Abstracts

Plenary Sessions

PS01. Progress in sequencing and annotating the human genome

J. Weissenbach
Genoscope; Evry, France
jsbach@genoscope.cns.fr

Despite the availability of most of the human genome sequence, the accurate identification of genes on the DNA sequence remains to be continuously improved and updated. This procedure relies on the combined use of a variety of tools including similarity searches and exon/gene prediction programs.

Similarity searches rely on a set of sequence data that has been increasing very substantially and rapidly during the past months. These include human cDNAs, mouse genomic DNA and cDNAs and puffer fish genomic sequences. With these resources, the definition of a vertebrate set of genes is progressively approaching completion.

The use of a compact vertebrate genome (the pufferfish, Fugu rubripes and Tetraodon nigroviridis) in protein similarity searches has proven to be extremely valuable for the identification of mammalian coding sequences. DNA sequences representing in total 1.3 X coverage of the genome of Tetraodon nigroviridis were used in TBLASTX searches and enabled us to detect sequence matches in 75% of human genes with a backround of false positive matches below 1%. These results will be compared to other similarity searches based on mouse genomic sequences that are actively being produced at present.

These searches have been completed by BLASTN comparisons using a set of full length cDNA sequences and applied to human chromosome 22. Results of these analyses have been matched to the updated annotation of this chromosome that has been released recently and will be discussed.

PS02. Protein Structures, Inherited Mutations and Diseases

J. M. Thornton
University College, Biochemistry and Molecular Biology Department and Birkbeck College; London, United Kingdom
thornton@biochem.ucl.ac.uk

The mechanisms whereby inherited DNA mutations cause disease are only beginning to be understood. These are best understood in the context of knowledge of the three dimensional structure of the relevant protein.

Therefore to improve our understanding of the molecular basis of inherited diseases we have mapped the disease related mutations annotated in the OMIM database onto protein structural data, when available.

I will present a preliminary review of the relationship between protein sequence, structure, function and disease, highlighting specific examples to illustrate our observations.

PS03. Mutations and Their Effect on Protein Structure

A.R. Fersht
Centre for Protein Engineering, University of Cambridge Cambridge, United Kingdom
hjt1001@cam.ac.uk

Missense mutations can knock out residues in proteins that are important for binding, catalysis or conformational change, or have subtle effects on stability or conformation. Because buried residues in proteins tend to be conserved and surface residues vary, it is generally thought that surface residues that are not directly involved in the function of proteins are unimportant, especially regarding their effects on stability. But, all residues in proteins make contributions to stability and structural integrity. Further, the stability of proteins has not been optimised in evolution to the maximum value, but proteins appear to have evolved to a stability that is sufficiently adequate and to be consonant with other factors such as requirements for degradation. Consequently, some proteins are just on the verge of being stable and are very susceptible to destabilising mutations. I will illustrate, using p53 as an example, how binding mutations can inactivate its function by a variety of effects from altering residues essential for function to changing structure and stability.

PS04. Pharmacogenetics; A Disruptive Technology For The Next Decade

A. Roses
Senior Vice President, Genetics Research; GlaxoSmithKline; London, United Kingdom
mdb4138@glaxowellcome.co.uk

The Genome Project has accelerated the availability of tools to apply pharmacogenetics to the development of personalized medicines. This differs from the long term [10-15 year] strategy that is relatively familiar; from disease gene identification to functional disease links to screenable targets to products. A near term [2-6 year] application of pharmacogenetics can affect markets during the real time careers of current CEOs. Using whole genome SNP maps to develop Medicine Response Profiles [MRP] for either efficacy or adverse events, it will be possible to test patients before prescriptions are filled to determine the right drug for the right patient. Proof of Principle experiments have demonstrated that SNP maps can define linkage disequilibrium that can rapidly localize and identify susceptibility disease genes. The application of linkage disequilibrium MRPs for identifying individuals who are more susceptible for troublesome adverse effects related to specific drug treatments will be presented, as will the potential effect on the competitive markets for those drugs.

PS05. Predictive Testing for Huntington Disease - 15 Years Later

M. R. Hayden
Centre for Molecular medicine & Therapeutics, Department of Molecular Genetics, University of British Columbia; Vancouver, BC, Canada
mrh@cmmt.ubc.ca

Predictive genetic testing (PT) for Huntington Disease has now been offered for longer than for any other late onset genetic illness. The significant number of persons at risk, who have participated in testing, now allows insights to be derived that can be generalized for PT for HD worldwide. Long term (5 year assessment) of the psychological effects of PT have revealed that patients receiving a decrease risk have improvement in well-being, associated with less depression, that is maintained for at least 5 years after receiving results of PT. By contrast, persons receiving an increased risk result have an immediate increase in anxiety and depression, followed within 6 months by improved in well-being that is not sustained. By 5 years, these persons have no obvious change in psychological profiles from that seen prior to receiving a predictive test result. Despite fears, the incidence of catastrophic responses to PT has been low (less than 1%), and the concerns that persons at increased risk would have a high frequency of suicide, has fortunately not been realized. The advent of a direct genetic test for HD, without requiring DNA from family relatives, resulted in a significant increase in the acceptability of PT. At the present time, in countries such as Canada and the United Kingdom, approximately 10% of the number of persons estimated at risk, have now participated in PT programs. The advent of a genetic test for Huntington. Disease has also resulted in numerous new insights, which include the recognition that there is a much higher mutation rate for HD than previously had been recognized, that incomplete penetrance for HD is most frequent, particularly in the lower ranges of CAG repeats, and that there is significant underascertainment of persons with CAG repeats less than 40. While PT is generally been accepted, there has been little demand for prenatal testing for HD. The involvement in large number of persons in PT programs, together with the advent of a genetic test for the diagnosis of HD, has also allowed refinement of the relationship between CAG repeat length and age of onset. Using the largest cohort of HD patients analyzed to date (involving 3500 patients from 39 centers on four continents), a model to accurately predict probability of disease onset (with mean 95% confidence, intervals of 1%) has been developed. Use of this survival model, will allow for the design of clinical trials which will significantly limit the number of persons necessary, and therefore, will reduce the cost, as well as the duration of such a trial, thus more quickly bringing such therapies into clinical practice. Affected families and professionals have worked together to develop guidelines for PT, which is likely to be a significant reason for the successful integration of PT into the health care system in different parts of the world.

PS06. The SNP Endgame

N. Risch
Department of Genetics, Stanford University Medical Center, Stanford, CA United States
risch@lahmed.stanford.edu

With the recent announcement of a rough draft of the human genome sequence came also the identification of between 1.4 and 2.1 million SNPs, from the private and public efforts. An interesting question is whether these SNP databases contain the variants that underlie genetic susceptibility to a variety of common, complex diseases, or are at least in linkage disequilibrium with them, and if so, how to identify them. At present it appears that testing such a large number of SNPs either in family-based or case-control association studies is beyond the scope of existing resources, so strategies need to be developed to prioritize the SNPs to be tested. Considerable discussion has evolved around the issues of how many and which SNPs are optimal, what study designs should be

employed, and which populations should be studied. Attempts at answers to these questions can be obtained by considering the plausible genetic basis for the diseases being studied, as well as what is currently known about human evolution, the characterization of genetic variation within and between human populations, and the epidemiology of the diseases of interest. It is likely that no single strategy will be successful in all cases, and that a broad perspective including a variety of approaches be maintained.

PS07. Candidate Gene SNP-Based Association Studies; Empirical Findings, Problems, and Possible Solutions

A. Brookes
Center for Genomics Research, Karolinska Institute; Stockholm, Sweden
Anthony.Brookes@cgr.ki.se

There is considerable debate about whether or not genetic association analysis based upon single nucleotide polymorphisms (SNP) will help decipher the causative basis of complex disease states. Success will ultimately depend upon the existence of common genomic sequence variations that have a significant disease-predisposing effect upon cell/organism function. To add practical input to the theoretical debate, a study shall be presented that explores the feasibility of using association analysis to study non-Mendelian Alzheimer s Disease (AD). For this investigation, several new research tools were developed, and shall be described. These include the Human Genic Bi-Allelic SEquences (HGBASE) SNP database (http;//hgbase.cgr.ki.se) and the Dynamic Allele Specific Hybridization (DASH) genotyping method.

The AD study was large-scale compared to many contemporary efforts, and designed to try to circumvent a number of typical pitfalls of association analysis, such as i) disease heterogeneity, ii) population stratification, iii) identification of type I errors, and iv) dependence upon LD (we tested principally coding sequence and promoter SNPs). A total of 60 SNPs were evaluated, taken from candidate genes (N=55) from processes strongly implicated in neurodegenerative illness (oxidative stress and apoptosis/inflammation). We also included 13 variants previously claimed to show significant AD association. Upon completion of this investigation, only two polymorphisms showed replicable disease association, but these signals would not survive multiple-test correction for 60 markers. None of the 13 prior candidates showed disease association. One of these two replicated signals entailed a promoter variation in a key apoptosis regulator gene (TNFRSF6), and showed evidence for an interaction with APOE alleles. This implies for a role for apoptosis control in the causation of AD, and may help explain the mechanism by which the E4 allele of APOE mediates an increased AD risk.

Overall, these empirical findings emphasize the doubtfulness of previous positive findings, and highlight the low success rates one should expect for typical current investigations. Multiple-testing, study power, and sample-size issues are central to these problems. From this experience, ways to improve the execution of association studies in the future will be proposed.

PS08. Systematic Mutagenesis in Mice: Models for Human Diseases

R. Balling[1], *E. Wolf* [2], *M. Hrabe de Angelis*[3]
[1]*GBF, German Centre of Biotechnology; Braunschweig, Germany;* [2]*Institute of Animal Breeding, LMU Munich; Munich, Germany;* [3]*Institute of Experimental Genetics, GSF-Research Center of Environment and Health; Neuherberg, Germany*
balling@gbf.de

One of the goals of the Human Genome Project is the systematic functional analysis of genes that are involved in the pathogenesis of human disease. Due to the similarity in their genomes, developmental pathways and physiology, the mouse has become a major model to study the genetics and pathogenesis of human diseases. An important tool to obtain insight into the function of genes is the use of mutants. Mouse mutants have occured spontaneously in breeding colonies or were chemically induced. Mutants produced by gene targeting and transgenesis play an increasing role in the dissection of biological pathways. The use of embryonic stem cells and homologous recombination allows a gene driven analysis of gene function. A prerequisite for this approach is the availability of the gene(s) whose function is to be investigated. Equally important and complementary is a phenotype driven approach, in which as many mutants as possible are obtained with specific diseases or defects and the responsible genes are then identified through positional cloning or other strategies. The validity and success of this approach- was demonstrated in the course of the genetic and molecular dissection on the pathway that set up the Drosophila body pattern. Although a large number of knockout mutants will be produced in the coming years, most of this mutants will be insertions that interrupt gene function and in most cases produce null alleles. The full power of a genetic analysis of gene function however requires the availability of multiple alleles of the same gene or of the different genes involved in the pathogenesis of the same disease. This include hypomorphs, alleles of different strength and gain of function alleles. Such alleles can be obtained after chemical mutagenesis with ENU which induce mainly point mutations. ENU is currently the most powerful mutagen for the production of mutants in mice and other species. We have set up a large scale ENU-mutagenesis screen at the GSF-Research Center. A core facility has been created in which mice are treated with ENU. Offspring of the G1 and G3 generation are then screened for dominant and recessive phenotypic abnormalities. Within the last 4 years we have screened more than 20000 mice and isolated over 200 genetically confirmed new mouse mutants. These include more than 60 mice with skeletal abnormalities and many with obesity, diabetes, hypercholestolemia, deafness, B-cell deficiency or hyper-IGE. Our goal is the production of a large number of new animal models for the study of human disease. Future work will involve the cloning of the corresponding genes and the detailed phenotypic characterization of the mutants obtained.

PS09. Public and Private Domains in Genomics: Conflict or Coorporation

Moderator: H. Galjaard[1]
Discussants: D. Cohen[2]*, H. Yang*[3]*, A. Kent*[4]*, M. C. Freire*[5]*, M. Oborne*[6]
[1]*Department of Clinical Genetics, Erasmus University; Rotterdam, The Netherlands;* [2]*Novartis Pharmaceutical Corp.; Summit NJ, United States;* [3]*Human Genome Center, Institute of Genetics; Beijing, China;* [4]*Genetic Interest Group; London, United Kingdom;* [5]*Office of Technology Transfer, National Institutes of Health; Rockville, MD United States;* [6]*Directorate for Science Technology and Industry; Paris, France*

How can you patent genes, the elements of the periodical system have not been patenhood?

How can you expect industry to invest enormously into the development of new drugs and vaccines without patenting?

We, representatives of developing countries are afraid of a widening gap in access to knowledge compared with the wealthy countries .

Patenting implies publicizing and modern ICT will further improve access to knowledge .

These are only four statements picked up during the many meetings and discussions on patenting genes and they illustrate differences in perception of the same theme. The more we learn about it, the more complicated it becomes.

Differences in definition of patenting between the USA and Europe, diversity in legislation and opinion among and within countries; emotions, sometimes based on inadequate knowledge of the facts.

In this session we hope to establish the facts and a choice between cooperation and conflict.

PS10. Chromosome Translocations in Leukemia; Impact on Cancer Biology

J. D. Rowley
Department of Human Genetics, The University of Chicago Genetic Services; Chicago, IL United States
jrowley@medicine.bsd.uchicago.edu

Chromosome analysis, particularly in leukemia and lymphoma, has had a dramatic impact on cancer biology. In acute myelogenous leukemia, leukemic cells with recurrent balanced translocations may have unique phenotypes. Several hundred recurring translocations have been identified and the breakpoints of about half have been cloned. Cloning translocation breakpoints has led to the identification of more than one hundred new genes including *BCL2, AML1, TEL, PML* and *MLL*. The mRNAs from the fusion genes are inframe and produce functional fusion mRNAs and unique, translocation specific proteins. Cloning translocation breakpoints has provided unique diagnostic probes used for fluorescence in situ hybridization or RT-PCR. Specific therapies based on an understanding of the altered function of these fusion genes are now available and more will be developed.

We need to define the alterations in gene expression in these different translocations using an unbiased genome-wide strategy. This will permit a more sophisticated approach to identification of the genotype of each specific translocation. We used a modified SAGE (Serial Analysis of Gene Expression) technique to analyze normal CD34+ and CD15+ myeloid cells. We identified 42,399 and 37,519 unique tags from CD34+ and CD15+ cells, respectively. Comparing changes in gene expression between CD34+ and CD15+ cells, 31,336 tags were absent and 20,054 new tags were present in CD15+ cells.

Information about the similarities as well as the differences in the pattern

of gene expression associated with each translocation will lead to a more precise understanding of the genetic differences induced by these fusion genes in leukemia. Identifying the unique gene expression pattern for each translocation will speed the development of a reliable, cost-efficient diagnostic DNA microarray. This will be critical as we treat each consistent translocation with its own genotype specific therapy.

PS11. Has gene detection benefited the cancer patient?

A. de la Chapelle
Human Cancer Genetics Program, Comprehensive Cancer Center, Ohio State University; Columbus, OH United States
delachapelle-1@medctr.osu.edu

Ideally, the detection of a gene whose mutations contribute to cancer should lead to the development of a drug or procedure that cures or prevents that cancer. So far, this sequence of events has occurred extremely rarely. A glimmer of hope is provided by the example of chronic myeloid leukemia (CML). Over 40 years ago a specific marker, the Philadelphia chromosome was found. Over 25 years ago the Ph1 was found to be caused by a translocation. Over 10 years ago the translocation was found to lead to the fusion of two genes creating a hyperactive tyrosine kinase. In a focused effort to provide an antidote to this kinase, hundreds or thousands of small-molecule compounds were tested by a drug company. After many years of endeavor the compound named STI 571 was identified that appeared to have the desired effect. Today, after some 3 years of clinical trials, STI 571 appears to induce complete remission in almost every patient with CML, even at advanced stages of the disease. There may even be hope for cure. What lessons does this story teach us? First, painstaking basic research is a prerequisite of any progress. This can best be done by academic researchers who are free to choose their projects. Second, drug development requires enormous resources that are only available to the industry. Imagination, discipline and hard core targeting is also required. Third, to get from gene discovery totherapy takes a long time. Fourth, the example shows that the scenario is possible. It should start happening more often.

My second example highlights the need of open-mindedness and imagination. Another leukemia, acute promyelocytic leukemia (APL) is often curable today through the combined use of trans-retinoic acid and chemotherapy. The disease occurs as a result of the fusion of the APL gene with the retinoic acid receptor alpha-gene. So one might think that the story resembles that of CML; the knowledge of the molecular event (in this case one interfering with the uptake of retinoic acid) hinting that treatment with retinoic acid might be useful. Not at all! Treatment of APL with retinoic acid preceded the molecular genetic findings. It was part of Chinese folk medicine! We learn again something that is hardly new in medicine. Imagination, open-mindedness and keen clinical observation are essential. How about the relatively recently detected genes that confer susceptibility to common cancers? BRCA1 and BRCA2 have been known for approximately 7 years. Their diagnostic implications are of course huge in affected families, and testing for mutations probably leads to better risk awareness and saved lives. Like in other cancers showing Mendelian inheritance, a major benefit is to those at-risk family members who are found to be mutation negative. But unfortunately, the BRCA genes have not yet provided real breakthroughs in our understanding of pathogenetic mechanisms. Curative, non-cytotoxic drugs are but a dream. Colorectal cancer looks somewhat brighter. Here the mismatch repair defect caused by mutations in several genes occurs not only in the main Mendelian form of colorectal cancer (HNPCC) but also in a sizeable proportion (up to 20%) of sporadic cancers. These mismatch-repair deficient colorectal cancer patients have a better prognosis than others. The basis of this phenomenon is not yet understood; however, epigenetic phenomena (methylation, loss of imprinting) seem to play a role. This gives hope that drugs and/or procedures may be forthcoming that interfere with these cancers. Already mismatch repair deficiency, as defined by microsatellite instability, is a tool in the large-scale molecular screening for HNPCC which in turn facilitates life-saving clinical surveillance.

In summary, the detection of cancer-related genes is beginning to impact the clinical handling of subsets of many cancers, but the road from gene to therapy and prevention is slow and unpredictable.

PS12. Classification and predictive modeling using gene expression analysis

J. Trent
Div.of Intramural Research, Cancer Genetics Branch, NIH/National Human Genome Research Institute; Bethesda, MD United States
jtrent@exchange.nih.gov

The use of gene expression patterns to target and predict functional pathways is an important goal in the post-sequence era. This lecture will provide a discussion of results from the investigation of both sporadic and hereditary cancers with the focused intent to integrate expression profiling with mutation status and pathway prediction. Information from both biologic and mathematical studies will be described to address the consequence of specific mutations on a cancer s gene expression profile. The results suggest the possibility for gene-expression based identification (classification) of mutation-positive cancers, as well as increased understanding of the functional consequences of gene mutations.

PS13. Gains and losses in skull and limb development

A. Wilkie
Weatherall Institute of Molecular Medicine; Oxford, United Kingdom
h.goodwin@iop.kcl.ac.uk

In the diagnosis of syndromes, malformations of the skull and limbs frequently occur in the same disorder. This suggests that similar molecular pathways are employed in development of these two evolutionarily distinct structures. These parallels will be illustrated from work in my laboratory to elucidate the molecular basis of skull (craniosynostosis, parietal foramina) and limb (syndactyly, brachydactyly type B) malformations. These disorders commonly show dominant inheritance and the causative genes that I will discuss encode proteins of two specific categories; transcription factors (MSX2, ALX4, HOXD13, TWIST) and receptor tyrosine kinases (FGFR1, FGFR2, FGFR3, ROR2). Heterozygous loss of function mutations are associated with abnormal phenotypes (haploinsufficiency) in the case of all four transcription factors but none of the four receptor tyrosine kinases; this reflects the frequent dosage sensitivity of transcription factors compared to most other proteins. In contrast, diverse gain of function mechanisms (dominant positive or negative) are commonly encountered in receptor tyrosine kinase mutations but more difficult to establish for transcription factors, although there is evidence for this type of mechanism in specific MSX2 and HOXD13 mutations. Homozygous mutations associated with complete loss of function are usually expected to be lethal, however in the case of ROR2 a distinct phenotype occurs, recessive Robinow syndrome. Much remains to be learnt about how these proteins are integrated in development, but elements of a molecular pathway in the cranial suture are beginning to emerge.

PS14. Transcription of Hox Genes Suggests a Link between Pattering and the Segmentation Clock

J. Z k ny[1], M. Kmita[1], J. L. de la Pompa[2], ***D. Duboule[1]***
[1]Department of Zoology and Animal Biology, University of Geneva, Sciences III; Geneva, Switzerland; [2]EMBL, Mouse Biology Programme; Rome, Italy
Denis.Duboule@zoo.unige.ch

During mammalian development, transcription of Hox genes is activated in presomitic mesoderm with a time sequence that follows the order of the genes along the chromosome. Consequently, newly formed somites contain specific combinations of HOX proteins that define their fates. I shall discuss the fact that Hoxd1 displays transitory stripes of expression within presomitic mesoderm, but not in somites. Stabilization of its transcripts through targeted modification uncovered sustained expression in somites, reminiscent of other Hox gene patterns, suggesting that cyclic activation in presomitic mesoderm may be a general phenomenon masked by slow transcript turnover. Accordingly, in addition to Hoxd1 and Hoxd3, we show that the promoters of both Hoxb1 and Hoxd11 transgenes can respond to this regulation. We propose that colinearity is associated with bursts of transcriptional activation of Hox genes every time a somite is about to form. This dynamic transcriptional behavior appears to depend upon Notch signaling, as mice deficient for the Su(H)/RBPJk/CBF1 gene, the effector of the Notch pathway, showed severely reduced Hoxd gene expression in presomitic mesoderm. These results suggest a tight link between Hox gene activation and the mechanisms behind the segmentation clock. Such a linkage would coordinate the production of novel segments with their morphological specification.

PS15. I met a traveller from an antique land : Genes as history

B. Sykes
Department of Cellular Science, University of Oxford, Institute of Molecular Medicine, John Radcliffe Hospital; Oxford, United Kingdom
bsykes@gwmail.jr2.ox.ac.uk

These days genes may aspire to predicting the future but they are also messengers from the past. Each gene has its own separate history and has made its own journey from the deep past to all of us in the present day. Interpreting the messages genes tell us about our ancestors, and with it the history of our species, has changed. It has shifted from the accumulation of summary statistics and the stale comparisons of one population

with another to the realisation that the history of our species is the history of individuals and their actions.
The agents of change have been the uniparental loci found on mitochondrial DNA and the Y-chromosome which trace the different histories of men and women. Robust phylogenies and accurate mutation rates introduce the fourth dimension of time into the interpretation of the patterns of genetic variation. The presentation will be illustrated by examples ranging from questions on a global dimension to what can be said of the individual behaviour and lives of our ancestors.

PS17. Genetic susceptibility to tuberculosis

L. M. Mitsos[1], L. R. Cardon[2], A. Fortin[1], L. Ryan[3], R. LaCourse[3], N. Jabado[1], S. Grinstein[4], R. J. North[3], ***P. Gros[1]***
[1]Department of Biochemistry, McGill University; Montreal, PQ Canada; [2]Bioinformatics and Statistical Genetics, University of Oxford; Oxford, United Kingdom; [3]Trudeau Institute; Saranac Lake, NY United States; [4]Research Institute, Hospital for Sick Children; Toronto, ON Canada
gros@med.mcgill.ca

Genetic factors play a key role in host response, disease severity, and ultimate outcome of infection with *Mycobacterium tuberculosis* in humans. Using a mouse model of susceptibility to Mycobacterial infections, we have isolated the *Nramp1* gene by positional cloning. Loss-of-function mutations at *Nramp1* in mice cause susceptibility to infection with unrelated intracellular parasites by impairing macrophage function. Recently, it was shown that polymorphic variants at the human *NRAMP1* gene are associated with susceptibility to tuberculosis and leprosy in certain endemic areas of disease. The Nramp1 protein is expressed in macrophages and neutrophils in a subcellular compartment that is rapidly recruited to the membrane of the phagosome, soon after phagocytosis of inert particles or live bacteria. Recruitment of Nramp1 to the phagosomal membrane interferes with the ability of intracellular parasites to inhibit phagolysosome formation. Microfluorescence imaging in intact cells of single phagosomes containing particles labeled with metal-sensitive fluorescent dyes demonstrate that Nramp1 protein functions as a pH-dependent, divalent cation efflux pump. To identify additional genes responsible for differences in host response to Mycobacterial infections, ninety five animals of an informative F2 cross derived from highly susceptible DBA/2J and resistant C57Bl/6J parents, were infected intravenously with *M. tuberculosis* (1×10^5 CFU) and duration of survival was used as a quantitative phenotypic measure of susceptibility in a whole genome scan. Quantitative trait locus analysis (QTL) showed that the genetically controlled susceptibility was multigenic. QTL analysis identified two significant linkages on the distal portion of chromosome 1 (LOD, 4.80) and on the proximal portion of chromosome 7 (LOD, 4.66) that each account for approximately 21% of the phenotypic variance. A third suggestive linkage was identified on the proximal portion of chromosome 3 (LOD, 3.93; additional 18% of the variance). At each locus, homozygosity for the parental C57Bl/6J alleles was associated with increased resistance to infection. The role of these three loci in regulating *M. tuberculosis* replication in the lungs is currently being validated in additional experiments, using aerosol infection and using bacterial replication as a quantitative trait. Such novel mouse loci provide the basis for evaluating a possible association of the corresponding syntenic chromosomal regions in humans with susceptibility to tuberculosis.

PS18. Preimplantation genetic diagnosis: an update

A. H. Handyside
Leeds PGD Centre, Assisted Conception Unit, Leeds General Infirmary and School of Biology, University of Leeds, Leeds, United Kingdom
a.h.handyside@leeds.ac.uk

Preimplantation genetic diagnosis (PGD) following *in vitro* fertilization (IVF), cleavage stage embryo biopsy and single cell genetic analysis has become increasingly established over the last ten years as a viable alternative to conventional prenatal diagnosis for both chromosomal and single gene defects (Handyside and Delhanty, 1997). The first pregnancies were established in 1989 in a series of couples at risk of various X-linked recessive diseases by identifying (unaffected or carrier) female embryos for transfer (Handyside et al., 1990). Towards the end of 1999, the European Society for Human Reproduction and Embryology (ESHRE) PGD Consortium reported the first detailed clinical data from 16 centres for 392 cycles completed in the period January 1997 to September 1998 (Geraedts et al., 1999). The overall clinical pregnancy rate for this series was 17% per oocyte retrieval or 22% per embryo transfer and data from 82 pregnancies and 79 children was collected. In the latest data, covering a period from 1994 to May, 2000, 1319 cycles from 25 centres are reported with the same overall pregnancy rate together with the details of 163 pregnancies and 162 children (Geraedts et al., 2000). The current status of PGD will be briefly reviewed together with prospects for future developments.

Geraedts, J., Handyside, A., Harper, J., Liebaers, I., Sermon, K., Staessen, C., Thornhill, A., Vanderfaeillie, A., and Viville, S. (1999). ESHRE Preimplantation Genetic Diagnosis (PGD) Consortium: preliminary assessment of data from January 1997 to September 1998. ESHRE PGD Consortium Steering Committee. Hum Reprod 14, 3138-48.

PS19. The spatial organisation of chromosomes and genes within the nuclei of human cells; implications for disease and genome plasticity

W. Bickmore
MRC Human Genetics Unit, Western General Hospital; Edinburgh, United Kingdom
wendy@hgu.mrc.ac.uk

We now know the sequence of DNA within the human genome. However, this linear information is an incomplete description of our genetic information. Many levels of chromatin structure are involved in regulating gene expression in a heritable (epigenetic) manner. Recently, attention has turned to the spatial organisation and functional compartmentalisation of chromosomes, and of the nucleus itself, in the quest to understand how the expression of complex genomes is regulated.
I will present evidence that individual human chromosomes occupy specific positions within the nucleus in both fixed and living cells. Gene-rich human chromosomes general localise towards the interior of the nucleus whereas gene-poor chromosomes are positioned towards the edge of the nucleus. Chromosome positioning in relation to cell-cycle changes and to certain disease states will be discussed. Relationships between nuclear organisation, and the occurrence of chromosome rearrangements in the human population will also be examined.
Evidence from model organisms suggests that the nuclear organisation of individual genes can be important in their regulation. I will present our studies that begin to reveal the nuclear organisation of genes from different parts of the human genome. I will show that individual genes occupy preferred sites within interphase chromosome territories but that some genes appear to occupy positions within the nucleus that are quite distinct form the space taken up by the rest of the chromosome to which they belong.

Concurrent Symposia

S01. Normal and abnormal CNS development in man and mouse.

K. K. Sulik
Department of Cell Biology and Anatomy, The University of North Carolina; Chapel Hill, NC United States
mouse@med.unc.edu

Similarities in the prenatal development of the mouse and human CNS have allowed for extensive extrapolation between species and a rapidly increasing understanding of factors involved in both normal and abnormal development. Of particular interest for this review are the formation and closure of the neural tube; events that occur during the third and fourth weeks of human embryogenesis. Neural tube closure is initiated at similar developmental stages in the mouse and human, but its progression differs. In the mouse there is an intermittent anterior closure site at the midbrain/hindbrain junction while humans lack this intermittent site. This has significant implications relative to our interpretation of the genesis of human neural tube defects.
Research utilizing mice has helped to reveal key cell populations and mechanisms that are involved in the genesis of neural tube defects as well as other CNS abnormalities including holoprosencephaly and hydrocephaly. Additionally, a range of causative environmental, genetic, and multifactorial insults have been examined that are relevant not only to major CNS malformations, but also behavioral abnormalities. For example, ethanol which is the most common nonhereditary cause of mental retardation, kills selective cell populations in the neural plate and early neural tube.
The pattern of apoptosis resulting from acute ethanol exposure is readily related to failure of the neural tube to close, deficiencies in the forebrain midline, and also to subsequent hindbrain abnormalities. Ethanol also kills selected CNS populations at fetal and perinatal stages of development. Another condition, cholesterol deficiency as underlies the human mental retardation/malformation syndrome, Smith-Lemli-Opitz (SLO), also can cause selective cellular loss. This selective vulnerability appears to be related, at least in part, to a signaling pathway involving sonic hedgehog and the involvement of cholesterol in hedgehog protein function.
Both Shh gene knockout and selective modification of the gene that is mutated in SLO are examples that will be presented to illustrate the utility of mouse models for the exploration of normal and abnormal CNS development. The following web site is recommended as a resource for micro-

graphs illustrating mouse and human embryogenesis

S02. LIS1 and DCX; the journey from rare human syndrome to essential components of cellular mechanics

W. B. Dobyns
Departments of Human Genetics, Neurology and Pediatrics, The University of Chicago; Chicago, IL United States

Classical lissencephaly (LIS) is a human cortical malformation caused by impaired neuronal migration, which results in reduced gyral formation and an abnormally thick, four-layered cortex. Subcortical band heterotopia (SBH) is a related disorder in which deficient neuronal migration results in a band of heterotopic gray matter between the cortex and ventricular walls, and an overlying six-layered cortex with shallow sulci.

Embryology. The cerebral hemispheres form at ~5 weeks gestation as diverticula of the rostral prosencephalon. The wall of the hemispheres develops a periventricular proliferative zone by ~6 weeks, which gives rise to billions of neuroblasts. Some of the early progenitor cells migrate outward to form the cortical plate, and are followed by billions of immature neurons in two major waves between approximately 7 and 13 weeks gestation, with residual migration continuing for several months thereafter.

Different types of lissencephaly. The most common type of LIS, known as classical LIS, is characterized by a very thick 15-20 mm cortex, and grossly normal brainstem and cerebellum. About 80% have mutations of either the LIS1 or DCX (XLIS) genes, with LIS1 accounting for most patients with LIS and DCX for most patients with SBH. In addition, most patients have a visible front to back gradient of LIS or SBH, which can be most severe posteriorly (P>A) or anteriorly (A>P). The P>A gradient occurs in patients with mutations of LIS1, while the A>P gradient is typical of patients with DCX mutations. Many rare forms of LIS have been observed, with the genetic basis of most unknown.

LIS1 protein function. *LIS1* encodes a noncatalytic subunit of platelet activating factor-acetylhydrolase, or Pafah1b1, and is part of a G-protein-like?1a2b heterotrimer, although it is not clear whether this is directly related to its? role in neuronal migration. Lis1 is a soluble protein with seven WD40 repeats forming a seven-bladed propeller-like structure involved in protein-protein interactions. It is a highly conserved homologue of the NUDF protein in Aspergillus, which it is required for nuclear translocation through interaction with a dynein motor and other proteins such as NUDC and NUDE. Lis1 also binds to dynein, and to Nude and Nudel, the mouse (and human) homologues of NUDE. mNudE and Nudel are both co-localized with Lis1 at the microtubule organizing center (centrosome), and bind multiple proteins that contribute to centrosome organization.

DCX protein function. *DCX* encodes a 40 kD soluble protein that has been detected only in neurons of the central and peripheral nervous system. It contains two internal repeat regions that bind to tubulin to promote precipitation and stabilization of microtubules. Thus, both LIS1 and DCX function appear critical for microtubule function in developing brain.

S03. The XLMR of mental retardation

J. Gecz
Department of Cytogenetics and Molecular Genetics, Women s and Children s Hospital; Adelaide, Australia
jozef.gecz@adelaide.edu.au

Mental retardation (MR) is a significant medical problem affecting ~2-3% of general population. MR is not a single disorder; rather, it is a large (genetically and clinically) heterogeneous group of disorders with a range of severity and associated dysmorphology. When the prevalence of MR between the two sexes (males and females) is compared, the excess of males over females at both ends of the normal IQ distribution is apparent. The existence of these differences in variance of IQ between males and females (males show consistently higher variance than females) was often interpreted as supporting evidence for the higher density of genes for mental retardation on the human X-chromosome. This issue still remains controversial.

X-linked mental retardation or XLMR is frequent with an estimated prevalence of 1.66/1000 males. Mental retardation can be a component of a syndrome, metabolic disorder, or neuromuscular disorder, or MR can be an exclusive phenotype affecting only the development of cognitive function.

There are numerous (>210) mental retardation entries in OMIM (syndromic and non-specific) associated with the human X-chromosome. Autosomal MR is much less characterised, especially the non-specific forms.

Currently there are 33 known XLMR genes. The majority of these are genes for syndromic types of XLMR (eg. fragile X syndrome, FMR1; alpha-thalassemia mental retardation syndrome; ATRX, etc.). The number of genes for non-specific X-linked mental retardation (MRX genes) has grown rapidly over the past three years. There are now at least eight such genes recognised. Based on currently available clinical and molecular data we recently estimated the minimum number of MRX genes to be approximately 22.

Some lumping of syndromic and non-specific phenotypes as a consequence of different disease causing mutations in the same gene (eg. ATRX, RPS6KA3 and MECP2 genes) may reduce the overall number of XLMR genes.

The scenario of many genes affecting normal development of cognitive function (eg. MRX genes) is appearing. A large proportion of idiopathic MR remains undiagnosed as a consequence of either the current inability to devise and carry out large scale tests, or the existence of as yet undiscovered cause(s) of MR. This represents a great challenge for future applications of large-scale genomics approaches like DNA chip and microarray technologies in particular.

S04. A genome-wide analysis of alternative exon-usage in the human transcriptome

S. J. De Souza
Ludwig Institute for Cancer Research; Sao Paolo, Brazil
sandro@bidu.ludwig.org.br

The completion of the human genome project has revealed a surprising low number of predicted genes. One of the consequences of this modest number is that we have to look carefully for mechanisms that would generate the complexity intrinsic to human development and homeostasis. One of these mechanisms at the transcriptome level is alternative splicing. One of the most common forms of alternative splicing is alternative exon usage where exons can be spliced out or skipped, which generates a pattern of exon usage. In my talk I will discuss results from an extensive analysis of alternative exon usage within the human transcriptome.

Our approach was centered in three aspects; mapping of all human cDNA information onto the draft of the human genome, a genome-based clustering of the cDNA information and the generation of a binary matrix representing all the variation found for each cDNA cluster.

The results from this analysis show that alternative exon usage occurs in more than 40% of known human genes. We have also been able to identify more than 100,000 variants. I will discuss several other aspects of this analysis including tissue distribution of variants and functional annotation.

S05. Computational analysis of microarray data

M. Vingron
Max-Planck-Institute for Molecular Genetics; Berlin, Germany
vingron@molgen.mpg.de

The analysis of gene expression data generated with DNA microarrays poses major computational challenges. In this talk we will review the necessary steps in processing such data. Image analysis is used to collect intensities, which then have to be normalized in order to determine ratios of change between mRNA abundancies under different conditions. Given results from many hybridizations, genes or conditions are grouped together by similarity using clustering algorithms. Additional knowledge about the conditions, e.g. tumor types, pose the problem of identifying characteristic features in the expression data that are associated with the those types. For several of these steps we will also suggest novel approaches and give examples of their application.

S07. Molecular Genetics of Circadian Rhythms in Flies, Mice and Humans

***M. Rosbash**, P. Emery, C. Kotarski, D. Lee, M. McDonald, P. Nawathean, Y. Peng*
Howard Hughes Medical Institute, Department of Biology, Brandeis University; Waltham, MA United States
rosbash@brandeis.edu

Genetic and molecular genetic analyses of the *Drosophila* circadian system originally identified the *period* protein as a clock molecule that contributes to circadian pacemaker function. The gene shows robust circadian rhythms of transcription, mRNA and protein expression, with strong evidence in favor of autoregulation. This raised the hypothesis that transcription and macromolecular metabolism, a transcription-translation feedback loop, was central to the mechanism(s) that generated circadian rhythms. This model is still viable today, and transcriptional regulation plays a prominent role in contemporary circadian studies. Subsequent work identified at least seven new *Drosophila* clock genes; *tim, Clock*, *cycle*, *doubletime, cry, pdf*, and *vrille*. There is now a good picture of what these different gene products do and how they collaborate during the 24 hr cycle. Most if not all of these genes have mammalian orthologs, which function in a very similar manner in mice. Therefore, the transcription-translation feedback loop hypothesis also appears to be central to mammalian clocks. Moreover, a recent human sleep disorder family has been described, with a mutation in a human *period* gene protein. Despite all of this substantial recent

progress, there are a large number of questions that remain unanswered. Prominent among these are how 24 hr timing is achieved and why the apparent differences in *cryptochrome* (*cry*) function between flies and mammals. New features of these and other issues will also be discussed. Finally, some evolutionary issues will be considered, in an attempt to provide a framework for considering together different circadian systems

S08. Molecular Mechanism of the Regulation of the Biological Clock in Mammals

H. Okamura
Department of Anatomy and Brain Science, Kobe University School of Medicine; Kobe, Japan
okamurah@kobe-u.ac.jp

In the eukaryotic circadian model systems, translocation of the oscillatory gene products into the nucleus is a key step for generation of a 24 hour cycle of the biological clock. We have examined nuclear import of clock proteins of the mammalian*period* gene family and the effect of serum shock, which induces a synchronous clock in cultured cells. We examined the nuclear import of mPER proteins in COS7 cells and found that nuclear translocation of mPER1 and mPER2 involves physical interactions with mPER3. This indicates that nuclear translocation of mPER1 also can occur under physiological conditions (i.e. in the intact mouse) in the absence of any CRY protein.

Recently we demonstrated that an accessory transcription loop exists helping to the core clock feedback loop. Transcript levels of DBP, a member of the PAR leucine zipper transcription factor family, exhibit a robust rhythm in the SCN. We report that DBP is able to activate the promoter of a putative clock oscillating gene,*mPer1*, by directly binding to the*mPer1* promoter. DBP and CLOCK-BMAL1 cooperatively activate the*mPer1* promoter. On the other hand,*dbp* transcription is activated by CLOCK-BMAL1 through E-boxes and inhibited by the mPER and mCRY proteins, as is the case for*mPer1*. Antiphase circadian regulated E4BP4, another member of leucine zipper transcription factor without PAR domain, antagonistically suppresses mPer1 transcription. Thus, a clock-controlled*dbp* and*e4bp4*genes may play an important role in the central clock oscillation. Using transgenic mice carrying the*mPer1*promoter fused to the luciferase (*mPer1-luc*) gene, we recently succeeded to monitor luciferase-mediated bioluminescence with a day-night variation in the SCN in brain slices and in living animals. We can record for several days oscillating photon emission with a periodicity and phase that accurately mirrored native*mPer1* mRNA expression. The real-time optical imaging of gene expression will be a new powerful tool to study mammalian brain function.

S09. Smith-Magenis Syndrome; A Circadian Rythm Disorder. From S ymptoms to Treatment

H. De Leersnyder
Department of Genetics, Necker Hospital; Paris, France
deleers@club-internet.fr

Smith-Magenis Syndrome (SMS) is a contiguous gene syndrome, ascribed to interstitial deletion of chromosome 17p11.2. The complex phenotype is thought to result from haploinsufficiency effects of contiguous genes that map within the critical interval. An alteration of the circadian rhythm of melatonin triggers sleep disturbance and causes advanced sleep phase in SMS. Normally, the secretion of melatonin, the main hormone of pineal gland is stimulated by dark and inhibited by light, photic information being transmitted from retina trough the suprachiasmatic nuclei of the hypothalamus and the sympathetic nervous system. How the chromosome 17 deletion causes an abnormal circadian rhythm remains unclear. It may result from; i) an alteration of the input/output-signaling pathway, ii) an alteration of the circadian time-keeping system (clock genes) of the suprachiasmatic nuclei or iii) the abnormal transmission of postganglionic fibers ascending to the pineal gland. Considering that behavioural problems correlate with the inverted circadian rhythm of melatonin and the night sleep insufficiency in SMS, we hypothesized that at least part of hyperactivity and attention deficit could occur because the patients struggle against sleep, when melatonin rises during the day. Beta1-adrenergic antagonists, which have been shown to reduce the production of melatonin, were given to 10 SMS children. A single morning dose of acebutotol suppressed the inappropriate secretion of melatonin during the day (n=10). A significant improvement of inappropriate behavior with increased concentration ability, increased hours of sleep and delayed sleep offset were also noted for all patients. This study suggests that a pharmacological approach of behavioral phenotype in SMS, as considered in part as the result of phase shift of melatonin, will hopefully help managing hyperactivity, reduce day naps and sleep attacks, enhance cognitive experience, and reduce sleep disorders in SMS.

