised criteria. However, to "lose" almost 50% of the patients raised eyebrows regarding the criteria. They implied over-diagnosing. "Correcting" such diagnoses may lead to a higher yield of MFS mutations in the laboratory but posed the question whether in patient care under-diagnosing is replacing over-diagnosing. This study raised concerns with regards to the diagnostic criteria and their usefulness particularly in the pediatric population.

Program Nr: 186 from the 1999 ASHG Annual Meeting

McKusick-Kaufman syndrome - phenotypic overlap with Bardet-Biedl syndrome necessitates modification of diagnostic criteria, management and genetic counseling. *A.M. Slavotinek[1], S. Durrani[1], R. Gorospe[1], T. Maygari[1], C.T.R.M. Schrander-Stumpel[2], L.G. Biesecker[1]*. 1) NHGRI/NIH, Room 4B75, 49 Convent Drive, Bethesda, MD; 2) Department of Clinical Genetics, University of Maastricht, The Netherlands.

We present two 'sporadic' female patients who were diagnosed with McKusick-Kaufman syndrome (MKKS) early in life because of classical findings of hydrometrocolpos (HMC) with vaginal atresia and postaxial polydactyly (PAP). In both children, later examination showed phenotypic features consistent with Bardet-Biedl syndrome (BBS) and one girl re-evaluated at 13 years of age had mild mental retardation, obesity and retinitis pigmentosa whereas the other girl had mild developmental delay and obesity at age three years. Further support for phenotypic overlap between these two syndromes can be found in the medical literature (Goecke et al., 1981; Chitayat et al., 1987; Schaap et al., 1998). We reviewd 90 published patients with MKKS and found that for the majority of cases, it was not possible to distinguish MKKS from BBS in children less than five years of age. Although the presence of renal or ovarian cysts may allow distinction between MKKS and BBS in the first year of life, to date reliable prediction of adult phenotype has not been possible for females with HMC and PAP without an affected relative. Allelic heterogeneity or a common genetic pathway could explain the shared clinical findings, but until the molecular mechanisms of the syndrome(s) are elucidated, we would propose that the diagnosis of MKKS based on the findings of HMC and PAP in the absence of a family history be provisional until at least the age of five years and that regular monitoring of growth and development and for the ocular, renal and other complications of BBS be performed in patients with provisional MKKS. [Goecke et al. Eur J Pediatr 1981;136:297-305. Chitayat et al. Am J Dis Child 1987;141:1133-6. Schaap et al. Eur J Pediatr 1998;157:170-1.].

Program Nr: 187 from the 1999 ASHG Annual Meeting

The Use of Unidentified Bright Objects on MRI for Diagnosis of Neurofibromatosis 1 in Children. *K. DeBella[1], K. Poskitt[2], J. Szudek[1], J.M. Friedman[1]*. 1) Medical Genetics, UBC, Vancouver, BC, Canada; 2) Radiology, BC Children's Hospital, Vancouver, BC, Canada.

Unidentified Bright Objects (UBOs) have been observed on cranial magnetic resonance imaging (MRI) in 45-80% of children with neurofibromatosis 1 (NF1). Because the NIH Diagnostic Criteria often do not permit unequivocal identification of NF1 in young children, UBOs have been proposed as an additional diagnostic criterion. We examined the sensitivity and specificity of UBOs for NF1 in 19 affected children and 19 age-matched controls. We measured the agreement of UBO recognition between two experienced pediatric neuroradiologists who independently examined the MRI studies on these patients. We also used data from 858 NF1 patients between the age of 2 and 21 from the NNFFIDB (NNFF International Database) who had cranial MRI scans to determine whether the addition of UBOs to the existing NIH diagnostic criteria would improve early diagnosis.

The overall sensitivity of UBOs for NF1 averaged 97%, and the specificity averaged 79%. Agreement between the two radiologists was 84% overall (95% C.I. 67-100%). We do not routinely obtain cranial MRI examinations on NF1 patients, so our subjects as well as our controls are more likely to have central nervous system pathology than unselected children. Adding UBOs as a diagnostic criterion would permit 85% rather than 80% of two-year old NF1 patients from the NNFFIDB to be diagnosed, a difference that is not statistically significant. UBOs occur in children who do not have NF1, but the frequency and clinical significance in normal children need to be defined. UBOs may be more reliable for diagnosis of NF1 if they can be better characterized and differentiated from similar lesions that occur in other patients. Our data suggest that requiring a patient to have three or more UBOs in the cerebellum, medulla, pons, midbrain, thalami or lentiform nuclei is a more specific criterion for NF1 than the simple presence of such lesions.

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Program Nr: 188 from the 1999 ASHG Annual Meeting

Logistic regressive models of associations among neurofibromatosis 1 (NF1) features. *J. Szudek[1], H. Joe[2], J.M. Friedman[1]*. 1) Dept Medical Genetics; 2) Dept Statistics; Univ British Columbia, Vancouver, BC, Canada.

Most severe features of NF1 are uncommon but unpredictable because of extreme clinical variability. We have previously shown that associations exist between some pairs of NF1 features. We now extend the analysis to associations of multiple different features.

Twelve features were analyzed in data from 2,797 probands in the National Neurofibromatosis Foundation International Database: freckling, discrete and plexiform neurofibromas, Lisch nodules, optic gliomas, seizures, learning disability, pseudarthrosis, scoliosis, macrocephaly, short stature, and neoplasms. Models were developed using half of the data, then tested on the remaining data.

Each of the 12 features was set as the output variable in a bivariate logistic regressive model to screen the remaining 11 features as potential main effects, with age as a continuous covariate. Significant features were entered in multivariate models, the importance of each variable reassessed, and significant variables used to revise each model. Interaction terms among the explanatory variables were then considered and significant terms added. Only models that fit both subsets by the Hosmer and Lemeshow statistic were considered valid.

The results reveal several associations. For example, Lisch nodules are 8.7 times more frequent in subjects with freckling, discrete neurofibromas and neoplasms than in subjects lacking these features. Optic glioma occurs 14 times more often in subjects with plexiform neurofibromas and neoplasms than in subjects who lack these features. Similar associations have been observed for several other features.

These findings demonstrate that some NF1 patients are far more likely than others to have certain features of the disorder. This suggests that fundamental biological differences exist between subjects who differ by particular constellations of features. Logistic regressive models may be useful for determining age- and sex-specific risks for complications of NF1 in clinically defined sub-groups of NF1 patients.

Program Nr: 189 from the 1999 ASHG Annual Meeting

Modeling human aneuploidy: studies of a nondisjunction-prone murine chromosome. *C. Bean, P. Hunt, E. Millie, T. Hassold.* Dept. of Genetics, CWRU, Cleveland, OH.

Despite the clinical importance of aneuploidy, we know little of the causes of mammalian nondisjunction. In part, this reflects the fact that, unlike lower organisms, segregation "impaired" chromosomes are virtually non-existent in mammals. To address this issue, we recently initiated studies of the murine Y chromosome on the BALB/cWt (Wt) inbred background, previously linked to hermaphroditism due to loss of the Y in gonadal tissue. In initial studies, we conducted cytogenetic studies at different developmental stages to determine 1) if nondisjunction occurs in both meiosis and mitosis and 2) if nondisjunction is restricted to specific developmental stages. In FISH sperm studies, we found similarly low levels of aneuploidy in Wt and control mice; thus the Wt Y is stable in meiosis. However, in studies of 2, 4, 8 and 16 cell pre-implantation embryos, we identified a remarkable level of mosaicism: indeed, by the 16 cell stage over 50% of Wt embryos are sex chromosome mosaics. Later in development, the Wt Y appears to "settle down"; i.e., studies of mid-gestation embryos indicate that, while almost all Wt males are sex chromosome mosaics, the level of mosaicism is virtually identical in various tissues of the same animal. Together with the data on pre-implantation embryos, this indicates that Wt Y missegregation is restricted to the early cleavage divisions. Further, it suggests that clinically relevant human mosaicism (e.g., CPM) may reflect the fact that the earliest cleavage divisions are especially vulnerable to nondisjunction. In other studies, we asked if there might be a genetic component to Wt Y nondisjunction. In initial studies, we analyzed F1 animals produced by crossing Wt males to Wt females or females of other inbred strains. In both instances, a proportion of offspring are sex chromosome mosaics - thus, other backgrounds do not rescue the Wt nondisjunction phenotype. However, the level of mosaicism is highly significantly increased in offspring of WtxWt animals. This provides strong evidence of a genetic influence on Wt Y segregation and is, to our knowledge, the first demonstration of a genetic effect on mammalian chromosome nondisjunction.

Program Nr: 190 from the 1999 ASHG Annual Meeting

Cell-wide reduction of genomic recombination in human eggs with non-disjoined chromosomes 21. *A.S. Brown[1], E. Feingold[2], K.W. Broman[3], S.L. Sherman[1]*. 1) Genetics, Emory University, Atlanta, GA; 2) Human Genetics,Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA; 3) Marshfield Medical Research Foundation, Marshfield, WI.

Altered recombination patterns along non-disjoined (NDJ) chromosomes (chrs.) is the first molecular correlate identified for non-disjunction in humans. In an earlier study we found strong evidence that specific recombination patterns along NDJ chrs. 21 are associated with an increased risk for most maternal meiotic errors. We have now extended our earlier study to determine the extent to which recombination may be altered in eggs with a NDJ chr. by asking if the altered recombination is a cell-wide phenomenon or if the effect is limited to the NDJ chr. 21. We attempted to enrich our study population for extreme deviations from normal variation in recombination by examining only Down syndrome cases due to maternal meiosis I errors that had no detectable chr. 21 recombination events (n=17). Female meiotic events from the CEPH families were used as controls (n=91). Using 366 genome-wide markers, we assayed for cell-wide disturbances in the amount of recombination. The genotypes from the maternal grandparents, parents, and case or control offspring were used in a conventional linkage study to detect recombination along maternally-transmitted chrs., excluding chr.21 and Y. A statistically significant reduction in total recombination was observed between the cases and controls, 35.9 ± 6.7 vs. 39.9 ± 6.8, respectively ($p < 0.02$). This reduction did not seem to be due to any particular chr(s)., rather it was cell-wide; however, the power to detect such variation was low. Our results provide the first evidence for a cell-wide reduction in the amount of recombination occurring in human eggs with NDJ, non-crossover chrs. 21. This novel finding advances the understanding of recombination-associated non-disjunction in humans by suggesting that a trans-acting factor(s) related to the meiotic recombination process, rather than a chr. 21 cis-acting factor, may underlie a subset of human meiotic non-disjunction events.

Program Nr: 191 from the 1999 ASHG Annual Meeting

Single sperm typing demonstrates that reduced recombination is associated with the production of aneuploid 24,XY human sperm. *R.H. Martin[1,2], Q. Shi[1,2], E. Ko[2], L. Barclay[2], L.L. Field[1]*. 1) Dept of Medical Genetics, University of Calgary, Calgary, AB, Canada; 2) Dept of Genetics, Alberta Children's Hospital, Calgary, AB, Canada.

To account for the increased proportion of paternal nondisjunction in 47,XXY males as compared to other trisomies, it has been suggested that the XY bivalent in males, with its reduced region of homology, is particularly susceptible to nondisjunction. Molecular studies of liveborn Klinefelter syndrome (47,XXY) individuals have reported an association between the absence of recombination in the pseudoautosomal region and nondisjunction of the XY bivalent. In this study we have examined single sperm from a normal 46,XY male to determine if there is any alteration in the recombination frequency of aneuploid disomic 24,XY sperm compared to unisomic sperm (23,X or Y). Two DNA markers STS/STS pseudogene and DXYS15 were typed in sperm from a heterozygous man to determine if recombination had occurred in the pseudoautosomal region. Individual unisomic sperm (23,X or Y) were isolated using a FACStar^{Plus} flow cytometer into PCR tubes. To identify disomic 24,XY sperm, 3-colour FISH analysis was performed with probes for chromosomes X,Y and 1. The 24,XY cells were identified using fluorescence microscopy, their position noted using a grid reference and each identified disomic sperm was scraped off the slide using a glass needle attached to a micromanipulator and then transferred by breaking off the tip of the needle in a PCR tube. Hemi-nested PCR analysis of the two markers was performed to determine the frequency of recombination. A total of 308 unisomic sperm and 108 disomic sperm have been typed. The frequency of recombination between the two DNA markers was 39% for the unisomic sperm, similar to frequencies previously reported. However the 24,XY disomic sperm had an estimated recombination frequency of 15%,a highly significant decrease compared to the unisomic 23,X or 23,Y sperm (chi square=34, p=.00001). This direct analysis of human sperm indicates that lack of recombination in the pseudoautosomal region is a significant cause of XY nondisjunction and thus Klinefelter syndrome.

Program Nr: 192 from the 1999 ASHG Annual Meeting

A postulated mechanism of human aneuploidy, precocious sister chromatid segregation, is influenced by genetic background. *C. Hodges, R. LeMaire-Adkins, P. Hunt.* Dept. of Genetics, CWRU, Cleveland, OH.

An estimated 10-25% of all human conceptuses are chromosomally abnormal due to chromosome missegregation at the maternal first meiotic division (MI). The underlying causes of these errors are unknown; however there is now considerable evidence that precocious sister chromatid segregation(PSCS) is a major contributor. To gain insight to the origin of PSCS, and thus human aneuploidy, we are using a mouse model. In previous studies of XO mice, we have demonstrated that the univalent X chromosome can segregate intact at MI or undergo PSCS. Unexpectedly, the propensity to undergo PSCS appears to be under genetic control. To determine the basis of this effect, we have tested two hypotheses: First, we tested the hypothesis that segregation is influenced by the self-synaptic behavior of the X chromosome during meiotic prophase. Previous studies have demonstrated that proteins involved in synapsis remain at the centromere until just prior to sister chromatid segregation at MII. These proteins have been postulated to influence the behavior of sister kinetochores at MI, facilitating reductional segregation. Using combined immunofluorescence and FISH, we evaluated the synaptic behavior of the single X chromosome at pachytene. No obvious differences in X chromosome synapsis were observed between the two genetic backgrounds. Second, we tested the hypothesis that MI segregation behavior is influenced by centromere structure. Studies in yeast have shown that premature segregation of sister chromatids at MI is a property of the centromere. Thus, we produced F1 hybrid XO females that were genetically identical but differed in the strain of origin of the X chromosome. The propensity for PSCS in oocytes from the two types of XO females was not different and was identical to one of the parental strains. This provides strong evidence that PSCS is the result of the action of modifying genes rather than differences in centromere structure or synaptic behavior of the chromosome. Further, we have observed striking differences in meiotic cell cycle kinetics that are correlated with the segregation differences, suggesting that genes involved in cell cycle control influence PSCS.

Program Nr: 193 from the 1999 ASHG Annual Meeting

Chromosome aberrations, aneuploidy, and abnormal cell division in Brca1-deficient mice and in the mammary tumors of Brca1 conditional mutants. *Z.A. Weaver[1], X. Xu[2], D. Larson[3], A. Wynshaw-Boris[3], C.-X. Deng[2], T. Ried[1].*
1) Genetics Department, Division of Clinical Sciences, NCI, NIH, Bethesda, MD; 2) Genetics of Development and Disease Branch, NIDDK, NIH; 3) Genetic Disease Research Branch, NHGRI, NIH.

Mice mutant for both alleles of the Brca1 tumor suppressor gene were generated by gene targeting, in order to study mammary gland development and subsequent neoplasia. These mutant mice die during early embryogenesis; therefore mouse embryonic fibroblast (MEF) cells were examined initially. The cells proliferate poorly in culture and exhibit a defective G2-M checkpoint accompanied by extensive chromosome abnormalities, which we have analyzed by spectral karyotyping (SKY). SKY is a multicolor FISH technique that eliminates many of the uncertainties in karyotyping of mouse chromosomes. Brca1-deficient MEF chromosomes exhibit multiple structural aberrations such as translocations and deletions, plus evidence of chromosome breakage such as quadriradial structures. By immunofluorescent detection we found that mutant fibroblasts also contain multiple, functional centrosomes, leading to unequal chromosome segregation, abnormal nuclear division, and aneuploidy. The results suggest that Brca1 is necessary to maintain genome stability through DNA damage repair and maintenance of the G2-M checkpoint. To investigate this hypothesis in adult mice, we inactivated the Brca1 gene specifically in mammary epithleial cells using the Cre-loxP recombination system. In these conditionally deleted Brca1 mice, tumors arise after a long latency period. As in the embryonic fibroblasts, tumor cells exhibit aneuploidy, multiple centrosomes and abnormal spindle pole formation during cell division. In contrast to the MEFs, structural aberrations found in the tumor chromosomes are clonal. Chromosome 11, which harbors the Trp53 gene as well as Brca1, is rearranged in all of the tumors karyotyped to date. Preliminary FISH analyses indicate that Trp53 is at or near the breakpoint region in chromosome 11. Comparison of chromosome losses, gains and breakpoints for human and mouse Brca1-deficient breast cancers will help to identify pivotal genes.

Program Nr: 194 from the 1999 ASHG Annual Meeting

Disruption of the murine *FANCC* gene results in abnormal chromosome pairing during meiosis I, germ cell apoptosis and impaired fertility. *M.S. Meyn, M. Carreau, W. Wang.* Department of Genetics, Hospital for Sick Children, Toronto, ON, Canada.

Fanconi anemia (FA) is an autosomal disorder characterized by bone marrow failure, cancer risk, congenital anomalies and sensitivity to DNA damaging agents. FA is caused by mutations in at least eight loci, including *FANCC*, which codes for a protein of unknown function. While gross gonadal abnormalities are uncommon in FA patients, disruptions of *FANCC* in mice leads to reduced fertility. To follow-up this observation we have used antibodies to SCP3 and other components of the synaptonemal complex (SC) to examine testes from normal and *FANCC-/-* mice.

Our studies confirm previous observations that FANCC is expressed in wild-type testes and that *FANCC-/-* mice have reduced numbers of germ cells. We now report aberrant processing of SCs in *FANCC-/-* spermatocytes. Leptotene and early zygotene nuclei from *FANCC-/-* mice appear normal. However, abnormal SCs with visible disruptions of SCP3 staining are observed in *FANCC-/-* pachytene spermatocytes, suggesting physical breaks in their SCs. These SC abnormalities are similar to those seen in mice carrying a disruption of *Atm*, the gene responsible for ataxia-telangiectasia. Unlike *Atm-/-* mice, Rad51 foci distribution does not appear to be abnormal in the spermatocytes of *FANCC-/-* animals. Diplotene nuclei are quite rare and progression of meiosis past diplonema is not seen in the testes of the *FANCC-/-* mice. Instead, wide-spread spermatocyte apoptosis is detected by TUNEL staining.

Our studies indicate that loss of FANCC function disrupts normal processing of SCs and that FANCC is required for progression of meiosis I past diplonema in mice. Our results suggest that *FANCC* may be involved in the processing of developmentally regulated double-strand breaks that occur during meiosis. Although meiosis ends in apoptosis for both *FANCC-/-* and *Atm-/-* mice, the presence of abnormal Rad51 foci only in *Atm-/-* spermatocytes suggests that Rad51 is a downstream component of an ATM-specific meiotic signaling pathway.

Program Nr: 195 from the 1999 ASHG Annual Meeting

Extremely skewed X chromosome inactivation is increased in women with recurrent spontaneous abortion. *W.P. Robinson[1], K.K. Sangha[1], M.D. Stephenson[2], K. Ochnio[1], C.J. Brown[1]*. 1) BC Res Inst for Children's and Women's Health, Dept. of Medical Genetics, Univ British Columbia, Vancouver, BC, Canada; 2) Children's & Women's Health Centre of B.C., Dept of Obstetrics and Gynecology, Univ British Columbia, Vancouver, BC, Canada.

Recurrent spontaneous abortion (RSA), defined as 3 or more consecutive losses at £20 weeks of gestation, affects 1-2% of couples trying to have a family. Although female carriers of certain mutations or rearrangements affecting the X chromosome can experience increased rates of spontaneous abortion, it is unclear what proportion of unexplained RSA this might account for. Germline mosaicism for a chromosome abnormality in either partner can also lead to recurrent pregnancy loss. It is difficult to detect the presence of either of these genetic abnormalities (X-linked mutations or germline aneuploidy); however, skewed X chromosome inactivation (XCI) is expected to be common in both situations. To assess the extent to which these genetic factors may account for RSA, a methylation sensitive assay at the androgen receptor locus was used to estimate the degree of XCI skewing in these patients. Extremely skewed XCI, defined as greater than 90% inactivation of one allele, was found in 19% of RSA patients (n=91) but in only 5% of controls (n=111) ($p<0.001$ Chi-square test). Thus genetic abnormalities associated with skewed XCI are involved in a substantial proportion of RSA cases. Among the group of women who had extremely skewed XCI, all had a normal 46,XX karyotype. However, 8 of the 10 spontaneous abortion specimens from this group that underwent karyotype analysis were aneuploid ($p<.06$ compared to abortuses of the non-skewed group), suggesting that germline aneuploidy may be most commonly involved. Conducting more detailed epidemiological studies in the women who are found to show extreme skewing will help to determine the cause of their RSA, and assist them in making informed reproductive choices in the future.

Program Nr: 196 from the 1999 ASHG Annual Meeting

Further evidence that nonhomologous Robertsonian translocations form predominantly during maternal meiosis. *S.A. Berend[1], S.L. Page[2], W.A. Atkinson[1], M.M. Madrigal[1], L.G. Shaffer[1].* 1) Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Section of Molecular and Cellular Biology, University of California, Davis, CA.

Robertsonian translocations (ROBs) are the most common chromosomal rearrangements in humans with a very high rate of de novo formation. Rob(13q14q) and rob(14q21q) are the most common, constituting about 85% of all ROBs. We hypothesize that recombination between homologous sequences in the short arms of chromosomes 13, 14, and 21 would give rise to these ROBs. A previous study of nine balanced de novo rob(13q14q) and rob(14q21q) showed that the parental origin was maternal in eight of the nine cases (Page and Shaffer, 1997). This study suggested that ROB formation most likely occurs during meiosis and predominantly during oogenesis. Breakpoint analysis lended support for a specific mechanism, unique to rob(13q14q) and rob(14q21q), that would account for their frequent occurrence. Recently, we have identified 32 additional balanced de novo ROBs, including twenty-two rob(13q14q), four rob(14q21q), three rob(14q15q), one rob(14q22q), and two rob(15q22q). Somatic cell hybrids were employed to isolate the ROB from its free-lying homologues so that the parental origin of the ROB could be unequivocally determined. To date, somatic cell hybrids have been constructed for 16 cases. By comparing polymorphic, microsatellite markers on the isolated ROB and the parental genomic DNA, the parental origin of the chromosomes involved in the ROB were determined. The parental origins of the ROBs have been eludicated for 15 of the balanced de novo ROBs, including seven rob(13q14q), three rob(14q21q), two rob(14q15q), one rob(14q22q), and two rob(15q22q). Fourteen of the fifteen ROBs were of maternal origin and one rob(14q15q) was of paternal origin. This provides additional evidence that ROBs predominantly form during maternal meiosis and that a distinct mechanism gives rise to the common ROBs.*de novo*.

Program Nr: 197 from the 1999 ASHG Annual Meeting

Molecular and cytogenetic analysis of de novo segmental aneusomy: Insights into parental origin. *D.A. Sirko-Osadsa, A.B. Zinn, S. Schwartz.* Department of Genetics and Center for Human Genetics, Case Western Reserve University and University Hospitals of Cleveland, Cleveland, OH.

Although the association of maternal origin with nondisjunction is well established, the etiology and parental origin of de novo segmental aneusomy is not well understood. To provide insight into the origin of de novo chromosomal aberrations that result in segmental aneusomy, we have studied 53 de novo chromosome abnormalities including 23 terminal deletions, 16 intrachromosomal duplications or interstitial deletions, and 14 interchromosomal duplications or deletions involving multiple rearrangements. No microdeletion syndrome cases were included in these studies. Initial detection of these abnormalities using high resolution chromosome analysis was followed by microsatellite studies to determine parental origin. These studies revealed that 65.2% (15/23) of the terminal deletions and 62.5% (10/16) of the intrachromosomal events were of paternal origin, while maternal and paternal origins were nearly equally represented among the interchromosomal events. Of the 53 cases studied, 62% (33/53) demonstrated paternal origin of the chromosomal abnormality, while 38% were of maternal origin. While more cases must be analyzed, these initial studies provide intriguing results. We can conclude that: 1) differences seem to exist between a predilection for parental origin and the type of de novo chromosome abnormality; 2) these differences may be dependent on the mechanism of formation; and 3) paternal involvement is much more common in these types of rearrangements than expected. These studies are important as they suggest that the mechanisms driving formation of the rearrangements may also drive the parental origin of specific classes of chromosomal abnormalities.

Program Nr: 198 from the 1999 ASHG Annual Meeting

Viable chromosomally unbalanced offspring of carriers of paracentric inversions. *L. Celle[1], A. Laufer-Cahana[1], D.M. McDonald-McGinn[1], S.H. Tucker[2], L. Rollings[1], N.B. Spinner[1], E.H. Zackai[1].* 1) Division of Human Genetics and Molecular Biology, and; 2) Department of Neurology,The Children's Hospital of Philadelphia, Philadelphia, PA.

Paracentric inversions (PAI) are a well-described but rare form of chromosome abnormality occuring in 1/2000 to 1/10,000 individuals. PAI are inherited in 60-70% of cases and carriers are at an increased risk for SAB as acentric and dicentric recombinants resulting from crossing-over in the inversion loop are typically non-viable. However, chromosomally unbalanced but viable recombinants may occur. We report 2 cases where a PAI in an unaffected parent led to an unbalanced karyotype in the offspring. Patient 1 came to attention at 14 months of age because of developmental delay, growth retardation and dysmorphia. The patient and his mother were found to have a similar abnormally banded chromosome 17, interpreted as 46,XX,inv(17)(q21.3;q23). Subsequent studies showed that the patient had a duplication of chromosome 17q between bands q21.31 and q21.33 in addition to the PAI. We hypothesize this resulted from unequal crossing-over directly outside of the inversion loop in his mother. Patient 2 was acertained through Endocrine clinic for work-up of short stature. She was non-dysmorphic, had normal intelligence and a mild learning disability. She was found to have a deletion of 18q21.2 to q21.3. Her father had a PAI of chromosome 18 between bands q21.1 and q21.3, leading us to re-evaluate the proband's karyotype. We determined that the patient had a recombinant chromosome 18 derived from malalignment within the inversion loop of the father's inversion. These cases provide further documention that carriers of PAI can have viable offspring with unbalanced recombinants. In some cases, the recombinant may appear cytogenetically similar to the parental PAI, requiring extensive cytogenetic or molecular analysis when the phenotype is abnormal. Patient 1 was originally thought to have the familial PAI, but in the presence of abnormal phenotype, further studies revealed a small duplication. Counseling carriers of PAI requires caution, as unbalanced recombinants may be difficult to identify cytogenetically.

Program Nr: 199 from the 1999 ASHG Annual Meeting

Molecular and cytogenetic analysis of two subtle rearrangements: Implications for increased risks to paracentric inversion carriers. *J.L. Winters, S.B. Cassidy, S. Schwartz.* Ctr Human Genetics and Department of Human Genetics, Case Western Reserve Univ, Cleveland, OH.

Paracentric inversions and insertions occur in the general population at a frequency of about 0.02% and 0.03% respectively. Meiotic recombination in either case may result in imbalances - acentric and dicentric recombinant chromosomes in the former case (with a low risk for unbalanced offspring), deletions or duplications in the latter (with a risk approaching 50% for imbalance). In this study we describe two families ascertained because of children with developmental delay and dysmorphic features. In each family, high resolution analysis and molecular cytogenetic studies revealed a parent carrying a subtle balanced rearrangement that led to a chromosomal imbalance in a child. In both cases abnormalities found in the children were reinterpreted after detection of subtle parental abnormalities. In case 1, a mother was found to have a small paracentric inversion in the long arm of chromosome 10 [46,XX,inv(10)(q25.2 q26.13)], while her 10-month-old son had a duplication of 10q26-q26.13. In case 2, two siblings were shown to have interstitial deletion within the short arm of chromosome 1 (p36.13-p36.33). Initial studies showed that the mother carried a paracentric inversion, but further analysis revealed that she in fact carried a subtle intrachromosomal inverted insertion [46,XX,inv ins(p34.3p36.33p36.13)].

These cases underscore the value of parental blood studies in both cases of deletions and intrachromosomal duplications, the use of high resolution techniques in conjunction with molecular cytogenetics and the high risk of abnormal offspring to insertion carriers. However, most importantly it indicates that while theoretic and empiric risk for paracentric inversion carriers is low, the actual risk may be higher due to the under ascertainment of parental abnormalities. Our study of two subtle parental rearrangements suggests that recombination may lead to deletions and/or duplication of the adjacent regions to the inversion breakpoints. Also, some of these inversions may be subtle insertions, which convey a much higher risk for recombination.

Program Nr: 200 from the 1999 ASHG Annual Meeting

Familial Testis Cancer: Three Candidate regions identified. *E.A Rapley[1], G. Crockford[2], D. Teare[3], D. Easton[3], D.T Bishop[2], M.R Stratton[1], presented on behalf of International Testis Cancer Linkage Consortium[4]*. 1) Cancer Genetics, Institute of Cancer Research, Sutton, Surrey, UK; 2) Genetic Epidemiology Unit, Imperial Cancer Research Fund, Leeds, UK; 3) CRC Genetic Epidemiology Unit, Cambridge, UK; 4) International Testis Cancer Linkage Consortium. (Includes groups from Australia, Canada, Germany, Ireland, Norway, and United Kingdom).

Testis cancer affects 1/500 men, is associated with undescended testis and is the most common cancer in males aged 15-35years. In 2% of cases another family member is affected. Within families brothers have a relative risk of approximately 8 of developing testis cancer while the relative risk to fathers is half of that to brothers; the reason for this difference is unclear but is probably due in part to decreased fertility among men who later develop testicular cancer or the effects of previous treatment for the disease. Familial cases tend to occur at an earlier age and are associated with a higher incidence of bilateral disease than sporadic testis cancer. The International Testis Cancer Linkage Consortium has been collecting families with two or more affected cases since 1992. A genome screen of up to 130 families using a 5-10cM marker map has now been completed. Exclusion analysis of this data demonstrates that familial testis cancer is not due to a single major gene but may be genetically heterogeneous or involve interactions between genes. Analysis using GENEHUNTER, identifies three regions suggestive of linkage (heterogeneity LOD scores > 1.3 or NPL p-value less than 0.05). These are now being further investigated. Preliminary analyses suggest that the presence of bilateral disease, undescended testis and early age of onset are strongly associated with linkage to one of these loci and therefore lend support to the existence of a gene in this location. These three clinical features are associated with each other within families and suggest a testicular cancer syndrome.

Program Nr: 201 from the 1999 ASHG Annual Meeting

Increased aneuploidy in morphologically normal testes spermatocytes adjacent to germ cell neoplasms. *J. de Lara[1], D. Lamb[1,2], E. Kim[1], L. Lipshultz[1], F. Bischoff[3]*. 1) Depts Urology; 2) Cell Biology; 3) OB/GYN, Baylor College of Medicine, Houston, TX.

Infertility after treatment of testicular neoplasms is common, prompting many affected men to cryopreserve sperm for future use. Semen quality is usually poor and few sperm may be available for freezing. Testicular sperm may provide an additional source for use with assisted reproductive techniques such as ICSI. However, it is unknown whether the tissue adjacent to tumor will provide a reliable source of normal sperm. METHODS-FISH was employed to evaluate the incidence of sex chromosomal aneuploidy in morphologically normal spermatogenic cells located adjacent to tumor tissue(n=3)as compared to unaffected normal controls(n=5). Nuclei were defined as diploid/primary spermatocytes or haploid/secondary spermatocytes based on the presence of two or one control signals. RESULTS-Distribution of normal and aneuploid cells scored in each of three patients was significantly different ($P<0.0001$) as compared to the controls (table); likely due to increased frequency of aneuploid cells among patients.