S10. The Population Dynamics and Genotype/Phenotype Relationships of the Thalassaemias

D. J. Weatherall
The Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital; Oxford, United Kingdom
liz.rose@imm.ox.ac.uk

The thalassaemias are the commonest monogenic diseases. As the countries in the developing world go through the demographic transition towards improved living conditions many babies with these diseases who, hitherto, would have died are now surviving. Recent studies have confirmed the long held belief that these conditions have reached their high gene frequency by natural selection acting on local mutations. These disorders have remarkably diverse phenotypes and it is now clear that this results from the action of a number of primary, secondary and tertiary genetic modifiers together with major environmental effects. Thus these diseases offer a model system for understanding the phenotypic diversity of monogenic disease, for underlining the problems that will be encountered when attempting to analyse multifactorial diseases, and for the application of the tools of molecular genetics to the problems of the developing countries.

S11. Disease Profiles in the Genetically Dinstinct Populations of South Africa

M. J. Kotze
Division of Human Genetics, Faculty of Health Sciences, University of Stellenbosch; Tygerberg, South Africa
mjk@gerga.sun.ac.za

The genetically distinct populations of South Africa provide valuable information for molecular research. Only a small fraction of the sequence changes that arose in Africa has spread to Europe and other countries. The indigenous Khoisan people and African Negro slaves contributed to the unique gene pool of the white Afrikaner population (~3 million), that originated from approximately 2000 settlers who emigrated from Holland, Germany and France in the late 17th and early 18th centuries, with a lesser increment from England in the 19th century. This group provides an excellent example of founder events in human evolution. The origin of the Coloured population of South Africa can be traced back to 1652, when marriages between European colonists and the two indigenous populations, the Khoi and the San, occurred relatively frequently. An additional gene flow to this gene pool came from the Indian traders and slaves from East Africa and Madagascar, who were either completely Negroid or Negroid with Malay or Indonesian admixture.

The high prevalence of several genetic diseases in the Afrikaner population resulted from a reduction in genetic variability due to geographic isolation and religious/cultural bonds, coupled to rapid population expansion over 10-12 generations. This was confirmed by molecular analysis of diseases such as familial hypercholesterolaemia, variegate porphyria and hereditary haemochromatosis. These studies illustrated the importance of genetic factors underlying population/ethnic differences in disease risk and defined the recently admixed Coloured population as a valuable candidate population for the identification of genes/mutations underlying complex diseases. This population is currently studied extensively, in an attempt to identify genetic factors associated with hypertension or modified risk of HIV infection and progression to AIDS. Information gained through the analysis of disease profiles in the African context has contributed to our understanding of why certain diseases affect different populations at different rates.

S12. Disease Gene Profiles Among Jewish Populations

H. Ostrer
New York University School of Medicine; New York, NY United States
ostreh01@endeavor.med.nyu.edu

The Jews are a people of Middle Eastern origin who have retained their religious and genetic identity over 2000 years of Diaspora. Estimates of historical admixture are as low as 0.5% per generation, suggesting that Jews remained endogamous. During the Diaspora, Jews established geographically dispersed communities in Central and Eastern Europe (Ashkenazim), North Africa, the Balkans, and the Levant (Sephardim), Iran and Iraq, Uzbekistan, Yemen and the Gulf states, and Italy, as well as elsewhere. Many Mendelian genetic conditions have been identified within these Jewish groups that share the feature of having founder mutations with heterozygote frequencies that exceed 1%. Mutations for some of these conditions, such as Tay-Sachs disease, Bloom syndrome, familial dysautonomia, phenylketonuria, and oculopharngeal muscular dystrophy, are found exclusively within certain Diapora groups and must have arisen during a period of relatively recent geographic isolation. Mutations for other conditions, such as familial Mediterranean fever, cystic fibrosis, GJB1

deafness, and Gaucher disease are shared between Jewish groups and non-Jewish groups of the Mediterranean basin, suggesting a more ancient origin. Mutations in the BRCA1 and Factor XI genes common to Ashkenazi and Iraqi Jews are likely to have arisen among the Jews of ancient Palestine in pre-Diaspora times. The observed intervals of linkage disequilibrium for these conditions tend to correlate inversely with the postulated dates of origin of the founder mutations. The presence of so many founder mutations facilitates genetic testing, including heterozygote screening for autosomal recessive conditions. For Ashkenazi Jews, multiplex heterozygote screening for 10 autosomal recessive conditions is now practical. Conversely, the observation of founder mutations occurring in linkage disequilbrium intervals that may span up to 7 cM suggests that association studies using genetic markers in linkage disequilibirum may facilitate discovery for new disease genes.

S14. Virtual Genomic Medicine in Developing Countries.

J. M. Cantu
Universidad de Guadalajara; Guadalajara, Mexico
jmcantu@koch.mb.udg.mx

The enormous advances in science and technology in the XX Century have facilitated the process of globalization with the aim of a better quality of life for all. Paradoxically, the gap between the rich and the poor, either between nations or persons, is constantly widening. Consequently, the access to health care and education, among other benefits, is everyday more limited for the poor. The actual trends in human genome research are leading towards a promising genomic medicine, which will be expensive and inaccesible for many, since the private funding for R&D have at least doubled the public ones in the last few years with the consequent bias in focusing on high prevalence disorders in G7 populations and neglecting the diseases of the poor.The abysmal investment differences in R&D between developed and developing countries (for instance, the 2.7% of the GNP in the USA versus the 0.4 % in M xico) is progresively increasing so it is not only difficult to keep the pace in research but also in the transfer of technology. In an attempt to optimize the scarce human and material medical genetic resources in M xico an initiative of a network, using internet as a means of communication and information has been created. The Mexican Network of Molecular Biomedicine aims to provide a program based on cooperation, high-quality service and patient care. Initial steps have succeded in the organization of a National Hemophilia Center for the molecular diagnosis of patients and possible carriers. The principles of our experience should be aplicable to any developing country. Nevertheless, although medicine has traditionally been viewed as a humane activity guided by altruism, the second half of the XX Century has seen it transformed into a profitable business with an inevitable greed. We need to recuperate the escence of Medicine so any health care system be based on a spirit of justice, equality and, above all, on compassion.

S15. The GeneTests and GeneClinics Experience Serving a Broadening Community of Users

R. A. Pagon
Children s Hospital and Regional Medical Center and University of Washington; Seattle, WA United States
bpagon@u.washington.edu

GeneTests (www.genetests.org) was established in 1993 with funding from the NIH to help geneticists identify clinical and research laboratories providing DNA-based testing for inherited diseases. GeneTests currently is comprised of a database of tests for ~800 inherited diseases, a directory of ~480 international genetics laboratories, a directory of over 900 US genetics clinics, educational materials on basic concepts in genetic counseling and testing, and PowerPointæ teaching tools. GeneClinics (www.geneclinics.org) was established in 1997 to provide geneticists with information on test usage in diagnosis, management and genetic counseling for specific inherited disorders. GeneClinics is comprised of over 100 expert-authored, peer-reviewed disease profiles that link to genomic databases, custom Medline searches, selected patient resources, and policy statements of the ASHG, ACMG and NSGC. Each day GeneTests is searched ~750 times and GeneClinics ~2500 times. Through the GeneTests registration system, Website user logs, and direct user feedback we have documented over time the expanding international scope and broadening audience of these resources. The laboratory directory has become increasingly international as researchers and clinical laboratories have come to depend on worldwide ascertainment of patients with rare inherited disorders. Patients, empowered by the consumer health movement, gather information to partner with their healthcare providers in medical decision-making. Educators have required students to use GeneTests and GeneClinics in case-based problem-solving in policy-making, laboratory medicine, and patient care. With the completion of the draft human genome sequence and the introduction of the revolutionary social and medical concepts of predictive and predispositional testing, the media regularly seeks aggregate information on genetic test availability and use. As genetic testing moves into mainstream medicine, US governmental agencies rely on centralized sources of genetic test data as they institute regulation of tests and more regulation of laboratories.

S16. Antenatal Diagnosis - The Indian Status

I. M. Thomas
Division of Human Genetics, St.John s National Academy of Medical Sciences; Bangalore, India
jato37@hotmail.com

The X-ray was the only technique used for antenatal diagnosis prior to the 1960 s. The introduction of Ultrasound [1970 s] and MRI [1980 s] improved the accuracy of diagnosis. The other non-invasive techniques developed include maternal serum studies and the study of foetal red cells in maternal blood[1990‘s].

Invasive procedures like Amniotic Fluid Cell Cultures [1960‘s] Chorionic Villi Biopsies [1970‘s], Cordocentesis, Fetoscopy, Embryoscopy, Amniography and various other Biopsy techniques [1980‘s] has further improved the prospects of diagnosis of foetal abnormalities and has added to the existing tests consisting principally of Chromosomal Analysis, Biochemical tests and Molecular methods. The introduction of Preimplantation Diagnosis in the1990‘s has helped to identify diseases and to select normal embryos.

India is a developing country and is trying to keep pace with these advances. While the non-invasive techniques, except fetal cells in maternal blood, are practiced most often, Chorionic Villi Biopsy, Placental Biopsy, Amniotic Fluid Cell Culture, Cordocentesis and other biopsies are undertaken less often. The tests on the material so obtained consist mainly of Chromosomal Analysis, a few biochemical tests and even fewer Molecular [DNA] tests which are being mainly done for Deuchenne Muscular Dystrophy and Hereditary Anemias [Thalassemia, Sickle Cell Anemia and Hemophilia]. These tests are available principally in urban-based private, non-governmental institutions and research centers. These are, therefore, not available to the vast majority of the population. Preimplantation diagnosis in India is, however, only being attempted in animals.

Antenatal diagnosis for sexing was started in 1978 and this led to female foeticide. Female foeticide exists because of ‘dowry‘ system. This ‘dowry‘ system is practiced in many areas of India, wherein money and valuables are given to the groom or his family mostly on demand during marriage. Although there is a law forbidding it and making it a criminal act, like the Antenatal Diagnosis Misuse Act of 1996 [which forbids sexing except for X-linked diseases] it is very difficult to implement these two laws, as there are no complainants.

In order to assess the exact status of antenatal diagnosis in India a questionnaire is being sent to various institutions. The results of these replies will be compiled and presented.

S17. Fetal DNA in maternal plasma

Y. M. D. Lo
The Chinese University of Hong Kong; Shatin, New Territories, Hong Kong Special Administrative Region of China
loym@cuhk.edu.hk

Recently, fetal DNA has been shown to be present in maternal plasma and serum. Using quantitative PCR techniques, fetal DNA in maternal plasma and serum has been demonstrated to be present in amounts which are easily detectable. This new source of fetal genetic material offers a powerful approach for the prenatal diagnosis of many fetal genetic characteristics, including blood group status and many paternally-inherited genetic traits. Diseases-causing mutations which have been detected in maternal plasma include myotonic dystrophy and achondroplasia. The finding that part of the fetal DNA in maternal plasma exists as intact fetal cells also offers new possibilities for the prenatal diagnosis of many chromosomal aneuploidies, including trisomy 21. Quantitative abnormalities of fetal DNA in maternal plasma and serum have also been found in many disorders, including pre-eclampsia, preterm labour and fetal chromosomal aneuploidies. These results suggest that fetal DNA measurement may become a useful tool for the detection or monitoring of these disorders. Apart from its diagnostic implications, circulating fetal DNA also opens up many previously unexplored research opportunities for understanding the fetomaternal relationship on a molecular level.

S19. Chromatin, cell cycle and §-globin gene transcription

F. Grosveld
Dept. of Cell Biology and Genetics, Erasmus University Rotterdam; Rotterdam, The Netherlands
grosveld@ch1.fgg.eur.nl

The Locus Control Region (LCR) is required for the activation of all of the human beta-like globin genes during development. The early embryonic developmental programme is executed in nucleated primitive red blood cells that express the epsilon and gamma genes. The foetal/adult programme takes place in a definitive red blood cells that first express the gamma and later the delta- and beta genes. This difference in expression programme correlates with changes in the chromatin structure within the beta globin locus. Repression of the early genes (epsilon and gamma) in late cells is achieved by as yet unknown factors acting on sequences flanking these genes. Superimposed on this is a mechanism in which the early genes (epsilon and gamma) suppress the late genes (delta and beta) by competition for the interaction with the LCR. In particular the latter mechanism has allowed a series of studies to examine the transcriptional process at the level of the single cell. These studies indicate that the LCR interacts with individual globin genes and that LCR/gene interactions are dynamic with complexes forming and dissociating continually. The levels of expression of each of the genes appear to depend on; (1) the frequency of interaction which is itself dependent on the distance of the gene to the LCR, (2) the affinity/stability of the LCR/gene complexwhich is dependent on the balance of transcription factors such as EKLF. When individual hypersensitive region in the LCR are deleted from a complete transgenic locus that is integrated in a pericentromeric region, the locus becomes sensitive to two types of position effects. One of these is classical Position Effect Variagation (PEV) while the other is a Timing Position Effect (TPE). The PEV can be modified by an increase in the concentration of EKLF which acts on the LCR and results in more cells expressing the locus, which is accompanied by a general increase in DNAse sensitivity throughout the locus. TPE is dependent on the cell cycle resulting in a limited period of expression in of all the red cells. Examination of single cells shows that both types of position effects involve the relocalisation of the transgenic locus in the nucleus. The implications of these experiments for the role of the LCR in the activation of the locus will be discussed.

S20. Regulation of Imprinting at the H19/Igf2 locus.

M. S. Bartolomei*, J. L. Thorvaldsen*
Howard Hughes Medical Institute and Department of Cell and Developmental Biology, University of Pennsylvania Medical School; Philadelphia, PA United States
bartolom@mail.med.upenn.edu

H19 and the oppositely imprinted *Igf2* gene are located 90 kb apart in mouse and share regulatory elements crucial to their imprinting. It is of particular interest to determine how and when the imprint that distinguishes these two alleles is determined. Two epigenetic modifications, DNA methylation and chromatin structure, are proposed to confer the allelic mark. Specifically, *H19* is hypermethylated on the inactive paternal allele in a 2 kb region 2 kb 5 to the start of transcription (this region is designated at the DMD or differentially methylated domain). This same region is hypersensitive to nucleases on the active maternal allele. Thus, it has been proposed that the DMD confers the allelic mark. To test this hypothesis, the DMD was deleted from the endogenous locus in mice. In the absence of the DMD, imprinted expression of *H19* and *Igf2* was perturbed on both parental alleles; on the paternal allele, transcription from the normally silent *H19* allele was activated and *Igf2* transcription was coordinately reduced whereas on the mutant maternal allele *Igf2* expression was activated and *H19* expression was coordinately reduced. These experiments prove that the DMD is required for imprinted expression on both parental chromosomes. It has been postulated that the DMD acts as a methylation-sensitive boundary element that isolates *H19* and the enhancers on the maternal chromosome, thereby preventing access of *Igf2* to the enhancers. On the paternal chromosome, *Igf2* is expressed because the DMD and *H19* gene promoter are methylated, preventing the boundary proteins from binding and *H19* from being expressed. It was recently demonstrated that a putative boundary protein, CTCF, binds to a conserved repetitive element in the DMD in a methylation senstive manner. While these experiments were performed *in vitro*, we are testing the role of CTCF and the conserved DMD elements *in vivo*.

S21. Dosage Compensation in Drosophila as a model for epigenetic regulation of gene expression

P. B. Becker
Adolf-Butenandt-Institute, Ludwig-Maximilians-University; Munich, Germany
pbecker@mol-bio.med.uni-muenchen.de

When it comes to their heteromorphic sex chromosomes, humans and fruit flies resemble each other. In both species males are characterized by an X, Y genotype, whereas females have two X chromosomes. Dosage compensation is a regulatory process that assures that X chromosomal genes are expressed to an equivalent extent from the single male and the two female X-chromo-somes. In humans, dosage compensation is achieved by inactivation of one female X chromosome through facultative heterochromatinization. Flies have chosen a different strategy; they express the genes on both female X chromosomes at a constant rate, but increase the transcription from the male X chromosomes by two-fold. Failure to activate their X chromosome is lethal for Drosophila males. Although dosage compensation strategies in flies and men are remarkably different, they are also resemble of each other. Both stategies tune the expression status of an entire chromosome through epigenetic means; chromatin structure is modified by targeted posttrans-lational modification of histones. Remarkably, both processes also involve non-coding RNA. The proteins that are involved in dosage compensation in flies are known due to the male-specific lethality of their loss-of-function mutation. They form a complex with noncoding roX RNA which associates with many sites on the X chromosome. We study the process of dosage compensation in Drosophila as a paradigm for the targeting of chromatin remodeling factors to a restricted chromosomal domain, the role of non-coding RNA in epigenetic regulation and the mechanisms by which histone modifications affect the higher order structure and function of chromatin.

S22. The limb-girdle muscular dystrophies- diversity of pathogenesis.

K. M. D. Bushby
Department of Human Genetics, University of Newcastle upon Tyne; Newcastle upon Tyne, United Kingdom
kate.bushby@ncl.ac.uk

The limb-girdle muscular dystrophies (LGMD) are a heterogeneous group of disorders which comprise both autosomal dominant and recessive diseases. A gene and protein based classification for these conditions is now possible, based on their underlying molecular pathology. Amongst the autosomal dominant forms of LGMD, mutations in the genes encoding myotilin (a component of the muscle sarcomere) caveolin 3 (a component of caveolae in the muscle fibre membrane) and lamin A/C, a nuclear envelope protein have been described, while mutations in the collagen VI genes account for a phenotypically overlapping condition, Bethlem myopathy.
Amongst the autosomal recessive types of LGMD, an important subgroup is caused by mutations in the genes encoding a, b, g and d sarcoglycan, proteins which, with dystrophin, form a complex of structural proteins in the muscle fibre membrane. The diseases of the dystrophin associated complex share some clinical and pathological similarities, though the mechanisms by which these diseases arise may in some ways be subtly different.
The other recognised forms of autosomal recessive LGMD are more diverse- mutations in calpain 3, a protease predominantly expressed in skeletal muscle cause a relatively common type of LGMD, while mutations in dysferlin, a membrane protein which contains multiple C2 domains cause a form of muscular dystrophy which may present either with predominantly proximal or distal disease. The latest gene identified as causing an autosomal recessive LGMD, telethonin, is a component of the muscle sarcomere.
With these diverse disease mechanisms, it is likely that a number of disease pathways can contribute to the causation of these diseases. In the meantime, improvements in the classification of this group have resulted in clear benefits to patients in terms of diagnosis and the provision of prognostic and genetic counselling information.

S23. Muscular Dystrophies; Same Mutation but Different Clinical Courses? The Next Challenge

M. Zatz*, M. Vainzof, M. R. Passos-Bueno*
Center for the Study of the Human Genome, Dep. of Biology, Inst. of Biosciences, University of S o Paulo; Sao Paolo, Brazil
mayazatz@usp.br

The autosomal recessive limb-girdle muscular dystrophies (AR-LGMD) and autosomal dominant facioscapulohumeral muscular dystrophy (FSHD) have been the subject of world-wide investigation.
Nine AR -LGMD (LGMD2A to LGMD2I) genes have been mapped and linkage analysis indicates that there is further genetic heterogeneity . The clin-

ical course is characterised by great variability, ranging from severe forms with early onset and rapid progression to milder forms with later onset. The gene product is known for 7 forms ; calpain 3 for LGMD2A , dysferlin for LGMD2B , alpha-sarcoglycan for LGMD2D , beta-sarcoglycan for LGMD2E , gamma-sarcoglycan for LGMD2C , delta-sarcoglycan for LGMD2F and the sarcomeric protein telethonin for LGMD2G.
Facioscapulohumeral muscular dystrophy (FSHD1) mapped at 4q35, accounts for most cases of FSHD. Affected patients have a deletion of a critical number of 3.3kb repeated units at D4Z4. The most likely hypothesis to explain the abnormal phenotype is a position effect.
We performed genotype-phenotype correlation studies in 216 LGMD patients classified from calpainopathy to telethoninopathy as well as 248 FSHD1 patients. In the LGMD group, a similar clinical course among patients with non allelic mutations while a discordant phenotype in patients carrying the same mutation was observed. For FHSD1 , in addition to the great intra and interfamilial variability a significantly greater proportion of affected males than females (due to a higher proportion of asymptomatic women carrying a small *Eco*RI fragment , < 35kb) was observed.
Understanding how patients with the same mutation may have a discordant phenotype, or why a greater proportion of women may be more protected from the deleterious effect of the FSHD1 mutation remains a major challenge. It will depend on future knowledge on gene-protein functions, on protein interactions and on identifying modifying genes and other factors underlying clinical variability. Supported by FAPESP, PRONEX, CNPq.

S24. Facioscapulohumeral muscular dystrophy

R. Frants
Department of Human and Clinical Genetics, Leiden University Medical Center; Leiden, The Netherlands
r.r.frants@lumc.nl

Facioscapulohumeral muscular dystrophy (FSHD) is the third neuromuscular disease with a prevalence of 1/25.000 in most populations. The sequence of muscle involvement, the high intra- and interfamilial clinical variability and lack of treatment have stimulated search for the molecular background of this syndrome. Being an autosomal dominant phenotype, a genome wide marker scan allowed mapping of the FSHD1 locus to #4q. The causative mutation in familial and sporadic cases turned out to be a deletion within a polymorphic repeat array only some 25 kb from the telomere. The molecular dissection of FSHD has been compromised, but also stimulated, by the extensive chromosomal dispersion of this region. The highly homologous locus on 10q has been particularly rewarding. In up to 20% of the population, an exchange between the subtelomeric regions of #4q and #10q can be observed. The presence of a supernumerary #4 type repeat on #10 seems to induce #4 instability. In most sporadic patients, a somatic mutation can be detected in either the patient or one of the parents. The detailed physical information on the 4q and 10q regions has improved the diagnostic repertoire. However, the molecular mechanism of FSHD remains elusive. The present prevailing model is that the FSHD mutation/deletion induces a change in the chromatin structure and accordingly the expression of genes in the region, basically according to position effect variegation. Several candidate genes have been identified in the region; FRG1, FRG2, TUBB4q and DUX4 all being localized within 100 kb of the D4Z4 repeat array. Implementation of various genomics approaches and (transgenic) animal models will be instrumental to reveal the molecular pathways necessary to open the FSHD Pandora box.
*On behalf of the Dutch FSHD Team.

S25. Intelligence

R. Plomin
Social, Genetic and Developmental Psychiatry Research Centre, Institute of Psychiatry; London, United Kingdom
r.plomin@iop.kcl.ac.uk

At the centre of the nature-nurture debate has been general cognitive ability (g), often called intelligence. Decades of genetic research comparing identical and non-identical twins and adoption studies consistently show substantial genetic influence on cognitive abilities such as verbal and spatial abilities and on cognitive disabilities such as reading disability and dementia. Genetic research on g has now gone beyond simply demonstrating genetic influence to ask questions about development, about relations between cognitive abilities, and about the environment. A surprising developmental finding is that genetic influence on g increases steadily during the life span. Concerning relations between cognitive abilities, genetic research has shown that the same genetic factors influence diverse cognitive abilities, suggesting that g is the genetic core of cognitive abilities. Research at the interface between nature and nurture shows that environmental factors that influence the development of g work very differently from the way we thought they worked.
An exciting new direction for genetic research is to identify some of the many genes that affect cognitive abilities and disabilities. We have conducted an initial scan of the genome using DNA pooling with two thousand DNA markers in order to find genes associated with g . Scientific and social implications of finding genes for g will be discussed.
Both nature and nurture are important for the development of g . The nature-nurture wars are over; For nearly all complex medical and behavioral traits, genetic factors are important but so too are environmental factors. Moreover, genetic and environmental factors interact and correlate. The issue is not nature *versus* nurture but rather nature *and* nurture.

S26. Genetic Influences on Alcohol Intake and Euphoria

T. J. Phillips
Veterans Affairs Medical Center and Portland Alcohol Research Center, Oregon Health Sciences University; Portland, OR United States
phillipt@ohsu.edu

Several behavioral genetic methods exist for studying human disease in animals. Mouse methods include special populations relevant for gene mapping such as recombinant inbred strains, genetically segregating populations, congenic strains and short-term selected lines, as well as other populations critical for disease phenotype analyses, such as long-term selected lines, panels of standard inbred strains, transgenic lines and knockout mice. All of these methods have been used to study voluntary alcohol intake in mice, a model thought to be relevant to human alcohol self-administration, ultimately associated with the development of alcohol abuse. Some of these methods have also been brought to bear on another common effect of abused drugs, locomotor stimulation. This motivationally relevant behavior has been described as a putative model of human drug-induced euphoria. Gene mapping efforts have produced convergent data indicating that genes on chromosomes 2, 3 and 9 influence voluntary alcohol intake. The region of interest on chromosome 9 harbors at least two interesting candidate genes; the dopamine D2 receptor gene, Drd2 and the serotonin 1B receptor gene, Htr1b. Genes influencing locomotor stimulant responses to several abused drugs, including cocaine, methamphetamine and ethanol (alcohol) have been provisionally mapped. The most definitive information has been gathered for ethanol, for which a strong association with a region on chromosome 2 has been found. Pharmacological studies in lines of mice bidirectionally selectively bred for extreme sensitivity (FAST) and insensitivity (SLOW) to ethanol s locomotor stimulant effects are another powerful tool for identifying the neurobiological determinants of alcohol sensitivity. Recent data in our lab suggests that gamma aminobutyric acid (GABA), and in particular, GABA-B type receptors in the ventral tegmental area, significantly influence ethanol s stimulant effects. Future efforts will be directed at further refinement of the biological circuitry and genetic regions associated with these important alcohol-associated traits. The ultimate goals will be identification of the several influential genes and discovery of the central structures and the component parts (e.g., cell types, neurotransmitters, receptor types) that modulate these traits.

S27. Perspective in psychiatric genetics; the example of the Tyrosine Hydroxylase genes and its implications for complex genetic traits

J. Mallet*, N. Faucon Biguet, V. Albanese, T. Aranyi, R. Meloni*
Laboratoire de G n tique de la Neurotransmission, CNRS, UMR 9923, H pital de la Piti -Salp tri re; Paris, France
suorsoni@infobiogen.fr

Having previously shown a positive association between the (TCAT)n microsatellite HUMTH01 located in the first intron of the Tyrosine Hydroxylase (TH) gene with bipolar disorder and schizophrenia as well as with disturbances in the catecholamine metabolism, we thereafter established that the alleles of this tetranucleotide repeat activate transcription driven by a minimal promoter. In addition, they are specifically recognized by nuclear proteins pertaining to the fos-jun family of transacting factors, as well as to a class of these factors that remained to be characterized.
Following the inroad opened by these results, the different HUMTH01 alleles were thus evaluated in the context of the TH gene. Specific transacting factors were then cloned. Finally, the methylation profile of CpG island flanking the repeated sequence was investigated to evaluate the role of epigenetic modifications in the transcriptional regulation of the TH gene.
The HUMTH01 alleles were found to differentially modulate TH gene activity in a quantitative manner. One of the specific transacting factors exhibits affinity for the HUMTH01 sequence correlated with variations in the number of (TCAT)n repetitions. TH+ and TH-human cell lines exhibited differential methylation patterns, and a new putative Ap2 site in the first exon of the TH gene was identified.
The (TCAT)n polymorphic sequence is widespread throughout the

genome. Thus, the characterization of the transduction pathway impinging on the HUMTH01 microsatellite and its flanking sequences may be relevant for the transcriptional regulation of the TH gene as well as that of other genes implicated in normal and pathological complex genetic traits.

S28. Quality in Molecular Genetic Testing — What is it, How can it be measured, How can it be achieved?

R. Elles
European Molecular Genetics Quality Network, C/o Regional Genetics Service; Manchester, United Kingdom
relles@smh5.cmht.nwest.nhs.uk

Molecular genetic testing is in a process of transition from a research based activity to a more routine service. Service providers are developing procedures to make tests more reliable, ways of measuring the errors in laboratory testing and mechanisms to manage the whole laboratory process for quality. Scientists leading genetic testing laboratories tend to think about quality first in terms of the accuracy and reproducibility of the analytical process, the reliability of mechanisms for handling materials and information and the quality of the information in the report from the laboratory. By contrast the perception of quality by the consumer (patients, their families and their physicians) may have a different emphasis. The consumers usually take the accuracy of the test for granted and think first about whether a test is available, whether they have easy access to the service and how long a result will take to be reported. Service providers need to place more consideration on the needs of the client and place their clients at the centre of the quality management process. Policy makers and regulators need to think about the problem of availability and access to testing given the rarity of many genetic conditions. They also need to develop simple but effective systems to promote quality management and to encourage service providers to comply with recognised international standards.

S29. Quality Control in a Home Brew Genetic Testing Environment

G. A. Miller
Genzyme Corporation; Framingham, MA United States
Glenn.Miller@genzyme.com

The number of Home Brew tests being introduced into the genetic testing market is increasing at a pace commensurate with the increase in genetic information resulting from the Human Genome Project. As more genes relevant to predisposition, diagnosis and prognosis are identified the pressure to bring clinically relevant genetic tests to the market will increase. The quality of these tests and the regulatory environment into which they are introduced will determine, in part, the success of the Human Genome Project in terms of its impact on human health in the near term. Genetic testing laboratories, both commercial and academic, are under increasing pressure to assure the quality of their testing facilities and technologies. The balance that is to be struck between appropriate regulation and a regulatory environment that would impede progress is currently under close study. In the interim, an emphasis on quality control for home brew testing remains a critical part of test development and practice. The fact that this effort is often driven by individual laboratory commitments to excellence in test development and practice will be discussed.

S30. Communicating genetic risk information

T. M. Marteau
Psychology and Genetics Research Group, King s College; London, United Kingdom
theresa.marteau@kcl.ac.uk

Genetic testing has most often been associated with predictive testing for untreatable conditions, such as Huntington s disease, or prenatal testing accompanied by an offer of termination of affected pregnancies. The isolation of genes associated with common forms of potentially preventable diseases such as heart disease, cancer as well as with risk factors such as smoking and obesity, raise the possibility of providing such information to those without family histories. It is envisaged that informing individuals of their genetic susceptibility to disease will motivate them to change their behaviour to reduce their risks. Drawing upon the few studies to date that have addressed this question, this paper will describe two possible barriers to behaviour change following the provision of genetic risk information and consider how these might be overcome.

1. Perceived non-salience of genetic risk information Acceptance of risk information depends in part upon the extent to which the information makes sense to an individual, ie fits with existing schema or representations of a health threat. Perceiving familial adenomatous polyposis as determined by factors other than genes was associated with expecting to continue bowel screening despite a negative result on genetic testing. This suggests the importance of eliciting and discussing with those undergoing such tests, their beliefs about the condition for which testing is being conducted.

S31. Ion channel mutations as a major cause in idiopathic epilepsy

O. K. Steinlein
Institute of Human Genetics, Rheinische-Friedrich-Wilhelms-Universit t; Bonn, Germany
osteinl@mailer.meb.uni-bonn.de

Mutations in several genes are known to be associated with subtypes of idiopathic epilepsy. Familial nocturnal frontal lobe epilepsy can be caused by a few site-specific mutations in the ion pore lining regions of the neuronal nicotinic acetylcholine receptor subunits CHRNA4 and CHRNB2. Other examples are the several different potassium channel mutations which have already been described in benign familial neonatal convulsions (BFNC). Most of these mutations are found in the voltage gated subunit KCNQ2, only a few are located in the homologous subunit KCNQ3. So far KCNQ2 mutations were shown to cause BFNC by haploinsufficiency of the ion channel. We recently found a family with an unusual KCNQ2 mutation which has a dominant negative effect on channel activity, and causes a formerly unknown syndrome characterised by BFNC and peripheral nerve hyperexcitability. The third example for an idiopathic epilepsy as a channelopathy is the syndrome of generalized epilepsy with febrile seizures plus (GEFS+), which can be caused by mutations in the voltage gated sodium channel genes SCN1A and SCN1B. GEFS has a wide spectrum of phenotypes, and many family members have an increased susceptibility for seizures during fever. These three epilepsies with known molecular defects support the hypothesis that ion channel mutations are a major cause of idiopathic epilepsies. They can serve as a models for further genetic studies which are more and more focusing on the common forms of the disease.

S32. From Mad Cows to Psi-chotic Yeast; A New Genetic Paradigm

S. M. Uptain, *S. L. Lindquist*
Department of Molecular Genetics and Cell Biology, Howard Hughes Medical Institute, University of Chicago; Chicago, IL United States
s-uptain@uchicago.edu

Our work supports and extends the hypothesis that a protein can serve as an element of genetic inheritance. This protein-only mechanism of inheritance propagates in much the same way as previously hypothesized for the mammalian prion protein, the infectious agent in the spongiform encephalopathies (such as Mad-cow disease). In yeast, however, the process produces heritable changes in phenotype, not disease. The yeast prion we study, [*PSI*$^+$], is a dominant, cytoplasmically inherited trait caused when a component of the translational termination factor, Sup35p, undergoes a conformational switch that causes it to aggregate. This reduces the efficiency of the termination factor and permits ribosomes to read through stop codons at an appreciable frequency. A crucial aspect of this process is that the prion form of Sup35p promotes conversion of newly synthesized Sup35p to the prion form; thus, Sup35p prion aggregates cause an inheritable change in phenotype, without a concomitant change in nucleic acid. We have provided genetic, cell biological and biochemical evidence in support of this revolutionary hypothesis and a molecular model for how protein-only inheritance might occur. Most recently, we have begun to determine the molecular basis of variants of [*PSI+*], which seem to be analogous to mammalian prion strains. Individual yeast strains can propagate different [*PSI*$^+$] variants which exhibit different nonsense-suppression efficiencies and stabilities. Similarly, mammalian prion variants can be maintained in isogenic mice yet are distinguished by differences in incubation time for disease onset, brain pathology and degree of species specificity. Our data shows that [*PSI*$^+$] variants differ in how efficiently Sup35p is converted to the prion form. This affects the balance of the soluble and prion form of Sup35p which determines the strength of nonsense suppression phenotype of [*PSI*$^+$] variants.

S33. The Genetics of Parkinson s Disease

A. Brice
INSERM U289, D partement de G n tique, Cytog n tique et Embryologie, H pital de la Salp tri re; Paris, France
brice@ccr.jussieu.fr

Several genetic epidemiological studies have suggested the existence of predisposing genetic factors in Parkinson s disease, which can be considered to be a multifactorial disorder. However, at least five monogenic forms have been recognized, which should help to understand the pathological mechanisms. Studies on the alpha-synuclein and Parkin genes have been

very fruitful.
The alpha-synuclein gene has been implicated in a small number of families with autosomal dominant Parkinson s disease with Lewy bodies. Subsequently, alpha-synuclein turned out to be a major component of Lewy bodies and Lewy neurites. The familial mutations increase oligomerisation of alpha-synuclein into fibrillar structures, which are found in Lewy bodies. Animal models over expressing normal and mutated alpha-synuclein reproduce some features of Parkinson s disease.
A wide variety of Parkin mutations are associated with autosomal recessive Parkinson s disease. They account for half of the early onset familial cases and a significant proportion of patients without family history but with early onset. In addition to early onset, Parkin cases are characterized by frequent dystonia at onset, excellent and sustained response to levodopa with frequent levodopa induced dyskinesias, and slow progression of the disease. It was recently shown that Parkin, which is expressed ubiquitously in the central nervous system, functions as an E3 ubiquitin ligase. Therefore, Parkin is expected to ubiquitinate specific substrates targeted for degradation by the proteasome. Interestingly, Parkin patients present severe nigrostriatal dopaminergic neuronal loss without Lewy bodies. This particularity might be related to the function of Parkin for which it is now crucial to identify the substrates.
It will also be necessary understand the relevance of these observations to idiopathic Parkinson s disease.

S34. Yeast genetics and human biology

P. Hieter
UBC Biotechnology Laboratory and Department of Medical Genetics, University of British Columbia; Vancouver, BC Canada
hieter@cmmt.ubc.ca

Yeast is arguably the most powerful experimental organism for learning about the basic function of genes involved in intracellular eukaryotic processes. The combination of classical genetics, recombinant genetics, and biochemistry offers a definitive approach to understanding protein function in cells. Furthermore, the availability of the entire yeast genomic sequence has spawned the development of genome-wide or system-wide approaches to the analysis of gene function in yeast. These functional genomic methods are of great value to yeast genetics research and also place yeast as a critical testbed for these emerging technologies. Links between human disease genes and yeast genes provide immediate protein functional data and an experimental paradigm for further analysis of disease relevant biology. They can also be used to accelerate the identification of disease genes. It is important to emphasize that the link to a yeast gene often provides connection to an entire genetic pathway, a multi-protein complex, or interacting gene products. These additional links can be key to an understanding of disease mechanism or development of a therapeutic strategy.
The discovery of a relationship between a human disease gene and a gene found in yeast is clearly advantageous, but how can these connections be established in a systematic fashion? Three general paradigms have emerged; 1) Human/yeast similarity searching, 2) Genome cross-referencing , 3) Homology probing . The worm and fly genome sequences make multi-organismal approaches for understanding human biology even more powerful. These organisms provide complementing experimental approaches and aspects of eukaryotic biology not approachable in yeast. Given the unique experimental advantages of the yeast system, yeast genetics/genomics approaches will continue to be a crucial component in medical genetics research for the foreseeable future.

S35. RNA intereference and transposon silencing in C. elegans

R. H. A. Plasterk
Hubrecht Laboratory; Utrecht, The Netherlands
plasterk@niob.knaw.nl

Genomes, like all other databases that are linked to other databases, are potentially in danger of infection by parasitic elements, viruses and transposons. The human genome consists for approximately 50% of transposons. Networked computers need to be protected against computer viruses by virus protection software. Likewise genomes are protected by a mechanism of transposon silencing. We have analysed this in the nematode C. elegans. The surprise finding is that the mechanism of transposon silencing in C. elegans is esentially the same in animals, plants and fungi. Thus we are beginning to understand an ancient mechanism that organisms use to protect themselves against invading selfish DNA. The current status of our understanding of the mechanism will be described.

S36. Molecular Genetics of Body Weight Regulation

R. L. Leibel
Division of Molecular Genetics and The Naomi Berrie Diabetes Center, Columbia University; New York, NY United States
RL232@columbia.edu

Three metabolic/behavioral phenotypes determine the body weight and composition of an organism; energy intake, energy expenditure, and the partitioning of stored calories among glycogen, protein and fat. The recent molecular cloning of rodent single gene obesity mutations, and the disruption of other genes by transgenic techniques, has identified some of the important molecular substrates for these processes. A variety of physiological and neurophysiological experiments indicate that the hypothlalamus receives humoral signals reporting the mass of adipocytes in the body, and uses this information to generate efferent neural signals that have coordinate effects on intake, expenditure and partitioning. An important afferent signal is the hormone leptin, produced and secreted primarily by adipocytes. This gene is mutant in the Lepob (obese) mouse, and its cognate receptor is mutant in the Leprdb (diabetes) mouse. Deficiency of the ligand or receptor is perceived physiologically as a deficiency of energy stores for which the organism compensates by coordinate increases in energy intake and reductions in energy expenditure. The model of leptin as a molecular signal of sufficiency of energy stores (for reproduction and survival of prolonged caloric restriction) is compatible with evolutionary arguments regarding the characteristics of a system designed to optimize survival/reproductive efficiency in hostile environments. Among the hypothalamic neuropeptides whose expression is altered by leptin are NPY, POMC, CART, HCRT/OREXIN, MCH, CRH, AGRP. The recently described chemical neuroanatomy of the neurons expressing these peptides is consistent with the long-standing observations that ablations of the ventromedial and lateral hypothalamic regions result, respectively, in hyper- and hypophagia. The precise roles of these neurons and neuropeptides in controlling body composition are not yet known. Obese humans with mutations in LEP, LEPR, LEPR (processed to ?-MSH) and mc4r (receptor for msh) have been reported. In some populations, up to 5% of severe obesity can be accounted for by haploinsufficiency of MC4R. However, in most instances, susceptibility to obesity is probably conveyed by the interactions among several of these (and other) genes and environmental circumstance. These gene-gene interactions may, in the aggregate, set the functional threshold for leptin s ability to provide a signal of energy sufficiency. Subtle coding and regulatory sequence variants may act additively and epistatically to alter this threshold.

S37. Clinical Genetics: The future

J. Burn
School of Biochemistry and Genetics, University of Newcastle; Newcastle upon Tyne, United Kingdom
John.Burn@newcastle.ac.uk

Genetics has become established in the popular view as exciting and threatening in equal measure. Its impact on future medicine is taken as a fact. Most commentators fail to distinguish between genetic technologies which will become incorporated in to general diagnostics, just as blood groups did in past years, and the application of genetics to inherited disease. The latter includes diagnosis, and assistance in reproductive choice and predictive testing. As a medical specialty, clinical genetics has followed two paths. The first involves clinical geneticists in small numbers acting as an academic focus with most applications dispersed to individual specialities. The second, as seen for example in the UK and the Netherlands, involves a large cohort of clinical geneticists supported by genetic nurses and counsellors acting as a focus for large scale application of genetic knowledge in medical practice. Chance, history, religion and money all play a part in determining which pathway is followed. The question is whether they can or should converge and if not, which will become the dominant model in the health services of tomorrow.

S38. Organization of Genetic Services in the Republic of Cuba

L. Heredero
National Center of Medical Genetics. Medical University of Havana; Havana, Cuba
lhere@infomed.sld.cu

The Island of Cuba with 11 million inhabitants, an infant mortality about 7/1000, life expectancy in 75 years, with a free national health system based in a well developed primary health care (more than 90% of population covered by family doctors) started a National Program for the Diagnosis and Prevention of Genetic Diseases in 1982, and all the island was covered by this service ten years later. After delineation of the most frequent genetic disorders and birth defects (2.2/live births with NTD«s, 3-7% of the populaqtion is a carrier of hemoglobinopathies (Hb S or Hb C), 4%

of pregnant women over 38 years old, 3% of the population is a carrier for CF) the development of appropriate local technologies and organizational clinical evaluation together with the Mother and Child Department of the Ministry of Health have permitted to offer a genetic service for all the population.
Starting by detection increased genetic risk using prenatal serum biochemical markers in close connection with the family health care and later over 500 family doctors were trained in the detection of increased genetic risk asking the family history at the community and its referral to every provincial medical Genetic center.
A net of Medical Genetic Centers was built all over the country with facilities for Hb electrophoresis, MSAFP determination, US fetal monitoring, amniocentesis, Chromosome analysis, and clinical genetics. The National Reference Center also offer DNA analysis. Enzymatic determination and neonatal screening for PKU and is engaged with postdoctoral medical training in medical genetics and evaluation of the procedures.
Some research about ethical aspects and evaluation of the services result in a positive acceptation of the population about this services. Still me need to be better in some branches and to increase more diagnostics options when the economical situation become better.

S39. Development of a Primary Health Care Genetic Services for Rural Southern African Communities; The Road to Equity and Equality?

P. A. Venter[1], *A. L. Christianson*[2], *J. Modiba*[3], *N. Raphahlelo*[3], *R. Shimange*[3], *M. Sithole*[3], *C. Hutamo*[3], *N. Ramatumbu*[3], *O. Tshivhase*[3]
[1]*Department of Health Sciences, University of the North; Sovenga, South Africa;* [2]*Department of Human Genetics, University of Pretoria; Pretoria, South Africa;* [3]*Genetic Clinics, Department of Health and Welfare; Northern Province, South Africa*
philipv@unin.unorth.ac.za
In 1990 genetic service were not considered to be part of Primary Health Care (PHC) in South Africa, specifically not in the underdeveloped poor rural African areas where very little epidemiological information/data existed on the incidence/prevalence of genetic disorders and birth defects. The general perception was that congenital disorders were not a health problem of significance in rural South Africa. This concept that a clinical genetic service was an inappropriate luxury in these developing countries obfuscated the fact that in such countries the incidence of congenital disorders was as high, and in certain circumstances higher, than in industrialized countries.
The Northern Province, South Africa is one of the poorest and most underdeveloped vast rural areas of South Africa, inhabited by 5, 2 million people of which 95% are indigenous black people from the Venda, Pedi, and Shangaan tribes while approximately 50% are still illiterate. Most households are still without electricity, running water, and sanitation, while public transport is almost non-existing with very poor rail and road infrastructure, even though most 1st World technology is on its doorstep but not accessible.
In 1989 a research project; Screening Programme To Diagnose Congenital/Genetic Abnormalities Among New Born Babies At Mankweng Hospital was started and is still continuing at several Hospitals in the Province. From this small beginning this project developed into a multiphase community-based PHC genetic/disability program that led to numerous developments and activities in many Hospitals, Institutions and Schools for the physical, visual, auditory and intellectually disabled.
The numerous facets of developments and activities will be discussed, including training of PHC workers, screening projects, research, counselling, service provision and establishment of parent support groups among the rural disadvantaged communities. It also provided for the first time information from rural South Africa on the incidence and frequency of congenital disorders in general and of specific common syndromes including Down syndrome, Albinism, and NTD s.

S40. Genomic and expression profiling of tumor genomes using DNA

P. Lichter
Deutsches Krebsforschungszentrum, Heidelberg; Germany
p.lichter@dkfz-heidelberg.de
Comparative genomic hybridization to microarrayed DNA fragments, an approach termed matrix-CGH, provides a powerful tool for the detection of genomic imbalances at a high resolution. Matrix-CGH allows the profiling and fine mapping of genomic imbalances in cell populations from patients with constitutional as well as somatic genetic diseases. Following the development and automation of matrix-CGH, we have applied this method to analyze human neoplasias in order to
I. fine map altered genomic regions facilitating the identification of genes, which play a role in tumor pathomechanisms,
II. generate profiles of copy number changes of known oncogenes and tumor suppressor genes uncovering biochemical pathways affected in a given tumor entity, and
III. design dedicated chips, by which all relevant imbalances of a given neoplasia type are detected, supporting diagnostic efforts to subclassify tumor patients.
Comprehensive expression profiling by DNA chip technology has the potential to uncover previously unknown pathogenic pathways and to possibly define new therapeutic interference points. We have performed genomic and expression profiling of human leukemias in order to elucidate disease pathomechanisms. Furthermore, applying statistical analysis and data mining approaches, these data were utilized for classification analyses within the investigated entities. Recent results will be presented, the different approaches will be compared and future perspectives will be discussed.

S41. Multi Colour COBRA-FISH; Applications in Clinical and Tumour Cytogenetics

H. J. Tanke*, V. Bezrookove, K. Szuhai, H. Engels, J. Wiegant, M. de Wind-van de Burg, C. Rosenberg, A. K. Raap*
Laboratory for Cytochemistry and Cytometry; Department of Molecular Cell Biology, Leiden University Medical Center; Leiden, The Netherlands
H.J.Tanke@lumc.nl
Present FISH karyotyping is based on the so-called combinatorial use of probes labelled with 5 distinct fluorophores. For such combinatorial labelling it has been shown that the number of targets (n) recognizable by FISH using (k) different fluorophores results in n= 2k-1 colours.
The principle of COBRA-FISH is based on the simultaneous use of combinatorial labelling and so-called ratiolabelling, which allows for simultaneous staining of many more targets than reported so-far. Three spectrally well-separated fluorophores are used pair wise for ratiolabelling, allowing a total of 12 colours to be distinguished. A second set of 12 probes, recognizing different targets are labelled exactly the same, but in addition are given a fourth label, resulting in a total of 24 colours. The fifth label is subsequently used to repeat this principle once again, to accomplish full staining of p and q arms, using arm specific probes. The fifth label may also be used to label any probe of interest to identify and locate a defined sequence in a FISH karyogram.
Multi-colour COBRA FISH was applied to detect cryptic translocations and abnormalities in patients with abnormal phenotype but normal Giemsa karyotype, to study HPV 16 integration sites in cervical cancer cell lines and to perform 48 colour staining in complex rearrangements karyograms from human solid tumours. Other work relates to the application of the same methodology for mouse paints to study mouse tumour models, and a full set of sub-telomeric probes for clinical diagnostic use.

S42. Chromosome Segregation one hundred years after Mendel«s Rediscovery

K. Nasmyth
Institute of Molecular Biology, University of Vienna; Vienna, Austria
BILIONI@imp.univie.ac.at
In eukaryotic cells, replicated DNA strands remain physically connected until their segregation to opposite poles of the cell during anaphase. This sister chromatid cohesion is essential for the alignment of chromosomes on the mitotic spindle during metaphase. Cohesion depends on a multisubunit protein complex called cohesin, which possibly forms the physical bridges that connect sisters. Proteolytic cleavage of cohesin s Scc1 subunit at the metaphase to anaphase transition is essential for sister chromatid separation and depends on a conserved protein called separin. We show here that separin is a cysteine protease related to caspases and that it alone can cleave Scc1 in vitro. By replacing one of Scc1 s cleavage sites by that for a different site specific protease, we show that cleavage of Scc1 in metaphase arrested cells is sufficient to trigger the separation of sister chromatids and their segregation to opposite cell poles.

S43. Is Disease Hereditary?

K. M. Weiss
Depts of Anthropology and Biology, Penn State University; University Park, PA United States
kmw4@psu.edu
There is widespread enthusiasm for mapping genes associated with almost any human trait. The hope is that the identification of common genetic variation with substantial effect will transform biomedicine, and dramatic claims have been about this to the public. But studies of complex traits have been frustrated by problems of genetic inference in the face of causal complexity. Some of these problems are fundamental, rather than

reflecting limitations on sample size, sample design, or biotechnology. The issues are understandable when considered from an evolutionary, population point of view. Etiologic heterogeneity is to be expected and probably is inherent in complex traits like chronic disease, for reasons that are well-known and supported by a wealth of existing data. I will talk about the history of ideas on the nature of heredity with regard to disease, the relevance of a population-evolutionary perspective on the problem, and some of the societal issues that arise in the study of the genetics of complex traits.

S44. Exploring the Genetic Structure of Isolated Populations

L. Jin
Institute of Genetics, Fudan University, Shanghai, China; National Genome Center at Shanghai, China; Human Genetics Center, University of Texas—Houston, Texas, United States
LJin@sph.uth.tmc.edu

Isolated populations that are founded recently by a small number of founders have been considered ideal populations for mapping genes underlying complex diseases. Among many other advantages such population may have, higher level of linkage disequilibrium, compared with those more outbred populations, is ideal for a genome-wide association study. In the last several years, we studied six putative isolated populations in China by typing microsatellites and single nucleotide polymorphisms for X and Y chromosomes, trying to understand the genetic structure of those isolated populations. We also conducted genome-wide scan on three of those populations which allows us to look into the fine detail of the populations. Those analyses will be reported in this presentation.

S45. Origins and evolution of the European gene pool

G. Barbujani
Department of Biology, University of Ferrara; Ferrara, Italy
bjg@unife.it

Patterns of genetic diversity in today s populations contain information about past demographic processes. Archaeological evidence suggests (1) that Europe has been first colonised in Upper Paleolithic times by people coming from the Levant; (2) that these populations contracted and then reexpanded in response to climate changes in Mesolithic times; and (3) that a new wave of immigrants came in from the Levant with the Neolithic spread of early farmers. It is not clear to what extent each of these three phenomena affected the current European gene pool. Establishing it is interesting per se, but it would also facilitate our understanding of the distributions of disease alleles.

Spatial autocorrelation analysis of both protein and nuclear DNA data shows that broad clines encompassing much of Europe are the rule. At the mitochondrial level, conversely, a gradient is evident only in the Mediterranean region, but not in northern Europe. Analyses based on admixture models identify the contributions of two founding populations, one Western and one Eastern, which one would be tempted to equate, respectively, with the descendants of Paleolithic hunter-gatherers and of the early Neolithic farmers. However, especially at the Y-chromosome level, there is also evidence of gene flow from Northern Asia into Europe.

The parallel clines observed at most loci studied, and the estimated admixture rates, suggest that much of the current European diversity has been shaped by a directional expansion from one extreme of the continent. That result, and the recent separation of the European gene pools estimated from microsatellite data, do not suggest that phenomena of Mesolithic reexpansion from glacial refugia have played a crucial role in determining the overall patterns and levels of diversity in the European gene pool.

S46. The Genetics of Deafness; A Model for Genomic and Biological Complexity

K. B. Avraham
Department of Human Genetics & Molecular Medicine, Sackler School of Medicine, Tel Aviv University; Tel Aviv, Israel
karena@post.tau.ac.il

Advanced genomic technologies has led to the isolation of many genes involved in human disease and the study of the human genome is now moving towards understanding the function of the proteins these genes encode (functional genomics). The intricate structure and multiple cell types of the inner ear requires a range of proteins with different functions, including maintenance of structural integrity, neuronal innervation, and mechanoelectrical transduction. There has been remarkable progress in the field of hereditary hearing loss over the past five years in elucidating the molecular basis of hearing loss. This has been no easy task, as human deafness is extremely heterogeneous. Not only is there great variability in the clinical features of human hearing loss (HL), but mutations in the same genes can contribute to syndromic, nonsyndromic, prelingual, and progressive deafness. I will discuss research being done in my own laboratory, putting our results in context of the field in general, including the unconventional myosin VI and Snell s waltzer mouse, the POU4F3 transcription factor and DFNA15 human deafness locus, and inherited connexin 26 mutations associated with human non-syndromic hearing loss. Emphasis will be placed on the advantages offered by the mouse as a model for human deafness.

S48. The genetics of inherited ocular disorders.

G. C. M. Black
Departments of Ophthalmology and Medical Genetics, University of Manchester; Manchester, United Kingdom
gblack@fs1.cmht.nwest.nhs.uk

Genetic factors contribute to a wide range of the disorders that cause blindness and visual disability. Inherited ocular diseases comprise the single most common group amongst those of childhood onset. Recent discoveries have underlined the complexity of genetic ophthalmic disease; currently over 135 genes and loci are defined causing retinal disease alone.

Locus heterogeneity is common, as illustrated by disorders of both anterior (e.g. anterior segment dysgenesis, autosomal dominant cataract) and posterior segments (e.g. retinal and macular dystrophies). The major steps taken to define the exact genetic basis of many of these conditions may now be used for diagnosis and counseling. In some cases this may be helpful in directing management and treatment of affected patients. This possibility has been demonstrated both in man as well as in animal models and will be illustrated with reference to the corneal dystrophies (honeycomb, Ries-Bucklers , granular and lattice dystrophies) anterior segment dysgenesis syndromes (aniridia, goniodysgeneses and Rieger syndrome) as well macular dystrophies (Stargardt disease).

Many multisystemic conditions (e.g. Phakomatoses, Norrie disease, Vitreoretinopathies) have an ocular component. The biochemical pathways involved in their aetiology are crucial to the development and maintenance of different organ systems. Combining the advantages of detailed ocular phenotypic definition with an expanding understanding of their molecular basis sheds light on molecular mechanisms of general significance. This will be illustrated with reference to the role of the hedgehog (Shh) pathways in vitreoretinal/neurodevelopment, as well as disorders of extracellular matrix construction.

Many of the commonest causes of blindness (cataract, glaucoma and macular degeneration) have important genetic components in their aetiology yet these remain unknown. Addressing this area remains a major challenge for the future.

S49. Sifting Sequence for Function: Exploiting the Mouse

E. Rubin
Lawrence Berkeley National Laboratory, University of California; Berkeley, CA United States
emrubin@lbl.gov

One of the major challenges in the post genomic era will be the identification of sequences participating in the regulatory circuitry controlling gene expression. For the analysis of genomic DNA, algorithms and databases are available facilitating the identification of those sequences encoding genes. While these sorts of computational aids are largely not available for the identification of noncoding sequences participating in gene regulation, cross-species sequence comparisons provide a robust means for identifying putative gene regulatory sequences. Using this approach to identify gene regulatory sequences we have examined orthologous regions of human 5q31 and mouse chromosome 11, DNA of biomedical importance due to the clustering of a several interleukin genes. The functional properties of the largest noncoding element (401 BP >85% conserved between humans and mice) located in the IL4, IL13 intergenic interval were examined in depth. To identify its function, knockout mice lacking this 401 BP element as well as YAC transgenics with and without the element were examined. Analysis of the animals revealed that this noncoding sequence plays a prominent role in the regulation of IL4, IL13 and IL5 genes that are spread over 120KB. The comparative genomic strategy used here for identifying noncoding sequence of biological import has led to the discovery of a regulatory element that acts over significant genomic distance to coordinate the expression of several genes involved in the inflammatory response. We have carried out similar studies in other regions of the genome that further illustrate the power of cross-species sequence analysis coupled with functional studies in mice to investigate the gene regulatory circuitry of mammals.

S50. The Rise and Fall of the Mammalian Y Chromosome

J. A. Marshall Graves
Research School of Biological Sciences, The Australian National University; Canberra, Australia
J.Graves@latrobe.edu.au

In mammals, sex is determined by an XY male; XX female system in which a male-dominant gene on the Y (SRY) determines sex. Sex chromosomes evidently evolved from an original autosome pair as the Y chromosome was progressively degraded. This view of a wimp Y is supported by our findings that even genes that are testis-specific, and have important functions in male determination or differentiation, have partners on the X from which they ultimately evolved. In particular, the SRY gene, which determines sex in humans and mice, has a partner (SOX3) on the X in all therian mammals from which it is thought to have been derived.

The mammalian Y chromosome must have arisen after the divergence of mammals from other vertebrates more than 300 million years ago, since birds and reptiles subscribe to completely non-homologous sex determining systems. To explore the origins of the mammalian Y chromosome and SRY, we compared the Y chromosomes of the three major mammal groups (eutherians, marsupials and monotremes). We find that some genes shared by the human X and Y chromosomes are also on the sex chromosomes in marsupials and monotremes, implying that part of the mammalian Y chromosome is at least 170 million years old. However, most human X-Y shared genes are located in autosomal clusters in marsupials and monotremes, implying that the human Y chromosome is largely derived from a region added to sex chromosomes recently in the eutherian lineage. Thus most of the original Y has degraded. In two rodent species (mole voles), the Y has completely disappeared.

The genes on the Y chromosome evolved from genes on the X, and those remaining have been retained because they evolved a vital male-specific function. In particular, the SRY gene, which determines sex in humans and mice, evolved from the brain-specific SOX3. However, SRY was not the original mammalian sex determining gene, since we now find that in monotremes there is no SRY and SOX3 is autosomal. Another sex determining gene must have pre-dated SRY. Nor is the SRY gene a permanent fixture in mammals, for the two species of mole voles with no Y chromosome have lost SRY in favour of an as yet unknown new sex determining gene. Thus SRY evolved in therian mammals less than 170 million years ago, and may be readily supplanted.

Thus the Y chromosome, and the SRY gene it bears, evolved relatively recently and its useful life is already running out.

S51. The Dynamics of Genome Evolution in Placental Mammals

J. Wienberg[1], *L. Fr nicke*[1], *R. Stanyon*[1], *S. M ller*[2], *M. Neusser*[2]
[1]*National Cancer Institute Frederick, Division of Basic Sciences; Frederick, MD United States;* [2]*Institut f r Anthropologie und Humangenetik, Universit t M nchen; Munich, Germany*
wienbergj@ncifcrf.gov

Many years ago S. Ohno postulated that the placental mammalian X-chromosome was evolutionarily conserved. He postulated that this conservation was due to the dosage compensation mechanism for X-linked genes that rely on the functional inactivation of X-chromosomes in somatic cells. Now it seems that most autosomes are also extensively conserved. This conclusion is supported by both comparative gene mapping and more recently by molecular cytogenetics showing, that huge tracts of the human genome are found in large syntenic blocks across different mammalian orders for tens of millions of years. Chromosome painting data with human chromosome specific probes are available for a good number of primates, tree shrew, various carnivores, artiodactyls, dolphin, whales, horse, bat, rabbit, squirrel, and the common shrew. Although one half of the about 19 placental mammalian orders have not yet been analyzed by molecular cytogenetic techniques it is probable that the ancestral placental mammalian karyotype had about 50 chromosomes. Many of these chromosome syntenies are still conserved in humans or differ only by simple reciprocal translocations. Since chromosome translocations are generally very rare events (about one translocation every ten million years in most phylogenies) they are useful markers to help establish phylogenies. Here, this will be demonstrated for New World primates were chromosome painting was especially helpful. The dynamics of chromosome rearrangements, however, is not linear over time in different orders. In some phylogenies we can observe sudden outbreaks of chromosome reshuffling. This will be shown for some primates, artiodactyls and carnivores. Chromosome painting can help in the understanding of these reshufflings that are often far beyond the resolution of classical banding patterns. With more chromosome painting data available from other mammalian orders and including the huge data set available from chromosome banding studies in mammals a deeper understanding of the gross architecture and evolution of the human genome can be obtained.

S52. Gene dosage, development, and disease; The unstable foundation of a robust response

S. J. Tapscott
Division of Human Biology Fred Hutchinson Cancer Research Center; Seattle, WA United States
stapscot@fred.fhcrc.org

There is increasing recognition that stochastic processes underlie the highly predictable patterns of gene expression in developing organisms. In many systems, the response of a gene to intermediate levels of an inducer has been shown to be a stochastic all-or-none response. Experimental data and inferential reasoning demonstrate that the balance between gene activation and de-activation rates is a critical variable for achieving specified levels of gene activity. Models of intermittent gene expression have implications for understanding human disease; gene dosage can protect against intermittent lapses in gene expression and increase the predictability of a response to a graded stimulus. Therefore, some haploinsufficiency syndromes might result from an increased susceptibility to stochastic delays of gene initiation or interruptions of gene expression. Although little is known regarding the relative on-rates and off-rates of gene expression, highly unstable gene expression provides a selective advantage both for signal discrimination and as a mechanism of limiting the prevalance of mutations in a population.

S54. Molecular Genetics of 22Q11 Deletion Syndromes — A Review

P. J. Scambler
Molecular Medicine Unit, Institute of Child Health; London, United Kingdom
p.scambler@ich.ucl.ac.uk

Deletions of human chromosome 22q11 occur in approximately 1;4000 live births and are associated with a number of haploinsufficiency syndromes, including DiGeorge and velocardiofacial syndromes. The deleted region — or DiGeorge Chromosomal Region (*DGCR*) — is entirely cloned and sequenced. Despite this, human genetic techniques in isolation have so far failed to unequivocally demonstrate that haploinsufficiency of any specific gene is required to produce this syndrome. As many genes at 22q11 show conservation of synteny in the mouse, several laboratories have addressed the role of single genes and groups of genes using mouse models. Direct deletion, and Cre-lox mediated deletion and duplication have been used to create a shortest region of deletion overlap for a murine *Dgcr* . 1n 1999 Baldini s group demonstrated that embryos hemizygous for the *Dgcr* have hypo/aplasia of the 4th pharyngeal arch artery at E10.5, which gives variably penetrant great artery and heart defects in live born mice. Recently, further deletion mapping, transgenic rescue and gene targeting experiments have identified the transcription factor *Tbx1* as vital for pharyngeal arch artery morphogenesis/remodelling. A second region contains a locus determining behavioural variation. The variable penetrance and expressivity of the murine deletion syndrome reflects the situation seen in man, yet the models are, unsurprisingly, an imperfect phenocopy. While cellular and animal models will undoubtedly be vital in understanding the developmental pathways affected by *Tbx1* haploinsufficiency and the role of other DGCR genes during embryogenesis, further human genetic investigations are required to assess the role of DGCR genes in the behavioural and cognitive deficits observed in this disorder.

S55. AAV-Mediated Gene Transfer for Hemophilia

K. High
Abramson Research Center, Children s Hospital of Philadelphia; Philadelphia, PA United States
HIGH@email.chop.edu

The goal of our work has been to establish an experimental basis for gene transfer as a method of treating hemophilia, an inherited bleeding disorder that results from the absence of functional Factor VIII or Factor IX. Using an adeno-associated viral vector derived from AAV serotype 2, we have shown in mice and in hemophilic dogs that we can achieve long-term expression (>3 years) of clotting factor at levels that would result in an improvement of clinical symptoms of the disease. A phase I trial of intramuscular injection of AAV-F.IX has been initiated to evaluate the safety of this procedure in patients with severe hemophilia B. To date, eight subjects have been enrolled at three doses, ranging from 2 x 1011 vg/kg to 2 x 1012 vg/kg. There has been no evidence of local or systemic toxicity in any of the subjects, including no evidence for inhibitor formation or for inadvertent germline transmission of vector sequences. Muscle biopsies have shown

unequivocal evidence for gene transfer and expression by PCR, Southern blot, and immunohistochemistry. In recent pre-clinical experiments, we have shown in mice that vectors derived from alternate serotypes AAV-1 and AAV-6 result in 10-20 fold higher levels of expression of F.IX compared to vectors derived from AAV-2, and we are currently investigating the performance of these vectors in hemophilic dogs. We have also shown that AAV-2 can be delivered to skeletal muscle via an intravascular approach, making transduction of large muscle groups much more feasible. Finally, we have administered AAV-F.IX into the portal veins of hemophilic dogs and shown that this results in high circulating levels of Factor IX, on the order of 5-14%, whereas delivery of similar doses to skeletal muscle results in factor levels of only 1-2%. Based on these results a trial of AAV-mediated liver-directed gene transfer for hemophilia B has been proposed.

S56. Ribozymes for Retinal Gene Therapy and Genomics

W. W. Hauswirth[1], *Q. Li*[1], *J. Liu*[1], *M. LaVail*[2], *J. Flannery*[3], *A. S. Lewin*[3]
[1]*Molecular Genetics and Ophthalmology, and Gene Therapy Center, University of Florida; Gainesville, FL United States;* [2]*Beckman Vision Center, UC San Francisco; San Francisco, CA United States;* [3]*Optometry and Neuroscience, UC Berkeley; Berkeley, CA United States*
hauswrth@eye1.EYE.UFL.EDU

We tested two hypotheses; 1. AAV-delivered ribozymes against an autosomal dominant mutant allele in a somatic retinal tissue can reduce the corresponding mRNA level sufficiently to rescue visual function in an animal model for autosomal dominant Retinitis Pigmentosa in which rod cells progressively die over an 8-12 month period after birth. 2. A similarly designed and delivered ribozyme against a wild type allele can create a somatic knockdown and lead to a retinal disease phenotype. Recombinant AAV-vectored ribozymes against a mutant P23H rod opsin gene in transgenic rats preserved 30-80% of the photoreceptors that would have been lost at 8 and 3 months respectively. Rescue was confirmed functionally by electroretinographic (ERG) analysis, cellularly by preservation of photoreceptor morphology and molecularly by specific reduction in mutant mRNA levels. To test the converse idea, that ribozymes targeted against wild type alleles might also create retinal disease, we attempted to produce Retinitis Pigmentosa (RP)-like rod cell loss in *rd/+* mice (heterozygous bPDE null mutation). *Rd/+* mice have an apparently normal retina at all ages in contrast to the homozygous *rd/rd* mouse that loses all rods within about one month. Ribozyme genes against wt bPDE mRNA were packaged into an AAV vector downstream of a proximal rod opsin promoter and injected into one eye of *rd/+* mice. At 4 and 8 months postinjection, we found 50%-80% fewer rod photoreceptors in ribozyme treated eyes relative to PBS treated contralateral control eyes. Ribozyme-treated rods die via an apoptotic pathway as they do in RP. ERG analysis in ribozyme treated and PBS injected control eyes confirmed that a profound functional vision deficit had also been created that paralleled the loss of rod cells in the treated eye. Extension of this knockdown strategy to normal +/+ mice showed a qualitatively identical effect, although at a lower level. This AAV-ribozyme technology can now be applied to a broad range of therapeutic and functional genomic questions in the retina and elsewhere.

S57. Multipotent Stem Cells for the Treatment of Genetic Diseases

M. Grompe[1], *X. Wang*[1], *M. Al-Dhalimy*[1], *M. Finegold*[2], *E. Lagasse*[3]
[1]*Oregon Health Sciences University, Portland, OR United States;* [2]*Baylor College of Medicine, Houston, TX United States;* [3]*Stem Cells Inc., Sunnyvale CA United States*
grompem@ohsu.edu

Orthotopic liver transplantation is the treatment of choice for several inborn errors of metabolism. Unfortunately, the supply of donor organs is limiting and therefore many patients cannot benefit from this therapy. In contrast, hepatocyte transplantation could potentially overcome the shortage in donor livers by use of cells from a single donor for multiple recipients. In classic hepatocyte transplantation, however, only <1% of the liver mass or less can be replaced by donor cells. Recently though, it has been shown in animal models that >90% of host hepatocytes can be replaced by a small number of transplanted donor cells in a process we term therapeutic liver repopulation . This phenomenon is analogous to repopulation of the hematopoietic system after bone marrow transplantation. Liver repopulation occurs when transplanted cells have a growth advantage in the setting of damage to recipient liver cells.

For human therapeutic application, cadaveric donors are the most likely source transplantable hepatocytes. This presents problems for the acquisition, distribution and quality control of these cells. Very recently it has been discovered that transplanted cells from extrahepatic sources such as the adult pancreas or bone marrow can also be used for liver repopulation. Because bone marrow donors are widely available, this finding raises the hope of therapeutic application of these cells in the future.

The current knowledge regarding therapeutic liver repopulation and the hopeful implications for treatment of liver diseases will be discussed.

S58. Molecular Mechanisms in Sphingolipidoses

K. Sandhoff
Kekul -Institut f r Organische Chemie und Biochemie der Universit t Bonn; Bonn, Germany
sandhoff@uni-bonn.de

Sphingolipids are components of plasma membranes of mammalian cells. Inherited defects in their lysosomal catabolism often result in fatal storage diseases. The molecular analysis of these neurodegenerative and dermal disorders and their respective mouse models lead to the discovery of principles of membrane digestion (Kolter T. and Sandhoff K., Angew.Chem. Int. Ed. 1999, 38, 1532-1568; Linke et al, J. Biol. Chem. 2001, 276, 5760-5768). Components of plasma membranes reach the lysosomal compartment for degradation on intralysosomal vesicles and membrane structures. The catabolism of the membrane-bound sphingolipids by water-soluble lysosomal exohydrolases is synergistically stimulated by two additional components, sphingolipid activator proteins (SAPs) and anionic phospholipids such as BMP (bis(mono-acylglycero)phosphate) (Wilkening et al, J.Biol. Chem. 273, 30271-30278 (1998)). The latter occurs only in inner membranes of the acidic components of the cell. Due to its physiochemical properties, the GM2-activator protein attacks the inner vesicle structures but apparently not the limiting lysosomal membranes (Giehl et al, Eur. J. Biochem. 261, 650-658, 1999). These observations may help to explain why intralysosomal membranes are digested and the limiting lysosomal membrane survives.

The analysis of the skin phenotype of SAP-knock-out mice indicates the importance of activator proteins for the formation of the epidermal water permeability barrier of land dwelling mammals. Ceramides, the main components of this extracellular lipid barier, derive in large part from hydrolysis of glucosylceramides mediated by the lysosomal enzyme §-glucocerebrosidase. In §-glucocerebrosidase- and SAP - deficient mice the epidermal permeability barrier is abnormal. Their epidermis accumulates various complex glucosylceramides as well as complex ϖ-hydroxylated glucosylceramides covalently linked to the cornified cell envelope of corneocytes in the stratum corneum. Their identification and metabolism will be discussed.

S60. Genetic defects in sterol metabolism

F. F. Moebius
Institute for Biochemical Pharmacology, University of Innsbruck; Innsbruck, Austria
Fabian.Moebius@uibk.ac.at

Genetic defects in sterol metabolizing enzymes have recently emerged as important causes of dysmorphogenetic syndromes. They affect enzymes required for the removal of methyl groups at C_4 (NSHDL), the shift of the double bond from C_{8-9} to C_{7-8} (3§-sterol D^8,D^7 isomerase/EBP, E.C. 5.3.3.5) and the removal of the double bond at C_{7-8} (D^7-sterol reductase/DHCR7, E.C. 1.3.1.21). Missense and nonsense mutations in *NSHDL* on Xq28 cause X-chromosomal dominant CHILD syndrome (congenital hemidysplasia with ichthyosiform erythroderma and limb defects, MIM308050), a rare inborn disorder which affects bone and skin in females and is presumably lethal in males. NSHDL is involved in C_4 sterol demethylation but its precise biochemical function remains to be clarified. Nonsense and missense mutations in the same gene (*Nshdl*) underly the Bare patches and Striated mutations in mice. Recurrent missense and nonsense mutations in *EBP* (Emopamil binding protein) on Xp11.22 are found in patients with X-chromosomal dominant chondrodysplasia punctata (Conradi-H nermann-syndrome, CDPX2, MIM302960). CDPX2 is characterized by skeletal (epiphyseal stippling, limb shortening, short stature) and epidermal (atrophy, ichthyosis, hyperkeratosis) manifestations as well as occasional sectorial cataracts. The mouse mutant Tattered carries a missense mutation in *Ebp*. The clinical differentiation between CHILD syndrome and CDPX2 is controversial. The morphological phenotypes of Bare patches and Tattered are similar to each other and ressemble CHILD syndrome as well as CDPX2 including lethality in male embryos. Cholesterol crossfeeding between cell patches in which either the X-chromosome carrying the wildtype or the mutant allele are active possibly explains why neither in CHILD syndrome nor in CDPX2 total cholesterol levels are reduced. In contrast substantially reduced total sterol concentrations are a hallmark of the more frequent autosomal-recessive Smith-Lemli-Opitz/RSH syndrome (SLOS, MIM270400) due to mutations in *DHCR7* in 11q13. SLOS is characterized by dysmorphogenesis (e.g. syndactyly, heart and lung defects), failure to thrive and mental retardation or autism. A recently described induced mouse mutant in *Dhcr7* had the biochemical character-

istics of the SLOS and most surprisingly symptoms of severe neurological deficits such as reduced physical activity and uncoordinated swallowing.
Our work is supported by grants from the FWF and the sterreichische Nationalbank

Concurrent Sessions

C001. Thrombocytopenia-Absent Radius Syndrome (TAR); A clinical genetic study.

K. L. Greenhalgh[1], *R. T. Howell*[2], *P. Ancliffe*[3], *A. Bottani*[4], *R. A. Newbury-Ecob*[5]
[1]*Department of Clincial Genetics; Bristol, United Kingdom;* [2]*South West Regional Cytogenetic Laboratorys; Bristol, United Kingdom;* [3]*Great Ormond Street Hopsital for Sick Children NHS Trust; London, United Kingdom;* [4]*Division of Medical Genetics, CMU; Geneva, Switzerland;* [5]*Department of Clinical Genetics; Bristol, United Kingdom*
lynn.greenhalgh@ubht.swest.nhs.uk
Information regarding the phenotype of TAR syndrome is largely based on single case reports. In order to delineate the phenotype, 32 patients diagnosed with TAR syndrome underwent clinical and cytogenetic analysis. All cases except 1 had bilateral radial aplasia. The thumbs were present in all cases but morphology and function were abnormal. Lower limb defects occurred in 47%, cardiac anomalies (e.g. tetralogy of Fallot) in 16%. Thrombocytopenia may be as aymptomatic and remain undetected until later life. Early detection is associated with low morbidity, highlighting the value of haematological investigation in patients with radial aplasia. Previous reports of urogenital abnormalities were rare, however in this study anomalies were found in 25% of cases. The association of TAR syndrome and cow s milk intolerance is well described, in this study 43% of cases showed cow s milk intolerance and 25% had experienced prolonged bouts of gastroenteritis. Three atypical cases were found. Two showed features of Robert s syndrome and the third had mental retardation and genitourinary anomalies suggestive of DK phocomelia. Four sib pairs were found. A female excess (23 female; 8 male; one case unknown) was observed. Chromosome breakage studies were normal in all cases tested. Two further cytogenetic abnormalities are under investigation and have provided useful candidate genes for further study based on role in limb development and haematopoiesis. This study suggests that the phenotype for TAR syndrome is wider than previously reported. The condition may be heterogeneous with implications for genetic counselling and gene identification.

C002. Heterozygous p63 mutations in LMS, EEC, Hay-Wells, ADULT syndromes, and in Split Hand/Foot Malformation reveal a genotype-phenotype correlation

P. H. G. Duijf[1], *J. A. McGrath*[2], *V. Doetsch*[3], *J. Celli*[1], *R. de Waal*[4], *A. Yang*[5], *F. McKeon*[5], *K. Vanmolkot*[1], *P. Propping*[6], *F. Gurrieri*[7], *G. Neri*[7], *M. Bamshad*[8], *B. C. J. Hamel*[1], *H. G. Brunner*[1], *H. van Bokhoven*[1]
[1]*Department of Human Genetics, University Medical Centre; Nijmegen, The Netherlands;* [2]*St. John s Institute of Dermatology, St Thomas Hospital; London, United Kingdom;* [3]*Department of Pharmaceutical Chemistry, UCSF; San Francisco, CA United States;* [4]*Department of Pathology, University Medical Centre; Nijmegen, The Netherlands;* [5]*Department of Cell Biology, Harvard Medical School; Boston, MA United States;* [6]*Institute of Human Genetics, University of Bonn; Bonn, Germany;* [7]*Institute of Medical Genetics; Rome, Italy;* [8]*University of Utah; Salt Lake City, UT United States*
p.duijf@antrg.azn.nl
EEC syndrome is a developmental disorder characterized by Ectrodactyly, Ectodermal dysplasia, and facial Clefting. We recently demonstrated that mutations in the p63 gene, a homolog of the tumor suppressor gene p53, cause EEC syndrome. An extended analysis revealed heterozygous p63 mutations in 34 of 37 unrelated EEC families, indicating that p63 is the major if not the only gene involved in EEC syndrome. EEC syndrome shows some overlap with several distinct syndromes, including Hay-Wells/AEC syndrome, Limb-Mammary Syndrome (LMS), ADULT syndrome, and Split Hand/Foot Malformation (SHFM). Mutation analysis of the p63 gene was performed to investigate the possibility of allelism for these disorders. Causative mutations were identified in AEC syndrome (8 mutations), ADULT syndrome (1 mutation), LMS (1 mutation), and SHFM (4 mutations). For EEC and AEC syndrome, the position and predicted effect of the mutations is clearly different. The majority of mutations in EEC syndrome are amino acid substitutions in the DNA binding domain and affect transactivational properties of p63. AEC syndrome is consistently caused by amino acid substitutions in the SAM domain and are predicted to affect protein-protein interactions. The effect of the mutations in SHFM, LMS and ADULT is less straightforward. To gain more insight in the functional consequences of the p63 mutations we are studying the characteristics of normal and mutant p63 proteins in transactivation assays, immunolocalization and protein-protein interaction studies. These approaches aim to find molecular explanations for these distinct disorders and hence establish a phenotype-genotype correlation for p63 gene mutations.

C003. Genotype-Phenotype Correlation in human GLI3 disorders

L. G. Biesecker
NIH-NHGRI; Bethesda, MD United States
leslieb@helix.nih.gov
Mutations in GLI3 have been associated with two malformation syndromes (Pallister-Hall and Greig cephalopolysyndactyly syndromes (PHS & GCPS)) and several types of polydactyly. Controversy persists regarding potential correlation of specfic mutation types, with some results suggesting there is no correlation of mutation type and phenotype. Here we show new results that strongly suggest that a correlation exists. The data comprise 19 families with PHS and 5 families with GCPS whose GLI3 gene mutation has been determined. We combine these data with published cases of PHS & GCPS where mutations have been determined. There are now more than 50 cases of known GLI3 mutations. In GCPS there are more than 10 large deletions, 5 translocations, 7 frameshifts 5 of the zinc fingers, 2 missense, 3 splice mutations, and 3 frameshifts 3 of the zinc finger. In PHS, 21 of 21 patients have frameshifts 3 of the zinc fingers. This is a non-random distribution of mutations that supports the hypothesis that GCPS is caused by functional haploinsufficiency of GLI3 and PHS is caused by 3 truncations. We will propose a hypothesis to account for the unusual 3 frameshift mutations in GCPS and review relevant animal model data.

C004. Acheiropodia is caused by a genomic deletion in C7orf2 the human orthologue of the Lmbr1 gene.

P. Tsipouras[1], *P. Ianakiev*[1], *M. J. van Baren*[2], *M. J. Daly*[3], *S. P. A. Toledo*[4], *M. G. Cavalcanti*[4], *J. Correa Neto*[5], *E. Lemos Silveira*[6], *A. Freire-Maia*[7], *P. Heutink*[2], *M. W. Kilpatrick*[1]
[1]*University of Connecticut Health Center; Farmington, CT United States;* [2]*Erasmus University; Rotterdam, The Netherlands;* [3]*Whitehead Institute; Cambridge, MA United States;* [4]*University of Sao Paulo; Sao Paulo, Brazil;* [5]*Private Practice; Sao Paulo, Brazil;* [6]*Private Practice; Porto Alegre, Brazil;* [7]*Universidade Estadual Paulista; Botucatu, Brazil*
ianakiev@neuron.uchc.edu
Acheiropodia is a unique condition presenting with bilateral congenital amputations of the upper and lower extremities and aplasia of the hands and feet. This severely handicapping developmental disorder appears to affect only the extremities with no other systemic manifestations reported. With the exception of two affected siblings in Puerto Rico the reported cases are of Brazilian origin. The incidence of acheiropodia in Brazil has been estimated to be approximately 1/250,000 births. Acheiropodia is inherited as an autosomal recessive trait and the heterozygotes are phenotypically normal. The vast majority of affected individuals are the offspring of consanguineous matings. A locus for Acheiropodia has been mapped to chromosome 7q36. To facilitate the identification of the gene responsible for this disorder, we first refined the acheiropodia critical region by haplotype analysis and subsequently identified a common mutation in C7orf2, the human orthologue of the mouse Lmbr1 gene, responsible for the disease. Analysis of a panel of five Acheiropodia families with fifteen polymorphic markers, narrowed the critical region to 1.3 cM, based on identity by descent, and to less than 0.5 Mb by physical mapping. Analysis of C7orf2, the human orthologue of the mouse Lmbr1 gene that has been implicated in mouse mutant Hemimelic extra-toes, identified a deletion in all five families thus identifying a common Acheiropodia mutation. The deletion was identified at both the genomic DNA and mRNA level. It leads to the production of a C7orf2 transcript lacking exon 4 and introduces a premature stop codon downstream of exon 3. Given the nature of the Acheiropodia phenotype, it appears likely that the Lmbr1 gene plays an important role in limb development.

C005. Deletions of the homeobox gene SHOX are an important cause of growth failure in patients with idiopathic short stature

G. A. Rappold[1], *M. Fukami*[1], *S. Schiller*[1], *B. Niesler*[1], *W. Zumkeller*[2], *M. Bettendorf*[3], *U. Heinrich*[3], *C. Francomano*[4], *T. Ogata*[5]
[1]*University of Heidelberg; Heidelberg, Germany;* [2]*Poliklinik f r Kinderheilkunde; Halle, Germany;* [3]*Department of Paediatrics; Heidelberg, Germany;* [4]*Department of Developmental Endocrinology; Bethesda, United States;* [5]*Department of Paediatrics; Tokyo, Japan*
gudrun_rappold@med.uni-heidelberg.de
With an incidence of 3 in 100, idiopathic short stature is a fairly frequent diagnosis in children. Idiopathic short stature refers to a heterogenous

group of patients which are short due to different reasons. Mutations of a human homeobox gene, SHOX, have been recently shown to underlie the short stature phenotype in patients with Turner syndrome and most patients with L ri-Weill dyschondrosteosis. Here, we address the question on the incidence and type of SHOX mutations seen in children with short stature of unknown aetiology, termed idiopathic. We have analysed the SHOX gene for intragenic mutations by single strand conformation polymorphism (SSCP) and for complete gene deletion by flourescence in situ hybridisation (FISH) in 820 and 150 patients, respectively. We have identified nonsense, missense and a small deletion in the coding region of SHOX (8/820). In 150 patients we were able to also carry out FISH analysis and detected 4 complete gene deletions (4/150). At least seven mutations (7/12) could be shown to be truly functional by comparing with the genotype and phenotype of the parents. Consequently, SHOX mutations have been detected in more than 2 % of children with idiopathic short stature. The spectrum of SHOX mutations is biased with the vast majority leading to complete gene deletions. Children with SHOX mutations have been shown in retrospect to often derive from families where older affected family members show mild skeletal features reminescent of the Turner skeletal features. Short patients with a normal karyotype and the presence of any of the Turner-characteristic dysmorphic skeletal features such as high-arched palate, short neck, abnormal auricular development, cubitus valgus, genu valgum, short 4th metacarpals and Madelung deformity or with frequent otitis media are therefore likely candidates for a SHOX gene disorder.