Cases	#Norm. Haploid/%	#Aneu.Haploid/%	#Norm. Diploid/%	#Aneu.Diploid/%
Controls (mean)	280/57%	133/27%	65/13%	15/3%
Patient 1	99/30%	192/58%	25/7%	18/5%
Patient 2	164/42%	133/34%	35/9%	58/15%
Patient 3	85/31%	101/38%	36/13%	50/18%

CONCLUSIONS-These preliminary results suggest that there is a possible increased risk of aneuploidy associated with ICSI. Studies are necessary to evaluate the use of the contralateral testes and regions further away from the tumor.

Program Nr: 202 from the 1999 ASHG Annual Meeting

Attenuated forms of familial adenomatous polyposis coli may be due to translational re-initiation within mutant mRNA. *T.M.F. Tuohy[2], K. Heppner Goss[1,2], J. Groden[1,2].* 1) HHMI; 2) Molecular Genetics, Biochemistry & Microbiology, University of Cincinnati College of Medicine, Cincinnati, OH 45267-0524.

Inherited mutation of the *APC* tumor suppressor gene predisposes to adenomatous polyps and colon cancer. Patients who inherit mutations in the first ~500 nucleotides of *APC* present with fewer polyps and later onset than patients with more distal mutations or cytogenetic deletions of the entire gene. To test the hypothesis that this attenuation of phenotype is caused by re-initiation of translation downstream of these 5' mutations, we have used green fluorescent protein (GFP) as a carboxy-terminal reporter for the production and analysis of amino-terminal truncations of the APC protein. Constructs were designed to express the first ~650 nucleotides of *APC* as *APC-GFP* fusions in COS-1 cells to facilitate detection by immunofluorescence and western analysis. Immunofluorescence studies showed that in spite of an in-frame stop codon, GFP is produced, albeit at a lower level than wild-type fusions. Comparable levels of fluorescence in constructs with two tandem stop codons ruled out the possibility of stop-codon read-through. Western blot analysis of cells transfected with wild-type fusions showed a GFP-containing protein of ~ 47kD. Cells transfected with mutant fusions showed a GFP-containing protein of ~28kD, as expected if re-initiation takes place at the first or second AUG codon downstream from the stop codon. Increasing or decreasing the number of available putative re-initiation codons confirmed their ability to function by appropriately affecting the number and sizes of products as determined by further Western analysis. To address the question of whether such re-initiation products retained normal APC function, full-length *APC*- and *AAPC-GFP* fusions were cloned and expressed in colorectal cancer cells to follow b-catenin levels. Both mutant AAPC and normal APC expressison down-regulated b-catenin similarly. These results suggest that translational re-initiation may produce a functional, albeit N-terminally truncated, derivative of APC that protects patients with 5' *APC* mutations from the consequences of early and complete loss of APC function.

Program Nr: 203 from the 1999 ASHG Annual Meeting

Frequency of microsatellite instability in tumors from HNPCC-suspected patients. *Y. Wu[1], M.J.W. Berends[2], R.G.J. Mensink[1], C. Kempinga[3], R.H. Sijmons[1], A.G.J. van der Zee[4], H. Hollema[3], J.H. Kleibeuker[2], C.H.C.M. Buys[1], R.M.W. Hofstra[1]*. 1) Medical Genetics, University of Groningen, Groningen, The Netherlands; 2) Gastroenterology, University Hospital of Groningen, Groningen, The Netherlands; 3) Pathology, University Hospital of Groningen, Groningen, The Netherlands; 4) Gynaecology, University Hospital of Groningen, Groningen, The Netherlands.

Hereditary nonpolyposis colorectal cancer (HNPCC), as defined by the (revised) Amsterdam criteria, is a disorder in which besides colorectal cancer also extracolonic tumors occur. Due to the defective repair mechanism, tumors of HNPCC patients often show microsatellite instability (MSI). To determine the frequency of MSI in tumors from HNPCC-suspected patients and to evaluate five consensus markers in HNPCC-related tumors, we performed MSI analysis in matched pairs of normal and tumor DNA from 180 HNPCC-suspected patients using two mononucleotide repeats (BAT26 and BAT40) and three dinucleotide repeats (D2S123, D5S346 and D17S250). These 180 patients participated in an ongoing prospective population-based study on the role of MMR gene mutations. In the 105 patients with colorectal carcinomas (CRC), we found that 30% (32/105) of the tumors displayed an MSI-high (MSI-H) phenotype. Amongst the 54 patients with endometrial carcinomas (EC), 33% (18/54) of the tumors exhibited an MSI-H phenotype. When comparing the performance of the different markers in the different tumor types, we found that mononucleotide repeats were much less informative in EC than in CRC. For CRC, MSI was observed with BAT26 in 75%, with BAT40 in 60%, with D2S123 in 65%, with D5S346 in 50% and with D17S250 in 83% of MSI-H cases. For EC these percentages were 35%, 41%, 81%, 76% and 47%, respectively. Our results show that a substantial proportion of HNPCC-suspected patients have an MSI-H tumor phenotype and that the different types of markers have different sensitivities for CRC and EC. This implies that for MSI analysis in endometrial and other extracolonic carcinomas (a) different set(s) of markers need(s) to be defined. [Supported by the Dutch Cancer Society, grant RUG 97-1544].

Program Nr: 204 from the 1999 ASHG Annual Meeting

The occurrence of MSH6 mutations in a large set of HNPCC-suspected patients. *M.J.W. Berends[1], Y. Wu[2], R.M.W. Hofstra[2], R.H. Sijmons[2], R.G.J. Mensink[2], H. Hollema[3], A. Karrenbeld[3], A.G.J. van der Zee[4], C.H.C.M. Buys[2], J.H. Kleibeuker[1]*. 1) Dept. of Gastroenterology, University Hospital of Groningen, Groningen, The Netherlands; 2) Medical Genetics, University of Groningen, Groningen, The Netherlands; 3) Pathology, University Hospital of Groningen, Groningen, The Netherlands; 4) Gynaecology, University Hospital of Groningen, Groningen, The Netherlands.

Hereditary nonpolyposis colorectal cancer (HNPCC) is characterized by germline mutations in DNA mismatch repair (MMR) genes, especially MLH1 and MSH2, and the occurrence at young age of gastrointestinal and urogenital cancers, particularly colorectal (CRC) and endometrial (EC) cancers. These tumors often show a high degree of microsatellite instability (MSI-high). Therefore, the MSI status has been proposed to select patients for mutation analysis. In a recent pilot study, we found involvement of MSH6 in a proportion of HNPCC-suspected patients with mainly MSI-low tumors (AJHG, 1998, A21, 107). To better determine the occurrence of germline MSH6 mutations, we analysed a larger set of patients, participating in an ongoing prospective population-based study on the role of MMR gene mutations. Inclusion criteria were (1) CRC or EC under age 50; (2) an HNPCC-related cancer and a first degree relative with CRC or EC, or vice versa, one of them under age 50; (3) more than 1 HNPCC-related tumor. Tumors were analysed for MSI. So far, 139 patients have been included. Thirteen patients (9%) had an MSH6 mutation (5 truncating, 6 missense, one nonsense, one intronic insertion). Of those patients, 7 had CRC, 3 had EC and 3 had multiple HNPCC-related tumors. From 10 of them tumor material was available for MSI analysis. Remarkably, 8 of the tumors were MSI-stable or -low. Two tumors were MSI-high, however, one patient also had an MLH1 mutation which can explain the presence of MSI. We conclude that MSH6 mutations are responsible for a substantial part of cancers in patients with a suspicion of HNPCC. Since the great majority of tumors in MSH6 carriers are MSI-low, MSI-low status should not be an exclusion criterion for mutation analysis of the MSH6 gene.

Program Nr: 205 from the 1999 ASHG Annual Meeting

Identification of individuals at risk of cancers due to defective DNA mismatch repair. *J.M. Cunningham, C.Y. Kim, E.R. Christensen, D.J. Tester, Y.R. Parc, L.J. Burgart, K.C. Halling, S.K. McDonnell, D.J. Schaid, C. Walsh Vockley, V. Kubly, V. Michels, S.N. Thibodeau.* Mayo Clinic, Rochester, MN.

Inheritance of defective DNA mismatch repair (MMR) genes is associated with the development of hereditary nonpolyposis colorectal cancer (HNPCC). The most commonly involved genes are *hMLH1* and *hMSH2*, and tumors from these cases are characterized by a high frequency of microsatellite instability (MSI-H). The best strategy for identifying individuals at-risk for HNPCC is not clear. Classically, a strict family history of early onset colorectal cancer (CRC) in multiple family members over several generations has been used to define this heightened risk (Amsterdam criteria, AC). However, a number of mutation carriers do not adhere to these criteria. Testing tumors for the presence of MSI-H has also been considered, but most positive cases are sporadic in origin. More recently, the Bethesda Guidelines (JNCI 89:1758,'97) were proposed to determine who should undergo MSI testing, to ultimately identify carriers of defective MMR genes. These guidelines use family history of cancer, the occurrence of multiple HNPCC-type cancers, and pathological characteristics of early onset cases. We sought to validate these guidelines in a prospective assessment of defective MMR in an unselected series of 257 CRC. In this series there were 51 patients with tumors showing defective MMR, expressed phenotypically by MSI-H and an absence of expression of either hMLH1 or hMSH2, resulting either from mutations or epigenetic silencing. Only 7 germline mutations (5 truncating/splice-site, 2 missense) were found among the 51 cases. Using the Bethesda guidelines, 38/257 individuals would have been tested for MSI. Overall, 10 of these 38 patients had tumors with defective MMR, 5 of which had germline mutations (all truncating/splice-site). Using the AC alone, 6 would have been tested, 3 having defective MMR and 2 of which had a germline mutation (both truncating/splice-site). Neither the AC nor Bethesda guidelines would have identified the 2 missense mutations. The Bethesda guidelines however, appear to significantly improve the predictive potential for identifying patients with defective DNA MMR genes.

Program Nr: 206 from the 1999 ASHG Annual Meeting

A 20-fold increase in risk of colorectal cancer with defective mismatch repair among current smokers who carry alpha-1 antitrypsin deficiency alleles. *P. Yang, J. Cunningham, J. Katzmann, T. Lesnick, K. Halling, L. Burgart, E. Wiegert, E. Christensen, R. Kyle, N. Lindor, S. Thibodeau.* Mayo Clinic, Rochester, MN.

Microsatellite instability (MSI) is a type of genomic alteration observed in approximately 15-30% of colorectal cancer (CRC). Three MSI phenotypes have been defined for CRC. MSI-H phenotype is characterized by MSI at ³30% of the loci, MSI-L by MSI at 1-30% of the loci, and MSS by an absence of MSI at any of the loci examined. MSI-H results from defective DNA mismatch repair (MMR). We report that MSI-H colorectal tumors are associated with alpha-1 antitrypsin deficiency carrier status (a_1AD-ht) and cigarette smoking history of the patients. Among 53 CRC patients with MSI-H tumors, the a_1AD-ht rate was 23% whereas among 108 patients with MSI-L/MSS tumors, the rate was 9% ($p=0.02$). A similar difference in the a_1AD-ht rate was observed between the 53 MSI-H CRC patients and 191 population-based controls who did not have CRC (9%). The estimated relative risk (via odds ratio) of having MSI-H CRC among a_1AD carriers was 3.3 (95% CI 1.3-8.2) compared to non-carriers. This was after adjusting for age, gender, and cigarette smoking history using multiple logistic regression analysis. Cigarette smoking, past and current, was associated with a 2.6- and 6.0-fold elevated risk, respectively, for MSI-H CRC (p£0.01); but the effects were not significant for MSI-L/MSS CRC. Compared to never smokers who were homozygous normal at a_1AD locus, the combined effect of being current smokers and a_1AD carriers on the risk of MSI-H CRC reached an odds ratio of 20. Based on the same multivariable model, women had a four-times greater risk to develop MSI-H CRC than men ($p<0.01$). In a case-case comparison, we have found that an over 3.5-fold increase in a_1AD-ht among MSI-H CRC patients versus MSI-L/MSS CRC patients was consistent in proximal versus the distal colon and rectum. These preliminary data suggest a possible etiologic link between a_1AD alleles and the development of CRC characterized by defective MMR.

Program Nr: 207 from the 1999 ASHG Annual Meeting

Aberrant DNA methylation occurs in multiple genes in esophageal cancer tissues. *T. Kubota[1], H. Seki[1], K. Wakui[1], W. Adachi[2], T. Nasu[1], Y. Fukushima[1].* 1) Dept. Hygiene and Medical Genetics; 2) 2nd Dept. Surgery, Shinshu University, Matsumoto, Japan.

Aberrant DNA methylation in CpG islands of certain cancer- associated genes is thought to be one of the genetic factors for carcinogenesis. However, it is not known whether the aberrant DNA methylation simultaneously occurs in cancer-associated genes and other genes. We have examined the methylation status in CpG islands of four cancer-associated genes and one non-cancer associated gene by methylation-specific PCR in pairs of cancer tissues and normal tissues of 148 cases with esophageal cancer. Of the 148 cancer tissues, 25 (17%), 1 (1%), 33 (22%) and 27 (18%) showed hypermethylation in the CpG islands of p16, p15, E-cadherin and H-cadherin genes, respectively, whereas only 0 (0%), 0 (0%), 2 (2%) and 1 (1%) showed hypermethylation in normal tissues. Five cancer tissues showed hypermethylation in three genes (p16, E-cad., H-cad.), and 11 cancer tissues showed hypermethylation in two genes out of the four genes examined. We also examined the methylation status in the CpG island of an imprinted SNRPN gene where the maternal allele should be completely methylated and the paternal alleles should be completely unmethylated in normal tissues. In 114 cancer tissues examined, 19 (17%) showed hypermethylation (methylated allele : unmethylated allele > 70:30) and 24 (21%) showed hypomethylation (methylated allele : unmethylated allele < 30:70). These results suggest that aberrant methylation occurs not only in the single cancer-associated gene, but simultaneously occurs in multiple genes in cancer tissues, and that the preservation system of DNA methylation at CpG islands established in normal tissues may be altered in cancer tissues.

Program Nr: 208 from the 1999 ASHG Annual Meeting

Familial Barrett Esophagus: A True Hereditary Cancer Syndrome. *H. Hampel[1], B.A. Poling[1], M. Curtis[2], M. Mascari[3], J.J. Fromkes[1], C. Eng[1]*. 1) Human Cancer Genetics Program, Arthur G James CA Hosp/Res Ins, The Ohio State University, Columbus, OH; 2) University of Arkansas for Medical Sciences, Little Rock, AR; 3) Hershey Medical Center, Hershey, PA.

There have been at least 15 case reports of familial Barrett esophagus (BE) and esophageal adenocarcinoma (EAC). It appears that this is an autosomal dominant condition with age-related penetrance. However, there has been great debate about the mechanism by which familial BE occurs. We present 3 new cases of familial BE, which provide evidence to strongly suggest that it represents a true hereditary cancer susceptibility syndrome. In Family 1, the proband has GERD and esophagitis. His mother and her brother have BE and his maternal grandmother died from EAC. In Family 2, the proband has had >10 colon polyps and BE. Her brother died from an unknown cancer at age 34. Her father had EAC and gastric cancer. There were 2 cases of colon cancer and one case of lung cancer in this family. In Family 3, the proband was diagnosed with a squamous cell carcinoma (SCC) of the tongue. His father and paternal grandfather had EAC. His paternal uncle had a SCC of the lower lip and the wrist as well as GERD, dysphagia and a hiatal hernia. There are 2 cases of breast cancer and another case of SCC of the tongue in the family. Given the variety of cancers found in Families 2 and 3, a review of the literature was performed to determine whether other families with Familial BE had extra-esophageal cancers. While the majority of case reports do not address other cancers in the family; three reported BE families include a total of 3 cases of gastric cancer, 1 case of colon cancer, 2 cases of head and neck cancers, basal cell carcinoma of the skin and ovarian cancer. It appears that families with BE may be at risk for more than just EAC. If so, this would argue strongly that familial BE is a premalignant condition that results from an underlying hereditary cancer predisposition. Notably, many of the cancers reported (eg. tongue, gastric, and colon) are part of the digestive tract. Ultimately, answers may only come when the responsible gene(s) are identified.

Program Nr: 209 from the 1999 ASHG Annual Meeting

The PTEN gene is methylated in endometrial carcinoma and associated with advanced disease and microsatellite instability. *S. Das[1], N. MacDonald[2], A. Ryan[2], I.J. Jacobs[2], E.D. Lynch[3], L.A. Akslen[4], H.B. Salvesen[1,4,5]*. 1) Dept Human Genetics, Univ Chicago, Chicago, IL; 2) Gynaecology Cancer Research Unit, Department of Gynaecological Oncology, St. Bartholomews Hospital, London, UK; 3) Department of Medicine, Division of Medical Genetics, University of Washington, Seattle, WA; 4) Department of Pathology , The Gade Institute, Bergen, Norway; 5) Department of Gynecology and Obstetrics, Haukeland University Hospital, Bergen, Norway.

The PTEN (MMAC1) gene is a candidate tumor suppressor gene associated with loss of heterozygosity on chromosome 10q23 that is deleted/mutated in a large number of human cancers in addition to being mutated in the germline in Cowden syndrome, a cancer susceptibility disorder. Abnormalities of the PTEN gene appear to be important in endometrial carcinoma as loss of heterozygosity for the 10q23 region and mutations in the PTEN gene are frequent in this disorder. Promoter hypermethylation has recently been identified as an alternative mechanism of tumor suppressor gene inactivation in cancer, but its importance in PTEN gene inactivation in endometrial carcinoma is unknown. We have performed methylation studies of the PTEN gene and correlated our results with clinicopathologic variables in a population-based series of endometrial carcinomas with complete follow-up. Presence of PTEN promoter methylation was seen in 26 of 138 patients (19%). Methylation was significantly associated with metastatic disease (p=0.01) and a microsatellite unstable phenotype (p=0.006). Our results suggest that PTEN promoter methylation is relatively frequent in endometrial carcinoma and its association with metastatic disease and microsatellite instability implicates its importance in the carcinogenesis of this disease. We are currently analyzing the PTEN promoter to obtain a detailed methylation profile of this region in our group of endometrial carcinoma patients in addition to performing mutation and deletion studies of this gene to obtain a better understanding of the relationship between the different mechanisms of gene inactivation.

Program Nr: 210 from the 1999 ASHG Annual Meeting

A population-based study of cancer incidence in relatives of prostate cancer patients in Finland: High risk of late-onset prostate cancer and association with stomach cancer. *M.P. Matikainen[1], R. Sankila[2], J. Schleutker[1,3], P. Koivisto[1], T. Tammela[1], E. Pukkala[2], O. Kallioniemi[1,3]*. 1) Lab Cancer Genetics, IMT, Univ of Tampere and TAUH, Tampere, Finland; 2) Finnish Cancer Registry, Helsinki, Finland; 3) Cancer Genetics Branch, NHGRI, NIH, Bethesda, MD.

As part of our genetic epidemiological study of hereditary prostate cancer (PCA) in Finland, we investigated the incidence of cancer in first-degree relatives of PCA patients by linking Finnish Cancer Registry data and population-based parish records on relatives. The aim was to determine the degree of prostate cancer-specific risk, as well as to search for associations with other cancer types. The study population was composed of 1st degree relatives (n=8628) of two groups of patients: 1) All early-onset (<60 years) PCAs diagnosed in Finland during 1988-1993 (n=557) and 2) a population-based sample of PCAs, excluding <61 year-olds (n=945). The standardized incidence ratio (SIR) of prostate cancer was elevated in both cohort 1 (SIR 2.5, $p<0.001$) and in cohort 2 (1.7, $p<0.001$). The risk of PCA was significantly elevated across most age groups. Highly elevated SIRs were observed even in 75-79 year-old relatives, suggesting that genetic factors may influence prostate cancer development even at an old age. The only other cancer type having a significantly (SIR 1.9, $p<0.001$) increased incidence in the relatives was stomach cancer. Stomach cancer risk was particularly high (SIR 7.1, $p<0.01$) among brothers of PCA patients diagnosed at a very early age (<55 years). In conclusion, our population-based study suggests the substantial contribution of hereditary factors in prostate cancer development even at an old age. This finding is compatible with the recent observation that HPC-X linkage is very common in Finland, and specifically contributes to old age of onset disease (Schleutker et al., 1999). Furthermore, the association of gastric cancer with prostate cancer has not been previously reported, and could reflect the influence of a novel locus predisposing to both cancer types in the Finnish population.

Program Nr: 211 from the 1999 ASHG Annual Meeting

Alu insertion polymorphisms and human genomic diversity. *M. Batzer[1], I. Nasidze[2], S. Sherry[1,3], G. Risch[1], M. Robichaux[1], M. Stoneking[2]*. 1) Dept. of Pathology, Louisiana State University Medical Center, New Orleans, LA; 2) Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany; 3) National Center for Biotechnology Information, National Institutes of Health, Bethesda, MD.

The amplification of Alu repeats is an ongoing process that has resulted in the generation of approximately 500,000 copies within primate genomes over the last 65 million years. The mobilization of Alu elements involves the duplication of a limited number of active elements and has given rise to a hierarchical subfamily structure. Most members of the recently integrated "young" Alu subfamilies (Ya5, Ya8 and Yb8) are restricted to the human genome and absent from nonhuman primates. Some of the recently integrated Alu repeats are bi-allelic polymorphisms for the presence or absence of an Alu insertion at a particular chromosomal location in the human genome. Alu elements offer several attractive features for the study of human genomic diversity. First, the presence of an Alu insertion represents identity by descent, since the probability that two different young Alu elements would integrate at the same chromosomal location is negligible. Second, they are stable polymorphisms that rarely undergo deletion, and even when a deletion does occur, it generally leaves a signature of the original Alu element. Third, the ancestral state of Alu insertion polymorphisms is known to be the absence of the Alu element, which can be used to root trees of population relationships. We have analyzed the distribution of 21 Alu insertion polymorphisms in an average of 1300 individuals from 27 population groups from the Americas, Caucasus, Africa, Sahul, Europe, and Asia. Preliminary results show that the average heterozygosity per locus is 0.29 (range 0.059-0.454) and the overall Fst value is 0.13 (range 0.06-0.26). The Caucasus populations displayed higher heterozygosity than the other European groups suggesting that they are either the source (ancestral) European populations or are admixed. Strong geographic clustering of the populations was evident in both principal components and neighbor-joining tree analyses of the data.

Program Nr: 212 from the 1999 ASHG Annual Meeting

A synopsis of the entire European Y-chromosome biallelic haplotype spectrum. *G. Passarino[1,2], O. Semino[1,3], A.S. Santachiara Benerecetti[3], L.L. Cavalli Sforza[1], P.A. Underhill[1]*. 1) Genetics Department, Stanford University, Stanford, CA; 2) Cell Biology Department, University of Calabria, Rende, Italy; 3) Department of Genetics and Microbiology, University of Pavia, Pavia, Italy.

Our laboratory has identified many biallelic polymorphisms associated with the non-recombining portion (NRY) of the human Y chromosome. Seven key markers (M9, M17, M26, M35, M170, M172, M173) appear to be highly informative for fractionating and cataloging the different components of the European NRY gene pool. The allelic status of several hundred representative European samples have been determined at these markers along with others previously reported (49,af Ht 15, the 49a,f Ht 11, the 12f2 8Kb allele, YAP). The haplotype data almost completely describe the entire underlining framework of NRY genetic diverstiy in Europe. Three components appear to have been present in Europe probably since the Paleolithic era: (M173/49af Ht15 and M170 in Western Europe; M173/49af Ht 11/M17 in Eastern Europe). Another haplotype, 12f28Kb/M172, represents the contribution of the demic diffusion of Neolithic farmers coming from the Near East after the invention of agriculture. Yet another component, represented by YAP+/M35 represents influences from North Africa which could have been spread many times during the last millennia. A simple correlation is observed between geographic NRY haplotype patterns and principal component synthetic maps based upon up to 95 autosomal genes. Since Y chromosome accumulate subsequent biallelic mutations changes without erasing past events, it is now possible to place European lineages in context with well resolved NRY haplotypes associated with other areas of the world. This makes it possible to reconstruct the origins and histories of contemporary Europeans to a new level of resolution and completeness. The data suggest that simple NRY biallelic haplotypes can act as a metaphor of overall population genetic diversity.

Program Nr: 213 from the 1999 ASHG Annual Meeting

Origins and diversity of Jewish Y-chromosome haplotypes. *M.F. Hammer[1], E.T. Wood[1], A.J. Redd[1], M.R. Bonner[1], T. Karafet[1], A.S. Santachiara-Benerecetti[2], A. Oppenheim[3], M. Jobling[6], H. Ostrer[4], B. Bonne-Tamir[5]*. 1) Laboratory of Molecular Systematics and Evolution, Univ Arizona, Tucson, AZ; 2) Dept. Genetics, Universita degli Studi di Pavia, Pavia, Italy; 3) Hadassah Medical School, Hebrew University of Jerusalem, Jerusalem, Israel; 4) Dept. Pediatrics, New York University Medical Center, New York, NY; 5) Dept. Human Genetics, Sacler School of Medicine, Ramat Aviv, Israel; 6) Dept. Genetics, Univ. Leicester, Leicester, England.

Haplotypes constructed from Y chromosome markers were used to examine the history and structure of populations from the Jewish Diaspora. A set of 17 biallelic polymorphisms were genotyped in 1378 males from 28 populations, including 8 Jewish and 11 non-Jewish populations from similar geographic locations. Jewish populations were characterized by a diverse set of 12 haplotypes that appear to have originated in Africa, Asia, and the Mediterranean region. Eight of these haplotypes were relatively common ranging in frequency from 5% to 35%. Principal components analysis placed six of the eight Jewish populations in a relatively tight cluster adjacent to Arab populations from Lebanon, Israel, and Saudi Arabia. The Jewish and Arab groups were more distantly surrounded by non-Jewish European and North African populations. Overall the results support the hypothesis that Jewish populations from North Africa, Europe, and West Asia are descended from common Middle-Eastern ancestors. A recent founder effect has been proposed to explain the high incidence of rare genetic diseases in Ashkenazi Jewish populations. In order to test this hypothesis, we estimated paternal genetic diversity in Ashkenazi and Finnish populations, as well as populations not thought to have experienced recent bottlenecks. There was no evidence for a reduction of Ashkenazi genetic diversity as assessed by both biallelic and microsatellite markers on the Y chromosome. We suggest that an alternative model involving a subdivided Ashkenazi population and multiple founder effects, may better explain both the observed frequencies of genetic diseases and the high levels of Y chromosome haplotype diversity.

Program Nr: 214 from the 1999 ASHG Annual Meeting

Y Chromosome Haplotypes Reveal Distinct Migration Patterns in Siberia and the Americas. *J.T. Lell, T.G. Schurr, R.I. Sukernik, Y.B. Starikovskaya, D.C. Wallace.* Center for Molecular Medicine, Emory University, Atlanta, GA.

Y chromosome haplotypes were analyzed for 533 individuals representing the native populations of the Americas and Siberia. A total of 112 distinct haplotypes (comprised of 7 biallelic markers and 4 microsatellites) were observed, and these haplotypes were further classified into haplogroups based on the the slower-evolving biallelic markers. Over 96% of the Native American Y chromosomes belonged to haplogroup A0 (defined by the M9 polymorphism) or A1 (M9/M3). Network analysis supported the conclusion that haplogroup A1 had a single origin during or immediately prior to initial entry into the New World. Additionally, the A0 haplotypes which probably gave rise to the A1 lineage were observed in the Chukchi, Kets, and Tuvans, in accordance with previous studies which traced the origin of this lineage to Central Siberia. Interestingly, a much more recently arisen lineage (A3), defined by the Tat polymorphism, was also shown to have its origin in Central Asia. The distribution of these lineages suggests that at least two major population expansion/migration events originated in the area of Lake Baikal, with the second event occurring after entry into the New World was blocked by glacial ice sheets. In contrast to the A1 lineage, a single origin for A0 in the Americas is not supported as discordance of A0 haplotypes was observed between North and South American natives. Nearyl 40% of the Siberian natives in this study did not harbor the ancient M9 polymorphism. The great majority of these individuals belonged to haplogroup B, defined by a polymorphism in the RPS4Y gene. Haplogroup B reached its highest frequencies in the native populations of Kamchatka and the Lower Amur and was also observed in a single Navajo individual (and at significant frequency in the Cheyenne and Tanana by Karafet et al. AJHG 64:817-931). We propose that the initial New World peopling event brought haplogroup A0 and A1 types from Beringia by way of Central Siberia, and that a later event contributed haplogroup B and distinct A0 types by way of a migration involving populations ancestral to the modern inhabitants of Kamchatka and the Lower Amur.

Program Nr: 215 from the 1999 ASHG Annual Meeting

Repeat polymorphisms versus Simple Nucleotide Polymorphisms: Implications for genome diversity studies. *R. Chakraborty[1], M. Kimmel[2], L. Jin[1], D.N. Stivers[1], Y. Zhong[1], D. Smelser[3], R. Deka[3].* 1) Human Genetics Ctr, Univ Texas Health Sci Ctr, Houston, TX; 2) Statistics Dept, Rice Univ, Houston, TX; 3) Dept Environmental Health, Univ Cincinnati, Cincinnati, OH.

With 3.3 million Simple Nucleotide Polymorphism (SNP) sites expected to be found on the human genome, it is anticipated that the human genome mapped by the tandem repeat markers will become denser with the arrays of SNPs. Obvious applications of SNPs will include their use in genome diversity studies. This research examines the comparative utility of SNPs versus repeat polymorphisms in addressing some key questions of genome diversity analysis. Through theoretical analysis and empirical data on repeat loci, we show that these markers differ individually in their rates and patterns of mutations. Consequently, we argue that for proper interpretation of the measures of within- and between-population variation, these loci are to be grouped by their rate and pattern of mutations. Further, higher mutation rates at repeat loci implies larger genetic distances between populations, although the coefficient of gene differentiation decays with increasing mutation rate. Ascertainment bias of selection of loci results in higher mean allele size in populations in which they are originally found. In contrast, SNPs are subject to lesser mutation pressure, resulting in inter-population differences produced largely by genetic drift. Smaller isolated populations exhibit larger allele frequency differences that may not entirely be due to their longer time of divergence. Ascertainment of SNP sites has a somewhat different effect, reflected in their allele frequency spectrum. Ascertainment bias of SNP loci may mimic the effect of overdominant selection even when they are neutral. As the alleles at repeat loci may be ordered by their repeat sizes, alternative measures of genetic variation at these loci offers opportunity of detecting past demographic history of populations, an advantage not directly enjoyed by SNP loci. These conclusions are illustrated with empirical data on world-wide diversity of human population populations at both types of loci. (Research supported by NIH grants GM 41399, GM 53545 and GM 45861).

Program Nr: 216 from the 1999 ASHG Annual Meeting

Significant variation in linkage disequilibrium among human populations. *K.K. Kidd[1], A.J. Pakstis[1], H. Zhao[1,2], J.R. Kidd[1]*. 1) Genetics, Yale Univ Sch Medicine, New Haven, CT; 2) Epidemiology & Public Health, Yale Univ, New Haven, CT.