C006. Familial Combined Pituitary Hormone Deficiency caused by a Mutation in PROP-1 Gene

J. Crone[1], *R. Pfaeffle*[2], *D. Prayer*[3], *I. Gomez*[4], *H. Frisch*[5]
[1]*Paediatric Deartment, University Hospital; Vienna, Austria;* [2]*Paediatric Department, RWTH; Aachen, Germany;* [3]*Radiologic Department, University Hospital; Vienna, Austria;* [4]*General Hospital; Klagenfurt, Austria;* [5]*Paediatric Department, University Hospital; Vienna, Austria*
herwig.frisch@akh-wien.ac.at

Mutations of PROP-1, a paired-like homeodomain transcriptionfactor which is responsible for early embryonic pituitary development have been recently reported as a cause of combined pituitary hormone deficiency. We report the phenotype, long-term auxological data and MRI findings in two families with 4 affected members. All patients had a complete deficiency GH, TSH, PRL, LH and FSH. ACTH deficiency was diagnosed in two probands only during the 3rd and 4th decade of life. Pituitary MRI showed hypoplastic adenohypophysis in 3 subjects, but an unspecific tissue accumulation which presented like a pituitary mass lesion in another patient. The affected boy from family 2 was continuously treated with all hormons required and reached the familial target height. However, the 3 subjects in family 1 were treated only sporadically (GH-treatment between 1-3 years resp.); despite this insufficient therapy they reached a final height in the lower normal range. Longitudinal growth continued up to the age of 40 years in these subjects.

C007. Mitochondrial Genome Instability In Human Cancers

N. O. Bianchi, *M. S. Bianchi, S. M. Richard*
IMBICE; La Plata, Argentina
imbice@satlink.com

Malfunction of mismatch repair (MMR) genes produces nuclear genome instability (NGI) and plays an important role in the origin of some hereditary and sporadic human cancers. The appearance of non-inherited microsatellite alleles in tumor cells (microsatellite instability, MSI) is one of the expressions of NGI. We present here data showing mitochondrial genome instability (mtGI) in most of the human cancers analysed so far. The mt-DNA markers used were point mutations, length-tract instability of mono- or dinucleotide repeats, mono- or dinucleotide insertions or deletions, and long deletions. Comparison of normal and tumoral tissues from the same individual reveals that mt-mutations may show as homoplasmic (all tumor cells have the same variant haplotype) or as heteroplasmic (tumor cells are a mosaic of inherited and acquired variant haplotypes). Breast, colorectal, gastric and kidney cancers exhibit mtGI with a pattern of mt-mutations specific for each tumor. No correlation between NGI and mtGI was found in breast, colorectal or kidney cancers, while a positive correlation was found in gastric cancer. Conversely, germ cell testicular cancers lack mtGI. Damage by reactive oxygen species (ROS), slipped-strand mispairing (SSM) and deficient repair are the causes explaining the appearance of mtGI. The replication and repair of mtDNA are controlled by nuclear genes. So far, there is no clear evidence linking MMR gene malfunction with mtGI. Polymerase g (POL g) carries out the mtDNA synthesis. Since this process is error-prone due to a deficiency in the proofreading activity of POL g, this enzyme has been assumed to be involved in the origin of mt-mutations. Somatic cells have hundreds to thousands of mtDNA molecules with a very high rate of spontaneous mutations. Accordingly, most somatic cells probably have a low frequency of randomly mutated mtDNA molecules. Most cancers are of monoclonal origin. Hence, to explain the appearance of mtGI in tumors we have to explain why a given variant mt-haplotype expands and replaces part of (heteroplasmy) or all (homoplasmy) wild mt-haplotypes in cancer cells. Selective and/or replicative advantage of some mutations combined with a severe bottleneck during the mitochondrial segregation accompanying mitosis are the mechanisms probably involved in the origin of mtGI.

C008. Meta-analysis of losses of heterozygosity in breast, lung and colorectal cancer

R. J. Osborne, *M. Hamshere*
University of Nottingham; Nottingham, United Kingdom
sbxrjo@nottingham.ac.uk

We have devised a novel technique that integrates data from more than 750 studies on the loss of heterozygosity in breast, lung and colorectal cancers. By comparing the data, and integrating this with the newly released sequence from the Human Genome Project, we have been able to identify many new regions of the genome that probably contain candidate genes involved in tumor formation. This technique also allows us to identify regions of the genome that are tumor-type specific as well as finding regions that are lost in multiple tumor types. In addition, we appear to be able to identify regions of the genome that are essential for tumor growth in certain cancers, but which can be lost in others. Finally, this method also provides an insight into the pattern of background rates of losses of heterozygosity on each chromosome - this enables us to determine whether a particular locus, or region, is consistently lost compared to its local level. Data will be presented in the form of a new set of loss of heterozygosity maps and graphs, which will also be made available at the University of Nottingham LOH database (http;//www.nottingham.ac.uk/~pdzmgh/lohdb).

C009. Development of a molecular signature for ovarian epithelial cancer

D. I. Smith[1], *V. Shridhar*[1], *L. Hartmann*[1], *J. Chien*[1], *J. Lillie*[2]
[1]*Mayo Foundation; Rochester, MN United States;* [2]*Millenium Predictive Medicine; Cambridge, MA United States*
smith.david@mayo.edu

Ovarian epithelial cancer is the most lethal of the cancers unique to women. Seventy percent of women present with advanced disease, with a five-year survival in the 15-20% range even with aggressive treatment. In contrast, those women who present with Stage I disease (confined to the ovary) have a five year survival in the 80-90% range. Unfortunately, very little is know about the molecular events underlying the development of this lethal disease. We have used the technique of transcriptional profiling to begin to develop comprehensive molecular signatures for ovarian epithelial cancers. Twenty primary ovarian tumors, 7 ovarian cancer cell lines and a variety of cultured and non-cultured normal ovarian epithelium were analyzed for the expression of 25,000 genes on nitrocellulose membranes. To complement this, we also constructed subtraction suppression hybridization cDNA libraries from many of the same tumors that were analyzed on microarrays. We detected many more down-regulated than up-regulated genes in the ovarian tumors. Many of the consistently down-regulated genes were found to reside within common fragile site regions providing further support that these regions play an important role in cancer development. Most of the same genes were aberrantly regulated in both early (stage I/II) and late (stage III/IV), but our results do support a model where later stage tumors have additional alterations generally not present in the early stage tumors. To complement the transcriptional profiling, we also performed comparative genomic hybridization to analyze the gross chromosomal structure of the profiled ovarian tumors. This analysis revealed that chromosomal regions that were deleted appeared almost identical between early and late stage tumors. However, amplification events were almost never observed in the early stage tumors, but were frequently observed in the late stage tumors. A comparison between gene expression patterns and regions of chromosomal deletion or amplification has delineated several interesting chromosomal regions. Interestingly, several of these regions are bordered by common fragile sites, again implicating these regions in important events occuring during cancer development. We have used a number of techniques to validate the expression levels of genes that appear to be aberrantly regulated in the ovarian tumors. This includes Northern analysis of ovarian cancer cell lines, and RT-PCR analysis of primary ovarian tumors. Our most specific is the use of RNA in situ to analyze histologically characterized ovarian tumor specimens. We are therefore working on the generation of a comprehensive molecular signa-

ture for ovarian epithelial cancers. In addition, we are working to identify markers for the early detection of ovarian cancer, as well as markers that can differentiate chemosensitive versus chemoresistant ovarian tumors. Finally, we hope to better understand the biology of this poorly understood tumor through the comprehensive analysis of gene expression.

C010. Identification of novel overexpressed genes in the Her-2 amplicon at 17q12 using CGH and cDNA microarrays

P. Kauraniemi[1], *O. Monni*[2], *M. B rlund*[1], *A. Kallioniemi*[2]
[1]*Laboratory of Cancer Genetics, Institute of Medical Technology, University of Tampere and Tampere University Hospital; Tampere, Finland;* [2]*Cancer Genetics Branch, Human Genome Research Institute, National Institutes of Health; Bethesda, MD United States*
lopakau@uta.fi

DNA amplifications have often been attributed to the effects of a single target gene, such as HER-2 at 17q12 or MYC at 8q24. However, many genes have been implicated in some amplicons, such as AIB1, BTAK, ZNF217, NABC1 genes at 20q12-q13, or S6 kinase, PAT, RAD51C, TBX2 genes (Cancer Res 60;5340-5344, 2000) at 17q23. Here, we used CGH and cDNA microarray technologies to undertake a comprehensive survey of genes involved in the 17q12 amplification in 11 breast cancer cell lines. We constructed a chromosomal region-specific cDNA microarray containing over 200 EST clones from the 17q12 region (http;//www.ncbi.nlm.nih.gov/genemap99/). These cDNA microarrays were used in the analysis of both gene expression as well as copy number (CGH microarrays) which enabled direct detection of amplified and overexpressed genes at 17q12. Comparison with actual copy numbers determined by fluorescence in situ hybridization (FISH) indicated >90% concordance with the CGH microarray data. Altogether, there were 23 transcripts that showed both an increased copy number and expression in four or more cell lines. These included the HER-2, GRB7, MLN64, and MLN62 genes that all have been previously implicated in such amplifications. In addition, 6 other genes and 13 novel ESTs were also found to be involved. These results were verified using Northern and FISH analysis. In conclusion, parallel analysis of gene copy number and expression levels by microarray analysis can be used to quickly identify all candidate target genes involved in a high-level genomic amplification.

C011. Lack of MSH2 and MSH6 characterizes endometrial but not colon carcinomas in hereditary nonpolyposis colorectal cancer

R. Vierum ki[1], *P. Schweizer*[2], *A. L. Moisio*[1], *S. A. Kuismanen*[1], *J. Jiricny*[2], *P. Peltom ki*[3], *M. Nystr m-Lahti*[1]
[1]*University of Helsinki; Helsinki, Finland;* [2]*University of Z rich; Z rich, Switzerland;* [3]*the Ohio State University; Columbus, OH United States*
reetta.vierumaki@helsinki.fi

Hereditary nonpolyposis colorectal cancer (HNPCC) syndrome is characterized by susceptibility to colorectal cancer (CRC) as well as a variety of extracolonic cancers, notably endometrial cancer (EC). The incidence of EC even exceeds that of CRC (60% and 54%, respectively) in female mutation carriers. The syndrome is linked to germline mutations in DNA mismatch repair genes, mainly MLH1 (~50%) or MSH2 (~40%). Moreover, approximately 10% of mutations affect MSH6, and these families often display atypical hyperplastic lesions and carcinomas of the endometrium. However, the biological basis of the organ involvement remains unknown. To clarify the role of MLH1, MSH2, and MSH6 protein expression as possible factors contributing to the HNPCC tumor spectrum, we used immunohistochemistry to compare the expression patterns of these proteins in 42 ECs and 35 CRCs derived from either the same HNPCC patients or close relatives carrying the same mutations. Among MSH2 mutation carriers, MLH1 was expressed in both tumor types whereas MSH2 and in many cases also MSH6 were absent. Remarkably, among MLH1 mutation carriers, 54% of ECs (21/39) but none of CRCs (0/32) lacked the MSH2 and/or MSH6 protein, in addition to the lack of MLH1 protein expression. These results demonstrate a marked difference between HNPCC related colorectal and endometrial cancers, and suggest that the development of the latter tumors is selectively associated with the MSH2/MSH6 protein complex deficiency.

C012. Comparative Gene Expression Profiling Of Merkel Cell Carcinoma And Small Cell Lung Carcinoma

M. Van Gele[1], *H. Leonard*[2], *A. Cook*[2], *J. Vandesompele*[1], *K. De Preter*[1], *N. Van Roy*[1], *A. De Paepe*[1], *P. Parsons*[3], *G. Boyle*[3], *F. Speleman*[1]
[1]*Dept. of Medical Genetics, University Hospital Ghent; Ghent, Belgium;* [2]*Queensland Radium Institute Laboratory; Brisbane, Australia;* [3]*Queensland Institute of Medical Research; Brisbane, Australia*
mireille.vangele@rug.ac.be

Merkel cell carcinoma (MCC) is a rare, highly metastatic skin tumor of neuroectodermal origin. Little is known about the genetic background of MCC. In previous studies we observed nonrandom losses for 1p, 3p, 9p, 10q, and 13q by LOH analysis and by comparative genomic hybridization (CGH) of a large panel of MCC samples. Mutation analysis of candidate TSG genes in these regions provided no evidence for a pivotal role of these genes in MCC development. Interestingly, the resulting CGH pattern of MCC closely resembled that observed in small cell lung carcinoma (SCLC). Both are small cell tumor types sharing several clinical and immunophenotypical characteristics. Until now, the mechanisms of carcinogenesis in both tumors types and the basis of their therapeutic responsiveness remains poorly understood. In order to study the possible relationship between MCC and SCLC expression profiling of 8 MCC cell lines and 4 SCLC cell lines were performed on Clontech cDNA expression arrays representing a total of 1891 different genes. In a preliminary analysis, the mean of all normalized MCC cell lines was compared to that of the SCLCs using 2-D scatter plots. A total of 138 genes encoding several tyrosine kinase, interleukin and other receptor associated-proteins, growth factors and cytoskeleton proteins were at least 5-fold higher differentially expressed in MCC. In addition, 46 genes were at least 5-fold higher differentially expressed in SCLC. Detailed data mining including hierarchical clustering and principal component analysis and verification of differential genes using real-time quantitative RT-PCR will be presented.

C013. A genome-wide screen in families from the Colorado twin study of reading disability, with evidence that a quantitative trait locus on chromosome 2p influences multiple measures of developmental dyslexia.

C. Francks[1], *S. E. Fisher*[1], *A. J. Marlow*[1], *L. R. Cardon*[1], *Y. Ishikawa-Brush*[2], *I. L. MacPhie*[1], *D. F. Williams*[1], *J. Gay n*[3], *R. K. Olson*[3], *B. F. Pennington*[4], *S. D. Smith*[5], *J. C. DeFries*[3], *A. P. Monaco*[1]
[1]*Wellcome Trust Centre for Human Genetics, University of Oxford; Oxford, United Kingdom;* [2]*Department of Biochemistry, University of Texas Health Science Center; San Antonio, TX United States;* [3]*Institute for Behavioral Genetics, University of Colorado; Boulder, CO United States;* [4]*Department of Psychology, University of Denver; Denver, CO United States;* [5]*Center for Human Molecular Genetics, Munroe Meyer Institute, University of Nebraska Medical Center; Omaha, NE United States*
clyde@well.ox.ac.uk

Recent advances in high-throughput genotyping technology and quantitative statistical methods have made possible a new flexible approach for mapping genes involved in complex multivariate disorders. This involves using marker data to infer genetic similarity of many sibling-pairs along the lengths of their chromosomes and correlating this similarity with that of their phenotypic scores, via approaches such as Haseman-Elston regression (HE) or partitioning of variance components (VC). We performed a genome-wide search for loci that influence quantitative measures of dyslexia in a sample of 119 nuclear dizygotic twin-based families ascertained through at least one reading-disabled proband. Our strongest evidence for linkage was to a locus on chromosome 2p13-16, influencing phoneme awareness (p=0.0016 VC; p=0.0053 HE), word recognition (p=0.0069 VC; p=0.0006 HE) and orthographic coding (p=0.0058 VC; p=0.0221 HE). Suggestive evidence was also found for loci on chromosomes 4, 13 and 18. The whole-genome approach enables us to judge the significance of individual loci against the broader genetic background of dyslexia susceptibility in our sample, in contrast to the majority of genetic studies of reading disability which have investigated individual loci in isolation. Intriguingly, our chromosome 2p locus overlaps with the region showing the strongest evidence for linkage in a genome-wide screen of a single large Norwegian pedigree segregating a qualitatively defined dyslexic phenotype in an autosomal dominant fashion. Therefore the 2p locus influences dyslexia susceptibility in a manner which may be robust to the major differences in ascertainment strategy, phenotypic testing and statistical analysis that can exist between behavioural genetic studies.

C014. Systematic search for LD identifies a common haplotype conferring risk to Crohn s disease.

J. D. Rioux[1], *M. J. Daly*[1], *K. Lindblad-Toh*[1], *M. S. Silverberg*[2], *T. Delmonte*[1], *K. Kocher*[1], *E. J. Kulbokas*[1], *K. Miller*[1], *S. O Leary*[1], *E. Winchester*[1], *A. Bitton*[3], *A. H. Steinhart*[2], *R. S. McLeod*[2], *Z. Cohen*[2], *E. S. Lander*[1], *T. J. Hudson*[1,3], *K. Siminovitch*[2]
[1]*Whitehead Institute/MIT Center for Genome Research; Cambridge, MA United States;* [2]*University of Toronto, Mount Sinai Hospital; Toronto, ON Canada;* [3]*McGill University Health Center; Montreal, PQ Canada*
rioux@genome.wi.mit.edu

Crohn s disease (CD) and ulcerative colitis (UC) are chronic inflammatory bowel diseases (IBD) of unknown etiology. Our previous linkage study

identified two loci of genomewide significance; 5q31-q33 (IBD5) and 19p13 (IBD6) conferring susceptibility to CD and IBD, respectively. The IBD5 locus was of particular interest since the region contained a cluster of well-characterized immunoregulatory cytokine genes including IL4, IL13, IL5, IL3, and CSF2. Traditionally, the pursuit of linkage results to identify causal genes for common disease has been faced with many challenges and has met with limited success. This can be explained by the fact that, unlike mendelian diseases, narrowing down the critical region will likely have to rely on a probabilistic linkage disequilibrium approach since recombinants are unreliable in the context of a complex trait - underlying mutations will be neither necessary nor sufficient to cause disease. Therefore, in pursuing the evidence of linkage to IBD5, we adopted a systematic LD approach that would take full advantage of the resources made available by the Human Genome Project. Specifically, we examined the 18 cM under the IBD5 linkage peak with 64 microsatellite markers and identified a 5-marker haplotype, extending over a region of approximately 500 kb, that is associated with CD. We then performed single nucleotide polymorphism (SNP) discovery throughout this region, and have genotyped over 200 of these SNPs. At least a dozen of these SNPs are shown to have significant TDT results (pvalue < 0.0005 - 0.00001) and uniquely identify a common haplotype that confers susceptibility to CD.

C015. Genome scan for quantitative traits involved in cardiovascular disease in three independent populations.

M. Beekman[1,2], *B. T. Heijmans*[3], *N. Lakenberg*[3], *E. Suchiman*[3], *G. P. Vogler*[4], *N. G. Martin*[5], *J. B. Whitfield*[6], *N. L. Pedersen*[7], *C. Kluft*[1], *G. J. B. van Ommen*[2], *R. R. Frants*[2], *P. de Knijff*[2], *E. Slagboom*[3], *D. I. Boomsma*[8]
[1]*TNO Prevention and Health, Gaubius Laboratories, Dept of Vascular and Connective Tissue Research; Leiden, The Netherlands;* [2]*Dept of Human Genetics Leiden University Medical Center; Leiden, The Netherlands;* [3]*Dept of Medical Statistics, Leiden University Medical Center; Leiden, The Netherlands;* [4]*Center for Special Populations and Health, Pennsylvania State University; Pennsylvania, PA United States;* [5]*Queensland Institute for Medical Research; Brisbane, Australia;* [6]*Royal Prince Alfred Hospital; Sydney, Australia;* [7]*Karolinska Institute, Institute of Environmental Medicine, Division of Epidemiology; Stockholm, Sweden;* [8]*Dept of Psychology, Free University of Amsterdam; Amsterdam, The Netherlands*
M.Beekman@PG.TNO.nl

The genetic basis of cardiovascular disease (CVD) is highly complex. One strategy to dissect this is to study less complex intermediate phenotypes instead of clinical endpoints. For CVD, such intermediate phenotypes include blood pressure, plasma levels of cholesterol, apolipoproteins and triglycerides, of which more than 50% of the variation is attributable to genetic factors. The aim of our study is to map and identify genes with a major effect on these intermediate phenotypes in the general population. We are performing a genome-wide scan in population-based samples of healthy Dutch, Swedish and Australian twin pairs. We designed 80 multiplex PCR reactions randomly typing markers, with an average spacing of 18 cM, in sets of 3 to 5 chromosomes, thereby enabling statistical analyses of chromosomes during the search. Intermediate phenotypes for CVD were determined in all the twin pairs. We calculated multipoint maximum-likelihood scores using GENEHUNTER 2.0 on the data of the first scanned chromosomes. Suggestive linkage was found with total cholesterol with a maximum LOD score of 2.8 in the Dutch twin population (N=199 pairs). In the Swedish (N=53 pairs) and Australian (N=263 pairs) twin populations maximum LOD scores of respectively 1.6 and 1.0 were found in the same chromosomal region. These linkage results provide support for the presence of one locus contributing to variation of total cholesterol levels in three independent populations. It is explored how the power and QTL localisation are influenced by simultaneously analysing the populations and by genotyping parents and additional markers.

C016. A mouse model as a tool for the complex genetics of vestibular dysfunction

K. Cryns[1], *M. Van Spaendonck*[2], *K. Flothmann*[1], *G. Mortier*[3], *S. Lyonnet*[4], *P. Van de Heyning*[2], *J. Timmermans*[5], *G. Van Camp*[1]
[1]*University of Antwerp (UIA); Antwerp, Belgium;* [2]*University of Antwerp (UZA); Antwerp, Belgium;* [3]*University Hospital Ghent; Ghent, Belgium;* [4]*Hopital Necker-Enfants-Malades; Paris, France;* [5]*University of Antwerp (RUCA); Antwerp, Belgium*
kcryns@uia.ua.ac.be

Vestibular dysfunction is commonly encountered in otological practice. In most cases the aetiology remains obscure but the importance of genetic factors has been suggested on many occasions. A problem in the study of human genetic vestibular defects is the lack of large monogenic pedigrees that contain a sufficient number of affected family members to perform linkage analysis. The use of animal models is an alternative to get insight in the complex phenomenon of imbalance. We used the *Epistatic circler* mouse as a model for vestibular dysfunction. This mutant exists in a proportion of the F2-generation from the cross between C57L/J and SWR/J mice and shows circling behaviour. The results of our genome search indicated that circling behaviour is caused by a single recessive gene derived from SWR/J (the *Ecs*-gene, localised at chromosome 14) in combination with at least 3 different dominant genes derived from C57L/J (the *Ecl*-genes localised at chromosome 3, 4 and 13). This is the first dichotomous genetic defect known to be caused by simultaneous mutations in 4 different genes. Morphological examination of the inner ears of circling animals revealed a bilateral malformation of the lateral semicircular canal. Since the absence of the semicircular canals is the most specific change in patients with CHARGE association we hypothesised that the same genes may be responsible for the inner ear defect in the *Epistatic circler* as well as in these patients. Mutation analysis of *Otx2*, a candidate gene localised at mouse chromosome 14, is ongoing in *Ecl*-mice and in patients with CHARGE association.

C017. Two-locus inheritance of multiple sclerosis in a large American pedigree.

E. Vitale[1], *M. Devoto*[2], *R. Sun*[1], *C. Specchia*[3], *K. Subramaian*[1], *M. Rocchi*[4], *M. Schwalb*[1], *D. Nathanson*[5], *C. Rohowsky-Kochan*[1], *S. Cook*[1]
[1]*UMDNJ-New Jersey Medical School; Newark, NJ United States;* [2]*AI duPont Hospital for Children; Wilmington, DE United States;* [3]*University of Genoa; Genoa, Italy;* [4]*University of Bari; Bari, Italy;* [5]*Sacred Heart Hospital; Allentown, PA United States*
vitaleem@umdnj.edu

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system with a probable immune mediated pathogenesis. There is good evidence supporting the hypothesis that MS is determined by both genetic and environmental factors, but these factors remain largely undefined. The presence of a genetic component is strongly supported by the high rate of concordance in monozygotic (28%) versus dizygotic (5%) twins as well as familial recurrence risk. Linkage analysis and association studies have shown that susceptibility to MS is associated with the human histocompatibility leukocyte antigens (HLA) class II region. In particular HLA-DR15 shows linkage disequilibrium with MS. There is however as yet no convincing evidence of a common susceptibility locus. We have identified a unique large pedigree of Pennsylvania Dutch extraction, segregating MS and showing a dominant inheritance pattern compatible with an autosomal mode of inheritance. In addition MS in this family appears to be fully penetrant. We have collected and phenotypically characterized 16 members from this family, seven of whom show the typical signs of MS using clinical and magnetic resonance scanning (MRI) criteria. We have performed a wide-genome scan and found strong suggestive linkage to 12p12 with a maximum LOD score of 2.71 conditional on presence of the HLA-DR15 haplotype. Our data are the first to suggest a two-locus inheritance model for MS, requiring both the HLA-DR15 haplotype and the additional disease locus on chromosome 12.

C018. A voltage-gated ion channel is a major susceptibility gene in genetically complex epilepsies

K. Haug[1], *A. Ramirez*[1], *T. Sander*[2], *J. Rebstock*[3], *S. Horvath*[4], *K. Hallmann*[1], *J. Dullinger*[1], *B. Rau*[1], *F. Haverkamp*[5], *S. Beyenburg*[3], *H. Schulz*[2], *C. Kubisch*[1], *D. Janz*[2], *C. E. Elger*[3], *P. Propping*[1], *A. Heils*[3]
[1]*University Department of Human Genetics; Bonn, Germany;* [2]*University Hospital Charit ; Berlin, Germany;* [3]*University Department of Epileptology; Bonn, Germany;* [4]*University of California; Los Angeles, CA United States;* [5]*University Department of Pediatrics; Bonn, Germany*
k.haug@uni-bonn.de

Human epilepsy is a common brain disorder, which affects about 2-3% of the population worldwide. Idiopathic generalized epilepsy (IGE) accounts for 40% of all epilepsies and comprises seven clinically defined syndromes, which are characterized by recurrent unprovoked seizures without any detectable brain lesion. It is well established that common IGE subtypes including childhood absence epilepsy (CAE), juvenile absence epilepsy (JAE), juvenile myoclonic epilepsy (JME), and generalized tonic-clonic seizures on awakening are inherited as genetically complex traits. Based on the results of a recent genome scan we identified a voltage gated ion channel as a major susceptibility gene for IGE. We found three different mutations, which co-segregate with the disease status in three IGE-multiplex families. Furthermore, we detected a novel common coding polymorphism, which is associated with IGE as shown by the highly significant results of a case-control study (OR=3.49; 95% CI; 1.88-6.49) and a family-based study. According to Khoury et al. (1993), we calculated an attributable risk of 33% for the risk-variant. Thus, we identified the first gene, in which both, rare mutations and a common sequence variation,

confer a range of varying susceptibility effects to genetically complex epilepsies. Reference; Khoury, M.J., Beaty, T.H., Cohen, B.H. Fundamentals of Genetic Epidemiology (Oxford University Press, Oxford, 1993).

C019. Order of intron removal is a determinant of outcome of splice site mutations

P. H. Byers, *C. D. Kuslich, U. Schwarze, E. Valiente*
University of Washington; Seattle, WA United States
pbyers@u.washington.edu

Mutations at splice sites account for up to 30% of identified mutations in some human genes. Surprisingly, identical mutations in adjacent introns [e.g., in the fibrillar collagen gene COL1A1, IVS47+1G->A and IVS48+1G->A produce a mild and a lethal form of osteogenesis imperfecta (OI), respectively] can have phenotypic outcomes at the opposite ends of the spectrum. To understand some of the factors that determine the outcome of splice site mutations, we examined the role of intron splice order in almost a dozen splice site mutations in the COL1A1, COL1A2, COL3A1, and COL5A1 fibrillar collagen genes. We used intron-exon primer pairs to amplify cDNA synthesized from nuclear RNA following treatment of cells for 5 to 40 minutes with Actinomycin D to stop new transcription, and examined the products by gel electrophoresis (32P-end labeled) or after separation on the ABI310 (end-labeled with fluorescent nucleotides) to identify splice intermediates and to determine the relative splice order of up to 6 introns surrounding a region of known mutation. We found that, in general, if a donor site mutation occurred in an intron that was removed rapidly with respect to its upstream neighbor or an acceptor site mutation occurred in an intron that was removed rapidly with respect to a downstream neighbor, the outcome was an exon-skip. In contrast, if the same mutations occurred in introns that were spliced late with respect to their neighbors then cryptic splice sites were used or the intron remained. In some regions an intron was spliced along two pathways and the outcome of mutations appeared to reflect the relative frequency of the use of the alternatives. Furthermore, unexpected stability of mRNA species that contained premature termination codons could be explained by removal of downstream introns prior to splicing of the intron that generated the new termination codon. These results indicate that splice order is an important factor in predicting outcome of splice site mutations. Collagen genes tend to have relatively small introns and the role of splice order in predicting outcome of mutations in other genes is uncertain but warrants close investigation.

C020. Human L1 retrotransposition in germ cells of transgenic mice

E. M. Ostertag[1], *R. J. DeBerardinis*[1], *G. Gerton*[2], *H. H. Kazazian, Jr.*[1]
[1]*Dept. of Genetics, University of Pennsylvania School of Medicine; Philadelphia, PA United States;* [2]*Dept. of Ob.-Gyn., University of Pennsylvania School of Medicine; Philadelphia, PA United States*
ostertag@mail.med.upenn.edu

L1 retrotransposons are mobile DNA elements that can cause human disease by inserting into genes. We created a transgenic mouse model of retrotransposition to elucidate L1 biology and to estimate the frequency of L1-induced mutagenesis. Our transgenes contain an active human L1 element tagged with an EGFP retrotransposition cassette that is under the control of either the endogenous human L1 promoter or the endogenous promoter plus the mouse RNA polymerase II large subunit promoter (pPolII). Our negative control transgenic lines contain an L1 with two missense mutations known to abolish retrotransposition. Using RT-PCR to detect EGFP-tagged L1 transcripts, we demonstrated that the human L1 transgene driven by its endogenous promoter was expressed at a high level in the mouse testis and ovary but at a very low or undetectable level in several somatic tissues, while addition of pPolII resulted in higher expression levels in somatic tissues. RT-PCR performed on pachytene spermatocytes, round spermatids, and condensing spermatids demonstrated expression from both promoters in these cells. Importantly, we observed retrotransposition in the fractionated male germ cells of several lines by RT-PCR and PCR analysis. The data suggest a rate of retrotransposition of up to 1 in 1000 sperm. We also detected individual sperm that contained a retrotransposition event by visualization of the EGFP marker. We did not detect retrotransposition in negative control lines by any means. Future work to select and isolate sperm containing new retrotransposition events may offer a novel random mutagenesis system in mouse, obviating the requirement for ES cell-based strategies.

C021. The gene product underlying Opitz syndrome, MID1, triggers ubiquitin-dependent degradation of phosphatase 2A via binding to its regulatory alpha4 subunit

S. Schweiger[1], *A. Trockenbacher*[2], *V. Suckow*[1], *J. Foerster*[3], *J. Winter*[1], *S. Krauss*[1], *H. Ropers*[1], *R. Schneider*[2]
[1]*Max- Planck Institute for Molecular Genetics; Berlin, Germany;* [2]*Institute for Biochemistry; Innsbruck, Austria;* [3]*Department of Dermatologie, Charite; Berlin, Germany*
schweige@molgen.mpg.de

Opitz syndrome is a genetically heterogeneous disorder characterized by abnormal closure of ventral midline structures. We previously identified the gene responsible for X-linked Opitz syndrome, MID1, and showed that it codes for a microtubule-associated protein. We now describe a specific interaction of the MID1 protein with the a4 protein, a regulatory B-subunit of microtubule-associated phosphatase 2A. Disruption of the PP2A-a4/MID1 interaction, as seen in OS patients, results in a deficient ubiquitin-conjugation and a consecutive accumulation of microtubule-associated PP2A. We further show that the increased level of microtubule-associated PP2A causes a marked imbalance of the phosphorylation/dephoposphorylation pattern of microtubule-associated proteins. In summary, our data show that MID1 functions as a regulator of microtubule-associated protein phosphorylation and document that PP2A is regulated by ubiquitin-dependent degradation. In line with other microtubule-associated phosphoproteins that play a role in genetic disorders, such as lissencephaly Type I and Morbus Alzheimer, increased dephosphorylation of microtubules associated proteins seems to be the key event in the pathogenesis of Opitz BBB/G syndrome.

C022. Transcription of the FMR1 gene in Fragile X syndrome

F. Tassone[1], *R. J. Hagerman*[2], *P. J. Hagerman*[3]
[1]*UCHSC; Denver, CO United States;* [2]*The Children Hospital; Denver, CO United States;* [3]*UCHSC; Denevr, CO United States*
Flora.Tassone@uchsc.edu

Fragile X syndrome is nearly always due to a large expansion of a CGG trinucleotide repeat in the CG-rich promoter region of the fragile X mental retardation 1 (FMR1) gene. Expansion into the full mutation range (> 200 repeats) is often associated with hypermethylation and transcriptional silencing of the FMR1 gene. In the conventional model for fragile X, clinical involvement arises as a consequence of silencing of the FMR1 gene, with the attendant loss of FMR1 protein (FMRP). However, it has recently been demonstrated that most males with premutation alleles, or with unmethylated full mutation alleles, have FMR1 mRNA levels that are higher than normal, despite reduced levels of FMRP. Here we present an extension of these observations using quantitative (fluorescent) RT-PCR on larger sample populations. We also demonstrate that the levels of FMR1 mRNA are elevated in females with premutation alleles although more varied than in the males, and are attenuated in a manner that is consistent with the fraction of normal alleles that are active in any given individual. Finally, we also observed that a fraction of males with full mutation alleles that are resistant to cleavage by methylation-sensitive enzymes produce FMR1 mRNA, with some mRNA levels approaching those found in normal individuals suggesting that the assumed relationship between enzyme-resistance and FMR1 gene silencing may not be generally valid. Although the mechanisms leading to increased FMR1 mRNA levels are not yet understood, the presence of FMRP deficits of varying extents, in the presence of elevated mRNA levels, strongly suggests that reduced efficiency of translation is mechanistically important. A defect in translation had been observed in the full mutation range and our own observations imply that a translational impairment may occur within the premutation range; thus the high levels of FMR1 mRNA could represent a response to the protein deficit. Although models in which FMRP level (or level of function) modulates transcriptional activity remain viable, other explanations for the elevated message levels, including direct (cis) effects of the CGG element on transcription, must also be considered.

C023. A mouse model for primary ciliary dyskinesia

J. Neesen[1], *M. Ochs*[2], *A. Schmiedl*[2], *C. M ller*[1], *T. N §lein*[3], *I. Adham*[1], *W. Engel*[1]
[1]*Institute of Human Genetics; G ttingen, Germany;* [2]*Centre of Anatomy; G ttingen, Germany;* [3]*Children s Hospital; Bochum, Germany*
jneesen@gwdg.de

Impaired ciliary and flagellar functions resulting in male infertility and recurrent respiratory tract infections are found in patients suffering from primary ciliary dyskinesia (PCD). In most cases, axonemal defects were present, i.e. PCD patients often lack inner and/or outer dynein arms in their sperm tails and cilia, supporting the hypothesis that mutations in dynein genes may cause PCD. However, until now it is unclear if mutations in dynein

heavy chain genes are responsible for impaired flagellar and ciliary motility in mammals. To elucidate the role of the Mouse Dynein Heavy Chain 7 (MDHC7) gene which encodes a component of the inner dynein arm, we have generated mice lacking this dynein heavy chain isoform. Both MDHC7+/- and MDHC7-/- mice are viable and show no malformations, however, homozygous males produce no offspring. In comparison to MDHC7+/- mice the spermatozoa of MDHC7-/- mice revealed a dramatic reduced straight line velocity and straight forward movement, resulting in the inability of MDHC7 deficient sperm to move from the uterus into the oviduct. Additionally, we measured the beat frequency of tracheal cilia and observed a decrease of the beat frequency of approximately 50% in MDHC7-/- mice. The reduction in both ciliary and flagellar motility is not correlated with any gross defects in the axonemal structure. The phenotype of MDHC7-/- mice is similar to that observed in some patients suffering from primary ciliary dyskinesia, and our data strongly suggest that this disease could in some patients be due to mutations in the homologous human gene DNAH1 (HDHC7).

C024. Molecular dissection of the contribution of single gene overexpression to the change in gene expression profile in the mouse model of Down syndrome.

R. Chrast[1], *R. Lyle*[1], *C. Gehrig*[1], *H. Scott*[2], *S. E. Antonarakis*[1]
[1]*Division of Medical Genetics, CMU; Geneva, Switzerland;* [2]*The Walter and Eliza Hall Institute of Medical Research; Melbourne, Australia*
chrast@pop.salk.edu

Down Syndrome (DS) or trisomy 21 is the most common autosomal aneuploidy with a complex clinical phenotype affecting many organs and tissues, the only invariant phenotype being mental retardation. Most DS phenotypes are probably due to an extra copy of a set of normal but dosage-sensitive genes on human chromosome 21 (HC21). The recently completed sequencing of HC21 revealed that it contains ~225 genes, however, it remains to be defined which are dosage sensitive. HC21 is largely syntenic with a region of mouse chromosome 16 and thus a partial trisomy 16, Ts65Dn, acts as a model of DS and shares many of the DS phenotypes. We have used Serial Analysis of Gene Expression (SAGE) and filter arrays containing ~25,000 mouse genes and ESTs to analyse changes in gene expression in whole brains of Ts65Dn mice. Both analyses revealed complex changes in gene expression. Several lines of evidence suggest that disregulation of the signal transduction cascades of Sim2 and Dyrk (Mnbh), a transcription factor and kinase respectively, are likely to be involved in development of DS phenotypes. To help dissect the contribution of the Sim2 and Dyrk to the Ts65Dn expression pattern, we have overexpressed their cDNAs in cell culture using adenoviral infection and analysed induced changes in gene expression using mouse filter arrays. A combination of these in vivo (Ts65Dn) and in vitro (Sim2 and Dyrk adenoviruses) data compared to controls should allow us to begin to decipher the molecular cascades leading from gene triplication to the complex DS phenotype.

C025. High Frequency Of Skewed X Inactivation In Young Breast Cancer Patients

M. Kristiansen[1], *A. Langer¿d*[2], *G. P. Knudsen*[3], *K. Calzone*[4], *B. L. Weber*[4], *A. L. B¿rresen-Dale*[5], *K. H. flrstavik*[3]
[1]*Institute of Medical Genetics, University of Oslo; Oslo, Norway;* [2]*Department of Genetics, Institute for Cancer Research, The Norwegian Radium Hospital; Oslo, Norway;* [3]*Department of Medical Genetics, National Hospital; Oslo, Norway;* [4]*Cancer Centre, University of Pennsylvania; Philadelphia, United States;* [5]*Institute for Laboratory Medicine, University of Oslo; Oslo, Norway*
marianne.kristiansen@ioks.uio.no

In females one of the two X chromosomes is inactivated in early embryonic life, thus making females mosaics for two cell lines. A skewed X inactivation is a deviation from a 50;50 distribution of these two cell lines. Patients with invasive ovarian cancer were recently shown to have a higher frequency of skewed X inactivation pattern compared to patients with borderline cancer and healthy controls, indicating that skewed X inactivation is a predisposing factor for the development of invasive ovarian cancer. We have analysed X inactivation pattern in peripheral blood from 181 patients with sporadic breast cancer aged 27-90 years. X inactivation was classified as skewed when 90% or more of the peripheral blood cells preferentially used one X-chromosome. Ten per cent of the patients had a skewed X inactivation. Since older females have a higher frequency of skewed X inactivation in peripheral blood cells than young females, young and old patients were analysed separately. The frequency of skewed X inactivation in 34 young patients (27-45 years) was significantly higher than in blood donors of the same age group (12% and 2% respectively, p=0.03). Among 34 old patients (73-90 years), 15% had a skewed X inactivation. This did not differ from the frequency in a population of control females of similar age (21%). Females with a skewed X inactivation pattern may be more susceptible to develop breast cancer due to an X-linked low penetrance susceptibility allele that is affected by the X inactivation pattern.

C026. A genome-wide scan for linkage in Finnish hereditary prostate cancer families identifies chromosome 11 and other putative regions of interest

J. Schleutker[1], *E. Gillanders*[2], *A. Baffoe-Bonnie*[3], *T. Kainu*[2], *M. Jones*[2], *C. Markey*[2], *M. Matikainen*[1], *P. Koivisto*[1], *T. Tammela*[4], *J. Bailey-Wilson*[5], *J. Trent*[2], *O. Kallioniemi*[2]
[1]*Laboratory of Cancer Genetics, University of Tampere and Tampere University Hospital; Tampere, Finland;* [2]*Cancer Genetics Branch, NHGRI, NIH; Bethesda, MD United States;* [3]*IDRB, NHGRI, NIH and Fox Chase Cancer Center; Philadelphia, PA United States;* [4]*Division of Urology, Tampere University Hospital; Tampere, Finland;* [5]*Inherited Disease Research Branch, NHGRI, NIH; Baltimore, MD United States*
johanna.schleutker@uta.fi

Several predisposition loci for hereditary prostate cancer (HPC) have been suggested (Smith et al., 1996, Berthon et al., 1998, Xu et al., 1998, Gibbs et al. 1999, Berry et al., 2000). Homogeneous populations, such as that of Finland, may help to reduce genetic heterogeneity that usually complicates linkage analysis of complex diseases. Here, we report results of a genome-wide linkage scan in 13 multiplex Finnish HPC families, selected on the basis of linkage informativeness from a total of 292 Finnish families (Schleutker et al. 2000). On average, 4 affecteds were genotyped per family (range 2-6), with a mean age of 68.9 years (range 44-99 years) at diagnosis. Altogether, 413 markers were analyzed with an ABI377 capillary sequencer. Two-point and multipoint LOD scores were calculated for all autosomes with FASTLINK, Genehunter and Genehunter-Plus. The results showed three chromosomal sites with two-point LOD scores greater than 1.5. The most promising area was at 11q, with a peak two-point LOD score of 2.85 (theta = 0.0), Genehunter HLOD of 3.28 (theta = 0.0) and NPL HLOD of 2.06 (p-value = 0.0098), all based on affected only analyses. Little, if any evidence for linkage was found for previously discovered candidate HPC-loci, such as HPC1, PCAP, CAPB and HPC20. The results suggest a novel chromosomal region at 11q with suggestive evidence of linkage in Finnish HPC families, and a few other regions that also deserve further study. Chromosome 11 linkage has previously been reported in prostate cancer (Gibbs et al., 2000), but not at 11q as found in this study.

C027. Functional analysis of pathogenic exon skipping in MLH1 gene unmask phenocopies in HNPCC

A. Stella[1], *N. Resta*[1], *S. M. Lipkin*[2], *H. T. Lynch*[3], *P. Watson*[3], *Q. Wang*[4], *F. Susca*[1], *I. Miccolis*[1], *B. Liu*[4], *G. Guanti*[1]
[1]*Sez. Genetica Medica DIMIMP Policlinico Universit di Bari; Bari, Italy;* [2]*Genetics and Molecular Biology Branch-NIH; Bethesda, DC United States;* [3]*Department of Preventive Medicine, Creighton University School of Medicine; Omaha, NE United States;* [4]*Pittsburgh Cancer Institute, University of Pittsburgh; Pittsburgh, PA United States*
alexst@medgene.uniba.it

Germline mutations in mismatch repair genes *MSH2* and *MLH1* occur in the majority of HNPCC families. One of the common mechanisms of these mutations disrupt splicing of *MLH1* and *MSH2* mRNA. The disruption creates aberrant mRNAs lacking specific coding exons (referred to here as exon skipping). Analysis of exon skipping in these genes is complicated by the fact that the identical aberrant mRNA transcripts also occur with low frequency in genotipically normal subjects. These observations make it difficult to interpret putative *MLH1* and *MSH2* splicing mutations in members of HNPCC families with the high specificity necessary for molecular diagnosis. Here, we report a causative exon skipping of *MLH1* that underlies the pathogenesis in three unrelated HNPCC families. Sequencing studies of RT-PCR products showed an aberrant *MLH1* mRNA transcript lacking exon 12 in all but two of the affected family members. The aberrant transcript produced a truncated *MLH1* protein of 353 amino acids as result of frame shift and premature termination. When tumor samples from the two affected individuals without exon skipping were examined for genomic instability and protein expression, they showed a RER- phenotype and normal MLH1 expression. In addition linkage analysis which previously demonstrated no linkage with chromosome 3, showed a lod score above 3 when these two individuals were excluded from the analysis. Therefore a SSCP analysis of the genomic region including exon 12 and proximal intronic sequences was performed on PCR amplified products. All of the patients with exon 12 skipping presented a variant conformer . The same PCR products were cloned in pSPL3 plasmid and used in an in vitro splicing system. Introduction of exon 12 genomic sequences from the mutant,

but not the wildtype allele of *MLH1*, disrupted normal exon splicing in an in vitro exon trapping system. The sequencing of both the PCR products showing variant conformers and clones generating abnormal splicing demonstrated the presence of an AAG to TAG nonsense mutation at codon 461 of exon 12. These results showed beyond any reasonable doubt that; a) exon skipping observed in the three HNPCC families is pathogenic and is the consequence of a nonsense genomic mutation in the exon 12; b) phenocopies in HNPCC are possible and molecular typing of the tumors arising in these families should be performed in order to correctly evaluate the genetic risk for each family member.

This work was supported by grants from AIRC and MURST Cofin98

C028. Association of two missense substitutions in HPC2/ELAC2 gene with prostate cancer

A. R kman[1], *N. Mononen*[1], *T. Ikonen*[2], *P. A. Koivisto*[1], *M. P. Matikainen*[1], *T. L. J. Tammela*[3], *O. P. Kallioniemi*[1], *J. Schleutker*[1]

[1]*Lab. Cancer Genetics, Inst. Medical Technology, University of Tampere and Tampere University Hospital; Tampere, Finland;* [2]*Lab.Cancer Genetics, Inst. Medical Technology, University of Tampere and Tampere University Hospital; Tampere, Finland;* [3]*Division of Urology, Tampere University Hospital; Tampere, Finland*

loanro@uta.fi

Several susceptibility loci, but few genes, have been reported for human prostate cancer. Recently, Rebbeck et al. (AJHG, 67;1014, 2000) reported an association of two missense variants (S217L and A541T) in the HPC2/ELAC2 gene (at 17p) with prostate cancer. They showed an increased risk of prostate cancer for carriers of the Leu217/Thr541 genotype (OR 2.37, CI 95% 1.06-5.29) as compared with the Ser217/Ala541 genotype. Here, we explored the significance of HPC2/ELAC2 gene in prostate cancer causation in the genetically homogenous Finnish population. The frequency of HPC2/ELAC2 gene missense variants was determined by RFLP in 154 unselected prostate cancer patients from the Tampere University Hospital. The results were compared with population allele frequencies determined from 169 blood donors from the same hospital. The results showed no increased risk of prostate cancer among the Leu217/Thr541 genotype carriers (OR 0.52, CI 95% 0.23-1.18, p=0.15). The same was true for the two variants analyzed separately. The highest risk seen was for the genotype S217/L217, which had a tendency towards increased risk as compared to genotype S217/S217 (OR=1.15, CI 95% 0.72-1.82, p=0.64). In conclusion, current evidence does not support a prominent role for the two variants of the HPC2/ELAC2 in prostate cancer causation in the Finnish population. Extension of these studies to include up to 430 unselected prostate cancer cases, 100 familial cases, 230 benign prostatic hyperplasia cases and 500 population controls are in progress.

C029. Segregation Analysis of 236 Families of Breast Cancer Cases without BRCA1/2 Mutations Provides Statistical Evidence for a Recessive Breast Cancer Susceptibility Gene with High Penetrance.

D. J. Kaufman[1], *J. P. Struewing*[2], *T. H. Beaty*[3]

[1]*National Cancer Institute, NIH; Bethesda, MD United States;* [2]*National Cancer Institute, NIH; Bethesda, MD United States;* [3]*Johns Hopkins University School of Public Health; Baltimore, MD United States*

kaufmand@mail.nih.gov

Between 5 and 10 percent of breast cancer is due to inherited cancer susceptibility genes. Mutations in the genes BRCA1 and BRCA2 account for two-thirds of these hereditary breast cancer cases leaving one-third unlinked. A positive family history remains a risk factor for the disease in women without BRCA1/2 mutations, suggesting that additional susceptibility genes exist. Using segregation analysis, families of cases without BRCA1/2 mutations were studied for statistical evidence of another major breast cancer gene. Three founder mutations account for 85% of BRCA1/2 mutations in Ashkenazi Jews. Age-of-onset of breast cancer was analyzed in 236 families of Jewish cases from an earlier, community-based study who do not carry the founder mutations. Of 602 female first-degree relatives, 78 had breast cancer. Pedigrees with any ovarian cancer cases (n=17) were excluded because they are more likely to carry undetected BRCA1 mutations. In the segregation analysis, extended logistic regression evaluated the likelihood of various genetic and non-genetic models. Sporadic (p=.004), environmental (p=.04), arbitrary and decreasing Mendelian genetic models (p=.0004 and 0.04 respectively) fit the family data poorly and were rejected. A Mendelian recessive model fit better (p=0.16) than dominant (p=.06) and co-dominant models (p=.08) though these three could not be rejected. The recessive model predicted that 4% of women would carry the high-risk genotype, and 85% of them would develop breast cancer by age 70. Cumulative incidence curves predicted by the model fit observed incidence among first-degree relatives. When the restriction of Mendelian transmission was lifted, transmission probabilities still took Mendelian values, suggesting the recessive model is robust. Heterogeneity was detected between the study families and 120 BRCA1/2 families from the same community-based cohort, implying that the observed recessive effect is not due to undetected BRCA1/2 mutations. The relatively small family sizes and large number of cancers in relatives may bias allele frequency and penetrance estimates upwards. To evaluate this, re-analysis will be undertaken on families of all 4,700 probands who tested negative for BRCA founder mutations. If the recessive model is validated in the larger data set, it can serve as the basis for parametric linkage studies to identify such a gene.

C030. Linkage disequilibrium in chromosome 22q13 in eastern Finnish breast cancer cases

J. M. Hartikainen[1,2], *H. Kujala*[2], *V. Kataja*[3], *V. Kosma*[4], *M. Eskelinen*[5], *A. Mannermaa*[2]

[1]*Department of Oncology, University of Cambridge; Cambridge, United Kingdom;* [2]*Department of Clinical Genetics, Kuopio University Hospital; Kuopio, Finland;* [3]*Deparment of Oncology, University of Kuopio; Kuopio, Finland;* [4]*Department of Pathology, University of Kuopio; Kuopio, Finland;* [5]*Department of Surgery, University of Kuopio; Kuopio, Finland*

jaana@srl.cam.ac.uk

About 5 % of all cases of breast cancer are thought to be due to high-risk susceptibility genes (BRCA1/2). However, these genes explain only about 15 % of the familial risk of breast cancer. Other loci for breast cancer susceptibility have been identified, but these are not important causes of familial breast cancer. Therefore, it is likely that low-penetrance genes for breast cancer susceptibility in the general population remain to be identified. In several studies chromosome 22q has been suggested as a possible location for a tumour suppressor gene. We are studying chromosome 22 to find further evidence for a breast cancer susceptibility/risk gene and therefore screened it for linkage disequilibrium (LD) using 18 polymorphic microsatellites spaced by 0.1-2 Mb. We used a set of 49 cases and 50 controls from the late-settlement area of eastern Finland. The cases and controls were matched for age and long-term area-of-residence and they did not have evident family history of cancer. Significance levels for comparisons between the allele frequencies of the cases and controls were computed by performing chi-squared tests using SPSS (Fisher s exact test). A significant (<0.05) p-value was detected with 4 markers within a region of 1.1 Mb in 22q13. Our results support the possible location of a breast cancer risk gene in chromosome 22q13, although suggesting a slightly more centromeric region than previous studies.

C031. FISH; quality control in diagnostic laboratories.

O. Bartsch[1,2]

[1]*Institut f r Klinische Genetik, Medizinische Fakult t, Technische Universit t Dresden; Dresden, Germany;* [2]*Sektion Molekulare Zytogenetik, Kommission f r zytogenetische Qualit tssicherung, Berufsverband Medizinische Genetik; Dresden, Germany*

obartsch@rcs.urz.tu-dresden.de

More than 90 private, university-based or other cytogenetic laboratories in Germany, Austria and Switzerland (some 80% of institutions) have included fluorescence in situ hybridization (FISH) into their diagnostic services. To evaluate the quality of cytogenetic testing by FISH, 27, 31, 35 and 29 laboratories from Germany, Austria and Switzerland participated in quality assessment (QA) trials in April 1998, February 1999, September 1999 and May 2000, respectively. One or two samples (metaphase spreads from blood, amniotic fluid or bone marrow) carrying either a normal karyotype, a deletion (22q11, 7q11.23 or 15q11-q12) or a supernumerary marker chromosome were sent with the request to test them using routine protocols. Results were reviewed by committees of experts from participating laboratories. Reviewing a diagnostic FISH test is a complex process - the test includes the generation of raw data, correct interpretation of data, the timely delivery of a concise written report, and should be done only with genetic counselling available. In each trial large numbers of mistakes ranging from inconsistencies in nomenclature (ISCN 1995) to unacceptable errors which could have serious consequences were identified. As a positive consequence of the QA scheme, many laboratories improved their procedures and reports over the study period. To improve the feed-back to laboratories, reviews were last performed using an assessment sheet and scoring points. Some participants requested specimens of rare aberrations (e.g. deletion of the CBP gene on 16p). Results demonstrate that FISH QA contributes to the efficacy and safety of cytogenetic testing and increases the awareness for good laboratory practice. A European concerted action for quality improvement in FISH diagnosis may further benefit services.

C032. A submicroscopic chromosome inversion as the basis for two macroscopic chromosome rearrangements.

S. Giglio[1], V. Calvari[1], G. Gimelli[2], T. Neumann[3], L. Voullaire[4], K. W. Broman[5], D. H. Ledbetter[6], O. Zuffardi[7]
[1]University; Pavia, Italy; [2]Ospedale Gaslini; Genova, Italy; [3]University; Muenster, Germany; [4]Murdoch Children Research Institute; Parkville, Australia; [5]J. Hopkins School of Public Health; Baltimore, MD United States; [6]University of Chicago; Chicago, IL United States; [7]University of Pavia; Pavia, Italy
giglios@unipv.it

Occurrence of de novo chromosome rearrangements has been considered a stochastic event. Alternatively, one could postulate that specific genomic characteristics might predispose normal chromosomes to undergo unequal crossovers leading to chromosome structural abnormalities. This hypothesis is supported by the finding that the Xp/Yp translocation responsible for XX males and XY females preferentially occurs on the Y chromosomes having the Yp submicroscopic inversion that was considered a neutral polymorphism (Jobling et al, Current Biology 1998, 8;1391-1394). The inversion, placing the PRKY gene in the same orientation as the homologous PRKX gene in Xp23.3, allows the occurrence of PRKY/PRKX recombination and, thus, to the transposition of SRY from the Y to the X chromosome. In fact, it has been demonstrated that the PRKY/PRKX recombination accounts for the most common class of XX males and a portion of XY females. Due to the frequency of submicroscopic genomic inversions and duplications, it seems likely that other chromosome rearrangements arise in similar way. We demonstrate, by experimental and in silico data, that unequal crossovers between two olfactory receptors (OR) gene clusters located in 8p23 at a distance of about 5 Mb, are responsible for the formation of two recurrent chromosome abnormalities. The first is the inverted duplication of 8p, inv dup(8p), associated with a distinct phenotype and severe mental retardation. The second is a supernumerary marker neocentromeric chromosome, +der(8)(8p23.1pter), that is also a recurrent rearrangement and is associated with minor anomalies. We demonstrate that it is the reciprocal product of the inv dup(8p), thus being always mediated by the two 8p-OR gene clusters. Since these rearrangements originate consistently in maternal meiosis (Floridia et al, Am J Hum Genet 1996, 58; 785-796; Giglio et al, submitted), we investigated the maternal chromosomes 8 in nine inv dup(8p) mothers and in one +der(8) mother with probes included between the two 8p-OR gene clusters. We found that all the mothers were heterozygous for an 8p submicroscopic inversion. In a population of European descent, the inversion was found in 19 of 72 controls (26%) in a heterozygous state and 9 (12.5%) in a homozygous state. This inversion may cause susceptibility to unequal recombination leading to the formation of the inv dup(8p) or to its reciprocal product, the +der(8p). Two observations suggest that heterozygous females have only a low risk to have children with inv dup(8p); 1) heterozygotes are common in the population whereas the rearrangement is rare and 2) none of the more then 50 inv dup(8p) subjects reported to date have sibs with the same rearrangement. These data demonstrate that occurrence of de novo chromosome rearrangements may not be a stochastic event but is rather due to specific genomic polymorphisms and indicate the possibility to develop a profile of the individual risk for having children with chromosome rearrangements.

C033. Screening for subtelomeric chromosome abnormalities in children with idiopathic mental retardation using the multiprobe telomere FISH and the new MAPH telomeric assays

P. C. Patsalis[1], J. A. L. Armour[2], J. Flint[3], C. A. Sismani[1]
[1]Department of Cytogenetics, The Cyprus Institute of Neurology and Genetics; Nicosia, Cyprus; [2]Institute of Genetics, University of Nottingham; Nottingham, United Kingdom; [3]Institute of Molecular Medicine, John Radcliffe Hospital; Headington, Oxford, United Kingdom
patsalis@mdrtc.cing.ac.cy

Subtelomeric chromosomal abnormalities are emerging as an important cause of human genetic diseases. The scopes of this investigation were to screen a selected group of children with idiopathic mental retardation for subtelomeric anomalies using the multiprobe telomeric FISH method and to develop and test in the same group of patients a new assay, the MAPH telomeric assay. The new MAPH telomeric assay used the recently published MAPH methodology that permits the measurement of locus copy number by hybridization and a specifically designed set of probes located at the end of human chromosomes. This study showed that subtle chromosomal abnormalities occurred with a frequency of 1.43% (1 out of 70; 95% confidence interval 0.5-6.6%) in the selected group of patients using the multiprobe telomeric FISH method. The new MAPH telomeric assay confirmed the same results in all normal and abnormal samples. Even though the prevalence of 1.43% is among the lowest found, this study demonstrated that screening for subtelomeric rearrangements is an extremely useful investigation as it appears to be one of the most common causes of idiopathic mental retardation. The new MAPH telomeric assay offers a new, fast and cost effective diagnostic tool for the investigation of mental retardation, the characterization of known chromosomal abnormalities, spontaneous recurrent miscarriages, infertility, hematological malignancies, preimplantation genetic diagnosis, and other fields of clinical and research interests.

C034. Loss of the Y chromosomal PAR2-region in four familial cases of satellited Y chromosomes (Yqs)

S. R ttger[1], H. K hl[1], H. Heilbronner[2], H. Enders[3], W. Schempp[1]
[1]Institut f r Humangenetik und Anthropologie; Freiburg, Germany; [2]Frauenklinik Berg, Institut f r Klinische Genetik; Stuttgart, Germany; [3]Institut f r Humangenetik und Anthropologie; T bingen, Germany
roettger@ukl.uni-freiburg.de

Applying flourescence in situ hybridization (FISH) of various Y chromosomal DNA probes to four familial cases of human Yqs we could demonstrate that the formation of Yqs must have occured via reciprocal translocation involving the short arm of an acrocentric autosome and the heterochromatin of the long arm of the Y chromosome (Yqh). Breakpoints map within Yqh and the proximal short arm of an acrocentric autosome resulting in the gain of a nucleolus organizer region (NOR) including the telomere repeat (TTAGGG)n and the loss of the pseudoautosomal region 2 (PAR2) at the long arm of the recipient Y chromosome. In no case the reciprocal product of an acrocentric autosome with loss of the NOR and gain of PAR2 could be detected. Using the 15p-specific classical satellite-III probe D15Z1 only for two of our four Yqs probands it could be shown that the satellited material originated from the short arm of chromosome 15. In contrast to the loss of PAR2 in Yqs chromosomes, another Y chromosomal variant (Yqh-) showing deletion of long-arm heterochromatin in Yq12 has retained PAR2 referring to an interstitial deletion of Yq heterochromatin in such deleted Y chromosomes.

C035. Interphase-FISH for detection of translocations affecting the HOX11/TCL3-locus in 10q24

S. Gesk[1], C. Kahl[1], L. Harder[1], J. Harbott[2], L. French[3], P. Deloukas[3], W. Grote[1], B. Schlegelberger[1], R. Siebert[1]
[1]Department of Human Genetics; Kiel, Germany; [2]Department of Pediatrics; Gie§en, Germany; [3]Sanger Centre; Hinxton, United Kingdom
gesk@medgen.uni-kiel.de

The translocation t(10;14)(q24;q11) and its variant t(7;10)(q35;q24), which are recurrent in acute T-cell leukemia, lead to activation of the HOX11/TCL3-gene in chromosomal region 10q24 by juxtaposing this gene to one of the T-cell-receptor loci. In the present study, we established a diagnostic assay for detecting these translocations by interphase fluorescence in situ hybridization (FISH). BAC clones flanking the HOX11/TCL3-locus were obtained from a fingerprinted BAC-contig of chromosomal region 10q24. BAC clones located proximal and distal of the HOX11/TCL3-locus were differently labeled and applied to interphase-FISH in seven normal controls and eight T-cell neoplasms with t(10;14)(q24;q11) or t(7;10)(q35;q24). In over 1600 nuclei of controls, a considerable split defined as separation of each one signal for the proximal and distal probe by more than three times the signal diameter was observed in only one cell. In contrast, all T-cell neoplasms with t(10;14) or t(7;10) contained at least 47% of nuclei with a signal split indicating a breakpoint in the HOX11/TCL3-locus. Thus, the established double-color FISH approach provides a new reliable and routinely applicable tool for diagnosing breakpoints in the HOX11/TCL3-locus. Supported by Deutsche Krebshilfe

C036. Chromosomal Aberrations In Early Stage-bilharzial Bladder Cancer As Detected By Fluorescence In Situ Hybridization

M. S. Aly[1], H. M. Khaled[2]
[1]Faculty of Science, Cairo University (Beni Suef branch); Cairo, Egypt; [2]National Cancer Institute, Cairo University; Cairo, Egypt
magdyaly@yahoo.com

In several developing nations in Africa and the Middle East, most predominantly in Egypt, bladder cancer is one of the most common types of malignancy in both men and women. It has several unique clinical, epidemiological, and histological characteristics that suggest that it is a distinct entity from bladder cancer in western countries. Genetic alterations in bilharzial related bladder cancer have been studied infrequently, and specially in the advanced sittings i.e. T3 and T4 stages. The objective of this study is to extend establishing the base line cytogentic profile of this type of malignancy to early T1 and T2 stages. For this purpose fluorescence in situ hybridization was applied to interphase nuclei of frozen-stored samples with biotinylated repetitive DNA probes specific for all chromosomes

to detect numerical chromosome changes. Thirty-five Egyptian patients with bladder cancer were included in this study. Eleven cases had squamous cell carcinoma, 24 with transitional cell carcinoma. Their median age was 56 years (range 20 - 82 years). They were 27 males and 8 females with a male-female ratio of 3.3;1. Grade I tumors were diagnosed in 11 cases, whereas 21 and 3 cases had grade II and III lesions, respectively. Data on pathologic disease stage were available for 34 cases. P1 lesions were encountered in 24 and P2 for 10 cases. Fluorescence in situ hybridization was successful in all cases studied. Most of the probes displayed a diploid spot distribution. Six out of 24 TCC cases had diploid chromosome count with all the probes. Numerical chromosome aberrations were detected in 18 cases (75%). In 12 cases, a loss of chromosome 9 was observed. In three cases, an additional loss of chromosome 17 was detected. One case demonstrated a loss of chromosome 10, whereas another two cases showed a gain of chromosome 7, next to a loss of chromosome 9. Loss of chromosome Y was observed in 9 of the 27 male cases studied (33.3%), from which one case as the only abnormality observed whereas 4 cases were detected next to loss of chromosome 9, one case with gain of chromosome 7. Five cases showed loss of chromosome 19 whereas gain of chromosome 4 was detected in 2 cases. Two out of 11 samples of SCC had normal diploid chromosome count with all the probes used. In 4 out of 11 cases (36.4%) underrepresentation of chromosome 9, compared with the other chromosomes, were detected. An additional loss of chromosome 17 and gain of chromosome 7, next to loss of chromosome 9, was detected in three cases. One case showed loss chromosome 17 as the only numerical aberration. Loss of the Y chromosome was detected in 3 cases of which one case was with gain of chromosome 7 and one case with loss of chromosome 19.

C037. Patients Views on Ethical Issues; Surveys in USA, Germany, France

D. C. Wertz[1], *I. Nippert*[2], *G. Wolff*[3], *S. Ayme*[4]
[1]*University of Massachusetts Medical School,; Waltham, MA United States;* [2]*University of Muenster; Muenster, Germany;* [3]*Institut f r Human-Genetik und Anthropologie, Albert-Ludwigs Universit t Freiburg; Freiburg, Germany;* [4]*INSERM; Villejuif, France*
dwertz@shriver.org

We surveyed patients ethical views at 12 genetics clinics in USA,2 in Germany,5 in France. 476(67%)in USA, 593(65%)in Germany,394(51%)in France returned anonymous questionnaires.Over 9/10 in USA and Germany were women, mostly married. Major findings; A. Family relations. Majorities (US 75%, Germany 76%, France 88%) thought warning relatives at genetic risk takes precedence over patient confidentiality. About half (53%,42%,55%) thought spouses had automatic rights to genetic information,without consent; 44%,32%, 41% favored automatic access for blood relatives. 61%,34%,45% would test children for genetic predisposition to Alzheimer. B. Autonomy. 60%,48%, 45% said patients had a right to any service they could pay for out of pocket; 69%, 46%, 48% thought refusal was denial of rights. Most believed (86-88%) in rights to referral within their country, but fewer (50%, 29%,33%)favored it outside the country. Most (87%,82%,70%) approved prenatal paternity testing on request for a woman with 2 male friends. C. Privacy. There was universal distrust of insurers and employers, but most (US 96%, France 92%) would tell a school system a diagnosis of XYY. Few (20%,11%,6%) would protect confidentiality of a bus driver at high genetic risk for heart attack. Most favored DNA fingerprinting for people convicted of (83-97%)or charged with (71-82%) serious crimes, armed forces (60-86%), but not passport applicants (50%,10%,12%) or newborns (73%,31%,47%). D. Prevention. Majorities (64%,49%,70%) thought people should know their genetic status before marriage, but fewer (31%,5%,29%) thought states should require carrier tests. Most (80-93%) thought women at high risk should have PND, but fewer (21%,43%,67%) thought they should abort if tests were positive. D. Disability. Most (78-90%) would respects parents wishes to refuse a life-saving operation on a handicapped newborn. 44%,48%,77% said bringing a child with a disability into the world was unfair to the child, if the birth could be prevented; 26%, 27%, 56% said it was socially irresponsible. 21% in US and 18% in France said laws should require sterilization for a blind woman on welfare. About 30% fewer in US would abort for each of 24 genetic conditions than in Germany. E. Preconception sex selection. 26%,10%,20% would use it; 15%,9%, 19% thought insurance should pay. On most issues, patients views differed from geneticists in their own country. It is time to examine reasons for these differences.

C038. The Disclosure of Genetic Information to Family Members

A. J. Clarke[1], *K. Featherstone*[2], *P. Atkinson*[3]
[1]*University of Wales College of Medicine; Cardiff, Wales, United Kingdom;* [2]*Cardiff University; Cardiff, United Kingdom;* [3]*Cardiff University; Cardiff, Wales, United Kingdom*
clarkeaj@cardiff.ac.uk

Genetic information about one individual may be of interest to other family members. When such information is relevant and important, genetic counselling clients are encouraged to make it available to these others. If professionals identify blocks to communication within families, they may be able to help individuals overcome them. A number of national professional bodies and ethics panels have announced that professionals should be willing to provide family members with such information if their relatives persist in refusing to do so and if the possible harm resulting from non-disclosure is thought to outweigh the harm of forced disclosure, i.e. of breaking confidentiality. We wish to challenge the framework within which such arguments are presented on several grounds. First, the concept of genetic harm depends upon an inadequate conception of individual autonomy. Secondly, the debate must be set within the context of family relationships, including who counts as family, patterns of communication, and understandings of inheritance and of practical kinship obligations. Our work on communication practices with families in South Wales demonstrates that the understanding of disclosure can be contested, and issues of disclosure may be influenced by ideas of the personal vulnerability of particular individuals. Both gender differences and practical barriers may also operate. We conclude that public policies or professional guidelines that promote the breaking of confidentiality or forced disclosure should be avoided. We need a much clearer understanding of current disclosure practices within families before deciding upon such policy questions.

C039. Knowledge Of Genetics And Genetic Tests Among Dutch General Practitioners.

M. J. H. Baars, *M. W. Langendam, L. P. ten Kate*
VU Medical Center; Amsterdam, The Netherlands
mjh.baars@azvu.nl

Objective To investigate knowledge of genetics and genetic tests among Dutch General Practitioners (GPs) and to determine factors influencing this knowledge. Methods The questionnaire of Hofman et al (Acad.Med 1993; 68 (8); 625-632) was translated in Dutch and adapted to the situation of the health care system in the Netherlands. The questionnaire was mailed to 200 GPs randomly selected by the Netherlands Institute of Primary Care Research (NIVEL) and for validation to 58 Clinical Geneticists (CGs). Next to questions about personal and practice issues the questionnaire covered knowledge of genetics concepts and facts, and awareness of the availability of genetic tests. Assigning one point for each correct answer to the 26 knowledge questions made a total knowledge score. Multiple linear regression was used to identify significant and independent predictors of total knowledge score. Results The response rate of GPs was 64% (124/195) and of CGs 84% (49/58). The total knowledge score could be computed for 122 GPs with a mean of 16.58 (64% correct answers), SD 3.62 and range 7.50-25.00 and for 49 CGs with a mean of 24.58 (95% correct answers), SD 1.06 and range 21.00-26.00. A lower GP s knowledge score was related to a higher age, not having taken an elective course in genetics during the study and a higher degree of urbanization. Comment The negative relation between the knowledge score and a higher degree of urbanization needs further assessment.

C040. Genetic discrimination experienced by Australian families

K. K. Barlow-Stewart[1], *D. Keays*[2]
[1]*Genetics Education Program of NSW; Sydney, Australia;* [2]*University of Melbourne; Melbourne, Australia*
kbarlow@med.usyd.edu.au

Discrimination in insurance and employment on the basis of a genetic test result for a range of genetic disorders has been reported in the USA and UK. Until recently no evidence was available of genetic discrimination in Australia. In May 2000 an anonymous survey was sent by Barlow-Stewart to genetic support groups (643), registrants on the NSW and ACT Hereditary Bowel Cancer Register (310) and individuals (337) to determine their needs and experiences following a diagnosis or genetic testing; one of these experiences included documentation of any instances of genetic discrimination. Of the 715 respondents, 43 reported discrimination in the areas of insurance, and employment. In addition Keays identified 5 additional cases through an independent survey of clinical genetics services in Australia. Discrimination reportedly occurred with different insurance packages; some respondents reported being discriminated in a number of

areas including refusal of life insurance, denial of an increase in life insurance for a pre-existing policy, refusal of income protection insurance, refusal of trauma insurance, reduction in superannuation and loading on the premiums for travel insurance. Two people reported that their applications for positions in the Public Service and the Armed Forces respectively were subject to a negative test result. In 3 other cases the employment was terminated or the subjects submitted their resignation following demotion of duties. Following public release of this evidence of genetic discrimination, the Australian Government has initiated several enquiries to determine the direction for law or policy development.

C041. What Are The Economic Stakes Of Gene Patents? Illustration Through A Cost-effectiveness Study (brca1 & 2)

C. S. Sevilla[1,2], *C. M. Julian-Reynier*[1], *F. Eisinger*[1,3,4], *D. Stoppa-Lyonnet*[5], *B. Bressac-de Paillerets*[6], *J. P. Moatti*[1,7], *H. Sobol*[3,4]
[1]*INSERM U379; Marseille, France;* [2]*Ecole des Hautes Etudes en Sciences Sociales; Paris, France;* [3]*INSERM E9939; Marseille, France;* [4]*Institut Paoli-Calmettes; Marseille, France;* [5]*Institut Curie; Paris, France;* [6]*Institut Gustave Roussy; Villejuif, France;* [7]*Facult des Sciences Economiques, Universit de la M diterran e; Marseille, France*
sevilla@marseille.inserm.fr

Genetic testing for breast cancer predisposition has been increasingly used on individuals with a high family risk since the identification of BRCA1 and BRCA2. Recognition of property rights by the United States Patent and Trademark Office on those genes has generated the creation of a monopoly position for BRCA1/2 testing on the whole US market. The European application for these same commercial rights is still pending (december 2000). The objective of our study was to compare the cost-effectiveness of technically available alternative strategies for BRCA1 mutation research to Direct DNA Sequencing (DS) of the entire gene which is currently used by the patent owner. The cost evaluation was based on a detailed observation of the different stages of each available strategy in three French laboratories. Twenty strategies, representing all the available combinations of techniques for the mutation research on BRCA1, were evaluated. The techniques considered were; DS, DHPLC, SSCP, DGGE, HA, PTT and FAMA. The cost-effectiveness analysis was undertaken in a theoretical population of 10,000 with a 15% prevalence of deleterious mutation for BRCA1. Five strategies were found to be simultaneously less expensive and more cost/effective than DS of the entire gene. Though gene patents are aimed at stimulating research, extensive patent coverage of all potential applications of an identified gene makes possible that an inefficient screening procedure becomes the unique standard of DNA testing practices.

C042. Influence of media on understanding and interpretation of prenatal diagnosis

P. Klein, *J. Pelz*
Institut fuer Humangenetik; Berlin, Germany
science2000uk@yahoo.co.uk

Articles in newspapers often report new findings in medicine and their actual or possible applications for the general audience. Prenatal diagnosis (pd) is an important application of human genetics. In a preliminary analysis we found that the information on the methods of pd in German newspapers is basically correct and thus may help people to understand the various procedures of pd. Therefore popular mass media may influence public opinion on the various procedures and on the conditions being screened for. In an interventional study more than 800 subjects were asked what they know and what they think about pd before and after reading a text, written in the format of a newspaper s article dealing with the various pd methods. This text existed in 4 different versions. Two favouring abortion of the foetus after a positive prenatal diagnosis of Down syndrome was made, two in which a woman chose to continue pregnancy. For both alternatives the prevalences mentioned in the article were given either as percentages or as natural numbers. We found that the article influenced the reader s attitude towards the use of pd and that the different presentations of the prevalences had an impact on the reader s understanding. Among other aspects we found that before reading the text subjects made a (hypothetical) choice of the methods of pd rather by default; 25% of them wanted all women to make use of all the available diagnostic techniques. After reading the text, this sub-group also decided against certain techniques that are not necessary in all cases (e.g. amniocentesis). This study might serve as a model for further research of the public understanding of genetic testing.

C043. Upregulation of WNT-4 Signaling; A New Mechanism for Dosage-Sensitive Sex Reversal in Humans

B. K. Jordan[1], *M. Mohammed*[1], *S. T. Ching*[1], *E. Delot*[1], *X. Chen*[1,2], *P. Dewing*[1], *A. Swain*[3], *P. N. Rao*[1], *B. R. Elejalde*[4], *E. Vilain*[1]
[1]*UCLA; Los Angeles, CA United States;* [2]*Cedars-Sinai Medical Center; Los Angeles, CA United States;* [3]*Chester Beatty Laboratories; London, United Kingdom;* [4]*University of Wisconsin MCC; Milwaukee, WI United States*
bjordan1@ucla.edu

WNT-4, a member of the Wnt family of locally-acting secreted growth factors, is the first signaling molecule shown to influence the sex determination cascade. In mice, a targeted deletion of Wnt-4 causes the masculinization of XX pups. Therefore, WNT-4, the human homologue of Wnt-4, is a strong candidate for sex reversal in humans. Recently, we have shown that in testicular Sertoli and Leydig cells, Wnt-4 upregulates expression of Dax1, a gene antagonizing the testis-determining factor Sry. Furthermore, this finding has elucidated the mechanism responsible for the human XY sex reversal associated with a duplication of 1p31-p35 encompassing the WNT-4 locus. Overexpression of WNT-4 leads to upregulation of DAX1, which redirects the fate of the developing gonad resulting in an XY female phenotype. Thus, WNT-4, a novel sex-determining gene, and DAX1 play a concerted role in controlling female development and in preventing maleness. Presently, transgenic studies are underway to evaluate the effects of WNT-4 dosage on the fate of the bipotential gonad. Taken together, these observations suggest that mammalian sex determination is sensitive to dosage at multiple steps in its pathway.

C044. Complex human Y-chromosomal HERV sequence structure in the AZFa region; new candidate genes for the control of early germ cell proliferation?

C. Kamp, *H. Ditton, K. Huellen, P. H. Vogt*
Institute of Human Genetics; Heidelberg, Germany
christine_kamp@med.uni-heidelberg.de

In humans, deletions of the AZFa region in the Y chromosome (proximal Yq11, D3-D6) are associated with a complete germ cell aplasia (Sertoli-cell-only syndrome; SCO) i.e. males with this interstitial Y deletion have no germ cells in their testes tubules (Vogt et al. 1996). Therefore, genes functional for early germ cell proliferation are expected in this Y region. Up to now, two AZFa candidate genes were isolated; USP9Y/DFFRY and DBY, because they were found to be completely deleted in each AZFa patient (Vogt, 1998). Interestingly however, a point mutation in USP9Y/DFFRY resulting in a truncated protein, does not result in germ cell aplasia but only severe oligozoospermia (Sun et al., 1999) and mature germ cells could be also found in a patient with deletion of the complete USP9Y/DFFRY gene (Sargent et al. 1999). Similarily, deletions of the complete DBY gene were shown to be associated with different infertility phenotypes including severe oligozoospermia although also the SCO syndrome (Foresta et al., 2000). This suggests that deletion of only one of the two AZFa candidate genes is NOT sufficient to cause the severe SCO phenotype and the question is raised as to whether at least both genes or even the whole AZFa region must be deleted to cause in men the SCO syndrome?. We were able to map the exact breakpoints of the AZFa region in six patients with the SCO syndrome and estimated a length of 792 kb for the deleted Y-DNA (Kamp et al. 2000). The breakpoints were found in the structure of a Human Endogenous Retrovirus (HERV15) present with two copies at the borders of the AZFa region (HERV15yq1, HERV15yq2). This strongly indicated that the AZFa deletion originates from an intrachromosomal recombination event between these two retroviral elements (Kamp et al. 2000). These data were confirmed by Sun et al. (2000) and Blanco et al. (2000). HERV15yq1 is part of a complex retroviral sequence structure (DYS11) expressed in human testis tissue (Leroy et al. 1987). We therefore currently analyse whether its deletion in all AZFa patients points to a causal relationship with the observed pathological AZFa phenotype. References 1. Vogt P.H. et al. (1996) Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. Hum. Mol. Genet., 5, 933-943. 2. Vogt, P.H. (1998) Human chromosome deletion in Yq11 AZF candidate genes and male infertility; history and update. Mol. Hum. Reprod., 4, 739-744. 3. Sun C. et al. (1999) An azoospermic man with a de novo point mutation in the Y chromosomal gene USP9Y. Nature Genet., 23, 429-432. 4. Sargent N. et al. (1999) The critical region of overlap defining the AZFa male infertility interval of proximal Yq contains three transcribed sequences. J. Med. Genet., 36, 670-677. 5. Foresta C.et al. (2000) Deletion and expression analysis of AZFa genes on the human Y chromosome revealed a major role for DBY in male infertility. Hum. Mol. Genet. 8, 1161-1169. 6. Kamp C. et al. (2000) Two long retroviral sequence blocks in proximal Yq11 cause AZFa microdeletions as a result of intrachromosomal recombination events. Hum. Mol. Genet.9, 2563-2572 7. Sun C. et al.. (2000) Deletion of azoospermia factor a (AZFa) region of human Y chro-

mosome caused by recombination between HERV15 proviruses. Hum. Mol. Genet. 9, 2291-2296 8. Blanco P. et al. (2000) Divergent outcomes of intrachromosomal recombination on the human Y chromosome; male infertility and recurrent polymorphism. J. Med. Genet. 37, 752-758 9. Leroy P .et al. (1987) Testis-specific transcripts detected by a human Y-DNA-derived probe. Development, 101, (Suppl.), 177-183.

C045. ELOVL4, a novel protein involved in elongation of fatty acids, is truncated in two related forms of autosomal dominant macular dystrophy

K. Zhang[1], *Z. Yang*[1], *Y. Li*[1], *Z. Yu*[1], *D. Figueroa*[2], *W. Li*[2], *R. Allikmets*[3], *P. Wong*[4], *P. Sieving*[5], *M. Metzker*[2], *M. Kniazeva*[6], *D. Zack*[7], *L. Kakuk*[8], *P. Lagali*[4], *I. MacDonald*[9], *M. Han*[10], *C. Austin*[2], *R. Gould*[2], *R. Ayyagari*[8], *K. Petrukhin*[2]

[1]Cole Eye Institute; Cleveland, OH United States; [2]Merck Research Labs; West Point, PA United States; [3]Columbia University; New York, NY United States; [4]University of Alberta; Edmonton, AB Canada; [5]University of Michigan; An Arbor, MI United States; [6]University of Colorado; Boulder, CO United States; [7]Johns Hopkins University; Baltimore, MD United States; [8]University of Michigan; Ann Arbor, MI United States; [9]Unversity of Alberta; Edmonton, AB Canada; [10]Unversity of Colorado; Boulder, CO United States

zhangk@ccf.org

Purpose; Stargardt-like macular dystrophy (STGD3, MIM 600110) and autosomal dominant macular dystrophy (adMD) are two inherited forms of macular degeneration characterized by decreased visual acuity, macular atrophy, and extensive flecks. We conducted molecular genetic study to identify the disease gene. Methods; Genetic linkage mapping and positional cloning approaches were employed. Results; Linkage and haplotype analyses revealed that all affected members in four independent STGD3 families and one adMD family shared a common founder haplotype. We limited the minimum genetic region (MGR) for STGD3 and adMD to a 0.6 cM interval and constructed a physical map. We characterized EST clusters and identified a novel cone and rod photoreceptor-specific gene called ElOVL4 within the MGR. ELOVL4 encodes a putative transmembrane protein with similarities to the ELO family of proteins involved in elongation of very long chain fatty acids. We found a single five base-pair deletion in the coding region of ELOVL4 which was present in every affected member of either STGD3 or adMD family (a total of 76 affected individuals) but absent in unaffected siblings or 300 normal controls. Conclusions; We have identified the gene for STGD3/adMD. Biosynthesis of polyunsaturated, long chain fatty acids (PUFA) in photoreceptors requires dietary consumption of the essential a-linolenic acid and a subsequent series of three elongation steps. We hypothesize that ELOVL4 may be involved in one or all of the three elongation steps required for PUFA biosynthesis. Our results are the first to implicate the biosynthesis of PUFA in the pathogenesis of at least two related forms of macular degeneration.

C046. Transient Neonatal Diabetes Mellitus, the spectrum of mutations in a cohort of 67 patients.

D. O. Robinson[1], *H. Cave*[2], *D. Mackay*[1], *A. Coupe*[1], *J. Shield*[3], *M. Polak*[2], *I. Temple*[4]

[1]Wessex Regional Genetics Laboratory; Wiltshire SP2 8BJ, United Kingdom; [2]Hopital Robert Debre; Paris, France; [3]Institute of Child Health; Bristol, United Kingdom; [4]Wessex Clinical Genetics Unit; Southampton, United Kingdom

d.robinson@dial.pipex.com

Transient Neonatal Diabetes Mellitus (TNDM) is a rare form of diabetes occurring in 1 in 400,000 births. It presents in growth retarded neonates within 6 weeks of birth with persistent hyperglycaemia. Endogenous insulin levels are extremely low or undetectable and patients usually require exogenous insulin therapy for a median period of 3 months. The condition is transient with recovery occurring by 18 months of age. Patients do not have islet cell antibodies or diabetes susceptible HLA haplotypes, which suggests a form of diabetes more akin to type 2 than to the classical autoimmune type 1 diabetes. Although the condition is transient in early life, about 40% of patients are known to relapse and develop type 2 diabetes later in life usually in the teenage years. A significant proportion of TNDM cases are known to be caused by abnormalities of chromosome 6 involving an imprinted locus at 6q24. We have analysed DNA from 67 patients. 15(22%) have paternal uniparental isodisomy of chromosome 6, 16(24%)have duplications of part of chromosome 6 including 6q24 and 5(7%) have abnormal methylation of an imprinted CpG island at 6q24 which forms part of the candidate TNDM gene ZAC. In a further 31(46%) cases no abnormality of chromosome 6 has yet been identified.