It is amply documented for classical markers, for STRPs, and for SNPs (RFLPs) that allele frequencies vary significantly among populations around the world. In contrast, variation in linkage disequilibrium (LD) is less well understood with very few loci studied on a global sample of populations. We have now assembled a unique, comprehensive dataset and present the first study of multiple haplotype loci in multiple populations. In addition to the haplotype data we have previously published (on CD4, DM, and DRD2), we now have data on an additional nine loci for 25-30 populations involving over 1500 individuals representing all of the major continental regions. Here we present the first analyses of the 25 biallelic markers (SNPs and indels) at 10 of these loci (2 to 4 sites per locus spanning molecular distances up to 70 kb) using a new statistic designed to be an overall measure of non-randomness of alleles across multisite haplotypes (Zhao et al., 1999, Annals of Human Genetics, in press). We find significant variation in LD among loci as well as significant variation among populations. These empiric data show that the human species is not homogeneous with respect to linkage disequilibrium--populations from different parts of the world have different amounts of linkage disequilibrium. A highly significant pattern for amount of LD, strongest for the multisite loci, shows Africa < Europe < East Asia < Americas, consistent with a single recent migration out of Africa and accumulation of random drift at the front of the population expansion. This pattern has implications for use of linkage disequilibrium for mapping complex disorder genes. Simulations designed to determine the "best strategy" need to take into consideration the population genetic complexities demonstrated by these empiric data. Supported in part by NIH grant P01-GM57672 to KKK & JRK and NSF grant SBR9632509 to JRK.<<.

Program Nr: 217 from the 1999 ASHG Annual Meeting

Linkage disequilibrium over short distances in the human genome. *S.N. Liu-Cordero[1], K.G. Ardlie[1], L. Kruglyak[2], M.J. Daly[1], E. Winchester[1], J.C. Barrett[1], E.S. Lander[1].* 1) Whitehead Institute/MIT Center for Genome Research, Cambridge, MA; 2) Fred Hutchinson Cancer Research Center, Seattle, WA.

Initial studies of human gene polymorphism demonstrate considerable variation in both the amount of nucleotide diversity and the level of linkage disequilibrium (LD) among polymorphic sites. The properties of LD throughout the genome may be a critical determinant for mapping of disease genes, in particular to the indirect strategy of association between the disease causing allele and neutral variants that are located nearby. LD tends to show a predictable decline with physical distance over large distances of 10's to 100's of kilobases, yet is highly variable over short distances. Recent studies of variation within genes have in some instances found strong LD over short distances. In other instances there has been a considerable lack of LD even over distances of several hundred base pairs, suggesting high levels of recombination. Thus, there is little consensus as to the extent and variation of LD over short distances in the human genome. To address this question, we have genotyped and haplotyped pairs of SNP's from over 70 STS's located randomly throughout the genome in a sample of 47 individuals from a global population and one chimpanzee. SNP pairs range in distance from 10 to 400 bp's apart. We compare our findings with those from simulations that derive an expectation based on recombination distance, sample size and SNP frequency.

Program Nr: 218 from the 1999 ASHG Annual Meeting

The effects of population history on linkage disequilibrium among SNPs. *M.T. Seielstad[1], A.P. Miller[2], N.J. Schork[1,3,4]*. 1) Program Population Genetics, Harvard Sch Public Health, Boston, MA; 2) Kiva Genetics, Inc., Mountain View, CA; 3) Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, OH; 4) Genset, La Jolla, CA.

Recent proposals for applying whole genome association studies to complex trait mapping have focused renewed attention on linkage disequilibrium (LD) in populations. A primary factor in determining the optimal density of a SNP map is the physical distance over which significant LD extends. While a strong correlation between LD and physical distance is expected, LD is affected by many other factors, including population history, admixture, allele frequency, mutation rates, and recombination rates. We have begun a systematic survey of the impact of these and other factors on the magnitude and behavior of LD among autosomal SNPs in 100 unrelated individuals from each of six subpopulations in Southeastern China (N=600), representing a range of demographic histories. In each genomic region, a SNP map of roughly 15kb density over 500kb was sought, by dHPLC screening of fully sequenced contigs in 16 individuals. The exact number of nucleotide sites separating each polymorphism is known. Genotypes were determined in the six subpopulations using a modified minisequencing protocol and a microchannel plate electrophoresis system. Offspring samples were available from several of the populations, allowing haplotypic phase of most autosomal SNPs to be determined directly. In other instances, haplotypes were inferred with maximum likelihood methods. We find significant LD extending over distances of several hundred kb in these populations, and a significant correlation between physical distance and LD over similar distances. LD is generally most extensive in the more isolated populations and slightly less so in the urban areas. The effects of an ascertainment bias and differing allele frequencies among populations will be assessed by typing the same SNPs in additional population samples such as those of the CEPH pedigree collections. Our data indicate that LD may frequently be sufficient to allow the successful application of genome wide association studies.

Program Nr: 219 from the 1999 ASHG Annual Meeting

The Haplotype Disequilibrium Test: A nonparametric haplotype based method for the genetic mapping of complex traits. *J.M. Akey, L. Jin, M. Xiong.* Human Genetics Center, University of Texas-Houston, Houston, TX.

The genetic dissection of complex diseases represents one of the most formidable challenges of modern human genetics. Recently, the proposition of conducting linkage disequilibrium (LD) based genome-wide screens to delineate the genetic architecture of complex phenotypes has received considerable attention. Integral to these endeavors is the development of multilocus measures of LD. One approach for capturing the maximum amount of inherent LD in a particular chromosomal region is to use haplotypes as opposed to individual marker data. To this end we propose a nonparametric method, the Haplotype Disequilibrium Test (HDT), which compares haplotype frequencies between cases and controls. A conventional c^2 statistic is used to test the null hypothesis of equal haplotype frequencies. Analytic formulas were derived and power calculations were performed to assess the utility of the HDT. Specifically, we investigated how the genetic distance between markers and disease locus, mode of disease inheritance, age of gene mutation, frequency of associated marker alleles, and levels of initial linkage disequilibrium affect the power of the HDT. In general, we demonstrate that the simultaneous use of marker data in the form of haplotypes results in a more powerful approach to map disease genes. For instance, when the markers are near the trait locus the sample size required to detect an association is dramatically reduced when haplotypes are used rather than individual markers. Furthermore, we show that the use of larger haplotypes does not necessarily increase the power of the HDT compared to the use of smaller haplotypes. Finally, we illustrate the utility of the HDT by applying it to published data from the hereditary hemochromatosis (HFE gene) region.

Program Nr: 220 from the 1999 ASHG Annual Meeting

Search for multifactorial diseases susceptibility genes in founder populations : hopes and limitations. *C. Bourgain[1], E. Genin[1], H. Quesneville[2], F. Clerget-Darpoux[1]*. 1) INSERM U155, Paris, FRANCE; 2) Laboratoire de Dynamique du Génome et Evolution, Institut J.Monod, Paris, FRANCE.

A new method (Maximum Identity Length Contrast, MILC) is proposed to locate susceptibility genes for multifactorial diseases in founder populations. It uses characteristics of such populations : linkage disequilibrium spanning over large regions and significant kinship coefficients. Affected individuals and their parents are typed for a set of linked markers and two groups of haplotypes are considered : those transmitted to affected individuals and those not transmitted, considered as representative of the population. The mean lengths of identity are contrasted between the two groups of haplotypes. MILC is based on the maximum of this contrast over all loci used in the analysis.

For a given disease genetic model and population history, the power of this method is evaluated through simulations as a function of marker map characteristics. With a dense map, this method is generally more powerful than a simple TDT test because it uses information on several markers. However there is a large variability of power for a given population history, a same genetic model and marker map. In fact, the power of our method strongly depends on linkage disequilibrium (LD) among markers that is highly determined by random processes in founder populations. In particular, we show that LD is not always a simple decreasing function of the genetic distance.

Our results suggest that one way to increase our power to detect genetic risk factor for multifactorial diseases in founder populations can be the use of markers showing strong LD one with the others. Preliminary population studies should then be required to use the best marker map.

Program Nr: 221 from the 1999 ASHG Annual Meeting

Covariates in linkage analysis. *J.P. Rice, P. Holmans, N.L. Saccone, J. Corbett, R.J. Neuman.* Dept Psychiatry, Washington Univ, St Louis, MO.

We have developed a model in which the probability that a sib pair shares an allele from a particular parent has a logistic regression on a set of covariates. In this setting, covariates may include indicator variables for diagnoses, indicators for the source of data in a meta-analysis, or genotypes at a particular locus. This formulation permits an overall test of linkage as well as individual tests of phenotypic and study heterogeneity. The use of multi-point data allows the estimation of gene location.

We assume that the probabilty of sharing at a disease locus has a logistic regression on covariates. Given a set of markers, the likelihood of the data can be expressed in terms of the location of the disease locus, the logistic coefficients, the sharing at the marker loci, and the recombination fractions derived from the map distances. Maximization of the likelihood provides estimates of disease gene location and logistic coefficients. Setting all the coefficients to zero (including the intercept) provides a test of linkage. Setting relevent subsets of coefficients to zero tests the importance of the individual covariates.

We use a version of MAPMAKER/SIBS modified by Jane Olson to dump IBD sharing probabiities with respect to the father and mother separately. This file serves as input to our program COVLINK, available upon request.

We discuss methodologic issues including the use of an ordinal phenotype (unaffected, mild, or severe), pooling of data from multiple linkage studies, and the conditioning on genotype at a particular locus as a covariate.

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Program Nr: 222 from the 1999 ASHG Annual Meeting

A major locus for vesicoureteric reflux and its nephropathy. *S. Malcolm[1], S. Feather[1,2], A.S. Woolf[2], V. Wright[1], D. Blaydon[1], C.J.D. Reid[3], F.A. Flinter[3], W. Proesmans[4], K. Devriendt[4], P. Warwicker[5], T.H. Goodship[5], J.A. Goodship[5].*
1) Clinical and Molecular Genetics Unit, Inst Child Health, London, England; 2) Nephrourology Unit, Institute of Child Health, London; 3) Division of Medical and Molecular Genetics, UMDS, London; 4) Dept of Medicine, University of Newcastle, Newcastle upon Tyne; 5) Centre for Human Genetics, Leuven, Belgium.

Primary vesicoureteric reflux (VUR) affects 1-2% of the Caucasian population and its associated reflux neuropathy (RN)causes up to 15% of chronic renal failure in children and adults. Genetics may play a role in the pathogenesis of VUR because of a 30-50 fold increased risk in immediate relatives of probands versus the general population. We now report the results of the first genome-wide search of VUR and RN in 7 European families with 5-8 affecteds who have an apparent dominant inheritance pattern. We used 350 polymorphic markers spaced at 10cM through the genome with affecteds-only parametic and non-parametric linkage analyses with the Genehunter programme. We identified 10 loci with $p<0.05$. The most significant spans 20cM on chromosome 1 between markers GATA176C01 and D1S1653 and has a non-parametric linkage score of 5.76 ($p=0.0002$). This same region was significant in analyses for VUR alone or RN alone. Thus lower tract malformations and kidney disease may have identical genetic determinants. Of note, no signifcant linkage was found to 6p, where a renal and ureteric malformation locus was previously reported, or to 10q where PAX2 mutations cause VUR in renal-coloboma syndrome. Our results support the hypothesis that VUR and RN are genetic disorders:work is now needed to define the mutation.

Program Nr: 223 from the 1999 ASHG Annual Meeting

Identification of a coding mutation in the norepinephrine transporter gene which predisposes a family to Orthostatic Intolerance. *N.L. Flattem[1], J.R. Shannon[1], J. Jordan[2], G. Jacob[3], I. Biaggioni[1], J.L. Haines[1], R.D. Blakely[1], D. Robertson[1]*. 1) Vanderbilt University , Nashville, TN; 2) Franz Volhard Clinic, Berlin, Germany; 3) Rambam Medical Center, Haifa, Israel.

The norepinephrine (NE) transporter (NET) is believed to play an important role in the inactivation of NE released at noradrenergic synapses in the CNS and PNS. Although drugs that act at NETs such as cocaine, amphetamines and tricyclic antidepressants exert a profound effect on systemic physiology and behavior, no alterations in hNET have as yet been linked to brain or autonomic disorders. Recently, defects in peripheral NE clearance, NET-dependent, tyramine-induced NE efflux and adrenergic receptor sensitivity have been recognized in some individuals with Orthostatic Intolerance (OI). OI patients, in general, exhibit markedly elevated heart rates upon standing, diminished cerebral perfusion, syncope and chronic fatigue that improves upon lying down. Given the prominent role of the NET in regulation of heart rate, blood flow and NE inactivation, we examined the NET gene of one proband for evidence of mutations that might disrupt NET expression or activity. We identified a coding mutation in the proband which converts a highly conserved transmembrane domain Ala residue to Pro. Analysis of the protein produced by the mutant cDNA in transfected cells demonstrates a >98% reduction in activity relative to the original NET isolated. Studies of the proband and her family reveal correlations of plasma NE, DHPG/NE and heart rate with the mutant allele. Our findings represent the first identification of a specific defect in OI and the first disease linked to a coding alteration in Na+/Cl- dependent neurotransmitter transporters. This research is supported by NIH award MH58921 to R.D.B. and awards PO1 HL56693 and NASA 9 19483 to D.R.

Program Nr: 224 from the 1999 ASHG Annual Meeting

Characterization of cirrin, a new extracellular protein gene that is associated with endocardial cushion defects in 3p- syndrome. *C.L. Maslen[1], P.A. Rupp[1], S.B. Olson[1], C.A. Reifsteck[1], K.L. Thornburg[1], R.W. Glanville[2]*. 1) Dept Molec/Med Genetics, Heart Research Center, Oregon Health Sci Univ, Portland, OR; 2) Shriners Hospital for Children, Portland, OR.

We have isolated and characterized a new gene that encodes a protein we have named cirrin. This highly conserved 45 kDa protein has some structural domains that are found in extracellular matrix proteins, leading us to speculate that this protein may be a previously uncharacterized component of the extracellular matrix (ECM). Experimental data demonstrates that cirrin is indeed a matrix associated extracellular protein, although biochemical analyses suggest it is not likely to be a structural component of the ECM. Its function is as yet unknown and lack of extensive similarity to other known proteins indicates that it is not a member of a recognized protein family. Characterization of cirrin has included studies of expression in early development. Whole mount in situ hybridization of chick embryos showed particularly high levels of expression in the endocardial cushions, atrial muscle and atrial and ventricular myocardium of the developing heart indicating that cirrin is involved in cardiac development, particularly valvuloseptal morphogenesis. Mapping of the cirrin gene to human chromosome 3p24.2-25 was additionally suggestive since this placed the cirrin locus in the region of breakpoints found in terminal deletions of the short arm of chromosome 3 (3p- syndrome), which is sometimes associated with cardiac septal/endocardial cushion defects. Examination of cell lines derived from children with 3p- syndrome using fluorescence in situ hybridization showed that the cirrin gene is deleted in 3p- syndrome with associated congenital heart defects, but is not deleted in cases of 3p- syndrome lacking a cardiac malformation. Together these data implicate cirrin as an important player in cardiac development and as a candidate gene for heart defects in 3p- syndrome and non-syndromic cardiac septal/endocardial cushion defects.

Program Nr: 225 from the 1999 ASHG Annual Meeting

Betwixt mouse and man: A chick model of DS heart disease. *J.R. Korenberg[1], G.M. Barlow[1], T. Person[2,4], I.N. Jongewaard[2,3], S.E. Klewer[2,3], R.B. Runyan[2,4].* 1) Medical Genetics, Cedars-Sinai Medical Center, Los Angeles, CA; 2) Sarver Heart Center, University of Arizona, Tucson, AZ; 3) Department of Pediatrics, University of Arizona, Tucson, AZ; 4) Department of Cell Biology and Anatomy, University of Arizona, Tucson, AZ.

DS-CAM (Down syndrome cell adhesion molecule), is located on human chromosome 21 within the region defined for DS congenital heart defect (CHD). It is a novel member of the Ig superfamily with a proposed role in cell-cell and potentially cell-matrix interactions. In view of the endocardial cushion defects observed in DS and the abnormal adhesiveness of cardiac fibroblasts derived from DS hearts, our hypothesis is that overexpression of DS-CAM in infants with DS results in abnormal cell adhesiveness and causes aberrant development of the atrioventricular (AV) cushions, septae and valves. We have generated a fragment representing the first Ig domain of the avian DS-CAM homologue (cDS-CAM) by using RT-PCR amplification of whole embryonic chick mRNA in a nested primer approach. Sequence analysis of this portion of the chick cDNA demonstrates 93% nucleotide; and 98 % amino acid identity to human DS-CAM, respectively. Using specific primers, cDS-CAM mRNA is detected in the embryonic chick heart beginning at Stage 16 and persists through Stage 35. We have examined the expression and functional role of cDS-CAM in the collagen gel system, an in vitro avian model of AV endocardial morphogenesis. Perturbations with five independent cDS-CAM anti-sense oligonucleotides, but not with scrambled or unrelated sequence control oligonucleotides, disturbed migration of mesenchymal cells derived from the AV cushions. Thus far we have demonstrated that: 1) cDS-CAM, the chick homolog for the candidate gene for CHD in infants with DS, is normally expressed in the embryonic chick heart during the period of AV valve development and remodeling; and 2) Decreased expression of DS-CAM due to antisense oligonucleotides produces defects in mesenchymal migration as proposed for DS-CHD. Ongoing studies will determine the molecular requirements and phenotypic effects of DS-CAM during AV canal morphogenesis.

Program Nr: 226 from the 1999 ASHG Annual Meeting

Genome-wide scan for familial combined hyperlipidemia loci. *P.E Pajukanta[1,2], J.D. Terwilliger[3], C. Ehnholm[2], M. Laakso[4], J.S.A. Viikari[4], M.-R. Taskinen[4], L. Peltonen[1,2].* 1) Human Genetics, UCLA, Los Angeles, CA; 2) National Public Health Institute, Finland; 3) Columbia University, Dept of Psychiatry and Columbia Genome Center, New York; 4) Depts of Medicine, Universities of Helsinki, Turku, and Kuopio, Finland.

Familial combined hyperlipidemia (FCHL) is a frequent genetic dyslipidemia predisposing to premature coronary heart disease (CHD). This complex disease, characterized by elevated levels of serum total cholesterol (TC), triglycerides (TG), or both, is most likely caused by interactions of multiple genes and environmental factors. With a prevalence of 1-2%, FCHL represents a major health problem exposing a significant number of affected individuals to premature CHD who remain poorly identified due to the lacking knowledge of the etiology. We recently localized the first FCHL locus on chromosome 1q21-q23 (Nat Genet 1998) and are now fine-mapping and restricting this region with the help of the syntenic Hyplip1 mouse region defined by Castellani et al. (Nat Genet 1998). To identify additional loci, we performed a genome-wide scan. In stage 1, we genotyped 368 markers in 35 clinically well-characterized FCHL families originating from the genetically isolated Finnish population. In stage 2, 26 more markers and seven additional FCHL families for the regions revealing lod scores higher than 1 were genotyped. Four relevant lipid traits, the FCHL and the different component traits, TC, TG, and apolipoprotein B (apoB), were analyzed using both linkage and affected sib-pair analysis. In this first completed FCHL genome scan we identified four novel loci for the component traits in addition to the FCHL locus on chromosome 1. For TGs, the chromosomal region 10p11.2 resulted in a lod score of 3.20 and 2q31 in a lod score of 2.25; for TC, 10q11.2-10qter produced a lod score of 2.59; and for apoB, 21q21 a lod scores of 2.24, respectively. Our data propose a hypothesis that these four loci would modify the TG, TC, and apoB traits and in combination with the FCHL locus on 1q21-q23 result in the complex FCHL phenotype. We are currently fine-mapping and tackling the candidate genes of all five regions to identify at least one FCHL gene.

Program Nr: 227 from the 1999 ASHG Annual Meeting

Generating a mouse model for Velo-Cardio-Facial Syndrome (VCFS). *R.S. Kucherlapati, S. Merscher, A. Puech, H. Sirotkin, B. Saint-Jore, A. Skoultchi.* Dept Molecular Genetics, Albert Einstein Col Medicine, Bronx, NY.

Velo-cardio-facial syndrome (VCFS) is a human developmental disorder that is characterized by heart defects, facial dysmorphology, cleft palate and learning disabilities. Most VCFS patients are hemizygous for a 3 Mb region on chromosome 22q11. Analysis of deletions in a large number of patients allowed the definition of a critical region that is 500 Kb in length. To fully understand the molecular mechanisms that lead to the complex phenotypes associated with VCFS, we generated mice that are hemizygous for a part of mouse chromosome 16 (MMU16) that has homology to human 22q11. The deletion spans a 500 Kb region and encompasses at least 12 genes. The deletion has the Idd gene as one of its boundaries and the Arvcf gene as its other boundary. To generate the deletion in mouse embryonic stem cells, a loxP site was introduced into each of Idd and Arvcf by gene targeting. We generated ES cells in which the two loxP sites were in cis or in trans. The cells were transfected with CAG-cre or CMV-cre and cells containing a deletion or deletion/duplication were selected using a new selectable system. A deletion of the same region was also obtained in vivo by mating mice carrying the Idd and Arvcf mutant alleles in cis with mice carrying a CMV- or Zp3-cre gene. Mice that are hemizygous for the deletion or that are partially trisomic are viable and appear normal. Mice that are homozygous for the deletion die during embryogenesis. The cause of this embryonic lethality and its relationship to VCFS associated phenotypes are being investigated.

Program Nr: 228 from the 1999 ASHG Annual Meeting

A genome scan and array-based candidate gene analysis identify loci for hand osteoarthritis. *J. Leppavuori[1], T. Pastinen[1], U. Kujala[2], J. Kaprio[3], J. Lohiniva[4], L. Ala-Kokko[4], L. Peltonen[1].* 1) Dept Human Molecular Genetics, NPHI, Helsinki, Finland; 2) Unit for Sports and Exercise Medicine, Institute of Biomedicine, University of Helsinki, Finland; 3) Dept Public Health, University of Helsinki, Finland; 4) Dept Medical Biochemistry, University of Oulu, Finland.

We have performed a genome scan for loci predisposing to distal interphalangeal joint osteoarthritis, a form of OA with the strongest genetic component, and found a locus on chromosome 2q. The intragenic marker of the interleukin 1 receptor type I gene (IL1R1) gave a maximum pairwise lod score of Zmax=2.34 (q=0, dominant mode). Multipoint analysis (SIMWALK 2.40, statistics B) yielded a p-value of 0.0001 near the locus IL1R1 and a p-value of 0.0007 ~20 cM telomeric near the marker D2S1399 and also provided evidence for two additional loci on 4q26-q27 (p=0.0001) and 7p15-p21 (p=0.001). As an extension of the scan, we have arrayed 43 intragenic SNP:s of 22 candidate genes for osteoarthritis, selected based on their relevant roles in cartilage structure or metabolism, earlier evidence of linkage or association to OA, and also the availability of established SNPs, identified either from the SNP-databases or based on sequencing data. From 2 to 4 SNP:s within each candidate gene were selected for the first-stage array, preferring potentially functional polymorphisms. When allelic frequencies were known, we typically selected both common and rare SNP:s within a gene for analysis. The DNA-microarrays were produced using a printing robot, and detection primers are immobilized on the silica surface. Our current procedure includes multiplex amplification of genomic DNA in 10-15l reaction volumes. Prior to microarray-genotyping the amplified templates are in vitro transcribed to RNA. The genotyping reaction is carried out using reverse transcriptase enzymes, fluorescent labeling and internal quality control for each spot. The reading is semiautomated using a commercial array-scanner with spot identification and quantitation software. The simultaneous analysis of multiple polymorphisms will allow the estimation of the effect of epistasis on the disease phenotype, intriguing in a complex disease like OA.

Program Nr: 229 from the 1999 ASHG Annual Meeting

A mutation in the human cannabinoid receptor (CNR1)gene in patients with Gilles de la Tourette syndrome. *M. Stuhrmann[1], D. Gadzicki[1], K. Mueller-Vahl[2].* 1) Inst Humangenetik; 2) Abt Klin Psychiatrie und Psychotherapie, Med Hochschule Hannover, Hannover, Germany.

Gilles de la Tourette syndrome (GTS) is a neuropsychiatric disorder of unknown etiology, characterized by multiple, fluctuating motor and phonic tics. The central cannabinoid receptor (CB1) mediates the pharmacological activities of cannabis, the endogenous agonist anandamide and several synthetic agonists. The successful treatment of GTS with D^9-tetrahydrocannabinol (THC), the major psychoactive ingredient of marijuana (Cannabis sativa) prompted us to screen the human cannabinoid receptor (CNR1) gene for mutations, which may predispose to GTS. In 3 out of 40 unrelated GTS patients we identified a single base substitution (G®T) in the proximal 3' untranslated region of the CNR1 gene immediately downstream from the stopcodon TGA. This mutation 1419+1G®C (numbering according to Gerard et al., 1990) was not present in 81 healthy German blood donors. In one family, 1419+1G®C was present in both the 8 years old female patient and her mother, who has a milder form of GTS. The second GTS patient (male, 17 years) with 1419+1G®C has a monozygotic twin, who is also affected and carries the mutation. No family members from the third patient (female, 39 years) had been investigated to date. Because of the statistically significant ($p<0.05$) association and cosegregation with GTS, we assume that the mutation 1419+1G®C in the CNR1 gene represents one of several, to date unknown predisposing factors in the etiology of GTS. Functional consequences of 1419+1G®C should be studied in brain tissue, since our RT-PCR analysis on lymphocyte CNR1-mRNA from all 5 mutation carriers and 5 healthy controls did not reveal any obvious influence of 1419G®C on mRNA stability or on aberrant splicing.

Program Nr: 230 from the 1999 ASHG Annual Meeting

Cloning of a candidate gene (ARG1) from the breakpoint of t(7;20) in an autistic twin pair. *R. Sultana[1], J. Yu[2], W. Raskind[3], C. Disteche[4], F. de La Barra Monsalvo[5], E. Villacres[1].* 1) Depts of Psychiatry; 2) Molecular Biotechnology; 3) Medicine; 4) Pathology, Univ of Washington, Seattle, WA; 5) Cytogenetics, Univ of Chile, Inta Santiago, Chile.

Autism is a pervasive developmental disorder characterized by markedly impaired social interaction and communication and a restricted repertoire of activity and interests. Evidence from sibling and twin studies supports a genetic basis for this disorder. A genome-wide search for susceptibility loci in autism has identified a region of interest on the long arm of chromosome 7 (International Molecular Genetic Study of Autism Consortium, Hum Mol Genet 7:571-578, 1998). In addition, several structural rearrangements of chromosome 7 have been observed in autistic individuals. These rearrangements include translocations involving 7q11.2 and 7q22 (Gordon et al., 1994; de la Barra et al., 1986) and a paracentric inversion (inv(7)(q22q31.2) (Pericak-Vance et al., 1998). Since structural chromosomal abnormalities can greatly facilitate the localization and cloning of disease causing genes, we studied a concordant autistic twin pair who have translocations involving chromosome band 7q11.2 (t(7;20)(q11.2;p11.2)). Using STS-content mapping and fluorescence in situ hybridization, a BAC contig of 7q11.2 was generated, and two BAC clones spanning the breakpoint of the translocation between chromosomes 7 and 20 present in our autistic twins were identified. Partial sequencing of these BACs and analysis of the available genomic DNA sequence revealed a gene, Autism-Related Gene1 (ARG1), that crosses the chromosome 7q11.2 breakpoint. Partial cDNA sequencing shows that ARG1 is a novel gene and spans a genomic region estimated to be at least 800 kb. Northern analysis showed that the ARG1 transcript is 7.5 kb and is highly expressed in adult and fetal brain. It is also expressed in lung, bladder, ovary, and uterus. The disruption of ARG1 in autistic twins suggests that it is a strong candidate gene for autism.

Program Nr: 231 from the 1999 ASHG Annual Meeting

A full genome scan for late onset Alzheimers disease. *J. Williams[1], F. WavrantDe Vrieze[2], R. Crook[2], P.G. Kehoe[1], W.S. Wu[3], A. Myers[3], I. Fenton[1], F. Rice[1], R. Wood[1], P. Holmans[3], S. Lovestone[4], N. Tunstall[4], J. PerezTur[2], M. Hutton[2], S. Shears[3], K. Roehl[3], J. Booth[3], J. Hardy[2], A. Goate[3], M.J. Owen[1].* 1) Neuropsychiatric Genetics Unit, Tenovus Building, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN, UK Dept Psychological Medicine, University Wales Col Medicine, Cardiff,S Glam, Wales; 2) Birdsall Building, Mayo Clinic, 4500 San Pablo Road, Jacksonville, FL32084,USA; 3) Departments of Psychiatry and Genetics, Washington University School of Medicine, 4940 Childrens Place, St Louis, MO 63110, USA; 4) Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF,UK.

We have genotyped 514 affected sibling pairs (ASPs) with probable/definite Alzheimers disease (AD) according to NINCDS-ADRDA diagnostic criteria with onsets above 65 years. The genome scan comprised two stages. In the first 292 ASPs were genotyped using 237 microsatellite markers separated by an average distance of 16.3cM. 16 peaks with a multipoint lod score (MLS) greater than 1 either in the whole sample, the e4 - positive or negative subgroups were observed on chromosomes 1 (two peaks) 2, 5, 6, 9 (two peaks), 10 (two peaks), 12, 13, 14, 19, 21 and X (two peaks). Simulation studies revealed that these findings exceeded those expected by chance, although many are likely to be false positives. The highest lod scores on chromosomes 1 (MLS 2.67), 9 (MLS 2.38), 10 (MLS 2.27) and 19 (MLS 1.79) fulfil Lander and Kruglyaks definition of suggestive linkage. In stage two we genotyped the 16 regions showing an MLS of greater than 1 in an independent sample of 222 ASPs, using the same markers as in stage one. Combined analysis of these data showed increased evidence for linkage in a number of regions, with at least two loci producing lod scores in excess of 3. These data will be presented in detail.

Program Nr: 232 from the 1999 ASHG Annual Meeting

A novel major psoriasis susceptibility locus identified in families of German origin . *A. Reis[1,3], Y.A. Lee[1], F. Rueschendorf[1], C. Windemuth[2], M. Schmitt-Egenolf[3], A. Brinkmann[4], M. Staender[5], T.F. Wienker[2], H. Traupe[4].* 1) Mol. Genetics & Mikrosatellitz., Max-Delbrueck-Centrum, Berlin, Germany; 2) Inst. of Medical Statistics, University of Bonn, Germany; 3) Dept. of Dermatology, and Inst. of Human Genetics, Charité, Humboldt University, Berlin, Germany; 4) Dept. of Dermatology, University of Muenster, Muenster, Germany; 5) Dept. of Dermatology, Fachklinik, Bad Bentheim, Germany.

Psoriasis vulgaris is a common chronic inflammatory skin disease in which genetic factors play a major etiological role. Previous studies have identified various chromosomal regions harboring susceptibility loci, mainly in the MHC region on 6p (PSORS1), 17q (PSORS2) 4q (PSORS3) and 1q . We now performed a genome-wide linkage scan based on 350 microsatellite markers in 244 individuals belonging to 21 extended families comprising 111 members diagnosed as having the disease. Analysis of all families using non-parametric methods (GENEHUNTER) gave only tentative evidence for linkage to PSORS1 on chromosome 6p (NPL =2.1, p=0.03), which is similar to that obtained in two previous genome scans. In contrast, we identified a novel susceptibility locus on proximal chromosome 19p (D19S 916, NPL = 4.1, p = 0.001), and suggestive regions on chromosomes 10q (D10S543, NPL= 2.9, p=0.007), 13q (D13S1316, NPL = 2.7, p = 0.01) and 8p (D8S277, NPL=2.5, p=0.01). No strong evidence for linkage was seen to any of the other previously described psoriasis susceptibility loci. Analysis of individual families demonstrated that the NPL peak on chromosome 10q mainly traces back to one large family with 8 affected persons. This family shows additional evidence for linkage to 8cen and distal 17q (PSORS2), with all 8 affected but none of the unaffected persons sharing the an identical haplotype on both chromosomes, compatible with a two locus hypothesis. In conclusion, our data confirms that familial psoriasis is highly heterogeneous and that at least in families of German extraction a major susceptibility locus for familial psoriasis is located on chromosome 19p.