C047. A novel MSP/DHPLC method for the investigation of the methylation status of imprinted genes enables the molecular detection of low cell mosaicisms.

A. Baumer, *U. Wiedmann, M. Hergersberg, A. Schinzel*

Institute of Medical Genetics, University of Zurich; Zurich, Switzerland

baumer@medgen.unizh.ch

We describe a new procedure for the analysis of the methlyation status of imprinted genes based on methylation specific PCR followed by DHPLC (MSP/DHPLC). The method offers a rapid and very reliable alternative to conventional methods such as Southern blots and methylation specific PCR (allele-specific MSP). The efficient resolution of the differentially methylated alleles is demonstrated for two human imprinted genes, namely SNRPN and LIT1. Abnormal imprinting of the two genes is associated with the Angelman / Prader-Willi syndromes and the Beckwith-Wiedemann syndrome, respectively. The MSP/DHPLC method is based on PCR amplification of gene segments which show parent-of-origin specific methylation. Genomic DNA is subjected to an in vitro bisulfite treatment prior to PCR amplifications using primers specific for modified DNA. Both alleles (theoretically amplified with equal efficiency) are represented by identically sized PCR products; they differ, however, at a number of positions within the amplified DNA segment. The DHPLC analysis allows a very efficient resolution of the two populations of PCR products. The high sensitivity and quantitative properties of the MSP/DHPLC method are illustrated based on its ability to reveal a low cell mosaicism in an infant with a maternal UPD15 (i.e. Prader-Willi syndrome patient). The minor cell line (approximately 8% in blood) was not detectable with conventional molecular analysis. Whilst the detection of low cell mosaicisms of structurally abnormal chromosomes usually relies on cytogenetic studies, the MSP/DHPLC method described here not only offers an alternative at the molecular level but may also reveal mosaicisms concerning structurally intact chromosomes.

C048. Truncating mutations in the glomulin gene cause glomuvenous malformations

M. Vikkula[1], *P. Brouillard*[1], *L. M. Boon*[2], *J. B. Mulliken*[3], *O. Enjolras*[4], *M. L. Warman*[5], *O. Tan*[6], *B. R. Olsen*[3]

[1]Christian de Duve Institute of Cellular Pathology; Brussels, Belgium; [2]Universit catholique de Louvain; Brussels, Belgium; [3]Harvard Medical School; Boston, MA United States; [4]H pital Lariboisi re; Paris, France; [5]Case Western Reserve University School of Medicine; Cleveland, OH United States; [6]Tufts University School of Veterinary Medicine; Boston, MA United States

vikkula@bchm.ucl.ac.be

Venous malformations (VMs), localized defects of vascular morphogenesis, are single or multiple bluish-purple lesions that occur mainly in skin and mucosa. They can be dominantly inherited and are among the most frequent lesions in centers that specialize in treatment of vascular anomalies (Vikkula et al., 1998). We previously identified the endothelial-specific angiopoietin receptor TIE2/TEK, located on 9p21, as the cause of mucocutaneous venous malformations (VMCM, MIM 600195) (Vikkula et al., 1996). We have also shown that GVMs (MIM 138000), cutaneous venous malformations with smooth muscle-like glomus cells, are linked to 1p21-22 (Boon et al., 1994). In this 4-6 cM VMGLOM locus, we identified linkage disequilibrium and narrowed the region to 1.48 Mbp (Irrthum et al., 2001, in press). Herein, we report on the identification of the mutated gene, glomulin, localized on the basis of our YAC and PAC maps (Brouillard et al., 2000). We report the complete cDNA sequence, the genomic structure of the gene, and 13 different mutations identified in 19 families. As all but two of the mutations cause premature stop codons, GVMs are likely to be caused by loss-of-function of glomulin. These data suggest that glomulin is important for the differentiation of vascular smooth muscle cells, and thus for vasculogenesis and angiogenesis. vikkula@bchm.ucl.ac.be

C049. Routes of expansion and founder effects in the evolutionary history of modern humans.

D. Labuda[1,2], *E. Zietkiewicz*[1], *V. Yotova*[1], *I. Arrieta*[3], *M. Batzer*[4], *D. E. C. Cole*[5], *J. Jaruzelska*[6], *D. Modiano*[7], *J. Moisan*[8], *R. Michalski*[9]

[1]Centre de Recherche, H pital Sainte-Justine; Montreal, PQ Canada; [2]D partement de P diatrie, Universit de Montr al; Montr al, PQ Canada; [3]UPV; Bilbao, Spain; [4]LSU; New Orleans, LA United States; [5]UT; Toronto, ON Canada; [6]Polish Academy of Sciences; Poznan, Poland; [7]ULS; Rome, Italy; [8]Centre Hospitalier R gional et Universitaire; Nantes, France; [9]Victoria Hospital; Prince Albert, SK Canada

damian.labuda@umontreal.ca

Our analysis of haplotypes consisting of 35 polymorphisms from an 8kb segment of the dystrophin gene on Xp22 indicates that African chromosomes descend from at least two lineages that have been evolving separately for a period of time. One of them underwent range expansion colo-

nizing different continents, including Africa where it mixed with the other one represented today by a large fraction of African-specific haplotypes. While explaining the general observation of the greater diversity of sub-Saharan populations this reveals a novel aspect of early human evolution, before expansion. The routes of expansion outside Africa can be retraced through the analysis of geographic distribution of the six most frequent haplotypes (representing more than 80% of the studied sample of 1815 worldwide chromosomes). The most ubiquitous haplotype B001 is found at the highest frequency in Eurasia and Americas. Haplotype B002 frequency distribution follows the southern route , linking Africa, South-East Asia, Indonesia and Papua New Guinea. In contrast, haplotypes B003 and B006 indicate northern route connecting Europe, Asia and Americas. A dramatic increase in B004 frequency in Amerindians from South and Central America and the absence of B005 from all Paleoindian and NaDene populations can be associated with the colonization of Americas. Our diversity data indicate that the dispersal of the expanding lineage was relatively recent (presumably in the Upper Paleolithic). They show as well that the peopling of the World by modern humans occurred through a series of founder effects. (Supported by the Canadian Institutes of Health Research)

C050. Microsatellite/RFLP haplotype analysis at the G6PD locus; Implications for the origin of G6PD A- and Med deficiency mutations

S. Tishkoff[1], *S. Abbes*[2], *G. Argyropolous*[3], *G. Destro-Bisol*[4], *A. Drousiotou*[5], *T. Jenkins*[6], *G. Lefranc*[7], *J. Loiselet*[8], *A. Piro*[9], *M. Stoneking*[10], *A. Tagarelli*[9], *G. Tagarelli*[9], *E. Touma*[8], *S. Williams*[11], *A. Clark*[12]

[1]*University of Maryland; College Park, MD United States;* [2]*Pasteur Institute; Tunis, Tunisia;* [3]*Med. Univ. of South Carolina; Charleston, NC United States;* [4]*University La Sapienza ; Rome, Italy;* [5]*Cyprus Institute of Neurology and Genetics; Nicosia, Cyprus;* [6]*S.A.I.M.R., University of the Witwatersrand; Johannesburg, South Africa;* [7]*Univ. of Sciences and CNRS; Montpellier, France;* [8]*Univ. St. Joseph; Beirut, Lebanon;* [9]*Institute of Experimental Medicine and Biotechnology; Cosenza, Italy;* [10]*Max Planck Institute for Evolutionary Anthro.; Leipzig, Germany;* [11]*Meharry Medical College; Nashville, TN United States;* [12]*Penn State Univ.; University Park, PA United States*

st130@umail.umd.edu

During the course of human evolution in regions where malaria is prevalent, naturally occurring genetic defense mechanisms have evolved which provide resistance against infection by the Plasmodium parasite. Glucose-6-phosphate dehydrogenase (G6PD) deficiency, resulting from mutations within the G6PD gene on Xq28, is the most common enzymopathy of humans and is thought to confer resistance against malarial infection. Three novel, highly variable microsatellite polymorphisms have been identified within 19 kb of the G6PD gene. These microsatellites have been analyzed as RFLP/microsatellite haplotypes on G6PD A , A- , and Med deficiency as well as non-deficiency chromosomes from Africa (n = 425), the Middle East (n = 135), the Mediterranean (n = 119), Europe (n = 15) and New Guinea (n = 25) in order to reconstruct the evolutionary history of the G6PD locus. The A/A- and Med deficiency variants exist on distinct haplotype backgrounds and are in very strong linkage disequilibrium (LD) with flanking markers out to 24 kb. The pattern of haplotype variability and LD is strikingly different on chromosomes with different deficiency variants. Analysis of microsatellite diversity on A- and Med chromosomes indicates that these G6PD deficiency mutations arose within the past 20,000 years, consistent with the hypothesis that malaria has had a major impact on humans since the introduction of agriculture. Funded by NSF Sloan postdoctoral and Burroughs Wellcome Fund Career Award fellowships to ST.

C051. Haplotype Analysis In European Smith-Lemli-Opitz Syndrome Patients Reveals Different Origins And Ages Of Common DHCR7 Mutations

M. Witsch-Baumgartner[1], *J. L ffler*[1], *U. Seedorf*[2], *J. Burn*[3], *G. Gillessen-Kaesbach*[4], *G. F. Hoffmann*[5], *P. Clayton*[6], *R. I. Kelley*[7], *M. Krajewska-Walasek*[8], *G. Utermann*[1]

[1]*Institute of Medical Biology and Human Genetics; Innsbruck, Austria;* [2]*Institute for Arteriosclerosis Research; M nster, Germany;* [3]*University of Newcastle upon Tyne, School of Biochemistry and Genetics; Newcastle upon Tyne, United Kingdom;* [4]*Institute of Human Genetics; Essen, Germany;* [5]*Department of Neuropediatrics and Metabolic Disease, University of Marburg; Marburg, Germany;* [6]*Institute of Child Health and Great Ormond Street Hospital; London, United Kingdom;* [7]*Kennedy Krieger Institute and Department of Pediatrics, Johns Hopkins University School of Medicine; Baltimore, United States;* [8]*Department of Medical Genetics, The Children«s Memorial Health Institute; Warsaw, Poland*

witsch-baumgartner@uibk.ac.at

Smith-Lemli-Opitz syndrome (SLOS [MIM 270400]) is an autosomal recessive multiple congenital anomaly/mental retardation snydrome caused by mutations in the D7-sterol reductase (DHCR7, E.C.1.3.1.21) gene. Analysis of the DHCR7 gene in 59 SLOS patients from Poland, Austria, Germany, and Great Britain revealed 35 different mutations some of which were common. Mutational spectra were significantly different across populations with each of the common mutations showing an east-west gradient (W151X, V326L) or vice versa (IVS8-1G>C). We have started to analyse the ages and origins of SLOS mutations in Europe. Using eight polymorphic sites localised in translated DHCR7 exons 12 different haplotypes were identified among 52 SLOS chromosomes. The 10 SLOS chromosomes carrying the most common mutation IVS8-1G>C shared the same haplotype suggesting a founder effect. This haplotype was also the most frequent on normal chromosomes suggesting that it is the original European haplotype. Mutations T93M and R404C which involve CpG islands were found on 4 and 3 different haplotypes respectively suggesting that they are recurrent. Surprisingly W151X which is the second most frequent mutation in Europe and the most frequent in Polish SLOS patients was detected on 4 different haplotypes including the most frequent one. The three other haplotypes could be derived from the original one by only three base substitutions. This may suggest that W151X is older than the mutations used for haplotype constructions and is the oldest SLOS causing mutation in Europeans. The data suggest an intriguing heterogeneity of the ages and origins of common DHCR7 mutations in Europe.

C052. Evaluation of Linkage Disequilibrium in Densely-Mapped Genomic Regions.

M. C. Karnoub, *M. J. Wagner, D. V. Zaykin, M. G. Ehm, Q. Nguyen, S. Chissoe, E. H. Lai, D. K. Burns*

Glaxo Wellcome, Inc.; RTP, NC United States

mac31876@glaxowellcome.com

Linkage analysis in family collections has resulted in identification of multiple potential susceptibility loci for a number of complex diseases. Resolution of linkage mapping is low, with the confidence interval for location of the susceptibility gene within a linkage peak extending over regions 10 Mbp or more. Association analysis using SNP markers distributed across these regions has the potential for refining the disease susceptibility locus to a region encompassing only one or a small number of genes. To employ this approach, we need high-density maps of SNPs across the chromosomal regions of interest, high-throughput genotyping methods and an understanding of the linkage disequilibrium between the SNP markers. We have identified, mapped and genotyped 450 markers in a 12 Mbp region having shown linkage to type 2 diabetes. The samples used for the analyses include 92 unrelated unaffected Caucasians. We have evaluated the pair-wise linkage disequilibrium between these markers. We have compared the results across different sections of this region. We concluded that for this region, a marker spacing of 30 Kb is needed. Moreover, a significance level of $p<0.001$ identified markers in LD due to close proximity. These data allow us to begin to understand the extent of linkage disequilibrium in an outbred Caucasian sample. They also help us define criteria by which to assess the significance of results that we obtain in our association studies for diabetes.

C053. A linkage disequilibrium map of human chromosome 22

A. Metspalu[1,2,3], *J. Zernant*[2], *T. Puurand*[2], *E. L hmussaar*[1,2], *N. T nisson*[2], *I. Valvas*[2], *H. Pavel*[2], *G. Slavin*[2], *R. Andreson*[1,2], *J. Vilo*[4], *A. Kurg*[1], *D. Cox*[3], *F. Canzian*[3], *E. Dawson*[5], *S. Kirby*[5], *D. Bentley*[5], *I. Dunham*[5]

[1]*University of Tartu, IMCB/Estonian Biocentre; Tartu, Estonia;* [2]*Asper Ltd; Tartu, Estonia;* [3]*IARC; Lyon, France;* [4]*EBI; Hinxton, United Kingdom;* [5]*The Sanger Centre; Hinxton, United Kingdom*

andres@ebc.ee

Sequence variants in the human genome are responsible for the genetic component of disease, individuality and drug response. In order to find associations between SNPs and phenotype, large sample sets need to be genotyped with high-density markers. Large-scale, population-based case-control studies have been limited because neither SNP maps nor genotyping technology have been adequate to fulfill these needs yet. With chromosome 22 fully sequenced and an SNP map constructed, we have performed genotyping on two population samples (CEPH families and Estonians) with 1396 SNP markers. The mean spacing of the SNPs used to construct this map is 22kb. An oligonucleotide array with 5600 oligonucleotides was constructed to genotype each SNP twice from both DNA strands simultaneously using APEX technology. Allele frequencies, Hardy-Weinberg equilibrium and heterozygosities were calculated for each typed marker. Software based on the EM algorithm (Arlequin) was used to calculate the standardized disequilibrium coefficient (D), and GOLD (Graphical overview linkage disequilibrium) plots were constructed. Preliminary

results demonstrate that LD is not continuous, and there are islands with high LD separated by low LD spots. Also, the extent of significant linkage is highly dependent upon the allele frequencies of the two markers in question. In principle, this approach can be extended to the whole genome, which makes genome-wide association studies feasible.

C054. Linkage disequilibrium in 50 candidate genes for cardiovascular diseases

L. Tiret, *O. Poirier, V. Nicaud, S. Barbaux, C. Perret, S. Herrmann, F. Cambien*
INSERM U525; Paris, France
tiret@idf.inserm.fr

In studies of complex diseases, the ability to detect association between marker alleles and disease depends critically on the extent of linkage disequilibrium (LD) between disease alleles and surrounding markers. Limited information is available about LD in candidate genes. We performed a systematic molecular screening of the coding, 5 and 3 regions of 50 candidate genes for cardiovascular diseases. The total length explored spanned 170 kb and 228 polymorphisms were identified. Most of them (87%) were single nucleotide polymorphisms (SNPs). All polymorphisms were genotyped in 750 European subjects. The average minor allele frequency was 0.19 – 0.16, not different between coding and non-coding regions. However, rare polymorphisms (frequency < 0.10) were more frequently seen among non-synonymous SNPs than among silent ones (54% vs 26%, p=0.02) and among nondegenerate sites than among fourfold or twofold degenerate sites (57% vs 29%, p<0.05). The average pairwise standardized LD was 0.78 – 0.31. The mean within-gene LD varied from 0.44 to 1. Across the 50 genes, LD was inversely correlated with the scanned length (r = -0.51). LD was higher between alleles of low frequency than between common alleles (0.84 vs 0.74, p<.001). Among coding polymorphisms, LD tended to be lower when both SNPs were silent than when at least one of them was non-synonymous (0.78 vs 0.86, p=0.06). The variability of LD between genes and according to the type of polymorphism suggests that LD mapping of complex diseases may require a detailed study of the overall sequence variation of candidate genes.

C055. Partial features of Williams-Beuren syndrome in a family with a novel 700 kb 7q11.23 deletion

M. Del Campo[1], *L. F. Magano*[1], *J. Martinez Iglesias*[2], *L. A. Perez Jurado*[1]
[1]*Genetics Unit, Dept of Experimental Sciences, Universitat Pompeu Fabra; Barcelona, Spain;* [2]*Centro de Salud Occidente; Cordoba, Spain*
migueldelcampo@airtel.net

Williams-Beuren syndrome (WBS) is a neurodevelopmental disorder with multisystemic manifestations characterised by distinctive facial features, mental disability with unique cognitive and personality profiles, supravalvular aortic stenosis and other vascular stenoses, growth retardation and occasional infantile hypercalcemia, caused by haploinsufficiency for genes deleted in chromosome band 7q11.23. The great majority of patients show a very similar ~1,6 Mb deletion that arises as a consequence of unequal crossing-over between highly homologous low-copy repeat elements, and includes a number of identified genes. However, with the exception of vascular stenoses caused by haploinsufficiency for the elastin gene (ELN), the other features of WBS have not been clearly attributed to specific genes. A phenotypic map for some clinical manifestations has been proposed based on a few atypical patients with smaller deletions and either a full phenotype (deletion including ELN to GTF2I) or partial phenotypes (several deletions surrounding ELN). We have characterised clinically and molecularly a three generation family with partial features of WBS who were ascertained due to supravalvular aortic stenosis. In addition, they presented with borderline mental functioning, gregarious personality, minor facial WBS features and absence of visual/spatial deficits. Molecular analysis has shown a previously unreported deletion of ~700 kb that includes all genes from ELN to GTF2IRD1. We are currently trying to precisely define the proximal deletion breakpoint. Along with previously reported atypical cases, our data indicate specific genes that are relevant for the cognitive profile and several physical features of the WBS phenotype.

C056. The elastin gene is disrupted in a family with a cytogenetically balanced t(7;16)(q11.23,q12.1) associated with Williams-Beuren syndrome

H. C. Duba[1], *A. Doll*[2], *M. Neyer*[1], *M. Erdel*[1], *C. Mann*[3], *I. Hammerer*[3], *K. H. Grzeschik*[2], *G. Utermann*[1]
[1]*Institut f. Medizinische Biologie und Humangenetik der Universit t; Innsbruck, Austria;* [2]*Medizinisches Zentrum f. Humangenetik der Universit t; Marburg, Germany;* [3]*Universit tsklinik f. Kinder- und Jugendheilkunde; Innsbruck, Austria*
hans-christoph.duba@uibk.ac.at

Williams Beuren Syndrome (WBS) is a complex developmental disorder with multisystemic manifestations including e.g. supravalvular aortic stenosis (SVAS) and a characteristic cognitive phenotype. Most WBS patients have a common microdeletion of the chromosomal region 7q11.23 but with the exception of the elastin (ELN) gene the contribution of other genes in the deleted region to the phenotype is unclear. One strategy to dissect the genetic causes of WBS is to investigate individuals with small deletions and translocations within the common deleted WBS region. We here describe a family with a cytogenetically balanced translocation t(7;16)(q11.23,q12.1) and an extremely variable phenotype. One female presented the full clinical spectrum of WBS, one male needed surgical treatment for severe SVAS and three members of the family presented only with distinct features of WBS. Molecular cytogenetic, molecular and DNA sequence analyses of the cloned translocation breakpoints showed that the cytogenetic rearrangements disrupted the ELN gene locus within intron 5 and the TM7XN1 gene locus on chromosome 16 within intron 1 in exactly the same manner in all translocation carriers. Disruption of TM7XN1 seems to have no obvious phenotype in heterozygotes. It is obvious that ELN - as it is deleted in all hitherto reported WBS individuals - takes a central position in the pathogenesis of WBS. The most likely explanation for the highly variable clinical phenotype in translocation carriers is a position variegation effect of the translocation breakpoint on neighbouring genes. (HCD, AD, KHG and GU contributed equally).

C057. MECP2 Mutations in Sporadic Cases of Rett Syndrome are of Exclusively Paternal Origin

R. Trappe[1], *J. Cobilanschi*[1], *M. Meins*[1], *P. Huppke*[2], *F. Hahnefeld*[2], *W. Engel*[1], *F. Laccone*[1]
[1]*Institut f r Humangenetik, Universit t G ttingen; G ttingen, Germany;* [2]*Abteilung Kinderheilkunde - Schwerpunkt Neurop diatrie, Universit t G ttingen; G ttingen, Germany*
rtrappe@gwdg.de

Rett syndrome (RTT) is known to be an X-linked neurodevelopmental disorder apparently lethal in male embryos. RTT almost exclusively affects girls and is in 99.5% of all cases sporadic due to de novo mutations in the MECP2 gene. Familial cases of RTT are rare and due to X-chromosomal inheritance from a carrier mother. We analysed the parental origin of MECP2 mutations in sporadic cases of RTT by linkage analysis between the mutation in the MECP2 gene and intronic polymorphisms in 27 families with 15 different mutations and found a high predominance of paternal origin of mutations in 26 out of 27 cases (p<0.001). The paternal origin was independent of the type of mutation and was found for single base exchanges as well as for deletions. Parental ages were not significantly increased in theses cases of RTT. We conclude that de novo mutations in RTT occur almost exclusively on the paternally derived X-chromosome and this is most probably the cause for the observed high female;male sex ratio in RTT patients. Affected males have been recently described in a few cases of familial inheritance. We recommend that RTT should be considered as a possible diagnosis in boys with an appropriate phenotype. Identification of parental origin can be useful to distinguish the sporadic form of RTT from a potentially familial form. This will allow geneticists to offer a more specific counselling and to discriminate between high (maternal origin) and low recurrence risk (paternal origin).

C058. A founder mutation in the dysferlin gene is associated with three different phenotypes of muscular dystrophies

A. Lasa[1], *J. Vilchez*[2], *R. Rojas-Garc a*[1], *E. Gallardo*[1], *M. J. Rodriguez*[1], *E. del Rio*[1], *M. Baiget*[1], *I. Illa*[1], *P. Gallano*[1]
[1]*Hospital Sant Pau; Barcelona, Spain;* [2]*Hospital La Fe; Valencia, Spain*
alasa@hsp.santpau.es

Dysferlin gene (DYSF), located on chromosome 2p13, has been identified as the gene responsible for limb-girdle muscular dystrophy type 2B (LGMD2B) and Miyoshi myopathy (MM). Recently, our group has described a new muscular dystrophy phenotype, distal anterior compartment myopathy (DACM), caused by mutations in the same gene. LGMD2B begins proximally in scapular and pelvic girdles, by contrast with MM in which the disorder begins distally in gastrocnemius muscles and with DACM, characterised by onset in the anterior tibial muscles. Here we report six dysferlinopathy patients; two of them were sporadic cases. All the patients came from the same village, Sueca (Valencia, Spain) and, apparently, they do not present consanguinity. The study of the dysferlin gene was performed using the SSCP technique and automated sequencing. All the patients were homozygous for the same mutation; a C to T transition at 6086 position of the cDNA sequence (R1905X). The mutated chromosomes shared the same 2p13 haplotype, suggesting a founder effect of the R1905X mutation in that region. Immunocytochemical analysis of dysferlin expression was undertaken in patients with the homozygous non-

sense mutation that would cause premature termination of traslation. All of them showed total absence of dysferlin. The immunohistochemical studies using antibodies to dystrophin and dystrophin-associated glycoproteins showed a normal pattern. The three phenotypes, LGMD2B, MM and DACM were observed in this group of patients, confirming the clinical variability of dysferlinopathies. These results also support the hypothesis that the DYSF gene itself is not responsible for the variability but additional factors must interact.

C059. A Mouse Model For X-linked Myotubular Myopathy

A. Buj-Bello, *V. Laugel, N. Messaddeq, J. Laporte, F. Blondeau, J. Mandel*
IGBMC; Illkirch, France
abb@titus.u-strasbg.fr

X-linked myotubular myopathy (XLMTM) is a severe congenital muscular disorder characterized by generalized muscle weakness leading in most cases to early postnatal death. Muscle biopsy reveals small rounded fibers with centrally located nuclei resembling fetal myotubes, suggesting that the disease results from an arrest in late myogenesis. We and others have recently shown that the MTM1 gene, mutated in the disorder, encodes a lipid phosphatase implicated in the metabolism of phosphoinositides. In order to understand the pathogenesis of XLMTM, we have generated a mouse model by deleting exon 4 in the MTM1 gene. Male knockout mice are viable and show no apparent phenotype at birth. However, they manifest a growth defect starting at around 4 weeks of age. Concominantly, a muscular deficit appears in the hindlimbs and progressively generalises to the forelimbs at around 5-6 weeks of age. KO mice present ciphosis and become paralysed from the hindlimbs. Death occurs at about 2-3 months of age, probably from respiratory failure and cachexia. No clinical phenotype has been observed in heterozygous females so far. We will present data on the histopathological analysis of the skeletal muscle in KO mice, which contains small fibers with centrally located nuclei. This model gives insights into the pathophysiology of the disease and shows that despite its ubiquitous expression, the MTM1 gene has a muscle specific function.

C060. Mouse models for Friedreich ataxia exhibit cardiomyopathy, sensory nerve defect and Fe-S enzyme deficiency followed by intramitochondrial iron deposits

H. M. Puccio[1], *D. Simon*[1], *P. Criqui-Filipe*[1], *N. Lagarde*[1], *L. Reutenauer*[1], *P. Rustin*[2], *J. L. Mandel*[1], *M. Koenig*[1]
[1]*IGBMC/ INSERM; Illkirch, France;* [2]*H pital Necker; Paris, France*
hpuccio@igbmc.u-strasbg.fr

Friedreich ataxia (FRDA), the most common autosomal recessive ataxia, associates degeneration of the large sensory neurons and spinocerebellar tracts, cardiomyopathy and increased incidence in diabetes. FRDA is caused by severely reduced levels of frataxin, a mitochondrial protein of unknown function. Yeast knock-out models, and histological and biochemical data from patient heart biopsies or autopsies indicate that the frataxin defect causes a specific iron-sulfur protein deficiency and mitochondrial iron accumulation leading to the pathological changes. These results suggest that frataxin has a role in controlling mitochondrial iron homeostasis and that FRDA might be caused by oxidative damage. A key tool for understanding Friedreich s ataxia pathology and for testing Friedreich s ataxia therapeutic protocols is the availability of a mouse model that most closely mimics the human disease. Through a conditional gene-targeting approach, we have generated in parallel a striated muscle frataxin-deficient line and neuron/cardiac muscle frataxin-deficient line, which together reproduce important progressive pathophysiological and biochemical features of the human disease, including cardiac hypertrophy without skeletal muscle involvement, large sensory neuron dysfunction without alteration of the small sensory and motor neurons, and deficient activities of complexes I-III of the respiratory chain and of the aconitases. To better understand the pathophysiology of the disease, we have performed detailed ultrastructure and biochemical studies of the cardiac phenotype. Time course experiments show an initial abnormal abundancy of variable size lipid droplets in the cardiac myofibrils with the presence of giant disorganized mitochondria. These lipid droplets disappear as the cardiac hypertrophy increases, and a compensatory proliferation of mitochondria occurs followed by a progressive intra-mitochondrial iron accumulation. Biochemical experiments reveal that the Fe-S enzymes deficiencies begin in the initial phase of the pathology, at around the time of lipid droplets accumulation, with a gradual decrease in their activities over the course of the disease. Our models, therefore, allow to demonstrate time-dependent intramitochondrial iron accumulation in a frataxin deficient mammal, which occurs after onset of the pathology and after inactivation of the Fe-S-dependent enzymes. These mutant mice represent the first mammalian models to evaluate treatment strategies for the human disease. We have initiated a pharmacological trial by administrating idebenone, a lipid-soluble antioxidant (a coenzyme Q) already used in preliminary human clinical trials, to the striated-muscle deficient mutant mouse line. We are following the clinical course and physiology of the treated and untreated mice, as well as assessing the effect of idebenone at different time point during the development of the disease in order to determine its prevention efficacy.

C061. The Study Of Gene-environment Interactions Using The Alspac Cohort.

M. E. Pembrey[1], *R. Jones*[2], *S. Ring*[3], *R. Hamvas*[1], *L. Tyfield*[3], *J. Golding*[2], *-. Alspac Study Team*[2]
[1]*Institute of Child Health, UCL; London, United Kingdom;* [2]*Institute of Child Health, University of Bristol; Bristol, United Kingdom;* [3]*Department of Molecular Genetics, Southmead Hospital; Bristol, United Kingdom*
mpembrey@picketts.u-net.com

ALSPAC (Avon Longitudinal Study of Parents and Children, formerly Avon Longitudinal Study of Pregnancy and Childhood) was designed to study the ways in which the physical and social environment interact, over time, with genetic variation to affect development, health and behaviour in children and the adults they become. 13,900 pregnant women reaching 20 weeks gestation (EDD 1.4.91 — 31.12.92) were enrolled, representing about 85% of the eligible population around Bristol. The cohort has been followed in great detail, including extensive hands-on assessment and clinical testing in special clinics (www.ich.bris.ac.uk/external/html). DNA is banked on the children, mothers and some fathers, and cell lines on ~10,000 children and 15,000 parents will be generated over the next 5 years. The central tenet of the ALSPAC approach is the hypothesis that most genetic influences in common disorders are conditional on the environment and many gene variants will exert their main effect at particular developmental stages. Early results from ALSPAC pilot studies (10% subset) support this view. Of the first 10 candidate gene studies with collaborators on early growth, allergy and infection, results support a prior hypothesis in 5. These include associations of size at birth/early growth with INS VNTR, mt16189v and ACE, and between eczema and IL4R depending on early infection or not. We believe the high hit rate with candidate gene association studies on just 10% of the sample reflects the precision and detail of longitudinal outcome data and the substantial information on environmental variables. Approaches about collaborations are welcome (see web site).

C062. Association between ancestral haplotypes and multiple sclerosis in Central Sardinia

L. Bernardinelli[1], *P. Bitti*[2], *C. Montomoli*[1], *B. S. Murgia*[3], *A. Ticca*[3], *R. Ferrai*[3], *M. Piras*[3], *L. Musu*[3], *A. P. Mander*[4]
[1]*Dep. Applied Health Sciences, University of Pavia; Pavia, Italy;* [2]*Centro Tipizzazione Tissutale, Ospedale San Francesco; Nuoro, Italy;* [3]*Divisione di Neurologia, Ospedale San Francesco; Nuoro, Italy;* [4]*Medical Research Council Biostatistics Unit, Institute of Public Health; Cambridge, United Kingdom*
luisa@fisher.unipv.it

Association and linkage studies have established the importance of the Major Histocompatibility Complex (MHC) in the susceptibility for Multiple Sclerosis (MS). In particular, association with HLA-A3, B7 and DR15 was first reported in patients of Northern European ancestry, while an association with DR3 and DR4 was reported in Sardinians. Recent genome scans confirmed the importance of the MHC region, highlighting the role of TNFa and of the DR region. Our work investigates the association between the A30B18DR3 ancestral haplotype and MS in the population of Central Sardinia, where malaria was endemic since pre-historical times in low laying areas. This is an isolated population, genetically distinct from others populations in the Mediterranean basin and characterized by high genetic homogeneity, high level of inbreeding, low migration, high prevalence of MS and high frequency of the A30B18DR3 haplotype. We carried out a case-control study, cases being MS patients and controls bone marrow donors from the bone marrow register covering the area. Cases and controls were serologically typed for the currently recognized HLA A, B and DR antigens. We used a log-linear approach to fit a wide class of models. We overcame the complication due to the unknown gametic phase using an Expectation-Maximization (EM) algorithm as estimation method. We found that (1) the ancestral haplotype A30B18DR3 was associated to MS after allowing for a possible genetic stratification in cases and controls; (2) DR3 was conditionally independent on disease status, given the A30B18 haplotype; (3) there was a tendency for Odds Ratios of the high-risk haplotypes to be higher in individuals originating from high past malaria prevalence areas. Results on other ancestral haplotypes will be also presented.

C063. A Polymorphism in the Gene for Insulin-like Growth Factor-I; Functional Properties and Risk for Atherosclerosis and Myocardial Infarction

N. Vaessen[1], *A. F. C. Schut*[1], *J. C. M. Witteman*[1], *P. Heutink*[1], *J. A. Janssen*[2], *L. Testers*[1], *A. Hofman*[1], *S. W. J. Lamberts*[2], *B. A. Oostra*[1], *H. A. P. Pols*[2], *C. M. van Duijn*[1]

[1]*Erasmus medical centre, genetic epidemiology unit; Rotterdam, The Netherlands;* [2]*Erasmus medical centre, dept. of internal medicine; Rotterdam, The Netherlands*

vaessen@epib.fgg.eur.nl

Evidence is accumulating that low levels of insulin-like growth factor-I (IGF-I) play a role in the pathogenesis of cardiovascular diseases. We examined the role of a genetic polymorphism in the promoter region of the IGF-I gene in relation to circulating IGF-I levels and body height, and we studied the relationship of this polymorphism with atherosclerosis and myocardial infarction. The relation between the IGF-I polymorphism, circulating IGF-I levels, body height and atherosclerosis was assessed in population-based sample of 900 subjects. Atherosclerosis was measured as rontgenographic detectable aortic calcifications, atherosclerotic plaques or increased intima-media thickness of the common carotid artery or a decreased ankle-arm index. To assess the risk of myocardial infarction, 477 patients with evidence for myocardial infarction at ECG and 808 controls were studied. A 192-bp allele was present in 88% of the population. Body height was on average 2.7 cm lower (p = 0.004) and serum IGF-I concentrations were 18% lower (p = 0.003) in subjects who did not carry the 192-bp allele. Although no significant association was found between the IGF-I polymorphism and atherosclerosis, non-carriers of the 192-bp allele had an increased risk for myocardial infarction (RR 1.7 [95% CI 1.1-2.5]). Further analysis demonstrated that the risk for myocardial infarction associated with the absence of the 192-bp allele was almost exclusively restricted to subjects with atherosclerosis (RR 3.4 [95 % CI 2.9- 5.6). Our study suggests that genetically determined exposure to relatively low IGF-I concentrations in subjects without the 192-bp allele, results in an increased risk for myocardial infarction, especially in subjects with profound atherosclerosis.

C064. Genetic determinants of calcium metabolism; Comparison of calcium-sensing receptor (CASR) and vitamin D receptor (VDR) polymorphisms.

D. E. C. Cole[1], *A. B. Scillitani*[2], *R. Vieth*[3], *B. Y. L. Wong*[4], *H. Trang*[1], *G. N. Hendy*[5], *L. A. Rubin*[6]

[1]*Banting Institute; Toronto, ON Canada;* [2]*Casa Sollievo Sofferenza; San Giovanni Rotondo, Italy;* [3]*Mount Sinai Hospital; Toronto, ON Canada;* [4]*University Health Network; Toronto, ON Canada;* [5]*McGill University; Montreal, PQ Canada;* [6]*St Michael s Hospital; Toronto, ON Canada*

davidec.cole@utoronto.ca

Serum calcium concentration is a continuous trait, maintained in a narrow range for each individual. Genetic factors are thought to account for more than half of the between-person variability, but biochemical covariates such as serum albumin are also important. Recently, we demonstrated that in a sample of young, caucasian healthy women, the calcium-sensing receptor (CASR) variant, A986S, exhibits a significant association with the free calcium fraction. Since vitamin D is involved in calcium homeostasis, we asked whether vitamin D receptor (VDR) polymorphisms might be co-contributing to the genetic variance of serum calcium levels. In 419 healthy Toronto women 18 to 35 years old, we characterized the silent Taq1 polymorphism in exon9 of the VDR gene [frequency of the variant t allele (f_t) = 0.39] and the translation initiation codon polymorphism detected by Fok1 digestion (f_f = 0.40). We also characterized three CASR polymorphisms A986S (f_S = 0.15), R990G (f_G = 0.08), and IVS5 T-88C (f_C = 0.31). Partial correlation coefficients (corrected for age, weight, height, serum phosphate, serum creatinine, serum albumin, and serum total globulins) show significant association of serum calcium with all three CASR polymorphisms; A986S (r = 0.291, p = 0.0001), IVS5 T-88C (r = -0.11, p = 0.032), and R990G (r = -0.10, p = 0.050). No correlation was observed with either of the VDR polymorphisms; Fok1 (p = 0.77) or Taq1 (p = 0.57). These results confirm that variations in extracellular calcium concentrations may be determined in part by CASR, but there is no apparent contribution of the VDR polymorphisms examined here. Since other studies have suggested that urinary calcium excretion is determined in part by VDR (Bone 1998;5;S248), it seems likely that multiple loci contribute co-ordinately to the genetic variability of calcium metabolism in humans.

C065. DNA variants in the NQO1, MPO, and CYP2E1 genes and the susceptibility to childhood acute lymphoblastic leukemia.

M. Krajinovic, *D. Labuda, D. Sinnett*

Ste-Justine Hospital; Montreal, PQ Canada

sinnettd@ere.umontreal.ca

Acute lymphoblastic leukaemia (ALL) is the most common paediatric cancer. Little is known about the genetic factors underlying the susceptibility to this disease. The enzymes CYP2E1, MPO and NQO1 are involved in the biotransformation of a variety of xenobiotics present in organic solvents, tobacco smoke, drugs, plastic derivatives and pesticides. They also control the level of the oxidative stress by catalysing formation of free radicals or by protecting the cells from their deleterious effect. DNA variants in these genes have been associated with an increase susceptibility to different adult cancers, including haematological malignancies. To investigate the role of these loci and in particular their genetic variants as risk modifying factors in childhood ALL, we conducted a case-control study on 181 ALL patients and 337 healthy controls, both of French-Canadian origin. We found that the carriers of the CYP2E1*5 variant were found at 2.8 fold (95% CI, 1.2-6.6) higher risk of ALL, and that NQO1 alleles *2 and *3 contributed as well to the risk of ALL (OR=1.7, 95%CI, 1.2-2.5). No such association was found with MPO alone. However, when the wild-type MPO allele was considered together with the CYP2E1 and NQO1 risk-elevating genotypes, the risk of ALL was increased further (OR=5.4, 95%CI, 1.2-23.4) suggesting a combined effect. These findings suggest that xenobiotics metabolized by these three enzymes might be implicated in the etiology of childhood ALL.

C066. DNA repair gene variants as candidate genes for low-penetrance breast cancer susceptibility

B. Kuschel[1], *A. Auranen*[1], *K. Thorpe*[1], *S. McBride*[1], *P. D. P. Pharoah*[1], *D. F. Easton*[2], *B. A. J. Ponder*[1], *A. M. Dunning*[1]

[1]*CRC Human Cancer Genetic Group; Cambridge, United Kingdom;* [2]*CRC Epidemiology Group; Cambridge, United Kingdom*

bettina.kuschel@srl.cam.ac.uk

Carcinogens such as ionizing radiation frequently generate DNA-double strand breaks (DSB). Two pathways are involved in their repair; The homologous recombination (HR) and the non-homologous end-joining (NHEJ) pathways. HR is thought to be the major pathway for repairing severe damage in mammals. In a British population-based breast cancer case-control series (~2000 cases and ~1000 controls) we are performing association studies by identifying and analysing polymorphisms of potential functional significance in genes involved in HR (NBS1, Rad52, Rad51, Rad54, XRCC2 and XRCC3) and NHEJ (Mre11, Rad50, Ku70/80, Ligase IV, XRCC4). In HR we have identified a polymorphism in XRCC3 (T241M) which appears to contribute to an increased risk of breast cancer [OR (MMvs.TT); 1.3; 95%CI; 1.0-1.7; OR (MTvs.TT); 1.1; 95%CI; 0.9-1.3]. This polymorphism is close to an ATPase activity domain and might have functional significance. Other polymorphisms in XRCC3 [A4541G (OR; 1.1; 95%CI; 0.7-1.7), A17893G (OR; 0.8; 95%CI; 0.6-1.1)] are not associated with a significantly increased risk. Nor are Rad51 variants [G135C (OR; 0.9; 95%CI; 0.3-2.5) and G172T (OR; 0.9; 95%CI; 0.7-1.2)] or C2259T (3 UTR) of Rad52 (OR 0.9; 95%CI; 0.7-1.1). In the NHEJ pathway Mre11, Rad50, XRCC4 and Ligase IV have been studied so far and non of these variants have a significant effect on breast cancer risk. We aim to define which genes in these pathways are most important in breast cancer risk and how they interact.

C067. Physical and transcript map of the hereditary sensory neuropathy type 1 locus on chromosome 9q22.

K. Bejaoui[1], *C. Wu*[2], *P. de Jong*[2], *R. H. Brown, Jr.*[1]

[1]*Massachusetts General Hospital; Charlestown, MA United States;* [2]*Pfizer Laboratory for Molecular Genetics; Alameda, CA United States*

bejaoui@helix.mgh.harvard.edu

Objective; Positional cloning of the hereditary sensory neuropathy type1. Background; Hereditary sensory neuropathy type 1 (HSN-1) is a familial peripheral neuropathy characterized by loss of temperature sensation, ulceration and osteomyelitis of the digits and slight distal motor involvement. HSN-1 is associated with severe loss of ganglion cells in the sacral and lumbar dorsal root ganglia, as well as degeneration of the sensory fibers in the dorsal roots. The mode of inheritance of HSN-1 is autosomal dominant with onset typically in the second or third decade. HSN-1 is a heterogeneous disease that involves at least three loci. We and others have previously mapped one of the defective genes to human chromosome 9q22 in large informative HSN-1 families of diverse ethnic groups. We subsequently constructed a 2.5-Mb yeast artificial chromosome (YAC) contig that spans the HSN-1 locus. Haplotype analysis of various HSN-1 families

identified the markers D9S1941, D9S197 as flanking markers of the HSN-1 locus. To help our search for the gene defect, we have cloned the affected part of chromosome 9 in bacterial and P1-derived artificial chromosomes Design/Methods; To isolate genomic clones that span the HSN-1 locus, we screened three genomic PAC libraries and one BAC library with 45 markers that have been roughly mapped within our region of interest on chromosome 9. Information for all markers were obtained from the web pages (http;//www.genome.wi.mit.edu/;
http;//www.ncbi.nlm.nih.gov/genemap/;
http;//www.shgc.stanford.edu/Mapping/rh/).
We labeled DNA markers either by random oligonucleotide-primer synthesis or overgo labeling (http;//informa.bio.caltech.edu/). We analyzed the expression profile of the putative transcripts by two complementary approaches; northern blot analysis of a set of 15 adult human tissues and amplification of the 5 end sequences by SMART RACE-PCR from a selection of mRNA tissues, including rat dorsal root ganglia. To prioritize our search toward the transcripts expressed in the DRG neurons we also screened all candidate transcripts for expression in a rat DRG cDNA library. Clones that both map within the HSN-1 contig and are expressed in DRG were the first to be analyzed for mutations. To identify overlapping cDNA clones that cover the entire transcripts, we used the EST assembly machine at Tigem (http;//hercules.tigem.it). We determined the genomic structure of novel genes by using both the unfinished and the finished Human Genome Sequences databases at the NCBI (www.ncbi.nlm.nih.gov/blast). We scrutinized the candidate genes for mutations using SSCP analysis and cycle sequencing, either from genomic DNA or RNA using RT-PCR. Results; We have established a bacterial contig flanked by markers D9S1841 and D9S197. This contig spans 1.6 Mb. It consists of 112 clones (32 PACs and 80 BACs) and 51 markers. We have identified within this contig a total of 26 transcriptional units, including four that are expressed in the DRG. Among these four, three are novel genes. We have determined the sequence of the full-length transcripts, determined the exon-intron boundaries and analyzed the exons in our HSN-1 patiens in these four genes. Our preliminary data suggest that one may be defective in some HSN-1 families. Conclusions; Using various resources available through the human genome project, and informative HSN-1 families of various ethnic groups we were able to; 1) map one HSN-1 gene to human chromosome 9q22, 2) establish a physical and transcript map of the candidate region, 3) precisely map 26 genes in this region, including four expressed in the DRG. Preliminary screening analyses suggest that one of these may be mutated in some HSN-1 families.

C068. Molecular Profiling Of Cells From Carriers Of The ATM Mutations.

J. Watts, *J. Burdick, R. S. Spielman, V. G. Cheung*
University of Pennsylvania; Philadelphia, PA United States
vcheung@mail.med.upenn.edu

Patients with ataxia telangiectasia (AT) have two mutated copies of the gene ATM (ataxia telangiectasia mutated) and occur approximately 1 in 40,000 births. In contrast, heterozygous carriers for mutations in the ATM gene constitute about 1-2% of the general population. ATM protein plays a key role in cellular response to DNA damage caused by ionizing radiation (IR) and the maintenance of genomic integrity. Studies show AT carriers are more sensitive to IR and have increased risk for cancer, compared to normal controls. At present, there is no diagnostic tool for identification of AT carriers. Our goal is to determine a set of genes differentially expressed in normal and AT carrier cells. This molecular fingerprint could then be used to determine AT carrier status. We have used cDNA microarrays to determine the mRNA expression level of several thousand genes in 12 AT carrier and 12 normal lymphoblastoid cell lines. Expression profiles were generated for cells at baseline and after treatment with 3-Gy gamma radiation. There were marked, statistically significant differences in the expression level of genes at baseline and at subsequent timepoints post-IR treatment. We will present our work in determining a molecular fingerprint, a set of differentially-expressed genes, from AT carriers and normal controls.

C069. Correlations among DNA sequence variants in 97 genes on human Chromosome 22

W. R. Newell, *T. Acharya, R. Jiang, T. Kalbfleisch, C. Nicholson, J. Schneider, S. Stanley, G. F. Vovis, J. C. Stephens*
Genaissance Pharmaceuticals; New Haven, CT United States
w.newell@genaissance.com

3509 SNPs were discovered and genotyped in 97 genes on Chromosome 22 in 93 individuals from four broadly defined ethnic populations (African-Americans, Asians, European-Americans and Hispanic-Latinos). The sequences comprise exons and flanking intronic sequences on 22q. They were assigned unique locations on the chromosome by sequence comparison against the chromosome 22 reference sequence (1). The most common genotypes were defined for each gene from a set of SNPs selected from the most variable SNPs, accounting for 80% of the total SNP variability, since these are the most informative of the major genotypes. Accordingly, the number of SNPs used to define genotypes varies by gene and by population. An average of 11 distinct genotypes were observed for each gene. The strength of association between the genotypes for each pair of genes was evaluated primarily by the uncertainty coefficient, U, based on Information theory. The value of U ranges from 0 (no association) to 1 (complete association). Of the 18624 comparisons, 27 were determined by Freeman-Halton-Fisher s exact test to be significant with $p < 0.001$. Of these significant associations, the average U is 0.71, indicating that knowledge of the genotype of one of the genes is highly informative of the genotype of the other gene. Possible functional mechanisms for these strong associations are discussed. The strength of association between the different genes has also been compared with the known physical distances between them. Mapping data from different sources have been integrated, enabling the assignment of unique chromosomal locations to each SNP. 1. Dunham, I., Shimizu, N., Roe, B.A., Chissoe, S. et al. 1999. The DNA sequence of human chromosome 22. Nature 402, 489-495.

C070. High-throughput SNP genotyping by MALDI-TOF MS

M. Kostrzewa[1], *T. Fr hlich*[1], *T. Wenzel*[1], *C. Franke*[1], *W. Pusch*[2], *K. O. Kr uter*[2], *Y. Stalgies*[2]
[1]*Bruker Saxonia Analytik GmbH, Bioanalytics; Leipzig, Germany;* [2]*Bruker Daltonik GmbH; Bremen, Germany*
AKR@bdal.de

One of the great challenges of the upcoming postgenomic era is the determination of sequence variations, in particular single nucleotide polymorphisms (SNPs). These polymorphisms which occur with a frequency of more than 1/1000 in the human genome are believed to have an enormous impact in diagnosis of diseases, improvement of drugs, and forensic analysis in future. In contrast to the increasing demand for SNP genotyping, there is still a lack of highly reliable high-throughput SNP typing techniques. Matrix-assisted laser desorption/ionization time-of —flight mass spectrometry (MALDI-TOF MS) is a powerful advanced technology capable of the accurate and cost-effective typing of many thousands of SNPs per day. The GOOD assay , a novel sample preparation procedure for MALDI-TOF typing of SNPs was recently presented (Sauer et al., Nucleic Acids Research, methods online, 2000, 28, e13). This preparation uses sensitivity enhancing, chemical modifications of the allele specific products. Main advantage of the GOOD assay over other SNP genotyping preparation methods with MALDI detection is that this protocol does not require solid phase purification of the products prior MALDI analysis. SNP typing starts with a PCR encompassing a region containing a known SNP. Thereafter dNTPs are digested with shrimp alkaline phosphatase. In the following primer extension reaction using a charge tag containing extension primer, a conditioned set of ddNTPs and a DNA polymerase of allele specific products are generated. The 5 part of the extension primer is removed by an exonuclease. By alkylation the backbone phosphate groups are neutralised. This results in small singly charged product molecules which are transferred onto non-protonating matrix on the MALDI target. Due to the lack of mass and charge tagged nucleotides, flexibility of the GOOD assay was limited. Therefore, we synthezised a set of propargylamine-linked nucleosides which allow the charge-tagging of each of the four different nucleobases. In addition, we introduced charge tag molecules of different masses. Thereby we significantly increased flexibility and multiplexing capability of the GOOD assay . Mass spectra of 384 samples can be acquired in less than one hour. Allele calling is performed automatically by the novel genotools SNP manager software, online during spectra acquisition or offline, after measurement of a set of samples. The resulting genotypes are stored in a table in the ASCII formate together with a quality assessment.

C071. Comparison of Human Genetic and Physical Maps

J. L. Weber[1], *K. W. Broman*[2], *A. Yu*[1], *C. Zhao*[1], *Y. Fan*[1], *W. Jang*[3], *N. Ghebranious*[1], *A. J. Mungall*[4], *P. Deloukas*[4], *N. A. Doggett*[5], *A. Olsen*[6]
[1]*Marshfield Medical Research Foundation; Marshfield, WI United States;* [2]*Johns Hopkins University; Baltimore, MD United States;* [3]*National Institutes of Health; Bethesda, MD United States;* [4]*Sanger Centre; Cambridge, United Kingdom;* [5]*Los Alamos National Laboratory; Los Alamos, NM United States;* [6]*Joint Genome Institute; Walnut Creek, CA United States*
weberj@cmg.mfldclin.edu

Completion of the draft human genomic sequence offered the best opportunity to date to compare genetic and physical maps. Using computer searches we identified BAC sequences which encompassed about 75% of the 8000 short tandem repeat polymorphisms (STRPs) on our most recent

comprehensive genetic maps (Broman et al. Am. J. Hum. Genet. 63;861-869, 1998). Physical distances between STRPs were obtained from the Golden Path draft sequence assemblies, Sept. 5, 2000 version (http;//genome.cse.ucsc.edu). Genetic distances were obtained from new maps rebuilt using CEPH family genotyping data and marker order from the draft sequence assemblies. Recombination rates were found to vary greatly along each chromosome ranging from 0 to at least 9 cM/mb. Mean recombination rates were 0.92, 1.30 and 1.68 cM/mb for males, sex-average, and females, respectively. In general, female and male recombination rates varied independently of each other. Among several sequence and marker parameters tested including G + C content, density of SINEs and LINEs, density of various STRs, and marker heterozygosity, only relative marker position along the metacentric chromosomes in males correlated strongly with recombination rate. We identified several chromosomal regions up to 6 mb in length which were especially low (deserts) or high (jungles) in recombination rate. Because only 184 meioses (92 in each sex) were used to construct the genetic maps, confidence intervals for both genetic distances and recombination rates were relatively broad. Nevertheless, linkage disequilibrium was much more common and extended to greater distances in the deserts compared to the jungles. Estimates of recombination rates for individual STRPs along with accession numbers for overlapping BACs are available from the Marshfield web site (http;//research.marshfieldclinic.org/genetics).

C072. Comparative gene and genome mapping in pufferfish and humans

F. Gruetzner[1], *H. Roest Crollius*[2], *J. Weissenbach*[2], *G. L tjens*[1], *H. H. Ropers*[1], *T. Haaf*[1]

[1]*Max-Planck-Institute of Molecular Genetics; Berlin, Germany;* [2]*Genoscope; Evry, France*

gruetzne@molgen.mpg.de

Because of its highly compact genome, the pufferfish has become an important animal model in genome research. Although the small chromosome size renders chromosome analysis difficult, we have established both classical and molecular cytogenetics in the freshwater pufferfish Tetraodon nigroviridis (TNI), which is relatively easy to obtain and to keep in aquaria. TNI, as well as the Japanese pufferfish, Fugu rubripes (FRU), serve as models for large-scale genomic sequencing and other genome projects. The comparative mapping of 49 TNI specific BAC clones, which have orthologous genes on human X chromosome and chromosome 9 on TNI chromosomes, revealed remarkable differences in conservation of chromosomal synteny in the pufferfish. 24 of 31 BACs derived from human X chromosomal genes were distributed on only 4 different TNI chromosomes, delineating 4 large segments of conserved chromosomal synteny. In contrast, only 4 of 18 BACs isolated with genes from human chromosome 9 were linked in TNI. Therefore, the extraordinary conservation of the X chromosome, can now be extended to lower vertebrate species like pufferfish and hence seems not only due to mammalian X inactivation. In addition, we have generated chromosome-specific DNA libraries of pufferfish chromosomes by microdissection. Southern hybridization of these libraries to gridded filters was used to identify chromosome-specific genes and genomic clones to improve our first-generation homology map. Since TNI and FRU diverged only about 18 million years ago, probes from one species can easily be hybridized on chromosomes or filters of the other species.

C073. Two Year Experience of Enzyme Replacement Therapy for Mucopolysaccharidosis I

J. Muenzer[1], *G. Tiller*[2], *L. Waber*[3], *J. Belmont*[4], *M. Passage*[5], *B. Izykowski*[5], *J. Phillips*[5], *R. Doroshow*[5], *I. Walot*[5], *R. Hoft*[5], *K. Ti Yu*[5], *S. Okazaki*[5], *D. Lewis*[5], *R. Lachman*[5], *J. Thompson*[6], *E. Neufeld*[7]

[1]*University of North Carolina; Chapel Hill, NC United States;* [2]*Vanderbilt University; Nashville, TN United States;* [3]*University of Texas Southwestern Medical Center; Dallas, TX United States;* [4]*Baylor College of Medicine; Houston, TX United States;* [5]*Harbor-UCLA Medical Center; Torrance, CA United States;* [6]*University of Alabama at Birmingham; Birmingham, AL United States;* [7]*UCLA School of Medicine; Los Angeles, CA United States*

muenzer@css.unc.edu

Mucopolysaccharidosis I (MPS I) is caused by a deficiency of the lysososmal enzyme, ?-L-iduronidase. Recombinant human ?-L-iduronidase has been studied as an enzyme replacement therapy with follow-up data available for the first 2 years. Ten MPS I patients representing a spectrum of severity were treated weekly with intravenous infusions of ?-L-iduronidase. Liver volumes after 104 weeks of treatment were reduced 24.0% ($p<0.001$) from pretreatment with a range of 17.2 to 33.2%. Spleen volumes were reduced 22.1% ($p<0.05$) from pretreatment. At 104 weeks, urinary glycosaminoglycan excretion was decreased 72.1% ($p<0.001$) from pretreatment levels compared with 62.8% ($p<0.001$) at 52 weeks. Modest further improvement in shoulder range of motion was observed relative to 52 weeks of therapy. Improved NY Heart Association cardiac function scores were maintained at 104 weeks compared to 52 weeks, and no significant changes were observed on echocardiograms. In prepubertal patients, height and weight increased 8.0% and 35.3%, respectively, over the 2 years of treatment with similar growth rates at weeks 52 and 104. Hypersensitivity reactions were the most common adverse events, and urticaria was most frequent. Reactions were managed with increased premedications and slower infusion rates. Antibodies to the product declined with time and clinical reactions resolved. One patient died just prior to the 104 week evaluations due to an illness not directly related to therapy. The two year follow-up data suggest that treatment of MPS I patients with recombinant human ?-L-iduronidase continues to be beneficial and appears safe.

C074. Enzyme Replacement Therapy Reverses the Cardiomyopathy of Fabry Disease; Results of a Randomized, Double Blind, Placebo Controlled Trial

K. MacDermot, *A. Brown, Y. Jones, J. Zuckerman*

Royal Free Clinical Trial Centre, Royal Free and University College Medical School; London, United Kingdom

tschuetz@tktx.com

Purpose; Fabry Disease is a lysosomal storage disorder caused by deficiency of the enzyme a-galactosidase A. Globotriaosylceramide (Gb3), the glycosphingolipid substrate of the enzyme, accumulates within multiple cell types. Deposition occurs within cardiac myocytes, neurons, and renal glomerular and tubular epithelial cells. Patients suffer from serious debilitating pain, progressive cardiomyopathy and renal failure leading to premature mortality in the fourth or fifth decade of life. Methods and Materials; Fifteen patients with left ventricular hypertrophy and classic Fabry Disease were randomised to receive 12 biweekly infusions of 0.2 mg/kg of Replagal (human a-galactosidase A) or placebo. Outcome measures focused on the cardiomyopathy of Fabry Disease and Gb3 levels. Results; There was a statistically significant decrease in cardiac mass in the Replagal treated patients and an increase in cardiac mass in the placebo patients. Replagal effected a 4.2% decrease in cardiac mass, but placebo was associated with an 8.8% increase in cardiac mass ($p=0.041$). Replagal corrected the metabolic defect of Fabry Disease as measured by a 50% decrease in Gb3 storage in plasma ($p<0.001$) and urine sediment ($p=0.047$). Enzyme infusions were extremely well tolerated. Two of 7 patients (29%) developed a low titre IgG antibody response to Replagal, and 1 of these patients developed immunological tolerance. Conclusion; Therapy with Replagal was demonstrated to be extremely well tolerated. Therapy with Replagal has been demonstrated to reverse the cardiomyopathy and correct the metabolic defect of Fabry Disease.

C075. Substrate Reduction Therapy in Type 1 Gaucher Disease with OGT918

C. Moyses

Oxford GlycoSciences; Abingdon, United Kingdom

chris.moyses@ogs.co.uk

Gaucher disease is a rare inherited deficiency of the lysosomal enzyme glucocerebrosidase, which results in glucocerebroside storage in the liver and spleen, haematological abnormalities and accompanying bone complications. Current treatment involves giving recombinant macrophage targeted beta-glucocerebrosidase by repeated intravenous infusion. N-butyldeoxnojirimycin (OGT918, Vevesca“), an imino sugar which partially inhibits the ceramide-specific glucosyltransferase, the enzyme which initiates the glycosphingolipid biosynthetic pathway and catalyses the formation of glucocerebroside, is currently being tested in the clinic as a potential oral therapy for this disorder. Results from the first clinical study of OGT918 in 28 patients with type 1 Gaucher disease have shown significant improvements in liver and spleen organ volumes, hematological parameters and other markers of disease activity.1,2. Trials are ongoing to investigate further OGT918 as a therapy for type 1 Gaucher disease including a monotherapy study to assess dose effect in an additional 18 patients, plus a randomised study of patients who have received enzyme replacement therapy (ERT) for a minimum of 2 years. In this study, 36 patients were randomised to remain on their existing ERT (N=12), switch to OGT918 (N=12), or add OGT918 to their current ERT (N=12). These studies are complete and comparative efficacy and clinical safety measurements between treatment arms will be presented. 1. Cox T et al. The Lancet 2000; 355; 1481-1485 2. Zimran A et al. Fourth European Working Group on Gaucher Disease, Jerusalem; September 2000

C076. Tissue specific inducible expression of the human acid alpha-glucosidase (GAA) gene in GAA knockout mice; implications for therapy.

N. Raben[1], *N. Lu*[1], *Y. Rivera*[1], *K. Nagaraju*[1], *J. Hopwood*[2], *K. Umapathysivam*[2], *P. Meikle*[2], *P. Plotz*[1]
[1]*National Institutes of Health; Bethesda, MD United States;* [2]*Women s and Children s Hospital; North Adelaide, Australia*
rabenn@arb.niams.nih.gov

Deficiency of acid alpha-glucosidase (GSDII, Pompe disease) results in generalized deposition of lysosomal glycogen and manifests as cardiomegaly and muscular weakness fatal in infancy. We have used a tetracycline-controllable system to rescue the phenotype of the GAA ko mice by expressing hGAAcDNA-IRES-EGFP either in their skeletal muscle (mck-GAA/-/-) or liver (albGAA/-/-). In the two mck lines, the levels of GAA activity in muscle were increased ~5-fold (low) and ~100-fold (high) compared to the GAA+/- controls. These levels were sufficient to provide phenotypic correction locally — no glycogen accumulation was observed in muscle. However, systemic correction was observed only in the high expresser line. Northern, RT-PCR, and immunoblot with anti-EGFP antibody demonstrated that the GAA activity detected in distant organs reflects the uptake of the enzyme. Although the levels of GAA expression in the low expresser line exceeded the levels in the wt mice, the secreted protein was not detected in circulation, and glycogen accumulation in the heart was similar to that in the ko mice. No enzyme was detected in the brain in either of the two lines. The data indicate that intramuscular gene transfer as a treatment for GSDII is doubtful because of the high level of expression required for cross-correction. GAA expression in liver (~100-fold increase) resulted in a significant amount of secreted GAA and a metabolic correction of muscle and heart. Doxycycline abolished GAA expression in all the lines. Turning the gene on at different stages of the disease will reveal if and when the disease is reversible.

C077. Prevention and reversal of muscular dystrophy in mdx mice

J. S. Chamberlain, *D. Hartigan-O Connor, S. Harper, C. DelloRusso, G. Crawford, J. Scott*
University of Washington; Seattle, WA United States
jsc5@u.washington.edu

Duchenne Muscular Dystrophy (DMD) is an inherited, X-linked disorder characterized by progressive muscle degeneration and weakness. The disease is caused by mutations in the dystrophin gene, which encodes a 427 kDa protein that links the actin cytoskeleton to the extracellular matrix. We have been exploring the feasibility of gene therapy for DMD by developing viral shuttle vectors that can deliver dystrophin expression cassettes to muscle. Two key limitations to this strategy have been the size of the dystrophin cDNA (14 kb) and the immune response elicited against many viral vectors with large cloning capacities. As a result, we have developed a gutted adenoviral vector system that has a 30 kb cloning capacity and a significantly diminished propensity to trigger a host immune response. We have also developed a series of mini and micro dystrophin clones that we show display a surprisingly high functional capacity. The gutted Ad vector have no viral genes and are grown in packaging cell lines that express the Ad proteins DNA polymerase and terminal protein. This cell line (C7) supports robust growth of gutted Ad vectors using helper viruses deleted for the POL and TP genes, further minimizing the immunogenicity of this system. We show that gutted Ad vectors that express human dystrophin still elicit a detectable CTL response, but one that does not lead to an obvious diminution of dystrophin expression. Vectors expressing mouse dystrophin, in contrast, do not display adverse consequences. Injection of the mouse dystrophin gutted virus leads to a significant reduction and reversal of dystrophic pathology in dystrophic mdx mice, even those more than one year old. We have also developed a series of small dystrophins by combining deletions in the C-terminal domain of dystrophin with deletions of the rod domain. Removal of the dystrophin C-terminal domain, or 16 of 24 spectrin-like repeats, has no detectable functional deficit in transgenic mice. Furthermore, expression cassettes made by removal of 20 of 24 repeats lead to near normal functional capacity in some clones, but not in others, suggesting that not all the spectrin repeats function in a similar manner. These microdystrophin clones are approximately 4 kb in size, enabling their delivery with other genes and regulatory cassettes in gutted Ad vectors, or by themselves in adeno-associated viral (AAV) vectors.

C078. Restoration of dystrophin synthesis in muscle cells from DMD patients through induced exon skipping in vitro.

J. C. T. van Deutekom, *M. Bremmer-Bout, A. A. M. Janson, H. B. Ginjaar, J. T. den Dunnen, G. B. van Ommen*
Leiden University Medical Center; Leiden, The Netherlands
deutekom@lumc.nl

Duchenne muscular dystrophy (DMD) is a lethal muscle disease usually caused by frame-shifting mutations in the DMD gene. A milder allelic form, Becker muscular dystrophy (BMD), exists in which despite a deletion or duplication of one or more exons, the translational reading frame is maintained and a largely functional dystrophin protein is generated. We have explored an alternative strategy towards DMD gene therapy based on correction of the reading frame through targeted modulation of dystrophin RNA splicing. Using antisense oligonucleotides (AONs) directed at putative splicing regulatory sequences, we aim to induce the specific skipping of target exons from the transcript. Recently, we were able to induce exon 46 skipping in mouse and human myotubes in vitro, confirmed through RT-PCR and sequence analysis. In muscle cells from two unrelated DMD patients carrying one of the most frequent DMD mutations, i.e. a deletion of exon 45, exon 46 skipping would restore the reading frame and induce the synthesis of a BMD-like dystrophin lacking both exons 45 and 46. To verify this, we performed immunohistochemical analyses on transfected patient-derived myotubes. Dystrophin expression was detected at almost normal levels in at least 75% of cells, and was sustained for several days. Moreover, the dystrophin signals were found at the myotube membranes, suggesting a functional role of the truncated protein. Our results underscore the therapeutic potential of AONs to restore dystrophin production in cells from DMD patients. This strategy may be applicable not only to a variety of DMD mutations, but also many other genetic diseases.

C079. Preimplantation genetic diagnosis for §-thalassemia major and sickle cell thalassemia in Greece; two years clinical experience

J. R. Traeger-Synodinos[1], *C. Vrettou*[1], *M. Tzetis*[1], *G. Palmer*[2], *M. Mastrominas*[2], *E. Kanavakis*[1]
[1]*Athens University, Medical Genetics; Athens, Greece;* [2]*IVF unit Embryogenesis; Athens, Greece*
jtraeger@cc.uoa.gr

About 10% of the Greek population are heterozygous for -thalassemia or HbS, and prevention programmes involving prenatal diagnosis (PND) are well established. However, couples with an unsuccessful reproductive history may prefer to avoid terminating an ongoing pregnancy. Preimplantation genetic diagnosis (PGD) allows selection of unaffected IVF embryos for transfer. To accomplish PGD for the wide spectrum of -thalassemia major genotypes in Greece, we applied an accurate, reliable strategy involving nested PCR and denaturing gradient gel electrophoresis (DGGE) analysis. DGGE is an advantageous method since it allows simultaneous detection of both alleles that contribute to the genotype, minimizing risk of midiagnosis. Thirty-two couples were counselled, of whom 26 initiated 36 PGD cycles. PGD was discontinued in ten cycles with <4 IVF embryos. In 26 completed PGD cycles, 184 embryos developed to cleavage stage and were biopsied, 72 embryos were diagnosed as unaffected, and 61 were transferred (2.3–1.0 per cycle). From the 26 complete cycles, 13 pregnancies were established, (50% success per cycle), including 11 singletons, 1 twin and 1 triplet (26% implantation rate for embryos transferred). The multiple pregnancies await CVS PND. Of the singleton pregnancies, 3 await CVS, 2 were selectively terminated (1 ectopic and 1 misdiagnosis detected by CVS PND), 1 miscarried in the first trimester and 5 were confirmed as unaffected with CVS PND, and have resulted in the birth of full-term healthy babies. For couples with an unsuccessful reproductive history and high risk of transmitting a (severe) genetic disease, PGD is a valuable and highly worthwhile procedure.

C080. Flow-Cytometric Sperm Separation in Preimplantation Genetic Diagnosis (PGD) of X-Linked Disease.

D. P. Bick, *G. L. Harton, H. J. Stern, L. A. Fallon, K. L. Blauer, K. Keyvanfar, E. F. Fugger*
Genetics & IVF Institute; Fairfax, VA United States
dbick@givf.com

To improve the ratio of female to male embryos in PGD for the prevention of X-linked disorders, we have used flow-cytometric sperm separation (MicroSort) to enrich samples for X-bearing sperm. Embryos created by IVF are tested for the presence of X- and Y- chromosome specific DNA and only embryos determined to be female are transferred to the mother s uterus. We have performed 14 IVF cycles for 12 patients with 6 different X-linked disorders.

Enriched X-bearing sperm fractions with an average purity of 85.5%, as determined by dual color X and Y fluorescent in-situ hybridization (FISH), were used to fertilize oocytes from patients. Multiplex nested polymerase chain reaction (PCR) using multiple genetic markers was used to determine the sex of each embryo. Of 118 embryos biopsied, 94 were determined to be female, 7 were determined to be male, and 17 were of unde-

termined gender.
Among embryos where a gender assignment was possible, 93% (94/101) were female. Without sperm separation, one would expect roughly 50% of embryos to be female and available for transfer. MicroSort increases the proportion of X-bearing sperm, as reflected in the number of embryos determined to be female after blastomere biopsy. This method allows couples undergoing PGD for X-linked disease significantly more female embryos available on the day of transfer, and may also result in one or more frozen transfer cycles without the need for additional IVF cycles. We believe this data demonstrates the advantage of the use of MicroSort in PGD for X-linked disorders.

C081. First trimester fetal sex determination in maternal serum using real-time PCR

J. Costa[1], *A. Benachi*[2], *P. Ernault*[1], *J. Jouannic*[2], *E. Gautier*[1], *Y. Dumez*[2]
[1]*H pital Am ricain de Paris; Neuilly, France;* [2]*H pital Necker Enfants Malades; Paris, France*
jean-marc.costa@ahp-paris.com

BACKGROUND Fetal sex prediction is achievable using PCR targeted at the SRY gene by analysing cell-free fetal DNA in maternal blood. The PCR method is a useful non-invasive approach in the management of pregnant carriers of X-linked genetic disorders. Unfortunately, this procedure has, to date, lacked sensitivity and reliability during the first trimester of pregnancy. Thus, it can not replace chorionic villus sampling. METHODS A new highly sensitive real-time PCR was developped to detect SRY gene sequences in maternal serum. Analysis was performed on 101 pregnant women during their first trimester of pregnancy. Results of this prospective study were compared with those obtained later for fetal sex. RESULTS Among the 101 pregnant women, 51 were bearing a male fetus and 50 a female fetus. SRY PCR analysis of maternal serum was in complete concordance with fetal sex. No false negative results were observed. Furthermore, no false positive results occurred although twenty-seven women carried female fetus during the current pregnancy, had at least one previous male-bearing pregnancy. CONCLUSIONS This study demonstrates that a reliable, non-invasive sex determination can be achieved through an analysis of maternal serum during the first trimester of pregnancy in place of invasive CVS procedure. It has great implications for the managment of pregnant women who are carriers of X-linked genetic disorders. New strategies are being developed for the prenatal diagnosis of these genetic diseases.

C082. Dystrophin expression in cultured non-muscle fetal cells; possible applications for prenatal diagnosis

F. Leturcq[1], *U. Nudel*[2], *O. Fuchs*[2], *N. Deburgrave*[1], *M. Zabot*[3], *D. R can*[1], *F. Niel*[1], *J. Kaplan*[1], *D. Yaffe*[2]
[1]*Hopital Cochin-Maternit s; Paris, France;* [2]*Weizmann Institute; Rehovot, Israel;* [3]*Hopital Debrousse; Lyon, France*
leturcq@icgm.cochin.inserm.fr

Reliable prenatal diagnosis of Duchenne Muscular Dystrophy (DMD), by analysis of the dystrophin gene in fetal DNA from amniotic or chorion villi, can be currently proposed in only 2/3 of the cases. In the remaining, helpless, cases a direct dystrophin analysis in fetal sample would be highly warranted, but this protein is supposed to be exclusively expressed in muscle and nerve cells, and early in utero fetal muscle sampling is not feasible. In 1993 Prigojin et al (FEBS Letters, 1993, 335, 223-230), using a very sensitive method of immuno-precipitation followed by western-blot analysis, found that minute amounts of dystrophin are detected in cultured cells from control amniotic fluid (AF) and chorionic villus samples (CVS), whereas dystrophin is not found in DMD fetuses. Using this technique in a double blind study, we examined cultured CVS and AF cells obtained from pregnant women from high risk families in which the defect in the dystrophin gene was already known. All 16 DMD affected fetuses were dystrophin-negative, and 28 out of 31 control fetal samples were positive. The proportion of false negatives (3 out of 31) complicates the use of this method for prenatal diagnosis. We currently investigate the molecular and cellular basis for the unexpected expression of dystrophin in non-muscle fetal cells, and for the phenomenon of false negative cells, in order to make the method applicable to diagnosis.

C083. The impact of the predictive test result upon subsequent reproductive decision making in families with Huntington s disease; a European collaborative study

G. Evers-Kiebooms[1], *K. Nys*[1], *P. Harper*[2], *M. Zoetewij*[3], *A. D rr*[4], *G. Jacopini*[5], *C. Yapijakis*[6], *S. Simpson*[7]
[1]*Center for Human Genetics; Leuven, Belgium;* [2]*Institute of Medical Genetics; Cardiff, United Kingdom;* [3]*Clinical Genetics Center; Leiden, The Netherlands;* [4]*INSERM-Unit 298; Paris, France;* [5]*Istituto di Psicologia; Rome, Italy;* [6]*Department of Neurology; Athens, Greece;* [7]*Clinical Genetics; Aberdeen, United Kingdom*
Gerry.Kiebooms@med.kuleuven.ac.be

The paper, based on a multidisciplinary European study, is aimed at analysing differences in post predictive test reproductive decision making of carriers and non-carriers and uptake for prenatal testing for HD in the carrier group in six European countries. The study group consisted of 451 persons (40% carriers and 60% non-carriers) at reproductive age when the predictive test result was given (period 1993-1998) and counseled in one of the participating genetic centers. The testees mean age at the communication of the test result was 31.5 years and half of the group had one or more pregnancies before the predictive test. Overall significantly more non-carriers than carriers had subsequent pregnancies ($p<0.001$). In the carrier group refraining from further pregnancies was the most frequently chosen option; prenatal testing occurred in about two thirds of the pregnancies. A more refined analysis in the subgroup who expressed reproductive motives in the pretest period and for whom at least three years elapsed between the communication of the predictive test result and the last follow-up contact, 39% of the carriers had subsequent pregnancies versus 69% of the non-carriers. An analysis of covariance, controlling for age and number of pretest pregnancies, revealed a significant impact of the predictive test result upon subsequent pregnancies ($p<0.01$). The sex of the prospective parent did not play a significant role in the carrier group. It will be concisely discussed to what extent the considerable differences between countries may be due to variability in counseling approach, cultural differences, legislation and other factors.

C084. Reproduction and multiple sclerosis; A counselling paradigm

D. Sadovnick, *E. Dwosh, C. Guimond*
University of British Columbia; Vancouver, BC Canada
sadovnik@infinet.net

Multiple sclerosis (MS) is the most common neurological disorder (1/500 lifetime risk) affecting Caucasian young adults. Females are affected approximately twice as often as males. The etiology of MS is still somewhat of a mystery but genetic factors are recognized to be important in both susceptibility to and the familial aggregation of the disease. There is no cure for MS. Current treatments are categorized either as symptom specific (e.g. high dose steroids) or disease modifying (e.g. interferons). The possible impact of MS treatments on a developing fetus is of concern to potential parents as well as to health care practitioners. Increasingly, clinical geneticists and genetic counsellors are asked to provide genetic and reproductive counselling for women (and men) who have MS and wish to have children. The purpose of this presentation is to provide a paradigm for such counselling, based on the experience at the Vancouver Multiple Sclerosis Clinic. Topics to be discussed include; (i) MS risks to offspring; (ii) Overall pregnancy outcome - do women with MS have a higher risk of stillbirths, spontaneous abortions, etc. compared to age matched women without MS; (iii) Teratogenetic concerns with special emphasis on MS therapies; (iv) The impact of gestation and delivery on maternal MS; (v) The longterm implications of MS on parenting. There are many factors such as MS clinical course in the woman prior to pregnancy, age of MS onset, etc. which can alter the information provided during a counselling session. The information presented will provide guidelines on the adaption of counselling to specific family situations.

C085. Genomic overview of meiotic recombination in the human female

M. A. Hulten, *C. Tease, G. Hartshorne*
MRI, University of Warwick; Coventry, United Kingdom
MHulten@bio.warwick.ac.uk

We have exploited the remarkable behaviour of the DNA mismatch repair protein MLH1 to pinpoint recombination events along the length of chromosomes to obtain a genome-wide overview of meiotic recombination (crossing-over) in human females. We determined the time of appearance of the meiosis-specific pairing structures, the Synaptonemal Complexes (SCs), and MLH1 foci (which we have termed RFs for recombination foci) in fetal oocytes. It was evident that such RFs are not randomly distributed along the length of individual SCs. In addition, in SCs with more than one RF, the RFs were spaced out along chromosomes, indicating positive interference. This pattern of RF distribution in oocytes differed from that previously described for human spermatocytes. In particular, there is a more regular near-terminal positioning of RFs in spermatocytes. Counts of RFs were performed in 95 oocytes at the pachytene stage from one fetus. The mean per oocyte was 70.3 (n=95, stdev=10.5, range=42-105) = 3,5151 cM, while the value for spermatocytes is 50.9 (n=46,stdev=4.4, range=41-59) = 2,545 cM.- RFs appeared to be more closely spaced at early

zygotene in comparison to pachytene. This suggests the occurrence of an editing machinery, operating during the process of maturation of exchanges, which precludes close spacing. In other words, there is an idication that cross-over interference is established during the progression of oocytes from early zygotene to pachytene. Further detailed investigations, including observation on in vitro maturing oocytes, should help to elucidate the still enigmatic mechanism underlying cross-over interference.

C086. The origin of abnormalities in recurrent aneuploidy

***W. P. Robinson**, D. E. McFadden, M. D. Stephenson*
University of British Columbia; Vancouver, BC Canada
wendyr@interchange.ubc.ca

Recurrent pregnancy loss as a consequence of sporadic aneuploidies may simply be a consequence of the dramatic increase of trisomic conceptions with maternal age. However, it is also possible that some couples are at increased risk of aneuploidy as a result of gonadal mosaicism, factors affecting chromosome structure and segregation, or increased sperm aneuploidy in the male partner. We report cytogenetic and molecular findings for 90 spontaneous abortions (SAs) from 40 couples ascertained as having two or more documented aneuploid or polyploid SAs. The mean number of SAs was 4.1 per couple and mean maternal age at time of aneuploid/polyploid loss was 38 years. Overall the distribution of abnormalities is similar to that expected for older women, with 84% due to autosomal trisomy. The most common abnormalities observed were trisomy15 (N=17), trisomy 16 (N=9), triploidy (N=9) and double trisomy (N=8). Molecular analysis to determine origin of trisomy is so far complete for 24 SAs, all of which were attributable to an error at maternal meiosis except a paternal meiotic trisomy 7 and a maternal somatic trisomy 5. These preliminary results exclude a major paternal contribution to recurrent aneuploidy and confirm that most is related to age-related changes in the maternal ovary. However, further analysis and follow-up is needed to determine if these women have an increased predisposition to trisomy as compared to age-matched controls and if some specific trisomies (e.g. trisomy 15) are over-represented.

C087. XRCC2 and XRCC3 homologous recombination repair genes maintain chromosome stability and correct chromosome segregation

***C. S. Griffin**, B. Deans, J. Thacker*
Radiation and Genome Stability Unit,MRC,Harwell; Didcot, United Kingdom
c.griffin@har.mrc.ac.uk

Chromosome instability and loss or gain of chromosomes are changes characteristic of many tumour cells and human disorders. However, the mechanism of these changes has not yet been fully determined. We have recently shown that hamster cell lines deficient in homologous recombination repair genes XRCC2 and XRCC3 have an elevated frequency of aneuploidy compared with wild type cells and mutant cells transfected with the human genes. In addition XRCC2 and XRCC3 deficient hamster cell lines have a high frequency of mitotic cells with abnormal centrosomes and spindle formation (1). We have now examined chromosomal changes in mouse embryonic fibroblasts (MEFs) from Xrcc2-/- knock out mice. Disruption of the Xrcc2 gene in mice gives early embryonic lethality with only 29% survival by day 12.5 (2). Metaphases 2hrs after tissue dissociation showed a high frequency of chromatid breaks and exchanges in Xrcc2 MEFs compared with MEFs from heterozygote and wild-type litter-mates. Using 3-colour FISH chromosome type aberrations were observed in Xrcc2-/- MEFs and these included both simple and complex types. Aneuploidy was not detected in early passage Xrcc2-/- MEF cells but spontaneously transformed Xrcc2-/- MEFs, at low passage, were found to have a very high frequency of aneuploidy. Abnormal fragmented centrosomes and multiple spindles were seen at high frequency in mitotic cells from transformed Xrcc2-/- MEFs compared to transformed Xrcc2+/+ MEFs. This suggests that disruption of the mitotic apparatus in Xrcc2 deficient cells leads to cell death in p53 competent cells but in the presence of aberrant p53 the cells rapidly become polyploid. These results demonstrate the importance of the XRCC2 gene in preventing chromosome rearrangements and chromosome mis-segregation, and reinforce the possibility that XRCC2 is a tumour suppressor gene. 1.C.C.Griffin,P.J.Simpson,C.R.Wilson and J.Thacker. Nature Cell Biology,Vol.2,p757-761,(2000).
2.B.Deans,C.S.Griffin,M.Maconochie and J. Thacker.EMBO in press.

C088. The assignment of HMGIY retrospeudogenes correlates with breakpoints of clonal aberrations observed in benign solid tumors

***C. Blank**, J. Bullerdiek, P. Rogalla*
Center for Human Genetics; Bremen, Germany
coblank@uni-bremen.de

Recently, we have described a mechanism by which after retrotransposition a processed gene, i.e., HMG1L3 becomes an exon of a pre-existing active gene. Similar mechanisms can easily explain as to how processed genes derived from tumor-associated genes may be activated by chromosomal rearrangements in neoplasms. Because genes of the high mobility group protein family HMGI(Y) are known to be involved in the development of a variety of benign solid tumors we have analysed breakpoints of clonal chromosome abnormalities in that group of benign tumors for a possible correlation with retropseudogenes of the HMGIY gene. Whereas the HMGIYL1 retrospeudogene has recently been mapped to Xp22.1, we assigned a further retropseudogene by FISH to 4q13 and database research revealed the assignment of a third retropseudogene to 12q24.1. Sequence analyses of these retropseudogenes showed high identity indices to the HMGIY gene and no frame shifts. Breakpoint information was obtained from cytogenetic aberrations in uterine leiomyomas, lipomas, pleomorphic adenomas, and pulmonary chondroid hamartomas because in all of these tumor entities cytogenetic subgroups involving genes of the HMGI(Y) family exist. Statistical tests revealed that the chromosomal bands harbouring HMGIY retropseudogenes were affected with a significantly higher frequency than expected under the assumption of purely randomly occurring breakages. These results further allow us to hypothesize that HMGIY-related retropseudogenes can be affected by chromosomal rearrangements in benign human tumors.

C089. Single Copy Hybridization Probes For Detection Of Chromosomal Rearrangements Derived By Genomic Sequence Analysis

***P. K. Rogan**, P. M. Cazcarro, J. H. M. Knoll*
Children s Mercy Hospital and Clinics; Kansas City, MO United States
progan@cmh.edu

Commercial probes currently exist for the most common translocations in hematopoietic malignancies. However, these probes often do not permit analysis of rare breakpoints or less common aneuploidies. We developed a technique that customizes and rapidly generates single copy probes for chromosomal in situ hybridization (denoted scFISH). The locations of single copy sequences are inferred from sequence analysis of final or draft genomic sequences in specific disease intervals. Target sequence intervals are identified by comparing the genomic sequence with a comprehensive database of repetitive sequence families. DNA fragments suitable as hybridization probes are generated by long PCR amplification of the adjacent single copy sequences, purified, labeled and hybridized to genomic sequences. Suppression of repetitive DNA hybridization in the probes was unnecessary due to their single copy composition. Single or multiple fragments were developed from each region and utilized to detect sequences in either metaphase or interphase cells. Probes were designed to detect hemizygous deletions in DiGeorge/Velocardiofacial, Prader-Willi, Angelman, and monosomy 1p36 syndromes, translocations in AML M2 [8;21], ALL [12;21], CML [9;22]; inversion in AML M4 [16], and aneuploidy for chromosomes 5 and 7. Probes to detect other rearrangements are being developed. Currently, a single, 2.3 kb labeled fragment has been adequately and reliably visualized by scFISH. Mixed combinations of DNA fragments from the same contig often had but more intense hybridization signals on metaphase chromosomes than the same fragments hybridized individually. To demonstrate the feasibility of using such probes in high-resolution FISH studies, we examined the distribution of 2.3 kb single copy sequences on the completely-sequenced chromosome 22. The minimum separation between single copy intervals is 35 bp and the maximum is 284 kb, with an average spacing of 22 kb. Thus, it will be feasible to develop custom probes, covering most of the chromosome 22 euchromatic region. If single copy sequences are similarly distributed on other chromosomes, scFISH will enable more precise delineation of common or rare chromosome rearrangement breakpoints, distinction of multigene family members, and identification of marker chromosomes.