Program Nr: 233 from the 1999 ASHG Annual Meeting

A mutation map of Finland: Array-based screening of Finnish mutations in population based samples. *T. Pastinen[1], P. Tainola[1], A-C. Syvanen[2], L. Peltonen[1,3].* 1) Hum Mol Gen, Natl Public Health Inst, Helsinki, Finland; 2) Mol Med, Uppsala Univ Hosp, Uppsala, Sweden; 3) UCLA Dept Hum Gen, LA, CA.

Finnish disease heritage refers to a group >30 rare mainly recessively of inherited diseases which are more prevalent in Finland than in other populations. In many of these diseases the defective gene and underlying mutation has been found and reveals a strong founder effect in all cases. The epidemiology of these diseases is characteristic for the population history of Finns as many disorders have clustered to specific regions of Finland due to regional population bottlenecks.The carrier prevalence studies of Finnish disease heritage and other recessive inherited diseases in Finland have been restricted to a subset of the diseases and have been carried out in the general population. In this study we have collected large number of samples representing not only the general Finnish population but also samples from distinct geographical regions representing internal isolates. A panel of 30 mutations was selected to be analyzed from these samples, which included major Finnish mutations accounting for: 15 Finnish disease heritage diseases, 9 recessive diseases prevalent in Caucasian populations and 2 polymorphisms causing susceptibility for venous thromboembolism. The generation of the nearly 100.000 genotypes was carried out by a novel DNA-array genotyping method, in which in-house produced arrays are used in DNA-polymerase mediated allele-specific extension reaction with fluorescently labelled dNTPs. This procedure provides for good discrimination of normal versus carrier individuals. All putative carriers were further subjected to verification in a reference reaction. Our results show that more than 25% of the general population carries one mutation included in our panel, the carrier prevalence varies significantly in different regions of the country and that certain Finnish disease heritage mutations have a distinct geographic pattern of occurrence. This work exemplifies the unique genetic features of the Finnish population and the use of new DNA-array tools to study genetic variation in large scale. It also provides basic information for targeted carrier screening programs.

Program Nr: 234 from the 1999 ASHG Annual Meeting

Clinical phenotype and outcome of mitochondrial acetoacetyl-CoA thiolase deficiency (beta-ketothiolase deficiency) in 26 patients. *T. Fukao[1], C.R. Scriver[2], N. Kondo[1]*. 1) Dept Pediatrics, Gifu Univ Sch Medicine, Gifu, Japan; 2) McGill University-Montreal Children's Hospital Research Institute, Montreal, Canada.

Mitochondrial acetoacetyl-CoA thiolase (T2) or 3-oxo thiolase (EC 2.3.1.4) deficiency is an autosomal recessive disorder of isoleucine and ketone body metabolism (OMIM 203750). The main clinical manifestations were intermittent ketoacidotic attacks and coma, sometimes followed by developmental delay. Only a few reports described long-term follow-up and outcome of this disorder. We have now analyzed the molecular basis of T2 deficiency in 26 enzymatically-proved patients and characterized most of the disease-causing mutations. Expression analysis of mutant cDNAs revealed that 7 patients have mutations which retain some residual T2 activity; 19 patients have null mutations; the former could have more mild clinical phenotypes, the latter more severe forms. We then analyzed correlations between in vivo clinical severity and genotype by a questionnaire. Genotype does not predict clinical severity; however low or absent urinary excretion of triglycine during ketoacidosis may predict a "mild" genotype. We found that T2 deficiency has a favorable outcome in general; most patients developed normally. One patient died (during the first attack); and two showed developmental delay. The frequency of ketoacidotic attacks fell as age increased; the last ketoacidotic attack in this cohort of patients was at 10 years of age; 11 patients had only one episode and 3 patients had none. This finding suggests that ketoacidosis in T2 deficiency is avoidable with diagnosis. Our study reveals the clinical importance of acute care management during early postnatal life. [We thank more than 20 collaborating physicians and investigators for their contribution to this work].

Program Nr: 235 from the 1999 ASHG Annual Meeting

Effects of fetal genotype on pregnancy outcome and validity of molecular prenatal diagnosis in families with mutations in mitochondrial trifunctional protein. *J.A. Ibdah[1], M.J. Bennett[2], Y. Zhao[1], B. Gibson[3], A.W. Strauss[3]*. 1) Wake Forest Univ Sch Medicine, Winston-Salem, NC; 2) Univ Texas Southwestern Med Center, Dallas, TX; 3) Washington Univ Sch Medicine, St. Louis, MO.

Mitochondrial trifunctional protein (TFP) consists of 4 a and 4 b subunits that catalyze the final 3 steps of long chain fatty acid oxidation. Most of the recessively inherited mutations in TFP cause an isolated deficiency of long chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD). Others cause complete TFP deficiency (all 3 enzymes are deficient). Recently, we characterized the genotypes and phenotypes in pediatric TFP defects and documented an association with maternal liver disease (Ibdah et al, N Engl J Med 1999; 340:1723-31). To further study the effects of fetal mutations in TFP on pregnancy outcome, we analyzed the pregnancy history and the offspring genotypes in 31 families with documented TFP mutations (26 isolated LCHAD-deficiency, 5 complete TFP deficiency). In 75 pregnancies, 8 had spontaneous abortions with unknown fetal genotype and 2 had induced abortions with affected fetal genotype. 21 completed pregnancies were complicated by a maternal illness (13 with acute fatty liver of pregnancy, 8 with HELLP syndrome and preeclampsia). All these complicated pregnancies were associated with affected fetal genotype. All pregnancies associated with wild-type or heterozygote fetal genotype were uncomplicated. Affected fetal genotype was also associated with premature delivery (21 pregnancies) and low birth weight (10 pregnancies). Molecular prenatal diagnosis was performed in 9 pregnancies (7 families) using single strand conformation variance and nucleotide sequence analysis on chorionic villous samples. Two fetuses were affected, and both pregnancies were terminated. Molecular analysis and enzymatic assays on the aborted fetuses confirmed the diagnosis. Two other fetuses had normal genotype and 5 others were heterozygotes. All 7 pregnancies were uncomplicated and all the offspring are alive and apparently healthy. These results document that fetal genotype affects pregnancy outcome in families with TFP mutations and demonstrate the validity of molecular prenatal diagnosis in these families.

Program Nr: 236 from the 1999 ASHG Annual Meeting

Broadening the spectrum of fetal fatty acid b**-oxidation (FAO) disorders causing liver disease in pregnant women.** *D. Matern[1], P. Hart[1], A.P. Murtha[2], G. Vockley[3], N. Gregersen[4], D.S. Millington[1], W.R. Treem[1].* 1) Dept. of Pediatrics and; 2) Dept. of Obstetrics & Gynecology, Duke University Medical Center, Durham, NC; 3) Dept. of Medical Genetics, Mayo Clinic, Rochester, MN; 4) Research Unit for Molecular Genetics, Aarhus University, Aarhus, Denmark.

The hypothesis that fetal long-chain 3-OH-acyl CoA dehydrogenase deficiency (LCHAD-D) causes severe pregnancy complications such as acute fatty liver of pregnancy (AFLP) has been proposed repeatedly by means of case reports since 1991. Recently, a larger retrospective case study postulated a correlation between the common LCHAD mutation (E474Q) and the obstetric complications (Ibdah et al. NEJM 1999;340:1723-31). Based on these publications molecular genetic analysis has been recommended for families with a history of such pregnancy complications to rule out the carrier status for the E474Q mutation. We report a patient who was diagnosed with another FAO disorder, short-chain acyl-CoA dehydrogenase deficiency (SCAD-D), and born following a pregnancy complicated with severe AFLP. The patient is the 1st child of unrelated parents. His mother developed severe AFLP during the 36th week of gestation prompting emergent C-section delivery of the male newborn. The infant was well, but work-up to rule out LCHAD-D revealed findings suggestive for SCAD-D (ethylmalonic aciduria, elevated plasma short-chain acylcarnitines). Molecular genetic analysis ruled out the presence of the common LCHAD mutation, but revealed homozygosity for a polymorphism (G625A) within the SCAD gene. Biochemical genetic analysis demonstrated SCAD deficiency in fibroblasts. The 1-year old patient is doing well on riboflavin supplements and avoidance of fasting, but required one hospitalization for IV glucose treatment due to hypoglycemia during an intercurrent gastroenteritis. This report suggests that not only LCHAD-D requires consideration in children born following such complicated pregnancies. We believe that any fetal FAO disorder poses a risk to the pregnant, obligate heterozygous mother to develop preeclamptic complications. Therefore, evaluation of the newborn and the parents should NOT be limited to the search for the common LCHAD mutation.

Program Nr: 237 from the 1999 ASHG Annual Meeting

Mitochondrial DNA mutations associated with neonatal hemochromatosis. *M.D. Brown[1], D. Chitayat[2], J. Allen[1], S. Hosseini[1], J. Litten[1], R. Babul-Hirji[2], D.C. Wallace[1].* 1) Center for Molecular Medicine, Emory University School of Medicine, Atlanta, GA; 2) Dept. of Genetics, The Hospital for Sick Children, Toronto, ON, Canada.

Neonatal hemochromatosis (NH) is a heterogeneous disorder of prenatal onset which is characterized by systemic iron accumulation, ultimately leading to severe hepatic disease. Although NH typically manifests as sporadic cases, there are several reports in the literature of mothers who had NH infants by different partners. Both of theses familial presentations are common in disorders due to mitochondrial DNA (mtDNA) mutations. Also, neonatal/early onset hepatic failure has been associated with oxidative phosphorylation defects. To test the hypothesis that mtDNA mutations can contribute to NH, we performed mtDNA sequence analysis on two families in which siblings (Family 1) or half-siblings (with a common mother; Family 2) exhibited NH. Family 1 contained a novel, homoplasmic, G to A missense mutation at nucleotide position (np) 13711 which changed an alanine to a threonine in the ND5 subunit of complex I. Family 2 harbored a T to C transition at np 9957 which converted a highly conserved phenylalanine to a leucine in the COIII subunit of complex IV. To determine if the 9957 mutation was heteroplasmic, we cloned the mtDNA from the mother of Family 2 and found low levels (roughly 2%) of wild-type mtDNA. Control cloning experiments suggested that this heteroplasmy was not due to PCR-generated artifact during the cloning process. Neither the np 13711 nor the np 9957 mutations have been found in control mtDNAs. The np 9957 mutation has, however, been reported to be heteroplasmic in a patient with early onset, progressive, mitochondrial encephalomyopathy. Thus, the np 9957 mutation has only been found in the heteroplasmic state and only in association with disease. This suggests that at least this mutation may contribute to the heterogeneous etiology of NH, potentially providing the first direct linkage between a mtDNA mutation and liver disease.

Program Nr: 238 from the 1999 ASHG Annual Meeting

CARBOHYDRATE-DEFICIENT GLYCOPROTEIN SYNDROME: REPORT OF 23 PATIENTS. *P. de Lonlay[1], N. Seta[3], S. Barrot[3], M. Cuer[3], G. Durand[3], G. Matthijs[4], J. Jaeken[4], A. Munnich[2], J.M. Saudubray[1], V. Cormier-Daire[2]*. 1) Departement de Pediatrie, Hopital Necker-Enfants Malades, Paris; 2) Departement de Genetique, Hopital Necker-Enfants Malades, Paris; 3) Service de Biochimie A, Hopital Bichat,Paris; 4) University Hospital Gasthuisberg, Leuven.

Carbohydrate-deficient glycoprotein syndrome (CDGS) is characterized by defects in glycoprotein biosynthesis. We report twenty-three patients with CDGS, including eighteen patients with CDGS type Ia (phosphomannomutase deficiency), two patients with CDGS type Ib (phosphomannose isomerase deficiency), two patients with CDG type I but normal phosphomannomutase and phosphomannose isomerase activities and one patient with CDGS type II (N-acetylglucoaminyl transferase II deficiency). In the type Ia, two distinct clinical presentations were recognized based on the predominant clinical feature, namely a neurological form with psychomotor retardation, cerebellar hypoplasia and retinis pigmentosa (n=7) and a multivisceral form (n=11) including cardiac, renal, liver or gastrointestinal involvement. Dysmorphic features, inverted nipples and abnormal subcutaneous fat were not constantly observed in the multivisceral form. Recurrence in sibs was observed in 3 families and within the same family, clinical presentation was homogeneous(hepatic failure, pericarditis, neurological form). The R141H mutation was present in 14/15 type Ia patients and the genotype R141H/I132T was found in 4/7 patients presenting with the neurological form. The two patients with CDGS type Ib presented with liver disease, enteropathy and hyperinsulinaemic hypoglycemia without neurological manifestation. One was successfully treated with oral mannose administration. The two patients with CDGS type I unclassified presented with psychomotor retardation and retinitis pigmentosa. The type II patient had psychomotor retardation associated with severe diarrhea, dysmorphic features and abnormal electroretinogram. The diagnosis of CDGS should be called to mind when dealing not only with neurological symptoms but also with multivisceral involvement. The successfull treatment of the type Ib patient emphazises the importance of making the diagnosis of PMI deficiency.

Program Nr: 239 from the 1999 ASHG Annual Meeting

Phenotypic correlation and diagnosis of urea cycle disorders with stable isotope infusions. *B. Lee[1], H. Yu[2], F. Jahoor[2], W. O'Brien[1], A.L. Beaudet[1], P. Reeds[2].* 1) Dept of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX; 2) Dept of Pediatrics and CNRC, Baylor College of Medicine, Houston, TX.

Urea cycle disorders are a group of inborn errors of hepatic metabolism in which enzymatic deficiencies result in often life-threatening hyperammonemia. Clinical and laboratory diagnosis is often difficult during asymptomatic periods or in cases of partial deficiencies. Moreover, correlation of phenotypic severity with either genotype and/or in vitro enzyme activity is often imprecise or unavailable. We report here specific correlations of rates of total body urea synthesis, urea cycle-specific nitrogen flux, and glutamine flux, with phenotypic severity and carrier status in urea cycle patients, as well as with the effects of increased protein intake and pharmacotherapy aimed at activating alternative waste-nitrogen excretion. For controls, we studied 18 adult and pediatric subjects. In our urea cycle patient cohort, we studied a total of 32 patients and relatives with different enzymatic deficiencies. All subjects were studied twice, first on a low protein diet, and then again on either higher protein intake, on treatment with an alterative route medication (Ucephan), or arginine supplementation. After stabilization on the assigned protein intake, flux measurements were determined from ^{15}N-urea, ^{18}O-urea, and ^{15}N-glutamine enrichments measured in blood during intravenous co-infusions of 5-^{15}N-glutamine and ^{18}O-urea. The ratio of ^{15}N-urea/^{15}N-glutamine distinguished: 1) normal control subjects, 2) late presenting urea cycle patients with partial urea cycle activity, and 3) patients who presented in the neonatal period ($p<0.001$). Moreover, this index distinguished heterozygous carrier parents of citrullinemia patients from normal controls. In subjects studied on medications, the absolute rate of total body urea production directly correlated with molar conversion of medications used to treat these patients. The ^{15}N-urea/^{15}N-glutamine ratio is a sensitive index of in vivo urea cycle activity, correlates with clinical everity and is a sensitive tool for evaluating efficacy of therapeutic modalities as well as for the diagnosis and management of urea cycle patients.

Program Nr: 240 from the 1999 ASHG Annual Meeting

A novel carbohydrate-kinase gene on chromosome 17p13, contiguous with *CTNS*, is homozygously deleted in approximately half the patients with Nephropathic Cystinosis (NC). *Y. Anikster[1,5], J.W. Touchman[2], E. Orvisky[3], D. Krasnewich[4], E.D. Green[2], W.A. Gahl[1].* 1) HDB, NICHD; 2) NIH Intramural Sequencing Center, NHGRI; 3) NSB, NIMH; 4) MGB, NHGRI; 5) HHMI-fellow, NIH, Bethesda, MD.

NC is an autosomal recessive lysosomal storage disease. The cystinosis gene, *CTNS*, codes for cystinosin, the presumed lysosomal membrane cystine transporter whose deficiency causes cystine accumulation. The most common mutation in *CTNS* is a 57 kb deletion in 17p13 whose 5' breakpoint is 1 kb upstream of EST T85505; the 3' breakpoint occurs at the end of exon 10. Mutation analysis of 108 American-based NC patients revealed that 48 (44%) were homozygous for the 57 kb deletion. We investigated whether any EST in the 5' region of the deletion represents an expressed gene. We sequenced the entire genomic segment encompassing the 57 kb deletion, revealing a novel gene within the deleted segment that contains 7 exons, spans 29 kb, and encodes 478 amino acids. The predicted protein shows homology to a family of sugar-kinases. We cloned the human gene's cDNA and found a 3.9 kb mRNA transcript in liver, kidney and pancreas by Northern blot analysis. The homologous mouse gene's cDNA was also cloned and used as a probe to demonstrate a similar pattern of expression.

Sugar kinases phosphorylate carbohydrate substrates within the cell. We investigated whether some free sugar substrate accumulates in NC patients with the homozygous deletion because the sugar kinase is absent. On carbohydrate analysis of the urine of 12 deleted NC patients, 10 non-deleted NC patients, and 4 normal individuals by high performance anion-exchange chromatography and pulsed amperometric detection (Dionex), a unique peak was found only in deleted patients. This peak was purified and appears to be a reducing sugar derivative that runs between xylulose and ribulose.

Absence of a sugar kinase in specific patients may allow us to determine the clinical and biochemical importance of the involved pathway. Some of the clinical variation among NC patients may be attributable to the presence or absence of the sugar kinase.

Program Nr: 241 from the 1999 ASHG Annual Meeting

Altered phosphatase activity in mutant PTEN proteins associated with cowden syndrome missense mutations.*PTEN*. *X.L Ping, H. Zhang, F.F. Chen, J. Tok, H.C. Tsou, A.M. Christiano, M. Peacocke.* Dept. of Dermatology, Columbia University, New York, NY.

PTEN has been identified as the susceptibility gene for two autosomal dominant inherited diseases, Cowden Syndrome (CS) and Bannayan-Zonana Syndrome (BZS). Sequence analysis of PTEN reveals homology to tensin, a protein associated with focal adhesions, and to auxillin, a cytoskeletal protein. Functional in vitro studies show that PTEN protein is a dual specific protein phosphatase. To further understand the functional significance of altered PTEN proteins found in CS and BZS, we set up an in vitro assay system to assess the phosphatase ability of wild type and mutant recombinant PTEN proteins. An acidic artificial peptide polyGlu,Tyr, was phosphorylated by Abl protein tyrosine kinase and used as substrate. We examined a total of 12 missense mutant proteins associated with CS or BZS. All recombinant proteins, except one, showed a significant decreased phosphatase activity (< 90%) when compared to the wild type protein. The G129E protein showed a phosphatase activity similar to the wild type PTEN protein. In addition, we also tested two non-missense mutations (an inframe insertion and a frameshift mutation) for their phosphatase activities. The one amino acid insertion was isolated from a CS individual with early onset breast cancer, and this mutant protein also showed a decreased phosphatase activity similar to that of the other mutant proteins. A similar result was obtained from a truncated protein resulting from a 13 base pair deletion in exon 8 (frameshift mutation), as described in CS individual with bilateral breast cancer and endometrial cancer. In summary, we have shown an altered phosphatase function of recombinant mutant PTEN proteins. These data demonstrate that an intact protein phosphatase activity, at least in part, is responsible for the tumor suppressor function of the PTEN gene, and the phenotypic findings of CS and BZS.*PTEN*.

Program Nr: 242 from the 1999 ASHG Annual Meeting

Phenotypic variability in a murine model for the dominant negative disorder Osteogenesis Imperfecta. *A. Forlino, J.C. Marini.* SCTD/HDB, NICHD/NIH, Bethesda, MD.

Osteogenesis Imperfecta (OI) is a connective tissue disorder caused by defects in Type I collagen. By gene targeting in ES cells we generated a knock-in mouse with a typical glycine substitution in the collagen triple helix. This mouse reproduces the molecular defect, the phenotype and dominant genetic transmission of human OI and is designated Brtl (Brittle) IV. The Brtl mouse carries a single base change in one col1a1 allele and reproduces the Gly349®Cys substitution described in an OI type IV patient. Generation of Brtl mice in C57BL/6 and 129Sv backgrounds produced a lethal outcome, whereas the CDI background decreased the phenotypic severity and produced Brtl mice with variable phenotypic outcome. Thus, these OI mice carrying a single copy of an identical mutation reproduce the phenotypic variability which is common characteristic of human dominant negative disorders. Non-lethal Brtl mice are 50-80% the size of normal littermates. On radiographs they have the generalized undermineralization and bony deformities of the classical OI phenotype. Full histological analysis of mutant mice revealed that the Brtl calvarium and vertebral bodies were composed of thinner, less mature and more disorganized bone matrix. Long bones were osteoporotic and had disorganized trabeculae. Dentinogenesis Imperfecta was evident. About half of the Brtl mice have a lethal outcome within hours of birth from respiratory distress. X-ray and skeletal staining reveal rib and long bone fractures, flared rib cage and shorter vertebral bodies. Cultured fibroblasts from both lethal and non-lethal Brtl mice were used to confirm the presence of the mutant transcript and collagen protein. Analyses of expression levels of mutant col1a1 allele as well as of different extracellular matrix proteins in RNA extracted from tissues and cultured cells have been performed to investigate the basis of the phenotypic variability. We hypothesize the presence of modifier genes in the CDI background which modulates phenotypic severity and may provide understanding of the variability detected in human patients.

Program Nr: 243 from the 1999 ASHG Annual Meeting

Positional cloning of the *pallid* gene reveals a novel protein involved in syntaxin 13-mediated membrane fusion. *L. Huang[1], Y. Kuo[2], J. Gitschier[1,2].* 1) Howard Hughes Medical Institute; 2) Department of Medicine and Pediatrics University of California San Francisco, San Francisco, CA.

The *pallid* mutant (*pa*), found in the wild in 1926, is one of the platelet storage pool deficiency (SPD) mouse mutants. It is an autosomal recessive mutant characterized by prolonged bleeding time, pigment dilution, kidney lysosomal enzyme elevation, serum a1-antitrypsin activity deficiency, and abnormal otolith formation. As in the other mouse mutants of this class, the constellation of findings in *pallid* suggest an organelle biosynthesis problem. A YAC contig which covers the *pallid* region was generated extending ~1 Mb from *Epb4.2* to b*2M*. A mouse liver cDNA library was screened with a YAC DNA from this contig. One gene, expressed at significantly lower abundance in the *pallid* mutant, was discovered to encode a ubiquitously expressed, highly charged 172-amino-acid protein with no homology to known proteins. A nonsense mutation (C to T substitution) was detected at arginine codon 69 of this gene in the *pallid* mutant, and we thus refer to it as *pallidin*. Antiserum was raised against a peptide corresponding to the C-terminal region of pallidin and was affinity-purified. Pallidin is undetectable in the pallid mutant by western blot assays. The possible function of pallidin was revealed by the results obtained from a yeast two-hybrid screen, in which it was discovered to interact with syntaxin 13, a member of the t-SNARE (target-soluble NSF attachment protein receptor) family that mediates the intracellular membrane fusion. The interaction of pallidin with syntaxin 13 was subsequently confirmed by co-immunoprecipitation assays using anti-pallidin and anti-syntaxin 13 antibodies. Immunofluorescence studies indicate that the cellular distribution of pallidin overlaps with that of syntaxin 13, an endosomal membrane protein. Whereas the SPD *mocha* and *pearl* mutants have defects in AP-3, a protein complex involved in clathrin-mediated vesicle trafficking from trans-Golgi to endosomes, our findings show that the *pallid* SPD mutant is defective in the more downstream event of vesicle trafficking, namely vesicle docking and fusion.

Program Nr: 244 from the 1999 ASHG Annual Meeting

Screening for EXT1 and EXT2 mutations in 40 Hereditary Multiple Exostoses families. *C. Francannet[1,2], A. Cohen-Tanugi[1], A. Munnich[1], L. Legeai-Mallet[1]*. 1) Dept Genetics, INSERM U393, Hôpital Necker, Paris, France; 2) Dept Medical Genetics, Hotel Dieu, Clermont-Ferrand, France.

Hereditary Multiple Exostoses (HME) is an autosomal dominant disorder characterized by growth of benign bone tumors. Three chromosomal loci have been involved in the disease : EXT1(8q24.1), EXT2(11p11-p12) and EXT3(19p11-p13). The EXT1 and EXT2 genes have been cloned, defining a new family of tumor suppressor genes with glycosyltransferase enzymatic activities. Thirty eight HME families with were studied for possible linkage to the EXT loci. A total of 35/40 families was consistent with linkage to one of the EXT genes : EXT1 (24 families), EXT2 (10 families) and EXT3 (1 family). Three unlinked families showed strong recombination with the three loci providing further evidence for a fourth locus. Thirty five probands were screened by SSCP and mutations segregating with the disease were found in 27 unrelated individuals, including 20 EXT 1 (75%) and 7 EXT 2 mutations (25%). Eleven novel mutations in EXT 1 and four in EXT 2 were identified in addition to the 12 previously described mutations. While missense mutations were relatively unfrequent (5%), non sense or frameshift deletions or insertions creating premature termination codons accounted for most HME cases further suggesting that the phenotype is caused by haploinsufficiency. This pattern of mutations offers clues for a better understanding of the pathophysiology of this skeletal disorder.

Program Nr: 245 from the 1999 ASHG Annual Meeting

Disruption of gastrulation and heparan sulfate associated hedgehog signaling in EXT1 deficient mice. *X. Lin*[1], *M.M. Matzuk*[2, 3, 4], *Z.Z. Shu*[2], *L. Dryer*[1], *D.E. Wells*[1]. 1) Department of Biology and Biochemistry, University of Houston, Houston, TX. 77204; 2) 2. Department of Pathology, Baylor College of Medicine, Houston, TX 77030; 3) Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030; 4) Department of Human and Molecular Genetics, Baylor College of Medicine, Houston, TX 77030.

Mutations in the EXT1 gene are responsible for human hereditary multiple exostoses (EXT) type 1, an autosomal dominant bone disorder characterized by the presence of multiple benign cartilage-capped tumors (exostoses). *In vitro* studies have shown that EXT1 is a glycosyltransferase required for cell surface heparan sulfate biosynthesis. In *Drosophila*, the EXT1 homologue, *Tout-velu*, is vital for the diffusion of hedgehog (Hh) protein. To study the function of EXT1 in mammalian development, we generated EXT1-deficient mice using gene targeting in embryonic stem (ES) cells. EXT1 homozygous mutants fail to gastrulate or express a mesodermal markers. In addition, at E6.5 they are smaller than their littermates with no organized extraembryonic tissue. To analyze this defect further, mutant and wild-type ES cell lines were generated. Differentiation of wild-type ES cell lines into embryoid bodies resulted in the formation of a large cystic cavity, whereas the homozygous embryoid bodies failed to undergo cavitation. Furthermore, RT-PCR analysis of markers for visceral endoderm and mesoderm development indicates delayed and abnormal development of endoderm. To further study the formation of endoderm and the interaction of EXT1 in Hh signaling, we analyzed Indian hedgehog (Ihh), an important mediator of extraembryonic endoderm differentiation during early mouse embryogenesis. Preliminary results, indicate that Ihh in undetectable in EXT1 null mice during early embyrogeneisis. Taken together, these results indicate that EXT1 is essential for gastrulation and heparan sulfate associated hedgehog signaling in early embryonic development.

Program Nr: 246 from the 1999 ASHG Annual Meeting

The gene for the camptodactyly-arthropathy-coxa vara-pericarditis syndrome (CACP) encodes a secreted proteoglycan that is essential to normal joint function. *J. Marcelino[1], J.D. Carpten[2], W.M. Suwairi[3,4], S. Schwartz[1], C. Robbins[2], R. Sood[2], I. Makalowska[2], A. Baxevanis[2], B. Johnstone[1], R.M. Laxer[3], L. Zemel[3], C.A. Kim[3], J.K. Herd[3], J. Ihle[3], C. Williams[3], M. Johnson[3], V. Raman[3], S.A. Bahabri[3,4], J.M. Trent[2], M.L. Warman[1,3].* 1) CWRU and Univ Hospitals, Cleveland, OH; 2) NHGRI, Bethesda, MD; 3) CACP International consortium; 4) KFSHRC, Riyadh, Saudi Arabia.

Alterations of synoviocytes, the cells that line joint cavities, occur in two common joint diseases, osteoarthritis and rheumatoid arthritis. To begin delineating biological pathways that can affect synoviocyte function, we identified the gene responsible for causing the autosomal recessive disorder CACP. The principal pathologic feature of CACP is synoviocyte hyperplasia. Clinically, affected individuals have congenital or infancy-onset camptodactyly and childhood-onset non-inflammatory large joint arthropathy. We constructed a BAC contig across the 2 Mb CACP candidate interval on chromosome 1q and then performed sample sequencing to identify known and novel genes. This permitted assignment of a previously cloned but unmapped gene, megakaryocyte stimulating factor precursor, to the candidate interval. A putative bovine ortholog of this gene encodes a proteoglycan, superficial zone protein, that is synthesized by synoviocytes and articular cartilage chondrocytes. Consequently, the human gene was considered a candidate for CACP. To date, we have identified four likely disease causing mutations in affected individuals. Three are frameshift mutations, which are homozygous in affected offspring of consanguineous kindreds; one is a nonsense mutation. The absence of clinical findings in obligate heterozygotes suggests that all mutations likely create functional null alleles. CACP protein is a novel proteoglycan whose domains share homology with other protein families. While highly expressed in joint synovium and cartilage, the gene is also expressed in non-skeletal tissues including liver and pericardium. Sequence similarity to other protein families and expression in non-skeletal tissues suggests that CACP protein may have broad biological activities.

Program Nr: 247 from the 1999 ASHG Annual Meeting

Identification of a new mutation in the gene CDMP-1 causing brachydactyly type C in an extensive Colombian pedigree. *R. Mendoza-Londono[1], T. Argûello[2], J.C. Prieto[2], L.G. Biesecker[3], M. Warman[4].* 1) Pediatrics, SUNY Brooklyn, Brooklyn, NY; 2) Instituto de Genetica Humana, Universidad Javeriana, Bogotá, Colombia; 3) NIH, NHGRI, Bethesda, MD; 4) Dept. of Genetics and Center for Human Genetics, Case Western Reserve University & UHC, Cleveland, OH.

We evaluated a Colombian family with Brachydactyly type C, characterized by brachymesophalangy of the first, third and fifth digits, hyperphalangy of the third finger, shortening of the first metacarpal and clinodactyly of the fifth finger. The malformation in this family is inherited in an autosomal dominant pattern with complete penetrance and variable expressivity. There are 14 affected members in four generations.

Brachydactyly type C shows locus heterogeneity, having being mapped to two different genomic regions: On chromosome 12q24,where the responsible gene has not been identified and on chromosome 20, region 20q11.2, where the gene CDMP-1 is located. CDMP-1 codes for the cartilage derived morphogenetic protein, a member of the TGF-b family involved in the development of the digits.

Linkage analysis with polymorphic markers that span both regions was performed. We excluded linkage to chromosome 12 region 12q24 by demonstrating negative lod scores with markers D12S1045 (Lod score -5.8, q: 0.01) and D12S392 (Lod score -4.4, q: 0.01). Two markers on chromosome 20, D20S470 and D20S477, suggested linkage with the disease locus given by a three point lod score of +2.06 at q of 0. We performed mutational analysis of the gene CDMP-1 by direct sequencing of exons 1 and 2 and found a deletion of a thymine residue at position 297 of exon 1. This deletion would result in a frameshift mutation that affects the amino acid sequence of the protein and leads to the creation of a premature stop codon. The truncated protein would be 85 AA whereas the normal is 501 AA. We predict that this deletion severely affects the protein function and results in an abnormal development of the digits. Further analysis will allow us to determine the nature of the translated protein and the biochemical consequences in the function of this morphogenetic factor.

Program Nr: 248 from the 1999 ASHG Annual Meeting

Generation and analysis of a mouse model of a lethal dwarfism, Thanatophoric Dysplasia type II. *T. Iwata[1], L. Chen[2], C. Li[2], C.A. Francomano[1], C. Deng[2]*. 1) NHGRI/NIH, Bethesda, MD; 2) NIDDK/NIH, Bethesda, MD.

Thanatophoric Dysplasia type II (TDII) is a common form of neonatal lethal dwarfism associated with a specific mutation, Lys650Glu, in fibroblast growth factor receptor 3 (FGFR3). Recently, several mouse models have been generated with Fgfr3 mutations that showed the phenotype of achondroplasia, a milder form of dwarfism. TDII pathophysiology, on the other hand, is more severe and involves earlier embryonic bone development and dominant inheritance. We have successfully generated a TDII mouse model using an *in vivo* cre-lox recombination strategy to overcome the lethal effect associated with the Lys650Glu mutation. Upon cre-mediated recombination, the mutant mice are designed to have an exact chromosomal organization as wild type animals except for the introduced point mutation. Mutant pups heterozygous for the dominant mutation showed reduced skeletal growth and neonatal death, a phenotype closely resembling that in humans. Detailed analysis of the embryonic skeleton revealed abnormal rib cage development, which may be the cause of neonatal lethality. We also found that macrocephaly was caused by abnormal growth of the brain prior to skull bone formation at E11.5, suggesting a hitherto unknown role of Fgfr3 in early brain development. Furthermore, delayed knee joint formation was observed in mutant pups as early as E12.5, indicating the involvement of Fgf signaling in early knee joint formation. Finally, we observed increased proliferation and delayed differentiation in mutant growth plate chondrocytes. Neither the expression of the PTHrP receptor nor that of *Ihh* were altered, while the expression of *patched*, a downstream signaling component, was induced in the mutant growth plate, suggesting a new regulatory mechanism of embryonic bone development by Fgfr3. In summary, the TDII mouse closely mimics human dysplasia and will be useful for clinical studies. Our current studies at both the morphological and molecular levels will provide insights into the mechanisms of TDII pathology and the roles of Fgfr3 in the early development of bone and other tissues.

Program Nr: 249 from the 1999 ASHG Annual Meeting

Mutations of the homeobox gene *MSX2* cause symmetric parietal foramina: contrasting effects of loss and gain of function mutations for skull development. *A.O.M. Wilkie[1,2], A. Tang[3], N. Elanko[1], S. Walsh[1], S.R.F. Twigg[1], J.A. Hurst[1], K. Chrzanowska[4], S.A. Wall[2], R.L. Maas[5], R.E. Maxson[3].* 1) Institute of Molecular Medicine and Dept of Clinical Genetics, Oxford Radcliffe Hosp, Oxford, UK; 2) Craniofacial Unit, Radcliffe Infirmary, Oxford, UK; 3) Center for Craniofacial Molecular Biology, USCSD, Los Angeles, CA; 4) Department of Medical Genetics, CMHI, Warsaw, Poland; 5) Genetics Division, Brigham & Womens Hosp, Boston, MA.

Parietal foramina (*PFM*: MIM 168500) are circumscribed defects of cranial vault ossification that present radiologically either as symmetric lucencies of the parietal bones or as a confluent midline skull defect. Inheritance is frequently autosomal dominant but no causative locus has been identified in familial cases. We have identified heterozygous mutations of *MSX2* (located on 5q34-q35) in three unrelated families with *PFM*. One is a deletion of >90 kb that includes the entire *MSX2* gene; the other two are mutations of the 60 amino acid homeodomain (deletion of Arg-Lys at positions 18 and 19, and Arg31His substitution). No other consistent clinical abnormalities were present in individuals with these mutations. Haploinsufficiency is the likely pathological effect of these mutations and follows directly in the case of the deletion. The intragenic mutations are predicted to disrupt critical intramolecular or DNA contacts made by the MSX2 homeodomain, which we have investigated by functional studies. The parietal foramina phenotype contrasts with that found in the only mutation previously identified in MSX2, Pro7His in the homeodomain, which causes craniosynostosis, Boston type (MIM 123101). This mutation was shown to exhibit enhanced binding affinity to target DNA sequences *in vitro*. Our results complement work on *msx2* knockout mice, in which -/- progeny have thin parietal bones and reduced cellularity of the osteogenic fronts, but +/-mice are phenotypically normal. These data indicate that human skull development is particularly sensitive to *MSX2* gene dosage and suggest that parietal foramina and craniosynostosis are the consequence of opposite perturbations of developmental pathways in cranial ossification.

Program Nr: 250 from the 1999 ASHG Annual Meeting

Identification of the gene causing autosomal dominant molar hypodontia. *D.W. Stockton[1], P. Das[1], M. Goldenberg[2], R. D'Souza[2], P.I. Patel[1]*. 1) Baylor College of Medicine, Houston, TX; 2) University of Texas HSC Dental Branch, Houston, TX.

Congenital absence of teeth, referred to as hypodontia or agenesis, is one of the most common developmental anomalies in man. The genetic bases of hypodontia are largely unknown. We have identified a large family segregating a unique form of hypodontia in an autosomal dominant pattern. The 19 affected family members report normal primary dentition but are all missing most permanent maxillary and mandibular molars. Some individuals also lack lower central incisors as well as maxillary and mandibular second bicuspids. A genome wide screen was conducted using simple sequence repeat polymorphism genotyping and two-point linkage analysis. Significant evidence for linkage was identified between the hypodontia phenotype and marker *D14S70* in chromosome 14q12. The candidate gene, *Pax9*, which had been previously mapped to this region was screened for alterations by DNA sequencing. This revealed a single base insertion in the second exon of the gene, resulting in a frame shift and premature protein termination 243 amino acids after the insertion. This is the first association of *Pax9* and a human phenotype, and our data corroborate previous studies in mice that showed a key role for *Pax9* in tooth mesenchyme signaling. Together these data provide compelling evidence that *Pax9* is a critical regulator of tooth development in mouse and human.

Program Nr: 251 from the 1999 ASHG Annual Meeting

Progressive pseudorheumatoid dysplasia (PPD) is caused by mutation in the CCN family member *WISP3*. *M.L. Warman, J.R. Hurvitz, W.M. Suwairi, W. Van Hul, H. El-Shanti, A. Superti-Furga, J. Roudier, D. Holderbaum, R.M. Pauli, J.K. Herd, E. Van Hul, H. Rezai-Delui, E. Legius, M. Le Merrer, S.A. Bahabri.* Progressive Pseudorheumatoid Dysplasia International Consortium.

PPD is an autosomal recessive skeletal disorder. Affected individuals are asymptomatic in infancy and in early childhood. Symptoms generally begin in mid-childhood and consist of stiffness and swelling of joints. Although rheumatoid arthritis is a frequent initial diagnosis in affected children, radiographic examination distinguishes PPD from rheumatoid arthritis by demonstrating widened epiphyses, joint space narrowing, and vertebral flattening. However, in contrast to many skeletal dysplasias, cartilage loss and destructive bone changes are progressive in PPD and frequently necessitate early joint replacement surgery. PPD was previously mapped to human chromosome 6q. Using genomic sequence from a BAC which contains a fully linked marker D6S416, we determined that *WISP3*, a member of the CCN family of extracellular signaling proteins, is within the PPD candidate interval. Because other CCN family members have been implicated in cell growth and differentiation, including chondrogenesis, we evaluated *WISP3* as a candidate for causing PPD. To date, sequence analysis of *WISP3* in 13 unrelated individuals with PPD has led to the identification of 7 likely disease causing mutations, including 3 frameshifts, 2 nonsense, and 3 missense changes which alter highly conserved cysteine residues. Affected offspring in consanguineous kindreds are homozygous for disease causing mutations. All mutations appear to cause functional null alleles, since obligate heterozygotes have no clinical findings. *WISP3* mRNA can easily be detected by RT-PCR in synoviocytes and chondrocytes, and in bone marrow-derived mesenchymal progenitor cells that have been induced to undergo chondrogenesis *in vitro*; in contrast, expression is barely detectable in fibroblasts and is undetectable in EBV-transformed lymphoblasts. These results suggest that *WISP3* is the PPD locus and that it is essential for normal post-natal skeletal growth and cartilage homeostasis.

Program Nr: 252 from the 1999 ASHG Annual Meeting

Autoimmune Lymphoproliferative Syndrome (ALPS): an Inherited Disorder of Apoptosis with Predisposition to Development of Diverse Lymphomas. *C.E. Jackson[1], J. Wang[1,2], R.E. Fischer[1], A.P. Hsu[1], J. Niemela[3], J.K. Dale[4], T.A. Fleisher[3], E.S. Jaffe[5], M.J. Lenardo[2], S.E. Straus[4], J.M. Puck[1]*. 1) Genet Mol Biol Branch/NHGRI; 2) Lab of Immunol/NIAID; 3) Clinical Center; 4) Lab of Clin Invest/NIAID; 5) Lab of Pathol/NCI, NIH, Bethesda, MD.

Autoimmune lymphoproliferative syndrome (ALPS) is a disorder of immune homeostasis and tolerance. Defective lymphocyte apoptosis in ALPS leads to spleen and lymph node enlargement, autoimmunity, accumulation of CD4-/CD8- T cells and high IL-10 levels. Now we have documented increased lymphoma incidence in ALPS. Of 34 ALPS kindreds studied at NIH, 27 (79%) have dominant heterozygous mutations in the APT1 gene encoding Fas, a major mediator of lymphocyte apoptosis. Among ALPS patients lacking Fas defects were 2 with mutations of caspase 10, a downstream protease in the Fas apoptotic cascade. We report here 9 new ALPS kindreds with Fas mutations, 4 extracellular, 1 in the transmembrane domain, and 4 intracellular. 65% of all reported Fas mutations affect the intracellular death domain (DD) of Fas. These produce the strongest dominant inhibition of apoptosis and are associated with greater penetrance of ALPS features in Fas-mutation-bearing relatives in ALPS (87%) as opposed to extracellular mutations (18%) ($p<0.01$). Severe autoimmunity and requirement for splenectomy in the NIH cohort has been restricted to relatives with intracellular mutations. Additionally, 13% (6/46) of NIH ALPS patients with intracellular Fas mutations have developed B cell lymphoma, an incidence 15-fold higher than seen in the general population. Histologies of ALPS- associated lymphomas were diverse, with 2 Hodgkins and 2 Burkitt lymphomas, 1 follicular lymphoma, and 1 T cell rich B cell non-Hodgkins lymphoma (NHL). Although somatic Fas mutations have been described in NHL, T-cell leukemias and multiple myeloma, this is the first instance of inherited defects of apoptosis predisposing to lymphoma.

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Program Nr: 253

Mutations in the vascular endothelial growth factor receptor (VEGFR-3; Flt4) cause hereditary lymphedema. *M. Kimak[1], M. Kärkkainen[2], K. Alitalo[2], E. Lawrence[1], K. Levinson[1], R. Ferrell[1], D. Finegold[1].* 1) Human Genetics, Univ. of Pittsburgh, Pittsburgh, PA; 2) Molecular/Cancer Biology Lab., Haartman Institute, Univ. of Helsinki, Helsinki, FI.

Hereditary lymphedema (HL) is a developmental disorder of the lymphatic system which results in disabling and disfiguring swelling usually of the lower extremities. HL is inherited as an autosomal dominant trait with reduced penetrance, variable expression, and variable age of onset. We recently demonstrated linkage of HL to human chromosome 5q34-q35. This location contains the gene for the vascular endothelial growth factor receptor-3 (VEGFR-3; Flt4), a known regulator of lymphatic development. We identified a putative disease-causing mutation in VEGFR-3 that segregated with the HL phenotype in one family. Here, we report four additional nucleotide substitutions which lead to non-conservative missense mutations in highly conserved amino acids of the VEGFR-3 gene product.

Mutations in VEGFR-3 causing Hereditary Lymphedema			
Exon	Nucleotide Position	Amino Acid Substitution	Function al Domain
17	G2569A	G857R	Kinase 1
22	G3122C	R1041P	Kinase 2
22	T3131C	L1044P	Kinase 2
22	G3145A	D1049N	Kinase 2
24*	G3341A	P1114L	Kinase 2

* previously reported (Ferrell, et al., Hum Mol Genet 7: 2073-2078, 1998)
These five mutations segregate with lymphedema in affected or at-risk family members in five independently ascertained HL families. None of the mutations were observed in over 300 alleles from a population based control sample. These amino acid substitutions localize in kinase domains 1 or 2 of VEGFR-3 and are predicted to alter either charge or structure of the catalytic domain. The P1114L variant of VEGFR-3 was generated by site-directed mutagenesis and expressed in 293T cells, which lack endogenous VEGFR-3 expression. VEGFR-3 autophosphorylation in response to VEGF-3 stimulation was completely absent in the mutant receptor. Coexpression of the wild type and P1114L alleles showed inhibition of autophosphorylation of the wild type allele by the P1114L mutant allele. This suggests a dominant negative effect of this mutation. Functional studies of the other four mutant alleles are in progress. These studies demonstrate that mutations in kinase domains 1 and 2 of VEGFR-3 are one cause of hereditary lymphedema in humans.

Program Nr: 254 from the 1999 ASHG Annual Meeting

Association between M694V and amyloidosis in Familial Mediterranean Fever. *N. Magal[1], T. Shohat[1], N. Stoffman[1], A. Mimouni[1], R. Lotan[1], A. Minasian[2], M. Krasno[1], T. Dagan[1], G. Ogur[3], A. Sirin[3], G. Halpern[1], J.I. Rotter[4], N. Fischel-Ghodsian[4], Y. Danon[1], M. Shohat[1]*. 1) Department of Medical Genetics, FMRC and Beilinson Campus, Rabin Medical Center and Sackler School of Medicine, Tel Aviv University, Israel; 2) Emergency Medical Scientific Center, Yerevan, Armenia; 3) Medical Genetics, Gata Medical Center and Immunology Department, Ankara University Medical Faculty, Turkey; 4) Medical Genetics Birth Defects Center, Cedars-Sinai Medical Center and UCLA, CA, USA.

The gene causing familial Mediterranean Fever (FMF), an autosomal recessive disease characterized by recurrent attacks of inflammation of serosal membranes has recently been found and several mutations identified. The most severe complication of the disease is amyloidosis, which can lead to renal failure. The aim of this study was to investigate the association between amyloidosis and the common mutations in the gene causing FMF (MEFV). We studied 371 patients from 5 ethnic origins living in different environments: North African Jews, other Jews, Turks, Armenians living in the US, and Armenians from Yerevan, Armenia. A significant association was found between amyloidosis and the most common mutation in exon 10 of the MEFV gene, M694V (RR = 1.77, p=0.008, 95% CI = 1.16 - 2.71). Amyloidosis was present in 44 out of the 171 homozygous FMF patients (25.7%), in 22 out of the 143 compound heterozygous FMF patients (15.4%), and in seven out of 57 patients carrying other mutations (12.3%). In patients who had not been treated with colchicine before the age of 20 years, the risks of amyloidosis developing prior to this age were 61.0%, 4.6%, and 21% in each of these genotype groups respectively. In our series there were no cases of amyloidosis in patients carrying the common mutation E148Q. We recommend that all children carrying the mutation M694V should be treated with colchicine, irrespective of the severity of the inflammatory attacks, in order to prevent their developing amyloidosis prior to age 20. Of the other mutations, mutation E148Q seems to carry no significant risk for this complication.

Program Nr: 255 from the 1999 ASHG Annual Meeting

Diabetes increases coronary artery disease (CAD) risk to relatives of female CAD cases. *M.T. Scheuner, M. Alarcon.* Medical Genetics, Cedars-Sinai, Los Angeles, CA.

Diabetes (DM) is an important CAD risk factor, especially in women where its presence abolishes the protective female gender effect. CAD risk is increased in relatives of female CAD cases, suggesting increased genetic risk in families of female cases. The aim of this study was to assess the effect of diabetes on family history reports of CAD by CAD cases. Personal and family histories were available from 872 subjects who had undergone coronary artery bypass graft surgery and were enrolled in a multi-center trial investigating effects of lipid-lowering medication. 8% of subjects were female and 95% were Caucasian. The average age of females and males was 65 (range, 39-77) and 66 (range, 40-80), respectively. Z-tests were used to compare frequencies between males and females. 20% of females and 14% of males had a personal history of diabetes, $p=.12$. Average pedigree size was similar for females and males, 8 vs. 9. 82% of females had at least one first degree relative with CAD and 34% had three or more, whereas the corresponding values in males were 68% and 18%, $p=.01$ and $p<.001$, respectively. Reports of DM were significantly increased in first degree relatives of females with a family history of CAD compared to males, 62% (36/58) vs. 39% (211/545), $p<.001$. The correlation of CAD and DM in relatives was significant even after adjusting for pedigree size, $p<.05$; however, it was greater in females ($r=.24$) than in males ($r=.10$). Excluding families with DM, the frequency of CAD reports in relatives was reduced from 82% to 71% in families of females, but remained unchanged in males. Thus, family history reports of CAD in females and males became similar, 71% vs. 68%, $p=.07$. In conclusion, women with CAD are more likely to have multiple family members with CAD if DM is also present. Family history reports of CAD by female cases are reduced and become equivlent to those of male cases if families with DM are excluded. This suggests that increased genetic risk for CAD associated with female cases may be due to diabetes-related genes. These results may have important implications for genetic counseling and CAD prevention efforts in relatives of female CAD cases.

Program Nr: 256 from the 1999 ASHG Annual Meeting

INCREASED TRANSMISSION OF THE PREMUTATED ALLELE COMPARED TO THE NORMAL ALLELE IN FEMALE CARRIERS OF THE FRAGILE X SYNDROME. *V. Drasinover, S. Ehrlich, N. Magal, E. Taub, V. Libman, T. Shohat, M. Shohat.* Department of Medical Genetics, Rabin Medical Center, Petah Tikva, Israel.

Fragile X syndrome (Fra X) is the commonest heritable genetic disease accounting for mental retardation and is caused by an expanded CGG repeat in the first exon of the FMR1 gene. Previous studies have indicated a possible genetic advantage manifesting as an increased fertility rate among carrier females. However, these reports, which were based on investigation of fragile X families, were subject to sampling bias because families with multiple affected individuals were more commonly studied. The aim of this study was to test whether there is non-random transmission of the FMR1 alleles in females carrying premutated alleles for Fra X. During the period 1995 - 1998 we conducted a screening program in which we tested 10,587 normal females for carrier status for Fra X. We identified 138 individuals who carried more than 51 CGG repeats in one of their FMR1 alleles. Of these, 110 underwent prenatal testing for Fra X in the fetus. We found that the rate of transmission to the fetus of the premutated allele was significantly higher than that of the normal allele (69 vs. 41; $p<0.008$). Among the women who carried premutation alleles, those with 51 - 60 CGG repeats had the highest rate of skewed transmission to the fetus. Out of 71 women who carried 51 - 60 CGG repeats, 50 transmitted the premutation allele as compared with 21 who transmitted the normal allele, $p<0.001$. These results therefore show a non-random increased transmission of those alleles whose number of CGG repeats is in the premutation range as compared with those alleles with a normal number of CGG repeats, and this suggests a genetic fertility advantage for those ova which carry Fra X premutation alleles. If these results are confirmed they could have important genetic counseling implications.

Program Nr: 257 from the 1999 ASHG Annual Meeting

MTHFR genotype, malformations and neurodevelopment in the fetal valproate, carbamazepine and hydantoin syndromes. *J.C.S. Dean[1], S.J. Moore[1], A. Osborne[1], S. Joss[1], P.D. Turnpenny[2]*. 1) Department of Medical Genetics, Aberdeen, Scotland; 2) Clinical Genetics Service, Royal Devon and Exeter Hospital, Exeter, England.

Seventy children exposed to anticonvulsants in utero ascertained through the UK National Fetal Anticonvulsant Syndrome Association and clinical referral have been assessed using a standardised questionnaire and clinical evaluation. Fifty-one were exposed to sodium valproate (37 valproate monotherapy), 18 to carbamazepine (10 carbamazepine monotherapy) and 10 phenytoin (5 phenytoin monotherapy). Fifty (71%) show delayed development, 34/48 of school age requiring special education or learning support, 47/65 aged greater than two years requiring speech therapy. Seventy-six per cent have a behavior disorder. Twenty-two (31%) have congenital malformations (three neural tube defects) typical of anticonvulsant exposure. Glue ear (27%), joint laxity (64%) and myopia (35%) were common. Overall, fetal anticonvulsant syndrome was associated with maternal homozygosity for the 677C>T mutation in MTHFR (Odds Ratio 2.7, 95%CI 1.1 - 6.6). For fetal valproate syndrome, c^2 analysis also suggested an association with the child's genotype (c^2=6.49, $p<0.05$), the numbers in the carbamazepine and phenytoin groups were too small for meaningful analysis. If the folic acid pathway is an important risk factor for fetal valproate syndrome, the nature of the neurodevelopmental disorders seen suggests that folate supplements should be taken throughout pregnancy, not just for the first trimester.

Program Nr: 258 from the 1999 ASHG Annual Meeting

A novel type of Hermansky-Pudlak syndrome in Puerto Rico. *J.R. Toro[1], S.J. Bale[1], R.A. King[2], J.G. White[2], W.A Gahl[3]*. 1) Genetic Studies Section, NIAMS, NIH, Bethesda, MD; 2) Univesity of Minnesota, Minneapolis, MN; 3) NICHD, NIH, Bethesda, MD.

Hermansky-Pudlak Syndrome (HPS) is an autosomal recessive disorder characterized by oculocutaneous albinism, a bleeding diathesis due to platelet storage pool deficiency, and lysosomal accumulation of ceroid lipofuscin. HPS occurs with a high frequency in northwest Puerto Rico (PR), where 1 in 21 individuals is a carrier. HPS patients from the northwest are homozygous for a 16-bp duplication in exon 15 of the HPS1 gene; this mutation is associated with significant risk of pulmonary fibrosis. The objective was to determine the phenotype and genotype of HPS Puerto Rican patients from several parts of PR. All patients had absence of platelet dense bodies by electron microscopy. DNA was extracted from peripheral blood leukocytes and the presence of the 16-bp duplication in the HPS gene was determined by polymerase chain reaction. For those patients who were found not to have the 16-bp duplication, single-strand conformational polymorphism analysis was performed, followed by sequencing of suspicious exons. Sixty-one of the 76 patients, all from northwest PR, displayed the 16-bp duplication. However, we found 15 Puerto Rican patients from 8 families, all from central PR, who lacked the 16-bp duplication. Two of these patients had no mutations in the HPS-1 gene by cDNA sequencing. The patients exhibited only mild visual defects, and mild pigment dilution of hair, eye and skin; by history there was no pulmonary fibrosis. These findings differed significantly from HPS individuals with the 16-bp duplication. The 16-bp duplication in the HPS1 gene appears to be limited to Puerto Ricans from the northwest PR. The 15 HPS patients from central Puerto Rico who lack the 16-bp duplication may have HPS due to a mutation of a gene different from the HPS1 gene. We found a significant genotype-phenotype correlation between Puerto Rican HPS individuals with and without the 16-bp duplication. The human homologs of the genes causing both pigment dilution and a platelet storage-pool deficiency in several mouse strains are prime candidates for HPS in the new group of patients we have identified.

Program Nr: 259 from the 1999 ASHG Annual Meeting

Mortality associated with neurofibromatosis 1 in the United States from 1983 to 1995: an analysis using data from death certificates. *S.A. Rasmussen[1], Q.H. Yang[1], J.M. Friedman[2]*. 1) Centers for Disease Control and Prevention, Atlanta, GA; 2) Dept. of Medical Genetics, University of British Columbia, Vancouver, BC, Canada.

Neurofibromatosis 1 (NF1) is one of the most common autosomal dominant disorders, occurring in 1 in 3,000 individuals; however, information regarding associated mortality is limited. For this study, we used Multiple-Cause Mortality Files, compiled by the National Center for Health Statistics from US death certificates, for the years 1983 to 1995. These files included International Classification of Diseases, Ninth Revision (ICD9), codes for the underlying cause of death and up to 20 other conditions listed on the death certificate. We selected all cases listing the code for neurofibromatosis. Since this code could also include neurofibromatosis 2 (NF2), which is much less prevalent but usually more severe than NF1, we excluded cases with codes for sensorineural hearing loss or benign neoplasms of the cranial nerves or meninges as probable NF2 cases. 3,253 presumed NF1 cases were identified among over 28 million deaths, a prevalence of 1 in 8,600, suggesting underascertainment of NF1 in this population. Mean and median ages of death for NF1 cases were 54.5 and 59 years, respectively, compared to 69.8 and 74 years in the general population, confirming previous findings of a 15-year decrease in life expectancy among those with NF1. Results of proportionate mortality ratio (PMR) analyses showed that decedents with NF1 were 38 times more likely (PMR=37.9; 95% CI=33.7-42.4) to have a malignant connective or other soft tissue neoplasm listed on their death certificate, compared to those without NF1. Overall, decedents with NF1 were only 1.2 times more likely (PMR=1.2; 95%CI=1.15-1.31) to have a malignant neoplasm than those without NF1. Since previous NF1 mortality studies have suggested that hypertension may be related to mortality, we evaluated hypertensive diseases; these were not more likely to be listed on death certificates of those with NF1 than of those without NF1 (PMR=1.1; 95%CI=0.97-1.28). This study provides population-based mortality data useful to clinicians caring for patients with NF1.

Program Nr: 260 from the 1999 ASHG Annual Meeting

The phenotypic spectrum of malformation caused by mutations in the human TBX5 gene and evidence for genotype-phenotype correlation. *R.A. Newbury-Ecob[1], S.J Cross[2], Y.H Ching[2], C.H Yi[2], L. Armstrong-Buisseret[2], Q.Y Li[2], J.D Brook[2]*. 1) Clin Gen, Inst Child Hlt, Bristol Royal Hosp Sick Child, Bristol, England; 2) Department of Molecular Genetics, University of Nottingham, Nottingham, England.

Mutations in human TBX5 have been shown to cause the Holt-Oram syndrome (HOS) characterised by congenital heart defects (mainly septal) and upper limb abnormalities affecting predominantly the radial ray. TBX5, a member of the tbox transcription factor family, is involved in cardiac morphogenesis and specification of forelimb/hindlimb identity. A large UK and European series of HOS cases underwent clinical and molecular genetic analysis. Linkage analysis showed genetic heterogeneity. TBX5 mutations were identified in 14 families. We present data on 10 novel mutations and the associated phenotypes. 8 nonsense mutations in 12 families were associated with a wide spectrum of cardiac and limb defects which did not vary according to the location within the gene. 5 unrelated families share the same mutation (C-T nt1500 TGA stop codon) and show a wide inter and intrafamial variation in expression, although none had complex heart defects or phocomelia. 2 missense mutations were detected, one (G-A nt1160 Gly to Arg) lies within the highly conserved tbox DNA binding domain, a second (G-T nt 1410 Ser to Ile) lies in the carboxyl end of the gene outside of the tbox. The former is consistenty associated with serious congenital heart defects but mild limb abnormalities in all affected individuals; the latter with an equal severity of limb and cardiac involvement. Where genotype-phenotype correlation occurs this may reflect the location of missense mutations within the DNA binding domain and the effect on target genes.

Program Nr: 261 from the 1999 ASHG Annual Meeting

Identification of a phenotype associated with loss of the DCC gene on chromosome 18. *R.J. Leach[1], M. Semrud-Clikeman[2], N.M. Thompson[3], P.D. Ghidoni[1], C.T. Gay[4], L.J. Hardies[1], J. Lancaster[1], R.L. Schaub[1], C.I. Kaye[1], J.D. Cody[1]*. 1) The University of Texas Health Science Center, San Antonio, TX; 2) The University of Texas, Austin, TX; 3) University of Washington, Seattle, WA; 4) University of Oklahoma Health Sciences Center, Oklahoma City, OK.

We are studying individuals with deletions of 18q in an attempt to identify genes involved in the pathophysiology of this disorder. There appears to be no evidence of breakpoint clustering on chromosome 18 in these individuals. In a group of over 60 patients with deletions of 18q, no two unrelated individuals were found to have identical breakpoints based on the molecular analysis of their DNA with polymorphic markers. Thus, this segmental aneusomy is ideal for genotype/phenotype correlations. In our attempts to identify regions of chromosome 18 whose loss is associated with specific phenotypes, we observed a consistant group of features in the 7 patients with the most proximal breakpoints on chromosome 18q. These features included severe mental retardation, microcephaly (<3 S.D. below mean normalized for height), and inability to walk. The region missing in this cohort of patients, which was not lost by other patients without these features, contains the gene coding for DCC (deleted in colon cancer). DCC was first identified by its high loss in colon cancer. DCC has been demonstrated recently to be a netrin receptor and has a role in neuronal migration. Interestingly, mouse models with homozygous disruptions of DCC are unable to walk. From these observations, we hypothesize that DCC plays a causal role in this phenotype in the seven patients with large deletions of chromosome 18q. This would imply that DCC is haploinsufficient in humans and appears to play an important role in neurologic development.

Program Nr: 262 from the 1999 ASHG Annual Meeting

Familial overexpression of extraglandular aromatase due to genomic instability at chromosome 15q21.1. Another case of genomic disease. *J. Joslin[1], S. Bulun[2], M. Shozu[3], R.A. Schultz[1]*. 1) McDermott Ctr Human Growth, Univ Texas Southwestern Med, Dallas, TX; 2) Reproductive Endocrineology, Univ Illinois, Chicago, IL; 3) Dept OB/Gyn, School of Medicine, Kanazawa, Japan.

Gynecomastia, the excessive development of male mammary glands, is a rare condition which is frequently secondary to increased levels of circulating estrogen. Familial cases of gynecomastia have been associated with the testicular tumors of Peutz-Jeghers syndrome, but are also seen when excessive peripheral estrogen formation is the consequence of an unknown cause. We have been characterizing the molecular nature of the defect in a family with two affected sibs, an affected father and a mother who died from ovarian cancer. The sib who was the proband in this study had more than 50 times the normal levels of estrogen, with very little accounted for through testicular secretion. Characterization of the aromatase (Cyp19) gene localized at chromosome 15q21.1 revealed no defects in localized genomic structure as evaluated by Southern blot , but RT-PCR did reveal the presence of a 5-prime untranslated leader sequence on cyp19 mRNA which was designated EJ. This EJ leader sequence was also found on an antisence transcript of the nearby SPL gene. Neither of these products was seen in controls. Further characterization of the region in control cells revealed EJ to represent promoter sequences associated with a chromosome 15q21.1 homologue of the tropomodulin gene. A partial BAC contig was developed to further evaluate normal genomic organization of the region and the origin of the aberrant transcripts. FISH results with BACs on G1 interphase nuclei are consistent with a complex inversion of sequences within this region. Interestingly, additional patients have been identified with altered aromatase expression. In conclusion, familial gynecomastia appears to represent yet another in a growing list of genetic disorders where the underlying mutational mechanisms involve significant changes in genomic architecture.

Program Nr: 263 from the 1999 ASHG Annual Meeting

Cranio-lenticulo-sutural dysplasia: Clinical and molecular studies of a new syndrome. *S.A. Boyadjiev[1], J. Zwaan[2,3], C.M. Justice[4], V.A. McKusick[1], M. Jabak[3], A.F. Wilson[4], E.W. Jabs[1]*. 1) Institute of Genetic Medicine, Johns Hopkins Univ., Baltimore, MD; 2) Dept. of Ophthalmology, Univ. Texas Health Science Ctr., San Antonio, TX; 3) The King Khaled Eye Specialist Hospital, Riyadh, Saudi Arabia; 4) NIH/NHGRI, Baltimore, MD.

We describe a new syndrome in an inbred Saudi Arabian family. The most prominent feature is failure of closure of fontanels and sutures. At birth the anterior fontanel is large due to open sagittal and metopic sutures. The fontanel ossifies late and incompletely. There is hypotrichosis of the scalp. The skin over the fontanel is dark and with capillary hemangiomas. Frontal bossing, hypertelorism and high orbital roofs are present. The patients have large, low set ears, large mouth, thin upper lip, high palate and mandibular hypoplasia. The second major feature is posterior Y-shaped sutural cataracts that develop over time or may be congenital. The cataracts are heavily calcified, they mature rapidly, necessitating removal within a few months. Chromosomal and biochemical studies were normal. The family shows a pattern of repeated double first-cousin matings. The parents were unaffected. Six of twelve children in two sibships were affected. A genome-wide screen was performed using 387 markers (10 cM intervals) at the Center for Inherited Disease Research (CIDR) on 21 DNA samples. Model-dependent and model-independent linkage analyses were performed using LINKAGE and S.A.G.E. Two-point lodscore analysis, assuming autosomal recessive inheritance, suggested linkage to chromosome 17q11 (LOD=2.43 at a recombination fraction q=0.0 for D17S1303) and to chromosome 21q22 (LOD=1.35 at q=0.0 for D21S2052). Model-independent sib-pair linkage analysis suggested linkage to D17S1303 ($p<0.001$) and possibly D21S1437 ($p<.1$). Candidate loci include b-A3/A1-crystallin on chromosome 17q11 in which mutations have been found in patients with zonular sutural cataracts, and a-A crystallin on chromosome 21q22.

Program Nr: 264 from the 1999 ASHG Annual Meeting

g*2-COP*, a novel imprinted gene on chromosome 7q32, defines a new imprinting cluster in the human genome. *N. Blagitko-Dorfs[1], U. Schulz[1], A.A. Schinzel[2], H.-H. Ropers[1,3], V.M. Kalscheuer[1]*. 1) Max-Planck-Institute for Molecular Genetics, Berlin, Germany; 2) Institute of Medical Genetics, University of Zürich, Switzerland; 3) Department of Human Genetics, University Hospital Nijmegen, The Netherlands.

A systematic search for expressed sequences on human chromosome 7q32, which carries the paternally expressed *MEST* gene, resulted in the isolation of a novel imprinted gene, g*2-COP* (nonclathrin coat protein). g*2-COP* consists of 24 exons and covers >50 kb of genomic DNA. Like *MEST*, g*2-COP* is ubiquitously expressed in fetal and adult tissues. Allele-specific expression analysis using a polymorphism in intron 22 revealed that in most fetal tissues, g*2-COP* is transcribed only from the paternally inherited allele, except for brain and liver which show biallelic expression. The predicted g2-COP protein is closely related to g-COP, a subunit of the coatomer complex COPI, suggesting a role of g2-COP in cellular vesicle traffic. Association of Silver-Russell syndrome (SRS) with maternal uniparental disomy for chromosome 7 (mUPD7) may indicate the involvement of an imprinted gene in the disease. We screened 49 patients with SRS and 9 patients with primordial growth retardation for mutations in the coding region of g*2-COP* including exon-intron boundaries. However, the only mutation detected was maternally derived, arguing against a role of g*2-COP* in the aetiology of SRS. Together, g*2-COP* and *MEST* constitute a new imprinting cluster in the human genome that may harbor other, as yet unknown imprinted genes.

Program Nr: 265 from the 1999 ASHG Annual Meeting

Analysis of an imprinting control center on mouse chromosome 7 by targeted deletions. *J. Bressler, T-F. Tsai, A.L. Beaudet.* Dept Molecular & Human Gen, Baylor Col Medicine, Houston, TX.

Prader-Willi syndrome (PWS) is a neurobehavioral disorder caused by paternal deficiency of human chromosome 15q11-q13. There is conflicting evidence from human translocations regarding the involvement of *SNRPN* in the pathogenesis of PWS, and it is not known if PWS results from loss of expression of a single imprinted gene or multiple genes. The human *SNRPN* and mouse *Snrpn* genes are paternally expressed. These genes have 10 exons, with a small open reading frame (ORF) (exons 1-3) encoding the SNURF protein and a larger ORF (exons 4-10) encoding the SmN spliceosomal protein. Rare PWS patients with microdeletions including the *SNRPN* promoter and exon 1 have imprinting mutations with defects in allele-specific gene expression and methylation. Since in some cases the microdeletion was present in the patient's paternal grandmother and caused PWS when transmitted by her son, it was proposed that these microdeletions define a *cis*-acting imprinting center (IC) involved in imprint switching during gametogenesis. In order to characterize the murine IC, nested deletions of 0.9 kb (incorporating exon 1 and 0.62 kb upstream and 0.22 kb downstream) and 4.8 kb have been generated which remove part of a differentially methylated CpG island. Mice inheriting the 0.9 kb deletion either maternally or paternally from germline heterozygotes have no obvious abnormalities, and show a methylation pattern at *Snrpn* intron 1 consistent with an intact ability to switch epigenotype. Although the deletion is expected to remove the *Snrpn* promoter located 5' of exon 1, analysis of *Snrpn* transcription by RT-PCR in paternal heterozygotes shows the presence of a product using primers amplifying either exons 4-5 or exons 9-10. Northern analysis shows that the *Snrpn* transcript is reduced by approximately 90%, indicating expression from another promoter either upstream of exon 4 or further upstream involving exons analagous to the reported *SNRPN* BD exons. The 0.9 kb region and exon 1 are not essential for imprint switching, since transmission of the deletion by a male who had inherited the mutation maternally was associated with normal methylation and phenotype in the progeny at weaning age.

Program Nr: 266 from the 1999 ASHG Annual Meeting

Isoform-specific imprinting of human *PEG1/MEST* gene. *K. Kosaki[1,2], R. Kosaki[1,3], W. Craigen[4], N. Matsuo[1]*. 1) 1Department of Pediatrics; 2) 2Pharmacia-Upjohn Fund for Growth & Development Research, Keio University School of Medicine; 3) 3Health Center, Keio University, Tokyo, Japan; 4) 4Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

The human homolog PEG1/MEST on 7q31.3 is monoallelically expressed from the paternal allele in a wide variety of tissues during prenatal and postnatal period and is considered to be responsible for growth retardation associated with maternal uniparental disomy of chromosome 7 [UPD7] and/or Russell-Silver syndrome. However, in lymphocytes, PEG1/MEST is expressed from both paternal and maternal alleles and is expressed in lymphoblastoid cell lines from patients with maternal UPD7. The purpose of this report is to delineate underlying mechanism of this apparent loss of imprinting in lymphocytes in order to better understand the imprinting control of the human PEG1/MEST gene. Considering that loss of imprinting can be due to transcription of non-imprinted or reciprocally imprinted isoforms and that there exist at least 6 EST clones which share a novel sequence joined to exon 2 of PEG1/MEST we aimed to characterized the alternative isoform using RT-PCR with primer pairs specific for either the original isoform (isoform 1) or the alternative one (isoform 2). Lymphoblastoid cell lines from a patients with maternal UPD7, one with paternal UPD7 and a normal individual were cultured and total mRNA was obtained. RT-PCR from UPD 7 cells lines revealed that the maternal UPD 7 cell line expressed isoform 2 but not isoform 1 while normal lymphocytes, the paternal UPD 7 cell line expressed both isoform 1 and isoform2. In retrospect, previous studies would not have identified such differential imprinting because primers used for RT-PCR in the previous studies do not distinguish the imprinted isoform the non-imprinted isoform. We conclude that human PEG1/MEST is imprinted in an isoform-specific manner rather than in a tissue-specific manner in lymphocytes. Moreover, we suspect that such phenomena as tissue-specific loss of imprinting or leaky expression could result from isoform-specific imprinting in some cases.

Program Nr: 267 from the 1999 ASHG Annual Meeting

Allele-specific gene expression patterns in 15q11-13 by RNA-FISH. *L.B.K. Herzing, D.H. Ledbetter.* Dept. of Human Genetics, University of Chicago, Chicago, IL.

Abnormalities in 15q11-13 can result in Prader-Willi Syndrome, Angelman Syndrome, developmental delay or autism, with the phenotype dependent upon the parent-of-origin of the abnormal chromosome. Accordingly, many of the genes in this region are imprinted and neurally expressed. In an initial survey of imprint status, we compared gene expression across 15q11-13 using RT-PCR and a panel of deletion and UPD patients and somatic-cell hybrid lines. However, in the hybrids we found markedly inconsistent patterns of gene expression in the distal portion of our region of interest, between *UBE3A* and *OCA2*, which includes the *GABR* gene cluster. This may be attributable to the presence of only a single chromosome in the hybrids.

To directly observe gene expression in 15q11-13, we have utilized RNA and RNA/DNA FISH. This provides information in a normal, biparental cell environment, which may be important for proper regulation of imprinted gene expression. The presence and levels of expression can be determined, which is especially useful when there is no known polymorphism, or a gene duplication. RNA was detected using 1.5-6 kb cDNA and intron probes derived from IMAGE clones, RT- and genomic PCR. Co-localization of genomic probes by DNA-FISH confirmed the unamplified RNA signals. Parental origin of the RNA signal was determined by co-detection of expression from known imprinted genes and by using translocation chromosomes of known parental origin. In fibroblasts, *SNRPN* and *NDN* exhibit expression solely from the paternal allele, as expected. *UBE3A*, however, although biallelically expressed, consistently exhibits several-fold higher levels of expression from the maternal allele. This increased maternal *UBE3A* expression can also be observed from both alleles in a maternally-derived inv dup(15) chromosome, demonstrating that the arrangement (1 paternal: 3 maternal copies in *trans*) does not significantly alter gene expression. Ongoing work includes utilization of human neuronal cell lines to address issues of tissue-specific imprints and further analysis of genes potentially involved in autism.

Program Nr: 268 from the 1999 ASHG Annual Meeting

Methylation pattern of SNRPN exon 1 in germ cell tumors: assessing loss of imprinting in cancer. *K.J. Bussey[1,3], H.J. Lawce[1], S.B. Olson[1], X.O. Shu[2,3], R.E. Magenis[1,3].* 1) Oregon Health Sci University, Portland, OR; 2) University of South Carolina, Columbia, SC; 3) Children's Cancer Group, Arcadia, CA.

Loss of imprinting (LOI) for genes such as H19 and IGF2 has been described in a variety of tumors, including adult testicular germ cell tumors (GCTs). However, unlike somatic cells, the primordial germ cells (PGCs) giving rise to GCTs may or may not have a somatic imprint depending on their stage of development. In order to determine what role LOI might play in these tumors, it is important to establish the imprinting stage of the PGC.

SNRPN is a maternally imprinted gene at 15q11-13. The gene is methylated in the 5'-untranslated region of exon 1 on the maternal allele, resulting in expression solely from the paternal allele. Studies in mice have established the timing of methylation erasure and re-establishment in the germ line and correlated this with expression. Additionally, SNRPN has not been implicated in cancer, making it an ideal candidate to assay for methylation changes associated with erasure and resetting of the imprint in the germ line.

If human germ cell development is similar to that of mice, we can make predictions about the expected methylation patterns of SNRPN in GCTs. Tumors located in the sacrum, which arose from PGCs that had yet to erase their imprint, will have both a methylated and unmethylated band after digestion with NotI/XbaI. GCTs in other extragonadal locations along the midline are speculated to have arisen from PGCs that erased the imprint and are expected to have only the unmethylated fragment present. GCTs in the gonads of males are predicted to have only the unmethylated fragment, while those in females are expected to have an unmethylated band in tumors from premeiotic germ cells and only a methylated band in GCTs from meiotic precursors. 15/21 tumors examined had the predicted results. These results suggest SNRPN can be useful in establishing the reference imprinting stage for analyzing LOI at other imprinted loci.

Program Nr: 269 from the 1999 ASHG Annual Meeting

ENU Mutagenesis Screen for Dominant Mutations of X Chromosome Inactivation. *I. Percec[1,2], R. Plenge[1], J. Nadeau[1], M. Bartolomei[2], H. Willard[1]*. 1) Genetics, Case Western Reserve University School of Medicine, Cleveland, OH; 2) Cell and Developmental Biology, HHMI and University of Pennsylvania School of Medicine, Philadelphia, PA.

X chromosome inactivation is the mechanism via which females inactivate one X chromosome in order to equalize X-linked expression to males. The X inactivation center (*Xic*) is an X-linked region required in *cis* for inactivation. A single gene, *Xist*, has been mapped to the *Xic*, and shown to be necessary and sufficient in *cis* for inactivation. Although little is known about the mechanism of X inactivation, the pathway is believed to involve additional factors. To identify such factors, we have developed an ENU mutagenesis screen for mutations of X inactivation using two assays.

One assay examines the X inactivation pattern by quantitating allele-specific expression at *Pctk*, an X-linked locus subject to inactivation. Females that deviate from the mean X inactivation pattern may harbor mutations in genes involved in X inactivation. We have assayed 346 G_1 females and discovered 18 greater than two standard deviations, and 4 greater than three standard deviations away from the mean. Progeny tests determine whether the skewed inactivation patterns are due to stochastic variation or to dominant mutations of X inactivation. To date, three G_1 and two G_2 females have transmitted skewed patterns to their offspring, suggesting the segregation of dominant mutations of X inactivation.

A second assay examines escape from inactivation by quantitating allele-specific expression at *Xe169*, a locus that escapes inactivation. Females that display an escape pattern different from the expected may harbor mutations in genes that regulate escape from inactivation. We have examined 246 females and discovered 25 that deviate which are being progeny tested. This ENU mutagenesis screen will enable the recovery of mutations that disrupt X inactivation. The identification of factors affected by these mutations will be crucial to the elucidation of the pathway of X chromosome inactivation.

Program Nr: 270 from the 1999 ASHG Annual Meeting

Alternative promoter usage on active and inactive X chromosomes delimits a junction between two epigenetically distinct domains. *L. Carrel, J. Dunn, H.F. Willard.* Case Western Reserve Univ, Cleveland, OH.

X inactivation silences most genes on one X chromosome in females. However, many genes have been identified that escape inactivation and are expressed from active (Xa) and inactive (Xi) Xs. That some of these genes are clustered suggests that their expression may be controlled at the level of chromosomal domains. We previously determined the inactivation status of 3 genes within a 50 kb region in Xp11.23 (Hum Mol Genet 5:391). Of these, UBE1 and PCTK1 are adjacent and escape inactivation, while DXS8237E, mapping upstream of UBE1, is subject to inactivation. We have analyzed in depth the region between UBE1 and DXS8237E to examine the hypothesis that sequences between these genes act as a domain boundary and control Xi gene expression. Sequence and expression analysis of UBE1 identified an upstream alternative promoter (P1) and untranslated exon, in addition to the previously identified promoter (P2) and untranslated exon. To test whether both P1 and P2 and their respective exons escape X inactivation, expression was assayed in somatic cell hybrids retaining a single human X using RT-PCR with exon-specific primers. While P2 was expressed from all 4 Xi hybrids, P1 was only expressed in hybrids retaining Xa chromosomes. Both P1 and P2 contain CpG islands, and methylation studies of 5 restriction enzyme sites in males, females, Xa, and Xi hybrids correlate with expression results at each promoter. Therefore, UBE1 demonstrates a novel pattern of Xi expression; one promoter is subject to inactivation, the other escapes X inactivation. Identification of P1 narrows the distance between the two epigenetically distinct domains to 2.6 kb that separates these two promoters. This region was sequenced to search for potential chromosomal elements that may be involved in Xi gene regulation. Sequences within this region are identified by computer algorithm as a matrix attachment site and include 100bp that is 80% AT-rich. Biochemical studies of this site will address the role that chromatin packaging plays in the epigenetic control of X-linked genes and test whether sequences within individual chromatin loops are coordinately regulated.

Program Nr: 271 from the 1999 ASHG Annual Meeting

The human X inactivation center in male ES cells and transgenic mice. *B.R. Migeon[1], A. Hughes[1], H. Winter[1], E. Kazi[1], C. Haisley-Royster[1], R.H. Reeves[1], H. Morrison[2], P. Jeppesen[2].* 1) McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins Univ Schl Med, Baltimore, MD; 2) Human Genetics Unit, Western General Hospital, Edinburgh.

We have recently identified DNA sequences from the putative human X inactivation center (*XIC*) region that were sufficient to initiate random X inactivation in cells of chimeric mice. The mice were derived from male embryonic stem (ES) cells carrying ~ 6 copies of the 480 Kb human *XIC* transgene on mouse chromosome 11 (*Genomics, in press*). Inactivation resulting from this transgene (ES-10) was extensive on the X chromosome, but more localized on chromosome 11, showing that initial inactivation and spreading of inactivation signals along the chromosome are independent events. Now we report studies of another transfectant, ES-5 with ~1 copy of a truncated human transgene which includes the entire *XIST* locus and promoter sequences, inserted into mouse chromosome 6C1-3. Chimeric mice carrying the ES-5 transgene were obtained. The ES-5 transgene produced far less fetal lethality than the ES-10 transgene and the level of chimerism was very high. Although human *XIST* was well expressed and focused over the autosome in differentiated ES cells with the transgene, the FISH RNA signal in somatic cells from the transgenic mice was more dispersed. Whereas the mouse *Xist* locus was not repressed in ES cells, it was repressed in somatic cells. We obtained no evidence that this *XIST* RNA was effective in inducing inactivation in somatic cells. It seems that one copy of an intact *XIST* transgene is sufficient for focalized *XIST* expression and persistence of mouse *Xist* expression in differentiated ES cells, but not enough to induce random X inactivation in somatic cells expressing the transgene. The search for essential components of the human *XIC* is facilitated by interspecies *XIC* transgenes.

Program Nr: 272 from the 1999 ASHG Annual Meeting

Analysis of the dynamic relationship between rearranged human mtDNAs species in transmitochondrial cell lines. *Y. Tang*[1], *G. Manfredi*[2], *E.A. Schon*[1,2]. 1) Dept. of Genetics & Dev; 2) Dept. of Neurology, Columbia University, New York, NY.

Large-scale mtDNA partial duplications (dup-mtDNAs) and deletions (D-mtDNAs) were found to coexist in tissues from two patients, one with Kearn-Sayre syndrome (KSS) and the other with late-onset myopathy. The dup-mtDNAs were topologically related to the D-mtDNAs, implying that both are generated through a common mechanism. It has been suggested that dup-mtDNA, although not pathogenic per se, could be the intermediate recombinant which gives rise to a pathogenic deletion.

Clones of transmitochondrial cells containing 100% dup-mtDNAs from both patients were passaged for 6 months. Using Southern blot analyses, we found that after 6 months, the originally 100% duplicated clones now contained varying mixtures of wild-type (wt) and rearranged mtDNAs, likely generated through intramolecular recombination events. The relative amounts of each mtDNA species were analyzed at multiple time points, in order to follow their dynamic relationships. Clones of transmitochondrial cells containing 100% D-mtDNAs from the KSS patient were passaged for 2 years. These clones, which originally contained a mixture of monomeric and dimeric forms of D-mtDNAs, underwent an intermolecular recombination event, resulting in clones containing only deletion dimers. When we passaged clones which originally contained 50% wt-and 50% D-mtDNAs, the ratio of wt : D remained constant over a 6-month period, suggesting that there was no replicative advantage for the D-mtDNAs molecules even though they are shorter than wt-mtDNAs. Surprisingly, we did not observe any dup-mtDNA arising in this clone. Also, the total mtDNA content remained constant over time in all the transmitochondrial cell lines examined.

In conclusion, this study provides strong evidence that: 1) mtDNA duplications can be considered to be "precursors" of pathogenic deletions; 2) mtDNAs can undergo both intramolecular and intermolecular recombination events; and 3) there is no replicative advantage for the deleted molecules over wild-type mtDNAs.

Program Nr: 273 from the 1999 ASHG Annual Meeting

Position effect in the mammalian genome can be mediated by interstitial chromosomal regions. *J.M. Greally[1], R. Alami[2], K. Tanimoto[3], S. Hwang[1], Y.-Q. Feng[2], J.D. Engel[3], S. Fiering[4], E.E. Bouhassira[2]*. 1) Department of Genetics, Yale University, New Haven, CT; 2) Division Of Hematology/Department of Medicine, Albert Einstein College of Medicine, Bronx, NY; 3) Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, IL; 4) Microbiology Department, Dartmouth Medical School, Hanover, NH.

While transgenes are very useful research tools, they are so subject to artefacts that their epigenetic organisation rarely reflects that of their native loci. One major source of artefact is the influence of the site of integration on the construct. This phenomenon is best described in yeast and *Drosophila*, where juxtaposition of normally eukaryotic genes with heterochromatin leads to suppression of expression. Similar position effects occur in mammalian genomes, attributed either to the absence of *cis*-acting elements in small constructs, or integration at sites of constitutive heterochromatin for large constructs. We analysed a large number of human b-like globin transgenes in mice to test these assumptions. The transgenes were either small constructs containing locus control region derivatives linked to individual globin genes, or 150 kb YACs containing the entire human b-globin domain. FISH studies allowed simultaneous detection of the transgene, mouse centromeric repeats and chromosome-specific probes. Combining the FISH data with single cell expression analyses of the transgenes, we were able to correlate the cytogenetic locations of transgenes with the presence of position effect. We found no correlation between the presence of position effect and integration at sites of constitutive heterochromatin. This was shown by occurrences of position effect for transgenes integrated at interstitial sites, as well as the more surprising corollary that a small b-globin transgene integrated in pericentromeric heterochromatin was not subject to position effect. We conclude that position effect can occur at sites distant from constitutive heterochromatin in mammalian cells, implying either the presence of heterochromatin in interstitial regions or a greater complexity to the phenomenon of position effect than mere proximity to heterochromatin.

Program Nr: 274 from the 1999 ASHG Annual Meeting

A General Transcriptional Repressor of Genes Involved in Human Lipid Metabolism. *B.R. Bowen[1], S. Wagner[2], M.A. Hess[2], P. Ormonde[2], J. Malandro[2], H. Hu[2], R. Kehrer[2], M. Chen[2], C. Schumacher[1], M. Beluch[1], C. Honer[1], M. Skolnick[2], D. Ballinger[2].* 1) Novartis Institute for Biomedical Research, Summit, NJ; 2) Myriad Genetics, Inc., Salt Lake City, UT.

Hypoalphalipoproteinemia (low HDL) is a common risk factor for early coronary disease. A genetic locus for familial low HDL was identified near 11q23 by linkage analysis of Utah pedigrees. The minimal recombination interval contains ZNF202, a gene which encodes a 648 aa protein containing eight zinc finger motifs, a Krueppel-associated box (KRAB) and a SCAN/LeR domain. Because these signatures suggest that ZNF202 acts as a transcriptional regulator, we used an affinity selection technique to identify a consensus DNA binding site for ZNF202. A BLAST search of a human promoter database revealed direct repeats of the ZNF202 binding site in the regulatory sequences of genes involved in lipid metabolism. Among these are the promoters for apoAIV, apoE, LPL, LCAT, HTGL, and PLTP. The motif is also found in the apoCIII enhancer, which regulates expression of apoAI, the main protein component of HDL. Gel shift and competition experiments using several of these promoter fragments confirmed that ZNF202 binds these elements with high affinity (K_d ~10nM) and specificity. In addition to its DNA binding specificity, we analysed the effect of ZNF202 on transcription from reporter gene constructs. Transient co-transfection assays in HepG2 cells showed that ZNF202 represses transcription from the apoE and apoAIV promoters, indicating that ZNF202 acts as a repressor of genes related to lipid metabolism. Based on its linkage to low HDL and its function as a repressor of genes crucial to lipid metabolism, we propose that ZNF202 is a candidate predisposition gene for hypoalphalipoproteinemia.

Program Nr: 275 from the 1999 ASHG Annual Meeting

Polymorphisms in the 5' regulatory region of presenilin 1 associated with an increased risk for early-onset Alzheimers Disease. *J. Theuns[1], J. Del-Favero[1], B. Dermaut[1], C.M. van Duijn[2], H. Backhovens[1], M. Van den Broeck[1], S. Serneels[1], C. Van Broeckhoven[1], M. Cruts[1]*. 1) Department of Molecular Genetics, Flanders Interuniversity Institute for Biotechnology (VIB), Born-Bunge Foundation (BBS), University of Antwerp (UIA), Department of Biochemistry, Antwerpen, Belgium; 2) Department of Epidemiology and Biostatistics, Erasmus University Medical School, 3000 DR Rotterdam, The Netherlands.

In recent years important progress has been made in uncovering genes implicated in Alzheimers disease (AD). Three genes have been associated with autosomal dominant early-onset AD (EOAD): the amyloid precursor protein (APP) gene, the presenilin 1 (PSEN1) gene and the presenilin 2 (PSEN2) gene. Together these genes cause AD in less than 10% of all patients since the majority of these mutations occur in familial EOAD cases. However, PSEN mutations were found in a patient with sporadic EOAD and in patients with an onset age above 65 years (Cruts et al., 1998). The identification of these mutations predicts that PSEN1 mutations with even milder effect on psen1 functioning may be present in sporadic EOAD or LOAD. We previously demonstrated that polymorphisms in the promoter and 5 upstream region of PSEN1 are associated with increased risk for EOAD (van Duijn et al., in press). In this study we sequenced approximately 7 kb upstream of the non-coding exon1B of PSEN1 and designed primers to systematically screen this region for sequence variations associated with AD. Six novel sequence variations were found, two of which are occurring only in one patient. The variations are located upstream of exon 1A and two of the novel variations are in linkage disequilibrium with the variation 48 bp upstream of exon 1A reported earlier (Cruts et al., 1998). The effect of these polymorphisms on the promoter activity of PSEN1 was studied in a transient transfection system. One of the polymorphisms showed a significant effect on the transcriptional activity of the PSEN1promoter in neuroblastoma but not in kidney cells, suggesting a potential promoter element involved in neuron-specific expression of PSEN1.

Program Nr: 276 from the 1999 ASHG Annual Meeting

The most common Jagged1 missense mutation in Alagille syndrome (R184?) causes alterations in Jagged1 glycosylation. *J.J. Dowhanick-Morrissett[1], R.P. Colliton[1], S. DeRocco[3], D. Piccoli[2], I.D. Krantz[1], N.B. Spinner[1].* 1) Dept Human Gen & Molec Biol, Children's Hosp Philadelphia, Philadelphia, PA; 2) Gastroenterology; 3) Department of Genetics, University of Pennsylvania, Philadelphia, PA .

Alagille syndrome (AGS) is a multisystem disorder caused by mutations in Jagged1 (JAG1), a ligand in the Notch signalling pathway. This conserved signal transduction pathway plays a role in cell fate determination. JAG1 is a cell surface protein that interacts with the Notch transmembrane receptor, releasing an intracellular form of Notch which stimulates transcription from downstream promoters. Jagged1 mutations are found in > than 70% of AGS patients. The majority of these are protein truncating and 9% are missense mutations. To date, 22 unique missense mutations have been found in 298 patients. The most common is an alteration of amino acid 184 (5 cases R184H; 1 R184C; 1 R184G). The mechanism by which JAG1 and the Notch receptors interact is unknown, but studies in other organisms have pinpointed regions of the protein that are essential for normal signalling. Amino acid 184 lies adjacent to one of these highly conserved regions, the DSL domain. We are currently studying missense mutations in AGS patients to: 1) demonstrate that they are in fact disease causing and 2) determine why these single amino acid changes obliterate normal functioning of the protein. Using two assays for JAG1 function, we have confirmed that mutant JAG1(R184H) is incapable of activating Notch, consistent with this being a disease causing mutation. Furthermore, we have demonstrated that R184H is incompletely glycosylated. Immunoblot analysis of R184H demonstrates that the mutant JAG1 runs at a lower molecular weight on SDS PAGE compared to wildtype JAG1. To understand why this mutant causes a mobility shift, we treated wildtype and R184H with a variety of protein modifying agents and found that treatment with an endoglycosidase produced wildtype and mutant JAG1 molecules with identical mobilities, leading us to conclude that R184H is an incompletely glycosylated form of the protein. This is the first demonstration that glycosylation of JAG1 is a key element for normal signalling of this molecule. *Jagged1*.

Program Nr: 277 from the 1999 ASHG Annual Meeting

The C-terminal domain of dystrophin is not required to prevent muscular dystrophy or assemble the dystrophin associated protein complex. *G.E. Crawford[1], J.A. Faulkner[2], J.S. Chamberlain[1,3]*. 1) Dept of Cellular and Molecular Biology; 2) Dept of Physiology; 3) Dept of Human Genetics, Univ Michigan, Ann Arbor, MI.

Duchenne muscular dystrophy is caused by defects in the dystrophin gene. Dystrophin binds peripheral and integral membrane proteins, which together comprise the dystrophin associated protein complex (DPC). The C-terminal region of dystrophin has a cysteine rich domain (CR) and a C-terminal domain (CT). The CR binds an integral membrane complex of dystroglycans, sarcoglycans, and sarcospan. The CT binds peripheral DPC members, syntrophin and dystrobrevin, at an alternatively spliced region and leucine zipper domains, respectively. The importance and functional significance of syntrophin and dystrobrevin in the DPC remain unknown although they may be involved in cell signaling pathways. Previous studies of transgenic mice expressing dystrophins deleted for the alternatively spliced region (exons 71-74) or the leucine zipper motifs (exons 75-78) demonstrated normal localization of syntrophin, dystrobrevin, and neuronal nitric oxide synthase (nNOS). Furthermore, these truncated dystrophins completely prevented muscular dystrophy in dystrophin negative *mdx*mice. Since syntrophin and dystrobrevin bind to each other as well as to dystrophin, it was proposed that removing only one of the two binding sites was not enough to sever the linkage to the DPC. To test this hypothesis, we generated a new dystrophin transgenic *mdx*mouse deleted for both the syntrophin and dystrobrevin binding domains (exons 71-78). This new mouse, D71-78, displays normal muscle morphology and has no evidence of dystrophy in diaphragm and EDL muscles. Surprisingly, both syntrophin and dystrobrevin are present at normal levels on the sarcolemma. However, D71-78 muscle microsomes enriched for the DPC exhibit reduced levels of syntrophin and nNOS, but normal levels of dystrobrevin. These results indicate that syntrophin, dystrobrevin, and nNOS assemble at the sarcolemma with the DPC in the absence of direct binding to dystrophin. The absence of dystrophy in the D71-78 mice provides further evidence that the peripheral DPC may play a signaling, rather than a structural, role in muscle.

Program Nr: 278 from the 1999 ASHG Annual Meeting

The presence of novel discovered targeting signals in some of the alternative spliced FXR proteins and their tissue specific localisation indicate additional functions. *A.T. Hoogeveen, F. Tamanini, C.E. Bakker, L. Van Unen, C. Bontekoe, J. Schonkeren, B.A. Oostra.* Dept Clinical Genetics, Erasmus Univ, Rotterdam, Netherlands.

Fragile X syndrome is caused by the absence of the fragile mental retardation protein (FMRP). FMRP and its structural homologous FXR1P and FXR2P form a family of RNA-binding proteins (FXR-proteins) which are believed to play a role in RNA metabolism. The FXR proteins sediment with active translating ribosomes in an RNA-dependent manner and are released from the polyribosomes by EDTA treatment as messenger ribonuclearprotein (mRNP) particles of >600 kDa. Since the three proteins are interacting in vitro it is suggestive that they play a role in the pathogenesis of the fragile X syndrome. The in vitro and in vivo interaction as well as tissue specificity and intracellular expression pattern of the three proteins was investigated. Our results show that the ratio of expression of these proteins varies in different tissues. We further demonstrated that all FXR proteins can shuttle between cytoplasm and nucleus and that their nuclear export is exportin 1 dependent. However, while FMRP shuttles mainly from cytoplasm to the nucleoplasm, FXR1P and FXR2P isoforms can shuttles between cytoplasm and the nucleolus depending of their targeting signal. We further demonstrated that FMRP, FXR1P and FXR2P are dissociated from polyribosomes as a homopolymeric rather than a heteropolymeric complex. The variable ratio of expression of the FXR proteins in the different tissues, the formation of homopolymeric complexes and the difference in intracellular routing suggests the transport of different RNA's and independent roles for the FXR proteins. The variation of a unique targeting signal together with a tissue specific localisation indicates for the first time different functions for the individual splice variants of the FXR proteins.

Program Nr: 279 from the 1999 ASHG Annual Meeting

The loss-of-function of a SMN frameshift mutation in Spinal Muscular Atrophy. *S. Lefebvre, S. Bertrandy, P. Burlet, A. Munnich.* INSERM U-393, Hopital des Enfants Malades, Paris, France.

Spinal Muscular Atrophy (SMA) is an autosomal recessive disorder characterized by degeneration of spinal motor neurons. SMA results from alterations of the Survival Motor Neuron (SMN) gene located in chromosome 5q13. The SMN protein is markedly deficient in tissues derived from SMA fetuses and patients. SMN is distributed in the cytoplasm and in new nuclear bodies called gems involved in mRNA metabolism, but its specific functions remain unclear. SMN has been shown to contain two highly conserved domains found to bind a novel protein, SMN interacting protein 1 (SIP1), and the Sm proteins (common spliceosomal snRNP proteins), respectively. Isolation of the zebrafish and C. elegans orthologues allowed to demonstrate that RNA-binding capacity was conserved across species. Deletions of the zebrafish SMN protein defined domains conserved in evolution. The deleted protein mimicking a SMA frameshift mutation shows a dramatic change in the RNA-protein interaction. To characterize the human mutation, we produced and purified E. coli recombinant proteins that correspond to the normal and mutated human SMN and performed binding assays in vitro. We found that the mutation abolishes RNA-binding activity despite the presence of the RNA-binding domain. These data indicate that deletion of the C-terminal end of SMN (which binds to the conserved Sm proteins and includes the SMN self-association domain) can affect the RNA-binding capacity of SMN. Therefore, the different binding properties of SMN might be closely related and serve in the assembly of ribonucleoprotein complexes. To test this hypothesis, we first demonstrate that the mutated SMN protein still binds in vitro to SIP1. Secondly, the normal and mutated SMN proteins were expressed in mammalian cell cultures. The full-length SMN localized in the cytoplasm and in gems, whereas the mutated protein exhibits altered subcellular distribution. The subcellular localization of different SMN-associated proteins is currently under investigations in cell expressing the mutated SMN. This cellular model should help to elucidate the function(s) of SMN, contributing to a better understanding of the pathogenesis of SMA.

Program Nr: 280 from the 1999 ASHG Annual Meeting

Mutations in the tumor suppressor gene Patched alter normal protein trafficking. *H.J. Karpen, J. Bukowski, J. Daehne, M.R. Gailani.* Pediatrics,Yale University , New Haven, CT.

The tumor suppressor gene patched (PTCH) is altered in most basal cell carcinoma (BCC) and germline mutations in PTCH cause Gorlin syndrome (GS), and AD disorder associated with developmental defects and BCCs. PTCH is part of the hedgehog (HH) signalling pathway and functions as a HH receptor. PTCH also associates with and inhibits the function of a second membrane protein, smoothened (SMO). When HH binds to PTCH, this inhibition is released and SMO transduces the HH signal. We hypothesized that PTCH/SMO receptor complex function could be studied by tagging each protein with the green fluorescent protein (GFP) and examining localization and trafficking in cell culture. Introduction of PTCH mutations described in tumors and GS patients would determine important functional domains. A full length PTCH and SMO cDNA were cloned into a GFP vector, transfected into COS cells and examined under confocal microscopy. Most of the PTCH protein localized to the golgi with movement to and from the cell membrane; SMO localized mainly to the ER. Co-transfection experiments showed more protein at the membrane suggesting the complex forms prior to insertion in the cell membrane. PTCH mutations were introduced by site-directed mutagenesis. Introduction of a mutation in the 9th transmembrane domain (TMD) of PTCH showed little protein at the membrane but co-transfection experiments with SMO rescued the normal pattern suggesting the complex was able to localize to the membrane. A stop codon introduced to remove the PTCH c-terminus also prevented the protein from localizing to the cell membrane despite the presence of SMO. Mutations introduced into the first EC loop and TMD 4 did not alter normal patterns. All mutated PTCH proteins were able to associate with SMO by co-IP, suggesting that none of these areas are necessary for PTCH/SMO protein interaction. The regions may be important in binding HH or altering the signal between PTCH and SMO in the presenc of HH. Future studies will examine the ability of these constructs to bind HH and transmit the HH signal. Understanding the function of the receptor complex may suggest targeted drug therapy for a common skin cancer.

Program Nr: 281 from the 1999 ASHG Annual Meeting

The cellular distribution of the C-terminus of CFTR is altered by interaction with NHERF via a PDZ domain.

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Cystic fibrosis (CF) is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR). Mislocalization of CFTR is the pathologic mechanism underlying most cases of CF. We have been interested in determining the process of events that direct CFTR to apical membranes of epithelial cells. PDZ proteins are involved in targeting other proteins to specific subcellular compartments or clustering them in multi-protein complexes. Therefore, the presence of a PDZ-binding motif at the carboxy-terminus of CFTR suggests that this region of the protein may play a role in CFTR biogenesis. To explore this possibility, we tested whether expression of NHERF (EBP50) or CAL, two PDZ proteins known to interact with the C-terminus of CFTR in vitro, affects the distribution of GFP-CFTR C-ter fusion proteins in polarized MDCK cells. Co-expression of NHERF, but not CAL, leads to redistribution of GFP-CFTR 1370-1480 from intracellular aggregates to the cytoplasm. This redistribution was dependent on the presence of the PDZ-binding motif, indicating a direct interaction between these two proteins. Moreover, apically located GFP-CFTR 1370-1480D1 (D1395-1403) fusion protein was redistributed to the cytoplasm by co-expression with NHERF, whereas CAL had no effect. This is the first evidence that a protein with a PDZ-binding motif interacts with the C-terminus of CFTR in vivo.

Program Nr: 282 from the 1999 ASHG Annual Meeting

***Ufd1l*, a Candidate Gene for DiGeorge Syndrome, is Essential but not Haploinsufficient in Mouse Embryogenesis.** *F. Vitelli, E.A. Lindsay, Y. Wang, A. Baldini.* Dept. Molecular & Hum Genetics, Baylor Col. of Med., Houston, TX.

Ufd1l was identified as a gene consistently deleted in DiGeorge syndrome (DGS) and as the human homolog of the S.cerevisiae *UFD1*, a gene thought to be involved in a ubiquitin-dependent protein degradation pathway (Pizzuti et al., *Hum Mol Genet* 6:259-65, 1997). More recently it has been shown that this gene is down-regulated in *dHand* -/- mice, and that its expression is consistent with a role in pharyngeal arch development. In addition, the gene is disrupted in a patient with a very small deletion which includes only this and one other gene, *Cdc45l* (Yamagishi et al., *Science* 283:1158-61, 1999). *Ufd1l* is included in the engineered mouse chromosome deletion (*Df1*) which is associated with DGS-like heart defects (see abstract by Baldini et al.). The exact function of *Ufd1l* in yeast or mammals is unknown. To understand the role of *Ufd1l* in the *Df1*/+ phenotype and / or in the development of organs and structures affected in DGS, we analyzed the phenotype of *Ufd1l* knock out mice that were generated in our laboratory. If *Ufd1l* deletion were solely responsible for the *Df1*/+ (heterozygous) heart defects, *Ufd1l*+/- mice should have the same phenotype. Conversely, we found that heterozygous mutants had no heart defects. The targeted mutation produced a null allele and, in *Ufd1l*+/- embryos, the *Ufd1l* mRNA was approx 50% of the amount present in wild type embryos. Hence, the deletion of *Ufd1l* is not sufficient to generate heart defects in mice. *Ufd1l*+/- mice were crossed to obtain homozygous mutants. However, no *Ufd1l*-/- embryos could be recovered, even in the early stages of gestation. Because *Ufd1l* is physically very close to *Cdc45l*, a gene putatively involved in DNA replication, we tested whether the mutated allele may cause down-regulation of the *Cdc45l* gene. However, Northern blotting analysis showed no reduction of the *Cdc45l* message in *Ufd1l*+/- mice, suggesting that lethality is not due to loss of function of *Cdc45l*. Our data demonstrate that *Ufd1l* is essential for mammalian development and show that its deletion is unrelated to, or not solely responsible for the DGS-like heart defects observed in our mouse model.

Program Nr: 283 from the 1999 ASHG Annual Meeting

Autosomal dominant polycystic kidney disease: Expression analysis of polycystin2 and description of perinatal defects in a new PKD2 (+/-;LacZ+/-) knockout mouse. *J. Horst[1], P. Pennekamp[1], B. Skyrabin[2], M. Wilda[3], N. Bogdanova[1], H. Hameister[3], B. Dworniczak[1]*. 1) Institut fuer Humangenetik, Universitaet Muenster, Germany; 2) Institut fuer Experimentelle Pathologie, Universitaet Muenster, Germany; 3) Abteilung fuer Medizinische Genetik, Universitaet Ulm, Germany.

ADPKD is one of the most frequently inherited monogenic diseases and affects up to 1:1000 individuals worldwide. It is characterized by progressive development and enlargement of fluid filled cysts in the kidneys that frequently result in chronic and end-stage renal failure. Approximately 8-12% of all patients requiring haemodialysis suffer from this disease. However it is not only restricted to the kidneys but can also manifest as a multisystem disorder, with symptoms including arterial hypertension, hepatic cysts, cardiovascular valve abnormalities, pancreatic cysts, cerebral aneurysms and colonic diverticuli. To analyse the function of the ADPKD genes in more detail we characterised the mouse Pkd2 gene, analysed its expression pattern in comparison to Pkd1 during development and created a mouse model for ADPKD2 by homologous recombination. Using a gene targeting vector we replaced a part of the Pkd2 gene by the LacZ gene. FISH and interspecific backcross analysis located Pkd2 to mouse chromo-some 5 proximal to D5Mit175. The gene spans at least 35 kb of the mouse genome and consists of 15 exons. The protein shows a 95% homology to human polycystin2. In situ hybridisation on mouse embryo sections using Pkd1- and Pkd2-cDNA probes showed mainly coexpression of both poly-cystins in all stages analysed so far. Heterozygous Pkd2 knockout mice do not show any phenotype so far, whereas the homozygous Pkd2 mutant mice are not viable and die perinatally with multiple defects. In mouse embryos the Pkd2 expression pattern can be easily visualised by monitoring LacZ expression. Here we show that Pkd2 exhibits a more complex expression pattern than previously suggested and that polycystin 2 obviously participates in many differentiation processes during embryogenesis. Preliminary data of our analysis will be presented and discussed.

Program Nr: 284 from the 1999 ASHG Annual Meeting

A genome-wide analysis of exon-intron structure: Implications for cellular and molecular mechanisms of mRNA splicing. *G.K.-S. Wong, D. Passey, M.V. Olson, J. Yu.* The Human Genome Center, Dept of Medicine, University of Washington, Seattle, WA.

With the growing accumulation of genomic sequence data in GenBank, it is now possible to perform a genome-wide analysis of exon-intron structure for a large and representative sampling of human genes. We have identified >8000 unique cDNA sequences and aligned >1000 of them against the >10% of the human genome sequence that is in finished form. A detailed BIOLOGICALLY-DRIVEN analysis of the resultant exon and intron distributions, their inter-relationships, their sequence content, and their functional significance, will be presented in the context of the prevailing models of mRNA splicing. Initial analysis has yielded surprising conclusions about genome organization, with implications for genome evolution, cellular organization, and gene regulation.

Program Nr: 285 from the 1999 ASHG Annual Meeting

Genetic Cluster Analysis with Polymorphic Markers. *B.A. Skierczynski[1], D. Cohen[2], J. Lichter[1], N.J. Schork[1,3,4,5].* 1) Genset Corporation, La Jolla, CA; 2) Genset, SA, Paris, France; 3) Department of Epidemiology and Biostatistics, Case Western Reserwe University, Cleveland, Ohio; 4) Program for Population Genetics and Department of Biostatistics,Harvard University of Public Health, Boston, Massachussetts; 5) The Jackson Laboratory, Bar Harbor, Maine.

It is well known that populations can vary considerably with respect to the number and frequency of genetic variants that they possess. Analogously, or consequently, individuals within such populations can vary considerably in terms of the composition of their genomes. Both of these facts contribute to the tremendous phenotypic variation exhibited among individuals within and between populations. In the absence of a priori knowledge of which, and to what degree, different populations may have contributed to the genetic variation within a pool or sample of individuals, one can attempt to assess the existence of genetically similar "clusters" of individuals within a sample through the use of multiple genetic markers and appropriate statistical analysis methodology. We describe and contrast two methods for performing genetic cluster analysis of this sort. Applications of the proposed methodologies include the estimation of immigration and admixture rates, genealogical reconstruction, and the assessment of genetic heterogeneity. We showcase the proposed methods using genotype data from a number of different studies, including an ethnic diversity study. We also discuss the limitations of our methods and areas for future research.

Program Nr: 286 from the 1999 ASHG Annual Meeting

Patterns of meiotic recombination across the human genome. *A.H. Lynn, C. Kashuk, A. Chakravarti.* Dept. of Genetics, Case Western Reserve University, Cleveland, OH.

The pattern of recombination between a pair of homologues during meiosis is affected by many factors including gender, chromosomal location, and proximity to other recombination events. Although recombination has been studied at the molecular level for decades, and many of the biochemical processes are now understood, we still have a poor understanding of how and why recombination varies between individuals. In order to evaluate and define recombination patterns across the human genome, we have constructed new high-resolution, high-fidelity meiotic maps. We show significant differences in male-female recombination across all human autosomes, as expected, however we are now also able to show that these differences vary significantly across chromosomal length as well. On studying gender differences by chromosome, distinct patterns related to length of the chromosome arm and shape of the chromosome (i.e., acrocentric versus non-acrocentric) are detected. To address whether male-female recombination differences arise because of fluctuations in one gender or both, we analyzed meiotic patterns against radiation hybrid maps of the human genome. We compared observed versus expected rates of recombination, given the null hypothesis that recombination occurs at a constant rate per unit physical distance. None of the chromosomes demonstrated a constant rate of recombination when compared to physical distance, and distinct differences were observed between the genders in their patterns of recombination. However, there were many regions that were “hotspots” or “coldspots” for both genders. The rate of recombination per unit physical distance appears to be chromosome-dependent, and does not demonstrate clear, generalizable patterns. Thus, recombination across the human genome is not a random event, but is dependent on the gender of an individual, the structure of the chromosome and the underlying sequence composition.

Program Nr: 287 from the 1999 ASHG Annual Meeting

Phylogenetic studies of the oculocutaneous albinism 2 gene and its potential role in immunity. *M.A. Schaldach[1], T. Ohta[1], T.A. Gray[1], R.P.M.A. Crooijmans[2], M.A.M. Groenen[2], R.D. Nicholls[1].* 1) Dept. Genetics, Case Western Reserve Univ., Cleveland, OH; 2) Wageningen Agriculturual Univ., The Netherlands.

Type II oculocutaneous albinism (OCA2) is caused by recessive mutations of the *P* gene. The P protein is a melanosomal membrane transporter, but its transport function and role in pigmentation are unknown. Despite strong evolutionary selection against OCA2 homozygotes, OCA2 frequencies reach 1/1,100 in some African populations indicating OCA2 heterozygotes most likely have a selective advantage. To determine the nature of this heterotic advantage, we examined the evolution of the *P* gene and its potential role in resistance to infectious disease. We cloned and sequenced *P* orthologous cDNAs from chicken (84% amino acid identity to human over ~510 residues) and *Drosophila* (52% identity). To identify potential mutant phenotypes we mapped the chicken *P* gene to chromosome 1q and are currently mapping the fly gene. We previously described a small family of P-related proteins, including one from *M. leprae*, and now identify P homologs in Archaea (*Pyrococcus horikoshii,* 34% identity), eubacteria (*Synechosystis* sp., 25%), two in *M. tuberculosis* (~33%), and two intronless paralogs in *Drosophila* (34-43%). Phylogenetic analysis indicates that all P-related proteins are potentially functional and derive from a single gene ancestral to all three kingdoms. We show that *P* is expressed in lymph node and other immune tissues, in addition to pigment cells, suggesting a role for the P protein in immunity. Hypopigmentation is a feature of mycobacterial infections, and we propose this may result from a host cellular autoimmune response against the P protein, provoked by the mycobacterial P homolog. Although speculative, the insect P proteins may play a role in the eumelanin-encapsulation innate immune response in *Drosophila* and mosquitos. Based on these observations, we propose that the OCA2 heterozygote advantage in man may arise from selection against mycobacteria and/or malaria, by an unknown mechanism(s). Functional and epidemiological studies will determine the role the P protein plays in diverse organisms and its impact on the evolutionary relationship between infectious agents and their hosts.

Program Nr: 288 from the 1999 ASHG Annual Meeting

Homologous DNA exchanges in humans can be explained by the yeast double-strand break repair model: A study of 17p11.2 rearrangements associated with CMT1A and HNPP. *J. Lopes[1], S. Tardieu[1], K. Silander[2], I. Blair[3], A. Vandenberghe[4], F. Palau[5], M. Ruberg[1], A. Brice[1], E. LeGuern[1].* 1) INSERM U289, Hôpital de la Salpêtrière, Paris, France; 2) Dept. of Medical Genetics, University of Turku, Turku, Finland; 3) University of Sydney, Clinical Sciences Building, Concord Hospital NSW 2139, Australia; 4) Unité de Neurogénétique Moléculaire, Hôpital de l'Antiquaille, Lyon, France; 5) Unitat de Genètica, Hospital Universitari "La Fe", Valencia, Spain.

Rearrangements in 17p11.2, responsible for the 1.5 Mb duplications and deletions associated, respectively, with autosomal dominant Charcot-Marie-Tooth type 1A disease (CMT1A) and hereditary neuropathy with liability to pressure palsies (HNPP), are a suitable model for studying human recombination. Rearrangements in 17p11.2 are caused by unequal crossing-over between two homologous 24 kb sequences, the CMT1A-REPs, that flank the disease locus, and occur in most cases within a 1.7 kb hotspot. We sequenced this hotspot in 28 de novo patients (25 CMT1A and 3 HNPP), in order to localize precisely, at the DNA sequence level, the crossing-overs. We show that some chimeric CMT1A-REPs in de novo patients (10/28) present conversion of DNA segments associated with the crossing-over. These rearrangements can be explained by the double-strand break (DSB) repair model described in yeast. Fine mapping of the de novo rearrangements provided evidence that the successive steps of this model, heteroduplex DNA formation, mismatch correction and gene conversion occurred in patients. Furthermore, the model explains 17p11.2 recombinations between chromosome homologues as well as between sister chromatids. In addition, defective mismatch repair of the heteroduplex DNA, observed in two patients, resulted in two heterozygous chimeric CMT1A-REPs which can be explained, as in yeast, by post-meiotic segregation. This work supports the hypothesis that the DSB repair model of DNA exchange may apply universally from yeasts to humans.

Program Nr: 289 from the 1999 ASHG Annual Meeting

A common molecular basis for rearrangement disorders on chromosome 22q11. *L. Edelmann[1], R.K. Pandita[1], B. Funke[1], N. McCain[1], E. Spiteri[1], R. Goldberg[1], H. Pan[2], F. Chen[2], B. Roe[2], R. Shprintzen[3], A. Skoultchi[1], R. Kucherlapati[1], B.E. Morrow[1]*. 1) Albert Einstein College of Medicine, Bronx, NY; 2) University of Oklahoma, Oklahoma City, OK; 3) SUNY Syracuse, Syracuse, NY.

The chromosome 22q11 region is susceptible to rearrangements that are associated with congenital anomaly disorders. Velo-cardio-facial syndrome/DiGeorge syndrome (VCFS/DGS), der(22) syndrome and cat-eye syndrome (CES) are associated with monosomy, trisomy, or tetrasomy, respectively, of an overlapping region in 22q11. To determine whether there are sequences surrounding the breakpoints that confer susceptibility to rearrangements, we performed FISH mapping, haplotype and somatic hybrid analysis. We found that the breakpoints for all three syndromes lie within low copy repeats (LCR22). The proximal and distal common 3 Mb VCFS/DGS deletion and the CES Type I and Type II breakpoints occur within the same LCR22s, whereas the der(22) breakpoints lie within a third LCR22, the site of less common VCFS/DGS breakpoints. Each LCR22 is approximately 250 kb in size and consists of a cluster of genes or pseudogenes flanked by inverted subrepeats. Based on haplotype and genomic Southern hybridization analysis, we present models to explain how the LCR22s might mediate different homologous recombination events thereby generating distinct rearrangements. A familial interstitial inverted duplication of 22q11 has been identified with breakpoints in the same LCR22s as the common VCFS/DGS deletion breakpoints. We identified five additional LCR22s within the 22q11 region. To distinguish which sequences might be mediating chromosomal rearrangements, we constructed high resolution sequence-ready physical maps of the LCR22s. Mapping low copy repeats, which often correspond to gaps in physical maps, will be instrumental in completing the sequence of the human genome. To establish contigs across the LCR22s, we developed a strategy based on single nucleotide polymorphisms between the repeated sequences. Automated sequence analysis is currently underway. Interestingly, the inverted subrepeat markers are not present in the five additional LCR22s, suggesting that their presence may stimulate recombination events.

Program Nr: 290 from the 1999 ASHG Annual Meeting

Characterisation of the heterochromatin/euchromatin boundary at 10q11 and identification of novel transcripts formed by repeat induced instability. *M.S. Jackson[1], M. Rocchi[2], T. Hearn[1], M. Crosier[1], J. Guy[1], L. Viggiano[2], S. Piccininni[2], A. Ricco[2], R. Marzella[2], N. Archidiacono[2], A. McMurray[3], J. Sulston[3], J. Rogers[3], D. Bentley[3], C. Spalluto[1,2]*. 1) Biochemisty and Genetics, Univ Newcastle, Newcastle, England; 2) Instituto di Genetica, Univ Bari, Italy; 3) Sanger Centre, Hinxton Hall, Cambridgeshire, England.

Pericentromeric sequences exhibit a wide spectrum of poorly understood rearrangements including microdeletions and interchromosomal duplications. We have recently completed 1Mb of contiguous sequence from 10q11 which is the first to link centromeric satellites to a gene of known function (*RET*) The proximal 200kb consists of short arrays of classical satellites separated by stretches of interchromosomally duplicated DNA which contain no transcripts. In contrast, the distal 800kb contain no satellites, 4 intrachromosomal duplications (25-250kb) and 4 genes. All 24 of our STSs from the proximal 200kb map to multiple centromeres (X=9.5), whereas 24 out of the 40 STSs in the distal 800kb are chromosome specific. Thus, there appears to be a single boundary between highly repetitive centromeric DNA and the chromosome arm defined by the most distal satellite array. This separates distinct sequence domains, subject to mechanistically different pericentromeric rearrangements, and we propose that it corresponds to a heterochromatin/euchromatin boundary.

Of the 4 transcripts in the distal region, three have been affected by pericentromeric instability. Two (*ZNF33B* and *ZNF37B*) are primate specific, produced by a 250kb duplication. Exons from a third gene of unknown function, *KIAA0187*, have been distributed to multiple genomic locations due, possibly, to the presence of linked telomeric associated repeats. We have analysed over 100 ESTs associated with *KIAA0187* and found that more than half are derived from paralogous loci. These transcripts can include intronic sequence and novel gene motifs not present on chromosome 10. While it remains to be determined if these are translated, it does suggest that repeat induced duplications may have the potential to create new genes.

Program Nr: 291 from the 1999 ASHG Annual Meeting

Sequence analysis of the breakpoints of a pericentric inversion distinguishing the human and chimpanzee chromosomes 12. *E. Nickerson, R.A. Gibbs, D.L. Nelson.* Dept. Mol. and Human Genetics, Baylor College of Medicine, Houston, TX.

Molecular analyses have suggested that humans and chimpanzees are similar enough to be sister species. Humans and chimpanzees are, however, phenotypically distinct and genetic disparities must account for the differences between the two primates. With only a 1—2% nucleotide divergence between the species genomes, informative molecular fossils of Hominoid evolution may be difficult to discover. Current genetic technology can, however, begin to accommodate ambitious projects in molecular archeology. A directed approach to identifying genetic changes conceivably important to evolution is to characterize breakpoints of gross chromosomal rearrangements that distinguish the karyotypes of humans and chimpanzees. Previously we mapped the p and q arm breakpoints in human (HSA) and in chimpanzee (PTR) of a pericentric inversion that distinguishes HSA chromosome 12 from the PTR chromosome equivalent (XII). A region of HSA 12q15 genetic material duplicated to both chromosome arms in PTR was also identified.

We have now sequenced the HSA 12q15 breakpoint and both PTR XIIp and XIIq breakpoints, as well as >450 kb total of flanking sequence. Each breakpoint region is repeat rich (~47%; repeats on average). L1 repeats are prevalent (expected:observed, 7.5%:22%) and *Alu* sequences more sparse (expected:observed, 10%;:5%). The duplicated region in PTR is ~24 kb and contains clusters of *Alu* and L1 repeats possibly critical to the mechanism of the rearrangement. Comparison between HSA markers and the duplicated copies in PTR shows an average divergence of 1% from the HSA sequence. PTR specific base pair changes shared by the two copies of the duplicated markers indicate that the HSA and PTR chromosomes 12 diverged from the ancestral state before the inversion/duplication event in PTR. Putative coding sequences have been identified in both HSA and PTR. PTR sequence corresponding to HSA 12p appears to contain numerous regions showing identity to EST sequences, several of which are clustered in the Unigene database. One gene product is likely expressed in the brain. The HSA 12q15 region mapping near the inversion breakpoint appears gene poor.

Program Nr: 292 from the 1999 ASHG Annual Meeting

A large scale mouse genetics programme to identify new phenotypes of relevance to human disease. *I.C. Gray[1], M. Bouzyk[1], D. Kelsell[1], D. Gale[1], I. Latham[1], J. Chase[1], S-K. Ng[1], P. Nolan[2], L. Vizor[2], S. Brown[2], A.J. Hunter[1], S. Rastan[1], N.K. Spurr[1]*. 1) SmithKline Beecham Pharmaceuticals, Harlow, Essex, UK; 2) MRC Mammalian Genetics Unit, Harwell, Oxfordshire, UK.

Mouse genetic approaches are an important route to modelling and investigating human genetic disease and identifying some of the key genetic pathways involved. The SmithKline Beecham/MRC Harwell/London Hospital/Imperial College consortium research programme is aimed at generating large numbers of new mouse phenotypes, many of which will carry disorders that model human genetic disease. The programme uses high throughput phenotypic screens in order to survey thousands of progeny of mice treated with the mutagen N-ethyl-N-nitrosourea (ENU) and identify those carrying novel dominant disorders (see http://www.mgc.har.mrc.ac.uk/mutabase/). Following confirmation of a monogenic dominant mode of inheritance, genome wide scans are performed using approximately 100 CA repeat markers with a spacing of £ 20cM and 40 backcross mice. Pooling DNA from a number of mice (³ 20) prior to PCR and quantifying the signal generated from each allele allows rapid detection of linkage. Two phenotypes have been mapped to date using this strategy. Strain GENA51 with a resting tremor phenotype has been mapped to chromosome 11 and GENA38 (small hunched phenotype) has been mapped to chromosome 7. Fine localisation is currently in progress. Generation of a mutant map of the mouse will complement the current genome sequencing efforts, allowing rapid association of gene and phenotype.

Program Nr: 293 from the 1999 ASHG Annual Meeting

Genomic interval engineering of mice identifies a novel modulator of triglyceride production. *Y. Zhu[1], M.C. Jong[2], K.A. Frazer[1], E. Gong[1], R.M. Krauss[1], J.-F. Cheng[1], E.M. Rubin[1].* 1) Life Science, Genome Sciences Dept, Lawrence Berkeley Natl Lab, Berkeley, CA; 2) TNO-Prevention and Health, Gaubius Laboratory, Leiden, The Netherlands.

To aid in the characterization of novel genes discovered in sequenced regions of the genome, rather than examining one gene at a time, we are creating large deletions in mice that include clusters of such genes. Phenotypic assessment of these animals followed by transgene complementation has been used to identify individual genes responsible for specific deletion associated traits. Here we describe the targeted deletion of a 450 kb region on mouse chromosome 11 which, based on computational analysis of murine sequences in the deleted interval, codes for 9 putative genes of yet to be defined function. Mice homozygous for the deletion had a variety of abnormalities including severe hypertriglyceridemia, hepatic and cardiac enlargement, growth retardation and premature mortality. Analysis of triglyceride metabolism in these animals demonstrated a several fold increase in hepatic, very-low density lipoprotein (VLDL) triglyceride secretion. This phenotype is of particular interest since, in humans, hypertriglyceridemia is primarily the result of increased VLDL triglyceride secretion, the genetic modifiers of which have been minimally defined. A series of human YAC and mouse BAC transgenes covering different intervals of the 450 kb deleted region were assessed for their ability to complement the deletion induced abnormalities. This approach revealed that OCTN2, a gene recently shown to play a role in carnitine transport, was able to correct the triglyceride abnormalities. Coupled with clinical studies that have previously suggested a relationship between triglyceride levels and carnitine metabolism, this functional genomics study in mice has deciphered the molecular basis for this relationship through its identification of OCTN2 as a modulator of triglyceride production.

Program Nr: 294 from the 1999 ASHG Annual Meeting

The proposed anonymous Icelandic Healthcare Database:risks and benefits. *J.R. Gulcher, K. Stefansson.* Decode Genetics,Lynghals 1, Reykjavik,Iceland 110,jgulcher@decode.is,kstefans@decode.is.

The expectation from the new genetics in medicine's future is that genetic information will lead to 1)earlier diagnosis leading to earlier treatment or change in lifestyle,2)tailor-made treatments specific to disease-subtype and which avoid side-effects,3)more informed, cost-effective disease management practices, and 4)the identification of more relevant drug targets leading to more specific drug therapies. That the new genetics can deliver is still a hypothesis and the demonstration of its effectiveness is a prerequisite for its application in medical care of the future. The idea behind the Icelandic Healthcare Database that would be built by Decode Genetics is to link together some medical data generated within the Icelandic health system to genealogy,genetic data,environmental data,and resource use data.The database bill proposed by the Icelandic Health Ministry passed Icelandic parliament in December 1998 and is consistent with EU directives and International law.Further group consent will be obtained through the licensing process, ethics committee, and collaborations with the hospitals and clinics before the database is constructed. The data would be coded in multiple steps, one irreversibly, and the use of the database monitored by the data protection and ethics committees in Iceland. The medical data would be brought in without informed consent but individuals may opt-out of the database at anytime. No genetic data would be added to the database without informed consent by the individual.Icelandic physicians and the health ministry would have free access to the database as long as the information is not funneled to a natural client of the database.Foreign scientists may also have access to the database through collaborations with Icelandic physicians or Decode Genetics.The population gains in high technology jobs that would help reverse the brain drain in Iceland.Decode Genetics pays a user fee in the form of upgrading the entire health care informatics systems.All of the operations of the company are in Iceland and the company is majority owned by Icelanders so that most value created accrues to Icelandic people and their government.

Program Nr: 295 from the 1999 ASHG Annual Meeting

Economic justification for the addition of Inhibin-A in second trimester screening for Down syndrome. *T.J. Beazoglou[1], P.A. Benn[2], D.R. Heffley[3]*. 1) Pediatric Dentistry, Univ of CT Health Center, Farmington, CT; 2) Div Genetics, Dept Pediatrics, Univ of CT Health Center, Farmington, CT; 3) Economics, Univ of CT, Storrs, CT.

Many labs providing the second trimester maternal serum "triple test" (AFP, hCG and uE3) are considering the addition of Inhibin-A (Inh-A) to their screening protocols. For this "quad test" to gain widespread acceptance, it is necessary to demonstrate economic as well as medical benefits.

Analysis of the quad test versus the triple test has been conducted using a previously described algorithm for estimating societal economic net benefit (Beazoglou et al, Prenat.Diagn.,18,1241;1998). Detection rates (DR) and false positive rates (FPR) were based on 1996 US pregnancies and assumed that 60% of patients have gestational age based on ultrasound dating prior to screening. Net benefits were calculated at each maternal age from <15 to 49 using 2nd trimester risk cut-offs ranging from 1:100 to 1:500.

Using a 1:270 cut-off, DR and FPR for the triple test were 75.1% and 8.8% respectively for women at all ages combined and 62.0% and 6.1% for women <35. Rates for the quad test were: 79.8% and 7.7% (all ages); 69.5% and 5.6% (<35). Both triple and quad tests were cost-effective over a broad range of cut-offs. Net benefits peaked at the 1:300 cut-off, although changing the cut-off had a relatively minor effect on net benefits. For all ages, the addition of Inh-A could be justified if the test cost no more than $28 ($23 for <35). At $25 for the Inh-A test, screening all pregnant women in the US could yield societal net benefits of $952 million per year.

Other advantages of an improved DS screening protocol are excluded from this analysis: (a) reduced amniocentesis-related fetal losses due to the lower FPR; (b) lower patient anxiety associated with fewer false positives; and (c) increased confidence in screening with a potentially higher utilization rate. We conclude that the quad test should be made more widely available if the Inh-A test can be provided at a reasonable cost.

Program Nr: 296 from the 1999 ASHG Annual Meeting

Need for Sweat Test Confirmation in CF Newborn Screening that includes genotyping (IRT/DNA): Experience with F508C and IVS8 5T/7T/9T in the Massachusetts CF Newborn Screening Program. *R.B. Parad[1,3], A.-M. Comeau[1,2]*. 1) New England Newborn Screening Program,; 2) University of Massachusetts, Boston, MA; 3) Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

A pilot program for CF newborn screening began 2/1/99 in Massachusetts (MA). The IRT/DNA algorithm involves genotyping of infants with elevated IRT levels. We use a reverse ASO assay (Roche Molecular, CA) to detect 16 CFTR mutations. The detection strips have probes bound specific not only for the normal and abnormal sequences at these 16 loci, but for polymorphisms (I506V, I507V, F508C) at DF508 and IVS8 5T loci. The MA CF Newborn Screening Workgroup (a consortium of newborn screeners and CF center clinicians) developed the screening algorithm, and chose to block primary screening for these polmorphisms, to avoid the complexity of counseling parents on such results. However, in the setting of two genotypes (DF508/DF508, and any mutation in conjunction with R117H), we have developed a protocol to confirm results for confounding polymorphisms prior to reporting to pediatricians. We have also emphasized to CF centers that a sweat test should be performed immediately in all screen positive newborns with two identified mutations, (i.e. assumption of CF diagnosis should not be made in the absence of sweat chloride data). Over 80,000 MA births are anticipated this year. In the first 3 months of this program, 17,000 newborns been have screened. We have detected 2 infants (out of over 1,700 genotypes) who appear to have 2 CFTR mutations, but are likely not to develop a classic CF phenotype. One infant with IRT=77 ng/ml initially appeared to have genotype of DF508/ DF508, but had a sweat chloride [Cl-] of 15 meq/L. Testing for the F508C polymorphism revealed a compound heteroztgote (DF508/F508C). A second infant with IRT=64 ng/ml and genotype DF508/R117H had an IVS8 genotype of 7T/9T, reported to yield a mild or normal phenotype. [Cl-] was 31 mEq/L, which we have defined as a "newborn borderline" value. High volume genetic screening will need to carefully consider these and other potential false positives that could lead to presenting parents with an inappropriate diagnosis or prognosis.

Program Nr: 297 from the 1999 ASHG Annual Meeting

Nucleic acid extraction, amplification, and detection in a integrated, self-contained system. *S.E. Beard, J. Gerdes, D. Kozwich, J. Marmaro, C. Roehl, S. Woronoff.* Molecular Innovations, Inc., Denver, CO.

Molecular Innovations, Inc. has developed a new approach to nucleic acid testing for forensics and paternity identity testing, infectious human disease testing (including bacterial warfare), the detection of food and environmental contamination, research and other clinical applications. Broader future applications include test systems for genetic predisposition to disease and the human diagnostic markets. A novel system has been created for integrating nucleic acid extraction, amplification, and detection into a single, self-contained field-usable device. This system, referred to as SCIP (for Self-Contained Integrated Particle assay), can be modified to work with blood, water, tissue, food, urine, and other specimens. There are a number of isothermal amplification schemes which can be used in this system, none of which require a thermocycler. Current projects, funded by grants and contracts, using this SCIP technology this include detection tests for: Yersinia pestis, Cryptosporidium parvum, E. coli 0157H, Listeria monocytogenes, and coliforms. The nucleic acid extraction component of this system, the Xtra Amp Extraction System, is a stand-alone product for combining nucleic acid extraction and amplification, via PCR or isothermal amplification, in the same microcentrifuge tube. This nucleic acid extraction technology is part of a project in collaboration with the National Cancer Institute for development of a system to measure gene expression in small populations of cells within a tumor isolated by laser capture microdissection (LCM). The goal of this system is to generate gene expression information from very focused tissue regions to provide a more complete picture of the patients prognosis. In addition, this system is capable of archiving the nucleic acid from the cells for extended periods, providing a means to compare the expression patterns of key genes throughout treatment.

Program Nr: 298 from the 1999 ASHG Annual Meeting

HMSN1: A UK-wide comparative study of dosage analysis methods. *D.E. Barton[1], J.S. Rowland[2], G.R. Taylor[2], C.M.G.S. Laboratories[3]*. 1) National Ctr Medical Genetics, Our Lady's Hosp Sick Children, Dublin 12, Ireland; 2) Regional DNA Laboratory, Ashley Wing, St.Jamess University Hospital, Leeds LS9 7TF, U.K; 3) Clinical Molecular Genetics Society, UK*.

A number of different approaches are used in diagnostic laboratories to detect the 17p11.2 duplication seen in approximately 70% of cases of hereditary motor and sensory neuropathy type 1 (HMSN1, CMT1A). The approaches in use include quantitative methods (Southern blot, fluorescent STS dosage, microsatellite analysis) and methods detecting rearrangements (long PCR of junction fragments, Southern blot and pulsed field gel electrophoresis). In addition to the different approaches different analysers and different methods of deriving qualitative conclusions from quantitative data are used. There are little data available on the relative merits of these methods, their sensitivity or specificity. To address these questions, 100 samples consisting of duplicated and non-duplicated cases has been collected, randomised and distributed to ten UK laboratories for testing. Each of five different methods has been used on all 100 samples in two independent laboratories. There were 10 (identical) results for 44 samples. Other samples were classified as duplicated or not duplicated, on the basis of the consensus result. In total 88 samples were classified by more than one method. These 88 samples were therefore compared for the 'comparison of the methods'. Of the remaining samples, 8 had discordant results for one of the methods and 1 is suspected of having a partial duplication. Results of the study indicate that the junction fragment methods will miss a significant proportion of duplications, and that STS dosage analysis is the most robust and reliable of the methods tested.

*Participating CMGS Laboratories: **Aberdeen:** Euan Stronach, Caroline Clark; **Birmingham:** Fiona MacDonald, Max Rindl; **Bristol:** Maggie Williams, Linda Tyfield; **Cambridge:** Patrick Tarpey, Elizabeth Buckridge; **Guy's Hospital, London:** Michael Yau, Liz Green, Vandana Nihalani; **Liverpool:** Rachel Butler, Janet Lewis; **Manchester:** David Gokhale, Rob Elles; **Newcastle:** Ann Curtis; **Oxford:** Anneke Seller; **Salisbury:** John Harvey, Claudia Wolf.

Program Nr: 299 from the 1999 ASHG Annual Meeting

Efficient identification of retinoblastoma gene mutations enhances health care. *J. Anderson[1], L. Han[1], J. Sutherland[1], D. Rushlow[2], K. Vandezande[3], B. Gallie[1,4].* 1) Cancer & Blood Research, Hospital for Sick Children, Toronto, Ontario, Canada; 2) Visible Genetics Inc, Toronto, Canada; 3) Simon Fraser University, Burnaby, Canada; 4) Ontario Cancer Institute/Princess Margaret Hospital, University Health Network, Toronto, Canada.

We report the largest-yet series of retinoblastoma (RB) gene mutations, indicating that RB mutation identification belongs in routine health care. We developed a specific, sensitive (74%) strategy for identifying RB gene mutations and tested 309 retinoblastoma (RB) probands. In order to identify RB mutations, we apply a series of tests to tumor samples (unilateral probands) or blood samples (bilateral probands), in an order selected to maximize discovery of mutations. The first test is Fragment Analysis (FA), a set of quantitative multiplex PCR reactions that detect large and small insertions and deletions (involving the whole gene, a single exon, and sometimes, a single base pair) as well as copy-number changes. FA identified mutations in approximately 32% of samples and resulted in the completion of 76 (25% of 309) cases. Proband DNA samples that are normal for FA (and tumors from unilateral probands where only one mutation is found) were then sequenced, two exons at a time, in an order determined by the probability of finding a mutation in each exon pair. Sequencing detected mutations in 75% of probands tested and resulted in the completion of 152 (49% of 309) cases. Using both FA and sequencing we found the causative mutation(s) in 74% of probands tested. In another 6% of the probands, we found changes that may cause disease, but which require further investigation. The mutations detected by FA included 48 multi-exon deletions, 4 single exon deletions and 52 deletions and insertions within an exon. The mutations detected by sequencing included 188 point mutations (116 nonsense mutations, 38 splice site alterations, 17 missense mutations and 3 promoter mutations) and 14 insertions and deletions (12 one base pair, one two base pair and one in-frame, three base pair deletion in a low penetrance family). Finding the RB gene mutation has had a positive impact economically and psychosocially on families.

Program Nr: 300 from the 1999 ASHG Annual Meeting

Direct detection of common mutations in the *BRCA1* and *BRCA2* genes by Amplified Created Restriction Enzyme Site (ACRES). *K. Nafa[1], J. Angell[1], L. Bonavita[1], R. Baum[1], M. Robson[1], K. Offit[1], N. Ellis[1], M-C. King[2], L. Luzzatto[1]*. 1) Dept Human Genetics, MSKCC, New York, NY; 2) Dept Medicine and Genetics, Uni Washington, Seattle, WA.

Mutations in the *BRCA1* and *BRCA2* genes are associated with a large proportion of both hereditary breast cancer and ovarian cancer. In women of Ashkenazi Jewish origin three relatively common mutations in these two genes are known: 185delAG and 5382insC in the *BRCA1* gene, and 6174delT in the *BRCA2* gene. None of these mutations result in modification of any restriction site: therefore, in most labs these mutations are currently identified either by sequence analysis or by allele specific oligonucleotide hybridization using radioisotopes. We have developed an-ACRES-based methodology that is able to detect these 3 mutations reliably and conveniently. In this technique, primers are specially designed to have a mismatch near the mutation of interest. As a result, a restriction site is introduced in the normal sequence, but not in the mutant sequence. The PCR products are then digested with the appropriate restriction enzymes and resolved by agarose gel electrophoresis. A similar approach has been reported by Rohlfs et al. in Clinical Chemistry 43:24-29 (1997), for detecting the 185delAG and 5382insC mutations. We have tested 287 blood samples from Ashkenazi Jewish women in the New York metropolitan area. 216 samples were collected through a research protocol (the New York Breast Cancer Study or NYBCS), in which participation is limited to breast cancer patients of Jewish ancestry, and 71 samples were obtained from persons requesting a clinical test. By ACRES testing, we found 43 heterozygotes: 18 for 185delAG, 8 for 5382insC, and 17 for 6174delT. All the samples classified as heterozygous by ACRES were confirmed by DNA sequencing. In parallel, the 216 samples from the NYBCS were all analyzed by DNA sequencing in Seattle, and entirely concordant results were obtained. Thus, this ACRES method, which does not require radioactivity, has been proven to be reliable and efficient for detecting these mutations and it is cheaper than sequencing.

Program Nr: 301 from the 1999 ASHG Annual Meeting

Practitioner characteristics and use of BRCA genetic tests for cancer susceptibility. *M.K. Cho[1], P. Sankar[2,3], P.R. Wolpe[2,4], L. Godmilow[5]*. 1) Center for Biomedical Ethics, Stanford Univ, Palo Alto, CA; 2) Center for Bioethics, Univ of PA, Philadelphia, PA; 3) Dept of Mol & Cellular Engineering, Univ of PA, Philadelphia, PA; 4) Dept of Sociology, Univ of PA, Philadelphia, PA; 5) Dept of Genetics, Univ of PA, Philadelphia, PA.

Purpose: To examine whether characteristics of practitioners in the U.S. who use BRCA1 and BRCA2 genetic tests are associated with test use or beliefs about testing. Methods: Mailed questionnaire to practitioners requesting information on BRCA tests from the Univ. of PA Genetic Diagnostics Lab between Oct 1, 1995 and March 1, 1998. Results: 327/489 (67%) of practitioners responded. 50% were genetic counselors, 39% were physicians. Physicians were primarily oncologists (35%), medical geneticists (25%), obstetrician/gynecologists (22%), internists (16%). 60% of practitioners had ordered a BRCA1/2 test. Of all practitioners, 42% believed that BRCA1/BRCA2 tests should be made available to any competent, counseled adult patient who wants the information, regardless of her/his cancer risk status. Physicians were significantly more likely to agree with this statement than genetic counselors (P=0.005), and men were more likely to agree than women (P=0.02). 60% of practitioners had a patient decline to be tested when offered. In logistic regression analysis, practitioner age (P=0.018) and gender (P=0.004) were associated with having at least one patient who had declined an offer of BRCA testing, while specialty was not (P=0.84).Discussion: A substantial proportion of practitioners felt that patients should be able to get BRCA tests regardless of the patients' risk status, implying that patient preferences should be the primary basis for decision-making. Practitioner characteristics were associated with both beliefs about availability of testing and with the probability of having a patient decline an offer of a test, and may affect how information about testing is presented. Thus, the impact of informed consent and patient decision-making about testing may be influenced by characteristics of the practitioner as well as by patient preferences and cancer risk.

Program Nr: 302 from the 1999 ASHG Annual Meeting

The power of beliefs in the construction of ethical norms in medical genetics. *C. Bouffard.* Social & Cultural Anthropology, Laval University, Quebec, Quebec, CANADA.

We have observed that the influential ethical mechanisms which are used to oversee medical genetics are directly related to the realm of beliefs. This observation stems from a multisite ethnographic research project in medical anthropology involving meetings with researchers, physicians and patients as well as participation in a ministerial policy committee of Quebec Province. In order to better grasp the incidence of ethics in such a techno-scientific sector, one has to understand that genetics, in joining domains traditionally controlled by social forces such as reproduction, health, economics and risk assessment, activates powerful symbolic forces. Powerful because genetics proposes not only to disrupt or to actualize current norms, but because they carry fears and warnings based in our ideologies, beliefs and myths. In order to understand these opposing forces, we identified through participatory observations, interviews and content analyses, the beliefs that underlie the development of medical genetics and the constructs of ethical norms which are linked to it. Thus, we distinguished four beliefs systems which support what appears to be two distinct but consequent ethical functions. One of these systems encompasses religious and mythical beliefs while a second includes fictional and prophetical representations. Associated with the potential of human genetics, these first two systems together induce a discursive ethical function tied to the survival of the species, to the responsibility for future generations, and, to protection of the integrity of the human genome. A third belief system regroups representations concerning the etiology of genetics diseases and the perception of risk factors while a fourth reveals ideological representations of domination over persons, society and human species. These latter two systems, when linked to the practice of medical genetics and to the warnings of discursive ethics, seem to produce a more normative ethics centered on the respect and dignity of the person, including his family or community, on the modalities of consent for research and medical care, and on confidentiality and equity in access to services.

Program Nr: 303 from the 1999 ASHG Annual Meeting

Telomerase mediated immortalization of human primary fibroblast cells. *C. Lipps, P.M. Jakobs, M. Grompe.* Molecular and Medical Genetics, Oregon Health Sci. University, Portland, OR.

Patient derived cell lines are vital reagents for the study of human genetic disease. Although EBV-transformed lymphoblasts are suitable for many applications, cells which grow in mono-layer cultures have many experimental advantages. They are superior for microcell-mediated chromosome transfer, immunocytochemistry and the isolation of transfected clones. Primary skin fibroblast are useful, but their finite life-span limits their value for many applications. In the past, it has been very difficult to immortalize human primary skin fibroblasts. We have previously reported an improved protocol, which consisted of transfection of fibroblasts with SV40 T-antigen in combination with ethylmethylsulfonate (EMS) mutagenesis. Using this approach, we were able to obtain immortal fibroblast-derived lines from 4/10 patients with Fanconi anemia, but we failed in 6 lines. Recently, the human telomerase catalytic subunit (hTRT) was cloned and reported to overcome senescence in human primary cells (1). We therefore transfected 6 SV40 + EMS treated cell lines, which had previously failed attempts at immortalization with either hTRT telomerase expression construct or a lacZ expression vector. These cells had already undergone 30-40 population doublings (PDL). As before, all 6 lacZ transfected cells senesced after 60-70 PDLs. In contrast, immortal clones arose in 4/6 cell lines transfected with the telomerase expression construct.

We conclude that the combined expression of SV40 T-antigen and hTRT results in a high likelihood of immortalization in human primary skin fibroblasts.

Reference: 1) Bodnar-A-G. et.al., Science (1998) 279: p. 349-52.

Program Nr: 304 from the 1999 ASHG Annual Meeting

Homozygosity mapping of an acromesomelic dysplasia on chromosome 9. *P. Ianakiev[1], M.W. Kilpatrick[1], M.J. Daly[2], A. Zolindaki[1], D. Bagley[4], G. Beighton[3], P. Beighton[3], P. Tsipouras[1].* 1) Pediatrics, UConn Health Center, Farmington, CT; 2) Whitehead Institute, Cambridge, MA; 3) University of Cape Town, South Africa; 4) Principal Medical Officer, St Helena.

The Acromesomelic Dysplasias (AMD) are a group of autosomal recessive disorders that primarily affect the middle and distal segment of the extremities. Homozygosity mapping is potentially a powerful approach for mapping rare recessive traits in inbred populations. The island of St. Helena in the South Atlantic has a population of 5,500 derived from a discrete number of founders. DNA from 15 members of four related nuclear families segregating an AMD was collected for gene mapping. The phenotype was characterised by dwarfism, mesomelic limb shortening and gross distortion of the digits. Genetic linkage to the CDMP-1 locus on chromosome 20 that has been shown to be mutated in both Grebe and Hunter-Thompson types of AMD was excluded using markers flanking CDMP-1. Six consecutive chromosome 9 markers, spanning approximately 5 cM showed identical homozygosity in all affected individuals thus identifying a region of homozygosity by descent. Multipoint analysis generated a maximum lod score of 2.85. These data localize the gene for this dysplasia to the pericentromeric region of chromosome 9. This localization overlaps with the recently defined critical region for the Maroteaux type of AMD (AMDM). The phenotype of the St. Helena type of AMD shares some features with AMDM but also includes additional features. A number of genes have been localized to the pericentromeric region of chromosome 9 including the forkhead domain containing FKHL9 gene, IL11RA which has been shown to be expressed in skeletal progenitor cells during murine development and BAG1 which has been shown to enhance growth factor-mediated protection from apoptosis. This study demonstrates the power of homozygosity mapping in the rapid localization of recessive traits. Only four affected individuals generated a lod score of 2.85. The identification of the gene responsible for this disorder may shed further light on the extent of heterogeneity within the AMDs and on the complex processes involved in limb morphogenisis.

Program Nr: 305 from the 1999 ASHG Annual Meeting

Localization of the Gene for a novel syndrome characterized by Nodulosis, Arthropathy, & Osteolysis (NAO) to 16q11.2-21. *M. Kambouris[1, 2], B.F. Meyer[1], S. Mayouf[1], K. Sheth[1], S. Bahabri[1]*. 1) King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia; 2) Yale University School of Medicine, New Haven, CT, USA.

A genome wide scan was performed using four consanguineous Arab families to localize the gene for NAO (Bahabri-Mayouf) syndrome. This is an autosomal recessive, early onset (mean age of onset is 3.4 years) disease characterized by distal arthropathy, nodulosis and osteolysis. Common findings include distal arthropathy with nodulosis, osteopenia with under tubulation of bones distally more than proximally and upper extremities more than lower, osteolysis in carpal and tarsal bones, branchycephaly, sclerotic suture of the calcarium, and broad medial clavicle. DNA was pooled from three affected siblings and a whole genome scan was performed using 440 microsatellite markers (CHLC, Research Genetics) spaced at approximately 10cM, with the aim of identifying regions homozygous by descent. Homozygosity in the pool was present for several markers. Analysis of consecutive markers identified only a single region of homozygosity. This region, on chromosome 16, was defined by the proximal D16S3253 and distal D16S2624 markers, a genetic distance of approximately 20 cM and included GATA67G11. All four families (seven affected individuals, four normal siblings and eight parents) were screened using markers (D16S753, D16S3396, D16S3253, GATA67G11, D16S2624) within or adjacent to the linkage interval. A maximum LOD score of 4.73 was obtained with marker D16S3253 (Hsa 16q12) at theta=0. The linkage boundaries were defined by the presence of recombinants at the distal GATA67G11 and proximal D16S3396 markers. High density mapping within the linkage interval is currently in progress. Multiple ESTs are present in the linkage interval to which no human diseases have thus far been assigned. The unique phenotype and localization to 16p11.2-21 is consistent with the identification of a novel disorder characterized by nodulosis arthropathy and osteolysis. Refinement of the linkage interval and identification of candidate genes may be useful in understanding the pathogenesis of more common arthropathies.

Program Nr: 306 from the 1999 ASHG Annual Meeting

Human chromosome 18q12-q21 and distal rodent chromosome 18 are associated with multiple autoimmune phenotypes. *T. Merriman[1], F. Corradu[2], F. Cucca[2,3], S. Broadley[2], S. Sawcer[2], A. Compston[2], P. Wordsworth[4], S. Bain[5], J. Jirholt[5], R. Holmdahl[5], D. Kono[5], J. Tuomilehto[5], M. Marrosu[3], D. Undlien[5], P. Pozzilli[5], J. Shield[5], F. Pociot[5], C. Polychronakus[5], I. Eaves[2], J. Todd[2]*. 1) Uni of Otago, Dunedin, NZ; 2) Uni of Cambridge, UK; 3) Uni of Cagliari, Italy; 4) Uni of Oxford, UK; 5) Address via T. Merriman.

Autoimmune diseases, such as type 1 diabetes, multiple sclerosis (MS) and rheumatoid arthritis (RA) share common features; autoantibodies, self reactive T cells, and a genetic association with the MHC. We have published evidence, from 1761 type 1 diabetic families, for association of markers in the *D18S487*-region of chromosome 18q21 with type 1 diabetes (Pc=0.01). We have now extended this work by genotyping two newly-characterised microsatellite markers, 1 Mb centromeric of *D18S487*, in 2383 diabetic families. A haplotype of these markers was associated with disease ($P=1x10^{-5}$, $Pc=4.2x10^{-4}$). The haplotype also showed some evidence of association with disease in both a data set of 936 MS families (P=0.01) and in 309 RA families (P=0.03). In the total 3628 families there was strong association with autoimmunity ($P=5x10^{-8}$, $Pc=2.1x10^{-6}$). Human chromosome 18q12-q21 is syntenic to both rat and mouse chromosome 18: a 25 cM segment of human 18q12-q21 has conserved gene order with 8 cM of distal rodent chromosome 18. Chromosome 18 was examined for linkage to autoimmunity in rodent models of disease. By meta-analysis of published genome scans we show linkage of rodent chromosome 18 to type 1 diabetes ($P=9x10^{-4}$) and autoimmunity in general ($P=1x10^{-8}$). Linkage was maximal in the area syntenic to chromosome 18q12-q21. Linkage was replicated, in the chromosome 18q21 region, in human autoimmune scans of chromosome 18 (P=0.02). Data from the association and meta-analyses are consistent with the presence of loci, on both human and rodent chromosome 18, that regulate tolerance and immune homeostasis. Because the *D18S487*-region of chromosome 18 associated with human autoimmunity is syntenic to the region of maximal linkage in rodent autoimmunity, it is possible that homologous loci influence disease susceptibility in human, mouse and rat.

Program Nr: 307 from the 1999 ASHG Annual Meeting

Functional and genetic evidence for a role of a non HLA gene (the S-gene) within the MHC in psoriasis. *C.D Veal[1], M.H. Allen[2], A. Faassen[2], S.H. Powis[3], R.W. Vaughan[4], J.N.W.N. Barker[2], R.C. Trembath[1]*. 1) Genetics, University of Leicester, Leicester, Leicestershire, UK; 2) St John's Institute of Dermatology, King's College London, St Thomas's Hospital, London, UK; 3) Department of Nephrology, The Royal Free Hospital, London, UK; 4) Tissue Typing Laboratory, King's College London, Guy's Hospital, London, UK.

Psoriasis is an inflammatory skin disease affecting approximately 2% of caucasian populations. We have recently refined a MHC susceptibilty locus to a 400kb region that includes the class I HLA-C gene (Balendran, N. *et al*). However, HLA-Cs involvement in antigen presentation to natural killer cells provides little functional support for a primary pathogenic role in psoriasis susceptibility. Of additional non HLA positional candidates, the S-gene encodes for corneodesmosin, a protein central to the process of keratinocyte cell adhesion . Degradation of corneodesmosin is required for normal keratinocyte desquamation.

We have performed a combined genetic and functional assessment of the S-gene, as a putative candidate for psoriasis susceptibilty. 152 trios from 99 independent kindreds were genotyped for 3 coding sequence polymorphisms. TDT analysis gave strong evidence for linkage disequilibrium (p-value 0.000003) for allele 5 (defined as T (619), G (1240), C (1243)). Importantly 38% of informative S-gene allele 5 transmissions were independent of the high risk Cw6 bearing haplotype. Direct sequencing has identified 18 SNPs enabling detailed LD mapping of the S-gene.

Subcellular corneodesmosin distribution was assessed by immunofluorescence using monoclonal antibodies and demonstrates aberrant processing of the protein comparing psoriatic to non-lesional skin. In combination these data provide strong evidence that the S-gene represents an important marker for genetic psoriasis susceptibility and points to alternative pathogenic mechanisms in disease causation.

Balendran, N. *et al*. Journal of Investigative Dermatology.1999 in press.

Program Nr: 308 from the 1999 ASHG Annual Meeting

Locus for antiviral enzyme 2',5'oligoadenylate synthetase (OAS) on chromosome 12q24 influences predisposition to Type 1 diabetes. *L.L. Field*[1], *V. Bonnevie-Nielsen*[2]. 1) Dept Medical Genetics, University of Calgary, Calgary, AB, Canada; 2) Dept Medical Microbiology, Odense University, Odense, Denmark.

Type 1 (insulin-dependent) diabetes results from autoimmune destruction of the insulin-producing pancreatic bcells in a genetically susceptible individual. The factor(s) which initiate the autoimmune process are unknown, but studies showing elevated enterovirus antibodies in new Type 1 diabetic patients suggest that viral infection may be involved. The enzyme 2',5'oligoadenylate synthetase (2',5'AS) is a key component of the antiviral immune defense system leading to degradation of viral RNA. In recent studies, we have shown significantly increased activity of this enzyme in both newly-onset and long-term Type 1 diabetic patients compared with controls (Bonnevie-Nielsen et al. submitted). Since the increased activity is persistent, we hypothesize that this reflects either a normal response to some chronic infection or an aberrant response to ubiquitous viruses. Genetic factors may underlie differences in host antiviral immune response. We therefore genotyped 356 diabetic sibpairs and their parents for a microsatellite marker in the OAS gene cluster (encoding 3 isoforms of 2',5'AS) on chromosome 12q24.2, and tested for linkage and association with Type 1 diabetes. There was no evidence for linkage using affected sibpair analysis. However, significant association ($p=0.0019$) was detected using the AFBAC program to compare alleles transmitted and not transmitted to the first diabetic child. The effect was stronger in alleles transmitted from fathers ($p<0.0001$) than in alleles transmitted from mothers ($p=0.55$), consistent with epidemiologic data showing higher risk of Type 1 diabetes in children of diabetic fathers than children of diabetic mothers. The finding of significant association (linkage disequilibrium) without significant linkage is characteristic of a common susceptibility gene that elevates risk but does not directly produce disease (*IDDM2* in the insulin gene region shows the same effect). We conclude that genetic differences at the OAS loci (encoding 2',5'AS antiviral enzyme) influence predisposition to Type 1 diabetes by altering host response to viral infection.

Program Nr: 309 from the 1999 ASHG Annual Meeting

Loci interaction providing susceptibility to the most common autoimmune disease, rheumatoid arthritis. *F.B. Cornelis*[1, 2]. 1) for ECRAF, the European Consortium on Rheumatoid Arthritis Families; 2) Pole Genetique des Maladies Autoimmunes, Universite Paris 7, Paris, France.

Rheumatoid arthritis (RA) prevalence in siblings of patients is approximately 5%, 5 times more than the prevalence in the general population. The HLA locus accounts for 1/3 of that susceptibility and other loci are yet to be identified. The first genome scan in 90 caucasian RA sib-pair (ASP) families, publicly available (www.genethon.fr), revealed 26 potential new loci. Further support was found from new caucasian families for the chromosome 1 locus, for which evidence of interaction with HLA was observed, and the chromosome 3 locus. We investigated the chromosome 18 locus suggested in the scan (D18S61, p=0.001). Fluorescent microsatellite genotyping data of 272 new ASP caucasian families were studied with the Analyze package. Interaction was tested by performing the ASP analysis at one locus following partition of the families according to the ASP identity-by-state for 3 markers at the other locus. RA linkage was observed in the new families: p<0.004. Analysis of the data combined with those of the scan reached the threshold of 10-4: p=0.00005. Evidence for interaction was observed with the RA-chromosome-3 locus (K3), K3-identical ASP sharing 74% of parental alleles at the chromosome 18 locus, vs 58% for other ASP (p=0.05). These data support the existence of a new RA locus on chromosome 18, with evidence for interaction with the chromosome 3 locus. This could represent a combination of RA loci, different from the HLA-chromosome 1 combination, predisposing to the same disease. Taking into account such interactions could help identifying susceptibility genes for RA and other complex diseases. *Analyze*

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