C090. Deletion analysis of 98 cases with the 5p- syndromes by genomic DNA microarrays (GenoChips)

X. Zhang[1,2,3], *E. Niebuhr*[4], *R. Segraves*[2], *G. Hamilton*[2], *A. Snijders*[2], *S. Blackwood*[2], *K. Hindle*[2], *N. Brown*[2], *A. Niebuhr*[4], *H. Yang*[3], *D. Albertson*[2], *L. Bolund*[1], *D. Pinkel*[2]

[1]*Institute of Human Genetics, Aarhus University; Aarhus, Denmark;* [2]*UCSF Cancer Center; San Francisco, CA United States;* [3]*Human Genome Center, Institute of Genetics; Beijing, China;* [4]*IMBG, Panum Institute, Copenhagen University; Copenhagen, Denmark*

xxzhang@humgen.au.dk

A genomic DNA microarray, or GenoChip, system has developed based on the concept of the comparative genome hybridization (CGH) on a high-density microarray with around 2300 human genomic BAC clones giving an averaged resolution of about 1.5 Mb, and a higher density for the short arm of chromosome 5 in this experiment. The sensitivity, resolution and reliability of the GenoChip have fully been demonstrated when applied to the analysis of 98 cases of the 5p- syndrome with cytogenetically known deletion breakpoints. Deletions, either terminal or interstitial, and duplication can be identified with high accuracy in consistence with the data given by conventional methods. The measurement precision indicates the potential application of the GenoChip that could replace most, if not all, of the cytogenetic and molecular-cytogenetic methodologies and provide a high-throughput and high-resolution diagnostic capability for all chromosomal abnormalities except for balanced translocations.

C091. Usher Syndrome 1D and Nonsyndromic Autosomal Recessive Deafness DFNB12 Are Caused by Allelic Mutations of the Novel Cadherin-Like Gene CDH23

J. M. Bork[1], *L. M. Peters*[1], *S. Riazuddin*[1], *X. Liu*[2], *R. J. H. Smith*[3], *A. J. Griffith*[1], *E. R. Wilcox*[1], *T. B. Friedman*[1], *R. J. Morell*[1]

[1]*National Institute on Deafness and Other Communication Disorders, NIH; Rockville, MD United States;* [2]*Medical College of Virginia; Richmond, MD United States;* [3]*University of Iowa; Iowa City, IA United States*

borkj@nidcd.nih.gov

Usher syndrome type 1 is a recessive neurosensory disorder characterized by congenital profound deafness, vestibular dysfunction, and early onset retinitis pigmentosa. The USH1D locus, one of six known Usher syndrome type 1 loci, was mapped to chromosome 10q21-q22 (Wayne S. et al., Hum Mol Genet 5;1689, 1996). This genetic linkage interval overlapped the previously mapped nonsyndromic recessive deafness locus, DFNB12 (Chaib H. et al., Hum Mol Genet 5;1061, 1996). We have refined the DFNB12 locus by linkage analysis of seven consanguineous families segregating non-syndromic autosomal recessive deafness. Markers D10S1694 and D10S1737 defined a critical interval of approximately 0.55 cM in a single Pakistani family. Analysis of genomic sequence from this interval revealed a novel cadherin-like gene. Mutations in this cadherin-like gene, CDH23, were found in families with USH1D and in families with DFNB12. We have shown CDH23 expression in the retina, by northern blot analysis, and in the cochlea, by polymerase chain reaction amplification. Families with USH1D had nonsense or frameshift mutations, whereas families with DFNB12 had missense mutations, suggesting a genotype/phenotype correlation. We have demonstrated that allelic mutations of the CDH23 gene cause both nonsyndromic hearing loss and Usher syndrome type 1D (Bork J.M. et al., Amer J Hum Genet, in press).

C092. The Molecular Genetic Basis of Total Colorblindness

B. Wissinger[1], *H. J gle*[2], *E. Zrenner*[3], *L. T. Sharpe*[2], *S. Kohl*[1]

[1]*Molecular Genetics Laboratory, University Eye Hospital; T bingen, Germany;* [2]*Laboratory of Psychophysics, University Eye Hospital; T bingen, Germany;* [3]*University Eye Hospital; T bingen, Germany*

wissinger@uni-tuebingen.de

Total Colorblindness (syn.; Rod Monochromacy, Achromatopsia) is an autosomal recessively inherited condition characterized by the complete absence of color discrimination, reduced visual acuity, photophobia and congenital nystagmus. By linkage analysis two genetic loci for Total Colorblindness (ACHM2 on chromosome 2q11 and ACHM3 on chromosome 8q21) have been identified. As part of a longstanding effort we have cloned the corresponding genes, CNGA3 and CNGB3, which encode the a- and b-subunit of the cone photoreceptor cGMP-gated cation channel (Kohl et al. 1998, Nature Genet 19;257-259; Kohl et al. 2000, Hum Mol Genet 9;2107-2116). Molecular analysis of more than 300 independent patients now allows us to draw some principle conclusions on the genetic basis of Total Colorblindness; i) detectable mutations in CNGA3 and CNGB3 account for ~75% of all cases, ii) exclusion of known loci by linkage analysis support further genetic heterogeneity, iii) CNGB3 mutations are about twice as frequent as CNGA3 mutations, iv) missense mutations predominate in CNGA3 in contrast to the preponderance of nonsense mutations in CNGB3, v) one particular frameshift mutation, 1148delC, accounts for ~ 75% of all CNGB3 disease alleles, vi) haplotype reconstructions indicate founder effects for the most frequent CNGA3 and CNGB3 mutations and vii) mutations in CNGA3 and CNGB3 does not necessarily lead to Total Colorblindness but can also be found in patients with incomplete achromatopsia showing residual cone photoreceptor function and color discrimination capabilities. Supported by grants of the Deutsche Forschungsgemeinschaft and the BMBF/IZKF T bingen Medical Faculty.

C093. Identification of the Gene Responsible for Hereditary Lymphedema-Distichiasis Syndrome

T. W. Glover[1], *R. P. Erickson*[2], *S. L. Dagenais*[1], *J. Fang*[1], *M. F. Arlt*[1], *M. W. Glynn*[1]

[1]*University of Michigan; Ann Arbor, MI United States;* [2]*University of Arizona; Tucson, AZ United States*

glover@umich.edu

We have identified the gene responsible for the autosomal dominant disorder lymphedema-distichiasis (MIM 153400). Lymphedema-distichiasis syndrome classically presents as lymphedema of the limbs with variable age of onset and distichiasis, or double rows of eyelashes. Other complications, exhibiting variable expressivity, include cardiac defects and cleft palate, suggesting a defect in a developmental gene with pleiotrophic effects. We had previously mapped a translocation breakpoint in a male with congenital lymphedema to 16q24.3. Lymphedema-distichiasis was subsequently reported to map to this region by linkage studies. By analysis of genes flanking our translocation breakpoint, we identified nonsense and frameshift mutations in the FOXC2 (MFH-1) gene in three of three families studied with lymphedema-distichiasis. The translocation lies 120 kb distal to FOXC2 and is believed to inactivate it by position effect. Additional families are currently being studied for mutations and correlation to phenotypic features. FOXC2 is a member of the forkhead/winged helix family of transcription factors, whose members are involved in diverse developmental pathways. FOXC2 knockout mice display cardiovascular, craniofacial, and vertebral abnormalities similar to those seen in lymphedema-distichiasis syndrome. Our findings show that FOXC2 haploinsufficiency results in lymphedema-distichiasis syndrome. FOXC2 is the second known gene, along with VEGFR-3, to result in a form of hereditary lymphedema. Interestingly, lymphedema-distichiasis is only the second hereditary disorder known to be caused by a mutation in the forkhead gene family whose members play pivotal roles in mammalian development. Little is known about development of the lymphatic system, and our findings provide a novel insight into this process.

C094. Genotype/phenotype comparisions in Axenfeld-Rieger patients with PITX2 and FOXC1 missense mutations.

M. A. Walter, *R. Saleem, K. Kozlowski, F. B. Berry*

University of Alberta; Edmonton, AB Canada

mwalter@ualberta.ca

Purpose; To compare the effects of missense mutations of PITX2 or FOXC1 with the phenotypes of Axenfeld-Rieger (AR) patients. Methods; Five missense mutations of FOXC1 found in AR patients were introduced into the FOXC1 gene by site-directed mutagenesis. Recombinant wildtype (wt) and mutant FOXC1 proteins were expressed in COS-7 cells and assayed for their abilities to localize to the cell nucleus by immunofloures-cence. Electromobility shift assays (EMSAs) were used to compare the DNA binding abilities of wt and mutant FOXC1 proteins. Activation of a luciferase reporter gene was used to test the transactivation abilities of wt and mutant FOXC1 proteins. Analogous studies had previously been completed for PITX2 (Kozlowski and Walter, Hum Mol Genet 9; 2131-9, 2000). Results; We had previously found that the amounts of residual PITX2 function (as determined by nuclear localization, EMSA, and reporter gene transactivation) correlated with the severity of the patient phenotype in which the PITX2 missense mutation was found. One FOXC1 missense mutation (I87M) reduced the stability of FOXC1. Two missense mutations (S82T and S131L) reduced FOXC1 DNA binding ability, while two missense mutations (F112S and I126M) did not. The latter two mutations, however, decreased the transactivation ability of FOXC1. Interestingly, the phenotypes in patients with the S82T missense mutation (with almost 60% of wt FOXC1 transactivation ability) were indistinguishable from those of patients with the I87M mutation (producing less than 5% of wt FOXC1 protein amounts). Conclusions; Unlike the finding in patients with PITX2 mutations, the amount of residual FOXC1 function did not correlate with the severity of patient phenotypes. Aberrant ocular development arising from PITX2 mutations appears to follow a different mechanism than that arising from FOXC1 mutations.

C095. A Pathological mutation in a chromosome 19 gene causes a new dominant basal ganglia degeneration which can mimic Huntington s Disease

J. Burn[1], *A. R. J. Curtis*[1], *C. Fey*[1], *C. M. Morris*[2], *P. Ince*[3], *L. A. Bindoff*[4], *M. Jackson*[5], *A. Coulthard*[5], *A. Curtis*[1]
[1]*Institute of Human Genetics; Newcastle upon Tyne, United Kingdom;* [2]*MRC Neurobiology Unit; Newcastle upon Tyne, United Kingdom;* [3]*University of Sheffield; Sheffield, United Kingdom;* [4]*Haukeland Sykehus; Bergen, Norway;* [5]*Newcastle Hospitals NHS Trust; Newcastle upon Tyne, United Kingdom*
john.burn@ncl.ac.uk

An extensive pedigree in North West England contains multiple individuals affected by a dominant fully penetrant but highly variable late onset degeneration of the basal ganglia which can mimic Huntington s disease (HD) but is not asociated with significant dementia. Chorea,athetosis, spasticity,rigidity and bulbar dysfunction are described in different individuals. There is late characteristic cavitation of the basal ganglia, neuroaxonal spheroids and iron accumulation. Having linked the disorder to a 2cM region on chromosome 19 we found, in a candidate gene,a single base insertion resulting in an out of frame extension predicting protein elongation.Immunohistochemistry confirms massive accumulation of the gene product. A search through other local query HD cases revealed 5 further apparently unrelated cases. We have traced the family back to the 18th century Fletchers, a border clan. There was extensive emigration in the past. One member of that family, Fletcher Christian, achieved fame as leader of the Mutiny on the Bounty and founded a settlement in the Pacific. Mutation searching this gene will be important in atypical neurodegenerative disorders.

C096. Novel mutations in the somitogenesis gene, DLL3, cause a consistent pattern of abnormal vertebral segmentation in spondylocostal dysostosis

P. D. Turnpenny[1], *J. Duncan*[2], *M. P. Bulman*[2], *C. McKeown*[3], *C. G. Woods*[4], *K. Kusumi*[5], *S. Ellard*[2]
[1]*Royal Devon & Exeter Hospital; Exeter, United Kingdom;* [2]*Institute of Clinical Science, University of Exeter; Exeter, United Kingdom;* [3]*Clinical Genetics Unit; Birmingham, United Kingdom;* [4]*Dept. Clinical Genetics; Leeds, United Kingdom;* [5]*National Institute for Medical Research; London, United Kingdom*
turnpenn@eurobell.co.uk

The spondylocostal dysostoses (SD) are a heterogeneous group of disorders characterised principally by vertebral malsegmentation and malaligned ribs. We have identified mutations in the Delta-like3 (DLL3) somitogenesis gene in 7 families, all showing a consistent radiological phenotype of vertebral segmentation defects throughout the spinal column with rib fusions and deletions. In 5 consanguineous families there is homozygosity for DLL3 mutations. In 4 of these premature protein truncation is predicted from frameshift mutations 593ins5, 945delAT (2 families), and 1365del17 in exons 5,7 and 8 respectively. The two 945delAT mutation families are of Pakistani origin but geographically separated within the UK. The other consanguineous family demonstrate the missense mutation G385D in exon 8, substituting a highly conserved glycine residue within epidermal growth factor (EGF) repeat 5. The non-consanguineous families demonstrate; 1) compound heterozygosity for mutations C207X and 1418delC in exons 5 and 8 respectively; 2) heterozygosity for 593ins5 with the second mutation yet to be identified. The geographical origins of the two 593ins5 mutation families are distinct and they may be independently occurring mutations since this lies within a repetitive tract. We have therefore identified 6 mutations in DLL3, including 3 novel mutations (1365del17, C207X and 1418delC). All 6 mutations are located within conserved extracellular domains, affecting the EGF repeats. The SD phenotype is very consistent but of interest is the presence of arthrogryposis and neurological abnormalities in the 1365del17 mutation family. It is not yet known if this is a separate recessive disorder in this family.

C097. A New Locus for Recessive Familial Hypercholesterolemia maps to Chromosome 1p36.3-p35

H. A. Al-Kateb[1,2,3], *K. Hoffmann*[1], *A. Vergopoulos*[1], *G. N rnberg*[1], *A. Aydin*[1], *A. Dresel*[2], *E. K. F. Bautz*[2], *M. Jouma*[3], *S. B hring*[1], *F. C. Luft*[1]
[1]*Franz Volhard Clinic and Max-Delbr ck-Center for Molecular Medicine; Berlin, Germany;* [2]*Institute of Molecular Genetics and Faculty of Medicine, Heidelberg University; Heidelberg, Germany;* [3]*Department of Biochemistry, Faculty of Pharmacy, Damascus University; Damascus, Syrian Arab Republic*
hussam-alkateb@usa.com

High low-density lipoprotein cholesterol (LDL) levels are a major risk factor for coronary heart diseases (CHD). Genetic factors contribute greatly to high blood cholesterol levels. Better understanding of the factors involved in cholesterol homeostasis can lead to new targets for therapy. Here we describe a Syrian, Druze family in which three children have high LDL levels of 515, 474, and 332 /dl respectively. The other three siblings and the parents all have LDL < 175 mg/dl, suggesting an autosomal-recessive mode of inheritance. A locus (ARH1) has been described earlier. The large extended pedigree permitted us to study 66 additional persons, most of whom have normal LDL values. Linkage to the LDLR, APOB, APOE and ARH1 was excluded. We performed a genome-wide scan in the core family and found linkage to chromosomes 15 and 1. The linkage on chromosome 15 was not significant; however, markers on chromosome 1 revealed a multipoint LOD score of 3.05 between 1p36.1-p35.3. We have as yet found no obvious candidate gene in the region of interest. We suggest that this locus contains a second gene responsible for autosomal recessive hypercholesterolemia, namely ARH2 .

C098. A second gene for otosclerosis (OTSC2) maps to chromosome 7q34-36

K. Van Den Bogaert[1], *P. J. Govaerts*[2], *I. Schatteman*[2], *M. R. Brown*[3], *G. Caethoven*[1], *F. E. Offeciers*[2], *T. Somers*[2], *F. Declau*[4], *P. Coucke*[1], *P. Van de Heyning*[4], *R. J. H. Smith*[3], *G. Van Camp*[1]
[1]*University of Antwerp (UIA); Antwerp, Belgium;* [2]*St-Augustinus Hospital; Antwerp, Belgium;* [3]*University of Iowa; Iowa City, IA United States;* [4]*University of Antwerp (UZA); Antwerp, Belgium*
kvdbogae@uia.ua.ac.be

Otosclerosis is a frequent cause of hearing loss in adults, caused by abnormal bone homeostasis of the otic capsule. Usually, the hearing loss is conductive, due to a fixation of the stapes footplate thereby preventing normal ossicular vibration in response to sound. An additional sensorineural hearing loss may be caused by otosclerotic damage to the cochlea. The etiology of the disease is unknown and environmental as well as genetic factors have been implicated. Autosomal dominant inheritance with reduced penetrance has been put forward, but large families are extremely rare. To elucidate the pathogenesis of the disease, identification of the responsible genes is essential. We completed linkage analysis over the complete genome in a Belgian family in which otosclerosis segregates as an autosomal dominant disease. We excluded linkage to a known locus on chromosome 15 (*OTSC1*), and found linkage on chromosome 7q with a multipoint lod score of 3.54. Analysis of key recombinants maps this otosclerosis locus (*OTSC2*) to a 16 cM interval on chromosome 7q34-36 between markers D7S495 and D7S2426. To investigate the importance of *OTSC1* and *OTSC2* in autosomal dominant otosclerosis, we recruited and analyzed 9 additional families. These families were too small to individually reach a significant lod score of +3, but in 2 of them suggestive linkage to *OTSC2* was obtained. In the 7 remaining families, *OTSC1* and *OTSC2* were completely excluded, providing evidence for one or more additional (currently unknown) otosclerosis loci. In parallel with sensorineural hereditary hearing impairment, otosclerosis is a genetically heterogeneous condition.

C099. A novel locus for Charcot-Marie-Tooth type 2 neuropathy maps to chromosome 7q11-q21 in an extended Russian family

S. M. Ismailov[1], *V. P. Fedotov*[2], *E. L. Dadali*[1,3], *A. V. Polyakov*[3], *V. I. Ivanov*[1,3], *A. L fgren*[4], *E. De Vriendt*[4], *V. Van Gerwen*[4], *P. De Jonghe*[4], *V. Timmerman*[4], *C. Van Broeckhoven*[4], *O. V. Evgrafov*[3]
[1]*Russian State Medical University; Moscow, Russian Federation;* [2]*Genetic Counselling Department of Voronezh Diagnostic Centre; Voronezh, Russian Federation;* [3]*Research Centre for Medical Genetics; Moscow, Russian Federation;* [4]*Flanders Interuniversity Institute for Biotechnology (VIB), University of Antwerp (UIA); Antwerpen, Belgium*
shoukhrat_ismail@mail.ru

Charcot-Marie-Tooth disease (CMT) is the most common inherited motor and sensory peripheral neuropathy. The axonal form of CMT, or CMT type 2 (CMT2), is clinically and genetically heterogeneous with assigned autosomal dominant loci on chromosomes 1p35-p36 (CMT2A), 3q13-q22 (CMT2B), 7p14 (CMT2D), 8p21 (CMT2E). CMT2C is characterised by vocal cord paralysis, but the locus is unknown. Only in CMT2E, disease causing mutations were identified in the neurofilament-light gene (NEFL). We performed clinical, electrophysiological and genetic linkage studies in a single large family with autosomal dominant CMT2 from Voronezh province of Russia. All patients in this family have similar clinical phenotype typical for CMT. The electrophysiological findings are characteristic for axonal neuropathy and support the diagnosis of CMT2. Linkage to the known CMT2, CMT1 and other CMT-related loci was significantly excluded. A genome scan was performed using a Weber set (version 6, Isogen Bioscience BV, The Netherlands) of short tandem repeat (STR) markers

with a mean spacing 10.52 cM. Significant linkage to the long arm of chromosome 7 was observed, with a maximum two-point LOD score of 5.72 with STR marker D7S2204 in the absence of recombinations. Haplotype analysis demonstrates that the disease gene maps at chromosome 7q11-q21 within a 15 cM region between the flanking markers D7S2435 and D7S806. Our results indicate that the CMT2 neuropathy in this Russian family is genetically different from the known CMT1 and CMT2 loci. The axonal type of CMT in this family represents a novel genetic entity that we designate as CMT2F.

C100. A locus for autosomal dominant form of hemochromatosis on chromosome 2q.

O. T. Njajou, *N. Vaessen, B. Berghuis, M. H. Breuning, W. P. T. Rutten, P. J. L. Snijders, J. Houwing-Duistermaat, A. Hofman, B. A. Oostra, L. A. Sandkuijl, C. M. Van Duijn, P. Heutink*
Erasmus University Medical Center; Rotterdam, The Netherlands
njajou@mailcity.com

Hemochromatosis is a common recessive disorder in populations of caucasian origin. The disease is characterised by iron accumulation throughout the body leading to widespread pathology including diabetes, cardiovascular diseases, stroke and cancer. A locus for juvenile hemochromatosis on chromosome 1q, mutations in HFE gene (6p22.1) and a locus on 7q22 have been described. Still, not all hemochromatosis patients carry these mutations. This suggests that other genes are involved. To localise other genes involved in hemochromatosis, we performed a genome search in an extended pedigree (96 subjects) with multiple affected individuals (12 patients). Affected individuals in this family did not have a known mutation. In this pedigree, hemochromatosis segragates as an autosomal dominant disease. 47 subjects (10 affected and 37 unaffected) were selected for a genome wide search and linkage analysis. Simulation studies were performed as described by Ott J (1989) and Weeks et al, (1990); yielding a maximum lod score of 3.24. A genome screen was performed using 400 polymorphic markers. Two point linkage analysis was performed using disease frequency of 0.001 and an age dependent penetrance of 2%, 60% and 80%. Evidence for linkage to chromosome 2q was found with a lod score of 3.1 at theta = 0. Haplotype sharing showed that patients share a 10 cM region which include several iron metabolism genes. Fine mapping and sequencing is in progress and will indubitably leads to other gene(s) involved in hemochromatosis.

C101. Mapping of a severe form of X-linked myopia locus to the pseudoautosomal region (PAR) of Xq28 and exclusion of SYBL1 and HSPRY3 genes for their involvement.

U. Radhakrishna[1,2], *R. Rawal*[3], *M. A. Morris*[4], *A. Paoloni-Giacobino*[2], *L. Bartoloni*[2], *J. L. Blouin*[5], *R. Singh*[6], *V. Abay*[6], *J. V. Solanki*[7], *S. E. Antonarakis*[2,4]
[1]*Green Cross Blood Bank and RIA Laboratory; Ahmedabad, India;* [2]*Division of Medical Genetics, University of Geneva Medical School; Geneva, Switzerland;* [3]*Department of Biochemistry, Gujarat Cancer and Research Institute, Civil Hospital,; Ahmedabad, India;* [4]*Cantonal Hospital; Geneva, Switzerland;* [5]*Cantonal Hospital, Geneva; Geneva, Switzerland;* [6]*Iladevi Cataract and IOL Research Centre, Raghudeep Eye Clinic; Ahmedabad, India;* [7]*Department of Animal Genetics & Breeding, veterinary College; Anand, India*
uppal@medecine.unige.ch

Myopia is a common eye disease partly influenced by hereditary factors. It may occur as an isolated genetic anomaly or associated with other syndromes. We have studied a large six generation Indian pedigree with isolated, non-syndromic X-linked recessive form of Myopia (OMIM 310460). The degree of myopia was variable, ranging from -6 to -16.5D (mean -13.33D). Fundus examination revealed myopic degeneration of all the examined affected patients; the average anterior chamber depth was 3.28mm. In order to study the responsible gene, linkage analysis followed by candidate region mutation screening were performed on 26 individuals including 9 affected and 17 normals. We genotyped multiple microsatellite markers covering the entire X-chromosome. Marker DXYS154, which is located in the PAR in distal Xq28, showed no recombination with the phenotype with a maximum LOD score of 3.99 at theta=0 under an autosomal recessive model. Other Xq28 markers outside of the PAR that showed no recombination with the phenotype included DXS1108, DXS8087 and F8i13. We have subsequently decided to follow a systematic candidate gene approach in the linkage interval in order to identify the myopia-causing gene. Four genes (HSPRY3, SYBL1, IL9R and CXYorf1) have been identified in the PAR (which spans <500kb). We have screened the SYBL1 and HSPRY3 for mutations by amplification and sequencing of coding exons and flanking intronic sequences. No significant variants were found in patients implying that these two genes are not associated with the severe form of X-linked myopia. Analysis of the remaining genes in the PAR region is in progress.

C102. Location of the first predisposing gene locus for Asperger syndrome on chromosome 1q21-22

I. E. Jarvela[1], *T. Ylisaukko-oja*[2], *T. Nieminen*[3], *E. Kempas*[1], *M. Auranen*[1], *L. Peltonen*[1]
[1]*National Public Health Institute; Helsinki, Finland;* [2]*National Public Health Insitute; Helsinki, Finland;* [3]*University of Helsinki, Dept of Child Neurology; Helsinki, Finland*
irma.jarvela@hus.fi

Asperger syndrome (AS) was first described in 1944 by a Viennese physician Hans Asperger who reported a group of boys with autistic psychopathy whose clinical features resembled autism with some modifications. It is characterised by difficulties in social interaction and stereotyped behaviour but in contrast to infantile autism by normal intelligence and usually normal language skills. In the pediatric age group the prevalence of Asperger syndrome is around 4-7/1000 whereas the corresponding figure for the infantile autism is 4/10000. Etiopathogenesis is unknown but there is evidence for a strong genetic component. Based on clinical observations AS is inherited in the same families as infantile autism but also as a separate entity often transmitted from male to male. We report the analysis of 13 candidate gene loci associated with autism and schizophrenia in 17 Finnish AS-families with autosomal dominant mode of inheritance. Linkage to the previously reported predisposing loci for autism could not be replicated with Finnish AS families. By contrast, evidence for linkage was obtained on 1q21-22 the region that has previously been linked to schizophrenia (Brzustowicz et al., 2000) with the maximum two-point lod score of 2.70 with marker D1S484. Two families shared a common haplotype within an 8.6 cM region between markers D1S2721 and D1S484. Our results suggest that AS and schizophrenia may have common genetic background.

C103. Glutamate Receptor Dysfunction in a Genetic RSH/Smith-Lemli-Opitz Syndrome Mouse Model

F. D. Porter[1], *P. Zhu*[2], *R. Stewart*[2], *C. A. Wassif*[1]
[1]*National Institute of Child Health and Human Development; Bethesda, MD United States;* [2]*National Institute on Alcohol Abuse and Alcoholism; Bethesda, MD United States*
fdporter@helix.nih.gov

Smith-Lemli-Opitz syndrome (SLOS) is a MCA/MR syndrome due to mutation of the 3§-hydroxysterol ˇ7-reductase gene (*DHCR7*) which causes impaired reduction of 7-dehydrocholesterol (7DHC) to cholesterol. We produced a mouse model of SLOS by disruption of *Dhcr7* in mouse embryonic stem cells. Similar to infants with SLOS, *Dhcr -/-* pups, demonstrated growth failure, dysmorphic craniofacial features, decreased movement, and feeding problems. Craniofacial anomalies included cleft palate (9%) and nasal plug retention (39%). Mutant pups also appear to have decreased mineralization of bone. Mutant pups did not spontaneously feed, had an uncoordinated suck, and aspirated formula when hand fed. Biochemically, this mouse model is similar to the human syndrome. 3§-hydroxysterol ˇ7-reductase activity in mutant fibroblasts was 0.3–0.15% of control levels. Tissue sterol profiles showed decreased cholesterol and increased 7DHC levels in *Dhcr -/-* pups. In brain tissue, *Dhcr -/-* embryos also had decreased levels of desmosterol and increased levels of 7-dehydrodesmosterol (7DHD). Neurophysiological testing showed that cortical neurons from *Dhcr -/-* pups exhibited a normal sodium current, were able to generate an action potential in response to a depolarizing current, and responded to GABA. In contrast *Dhcr -/-* cortical neurons showed minimal (- 4.9 pA) or no response to glutamate stimulation (0 pA 25%, 11 pA 75%). The control glutamate response was - 39 pA (-21 pA 25%, -67 pA 75%, $p<0.001$). Both Western Blot and RT-PCR analysis confirmed expression of glutamate receptor subunits in cortex from *Dhcr -/-* pups. Thus, we postulate that this impaired glutamate response is due to receptor dysfunction as a consequence of the substitution of 7DHC and 7DHD for cholesterol and desmosterol in neuronal plasma membranes. Glutamate receptor dysfunction is associated with impaired suckling, thus potentially explaining this aspect of the mutant phenotype. Neurological dysfunction including hypotonia, mental retardation, and behavioral problems are frequent clinical findings in SLOS. Perturbation of neurotransmitter function may underlie these problems. This mouse model will be useful in determining the biochemical and neurophysiological basis of the behavioral and learning problems seen in this MCA/MR syndrome as well as testing therapeutic interventions.

C104. Mice with a Targeted Disruption of Dhcr7 Explain why Cholesterol (CH) Metabolism is Abnormal in Children with Smith-Lemli-Opitz/RSH Syndrome (SLOS)

G. S. Tint[1], *H. Asaoka*[2], *G. Xu*[2], *K. K. Sulik*[3], *N. Maeda*[3], *S. B. Patel*[4], *G. C. Ness*[5], *F. F. Moebius*[6], *B. U. Fitzsky*[6]
[1]*VA Medical Center; East Orange, NJ United States;* [2]*UMDNJ-New Jersey Medical School; Newark, NJ United States;* [3]*University of North Carolina at Chapel Hill; Chapel Hill, NC United States;* [4]*University of South Carolina; Charleston, SC United States;* [5]*University of South Florida College of Medicine; Tampa, FL United States;* [6]*Institute of Biochemical Pharmacology; Innsbruck, Austria*
tintgs@umdnj.edu

SLOS is a severe birth defect-mental retardation syndrome characterized by a recognizable facies, multiple organ abnormalities, failure to thrive and reduced survival caused by a defect in 7-dehydrocholesterol (7DHC) delta 7-reductase (DHCR7), the final enzyme in CH biosynthesis. As a result plasma and tissue cholesterol levels are abnormally reduced while 7DHC concentrations are increased 1000-fold. Using stem cell gene targeting we mutated Dhcr7 in the mouse and created an animal without enzyme activity (1–1, 142–44 and 206–120 pmol/min/mg protein, respectively, in -/-, +/- and +/+ mice). Though we noted no prenatal lethality, newborn homozygotes barely moved, did not suckle, breathed with difficulty, appeared blueish in color, exhibited markedly immature lungs, distended bladders and cleft palates and died within 18 hours. Biochemical abnormalities in -/- mice recapitulated the abnormalities in SLOS; sterol levels in 6 -/- vs. 14 pooled +/+ and +/- mice were abnormal ($p<0.002$) with reduced CH (0.6–0.5 vs.3.2–0.8 mg/g in liver and 0.5–0.1 vs. 3.4–0.5 mg/g in brain) and total sterols (1.0–0.7 vs. 3.2–0.9 mg/g in liver and 2.5–1.1 vs. 4.2–0.6 mg/g in brain) and markedly elevated 7DHC levels (0.3–0.2 vs. 0.01–0.02 mg/g in liver and 1.2–0.2 vs. 0.03 – 0.02 mg/g in brain). Sterol levels were low because activity of the rate controlling enzyme for sterol biosynthesis, microsomal 3-hydroxy-3-methylglutaryl coenzyme-A reductase (HMGR), was reduced 10-fold in liver (10–8 vs. 101–75 pmol/min/mg protein, $p<0.02$) and 3-fold in brain (169–92 vs. 500–230 pmol/min/mg, $p<0.05$) in -/- compared to +/+ mice. The reasons for low HMGR activity were two-fold. High concentrations of 7DHC in microsomal membranes (1) prevented increased expression of HMGR and other genes important for CH metabolism that is usually triggered by low CH levels and (2) increased the rate of HMGR protein degradation 3-fold. We propose that inhibition of total sterol synthesis in SLOS may exacerbate developmental abnormalities, especially in children with mutations which leave some DHCR7 activity, and suggest that prenatal therapy which raises maternal plasma CH levels might be useful in such cases.

C105. Cathepsin B and L-deficient mice develop a CNS-specific neurodegeneration

U. Felbor[1], *B. Kessler*[2], *R. T. Bronson*[2], *H. L. Ploegh*[2], *B. R. Olsen*[3]
[1]*Institut f r Humangenetik; D-97074 W rzburg, Germany;* [2]*Department of Pathology, Harvard Medical School; Boston, MA United States;* [3]*Department of Cell Biology, Harvard Medical School; Boston, MA United States*
ute_felbor@hms.harvard.edu and
felbor@biozentrum.uni-wuerzburg.de

Cathepsins B and L are ubiquitously expressed cysteine proteases implicated in intracellular proteolysis and extracellular matrix remodeling by in vitro studies. Gene targeting has not yet validated these functions in vivo. Cathepsin B null mice show no overt phenotype. Cathepsin L-deficient mice present with mild abnormalities in the T cell compartment and recurrent hair loss. In striking contrast, we here show that the combined deficiency of cathepsins B and L leads to death at the end of the second week of life. Only double mutant mice develop a dramatic brain pathology including massive neuron death and brain atrophy thus far unprecedented in mice. This neurodegeneration is preceded by the occurrence of various variants of autophagic abnormalities in neuronal cell bodies, dendrites and axons. Biochemical analyses of the vacuolar system suggest a defect in vesicle maturation in cathepsin B-/-L-/- mice. Since the role of the autophagosomal/endosomal-lysosomal system in neurodegeneration and aging is not well understood, cathepsin B-/-L-/- mice provide an exciting model to study the basic pathogenetic mechanisms.

C106. Effects of Methyl Donor Supplementation on Survival and Metabolism in a murine Model of 5,10-Methylenetetrahydrofolate Reductase Deficiency

B. C. Schwahn[1], *Z. Chen*[1], *M. D. Laryea*[2], *C. Castro*[3], *U. Wendel*[2], *S. Lussier-Cacan*[4], *T. Garrow*[3], *R. Rozen*[1]
[1]*Depts. of Human Genetics and Pediatrics, McGill University - Montreal Children s Hospital; Montreal, PQ Canada;* [2]*Metabolic Unit, University Children s Hospital; Duesseldorf, Germany;* [3]*Dept. of Food Science and Human Nutrition, University of Illinois; Urbana, IL United States;* [4]*Clinical Research Institute of Montreal; Montreal, PQ Canada*
bernd.schwahn@sympatico.ca

Homocystinuria can be caused by deficiency of 5,10-methylenetetrahydrofolate reductase (MTHFR), which is required for folate-dependent homocysteine remethylation. We recently generated MTHFR-deficient mice (mthfr -/-), which are a good model for homocystinuria. To address therapy, we placed female mthfr +/- mice on oral betaine before mating to mthfr +/- males, until weaning of offspring. Betaine is an alternate methyl donor for homocysteine remethylation, through betaine homocysteine methyltransferase (BHMT). Survival and growth of pups were monitored and compared to a control group. We also performed studies in adult mice of the three mthfr genotypes. An unsupplemented diet or diets supplemented with betaine or dimethylsulfoniumpropionate (DMSP, a betaine analogue) were administered for 14 days. Plasma metabolites and liver BHMT activity were determined. Liver histology was evaluated in mthfr -/- mice. RESULTS; Survival rate (14.9%) and weight gain of mthfr -/- pups from untreated mothers were significantly lower than those from betaine-supplemented mothers (70.2% survival). In the study of adult mice, betaine and DMSP lowered homocysteine levels significantly, by >50% in all genotype groups. Mthfr -/- mice on control and DMSP diets had fatty livers; those with betaine did not. Plasma betaine levels increased with betaine, but not DMSP supplementation. BHMT activity was unchanged. CONCLUSION; In mthfr -/- mice, betaine significantly improves survival, growth, and hepatic morphology. Betaine and DMSP reduce homocysteine in mice of all three genotypes, but DMSP supplementation is associated with betaine depletion and fatty liver. In summary, betaine is a reasonable methyl donor when folate-dependent remethylation is compromised.

C107. 3-Hydroxy-3-methylglutaryl-CoA synthase deficiency is caused by mutations in the HMGCS2 gene

J. Zschocke[1,2], *F. G. Hegardt*[3], *N. Casals*[4], *J. M. Penzien*[5], *R. Aledo*[4], *J. P i* [3], *G. F. Hoffmann*[1], *E. Mayatepek*[1]
[1]*Univ. Children s Hospital; Heidelberg, Germany;* [2]*Institute of Human Genetics; Heidelberg, Germany;* [3]*Dept. of Biochemistry, Univ. of Barcelona; Barcelona, Spain;* [4]*International University of Catalonia; Barcelona, Spain;* [5]*Children s Hospital, Zentralklinikum; Augsburg, Germany*
johannes_zschocke@med.uni-heidelberg.de

Hepatic production of ketone bodies during fasting periods requires mitochondrial 3-hydroxy-3-methylglutaryl-(HMG-)CoA synthase. A genetic deficiency of this enzyme has been previously reported in two children with hypoketotic hypoglycaemic coma, but enzymatic confirmation was not possible, and molecular studies were not performed. We report the clinical, biochemical and molecular findings in two other children with HMG-CoA synthase deficiency. Both suffered from acute hypoketotic hypoglycaemic coma during a febrile infection with prolonged fasting in infancy. There was no other specific organ dysfunction. Urinary organic acids showed a characteristic metabolite pattern, reflecting a fasting state but without evidence of ketogenesis. Acylcarnitines were consistently normal, an important diagnostic feature that distinguishes the disorder from beta oxidation defects. Molecular studies in the first patient revealed compound heterozygosity for mutations G212R and R500H in the HMGCS2 gene; G212R was also identified in the second patient. Neither mutation was detected on 200 control chromosomes. Further analyses including expression studies are in progress. With avoidance of prolonged fasting periods the children are developing normally. HMG-CoA synthase deficiency is the only known disorder that exclusively affects mitochondrial ketogenesis, explaining both the clinical features as well as the characteristic biochemical findings.

C108. Differential utilization of systemic vs. enteral sources of nitrogen for urea synthesis in control subjects and ornithine transcarbamylase deficiency females

B. Lee[1], *W. O Brien*[1], *J. Henry*[2], *J. Rosenberger*[2], *P. Reeds*[2], *F. Scaglia*[1]
[1]*Department of Molecular and Human Genetics, Baylor College of Medicine; Houston, TX United States;* [2]*Children s Nutrition Research Center and Department of Pediatrics, Baylor College of Medicine; Houston, TX United States*
blee@bcm.tmc.edu

We have observed that while many ornithine transcarbamylase deficiency (OTCD) females exhibit little dietary protein sensitivity, they may be more sensitive to intercurrent stress including fasting, trauma, or infection. Our previous observation that OTCD females maintain a relatively normal rate of total urea synthesis but derive less of this urea from glutamine implies that other sources of nitrogen are more important to nitrogen homeostasis in these individuals (Lee et al, 2000). We hypothesize that nitrogen containing compounds generated from the intestinal catabolism of dietary

amino acids are the most important source of ureagenesis in OTCD females. We have previously used stable isotopes to correlate *in vivo* urea cycle activity with clinical severity in null and partial activity urea cycle patients and controls. Using these methods we have now tested whether intestinally generated ammonia is a more effective precursor for urea synthesis than peripherally generated glutamine. By administering ^{15}N-ammonium chloride ($^{15}NH_4Cl$) either intravenously or enterally with a simultaneous I.V. infusion of $^{18}O/^{13}C$-urea, we measured the efficiency of ^{15}N transfer from a nitrogen source into urea in 6 OTCD females and 6 controls. Interestingly, the utilization of enteral $^{15}NH_4Cl$ was similar between OTCD and control subjects. In contrast, OTCD females utilized peripheral $^{15}NH_4Cl$ less efficiently at a rate of 46% that of controls, suggesting that OTCD females rely more on enteral precursors as the most improtant source of ureagenesis. These approaches represent an important tool to optimize the nutritional management of urea cycle patients especially during critical illness or catabolic states.

Posters

P0001. Differential expression of a novel small intronless gene in sporadic breast cancer

S. Singh[1], *E. Preddie*[2]
[1]*Institute of Human Genetics, University of Hamburg,UKEH; Hamburg, Germany;* [2]*Altgen; Wilmington, DE United States*
singh@uke.uni-hamburg.de

We have discovered an unique intronless gene located at 17q21 in the region of BRCA1 gene encompassing a protein of only 30 amino acids. Mutation analysis of this gene was preformed in blood and tumor tissue of 50 patients with sporadic breast cancer by employing SSCP and direct sequencing. No mutations were found. The expression of this gene was looked at mRNA and protein level. The gene is normally not expressed or expressed at a very low level in serum, cultured fibroblasts and breast tissue of normal females. A comparatively three to four times higher expression of mRNA was noted in the tumor tissue of the patient by northern and RTPCR techniques . ELISA tests were developed to monitor the expression of this protein and presence of an antibody to it in sera and other tissues including fibroblast and tumor tissue cell cultures . An investigation of a large sample of the patient sera has revealed that this protein is expressed at considerable level in sera of patients and also antibody against this protein can be traced in the sera of patients with breast cancer. In about 7% of normal controls the values in range of the affected patients were also observed. This assay promises a good potential hope to develop a preclinical test and therapeutics for sporadic breast cancer. This protein contains a strong homology to BRCA1 and BRCA2 gene and can be predicted to block the sulfatation of BRCA1 protein and glycosylation of BRCA2 protein and thus prevent their proper maturation and functioning.

P0002. Significant expression of Human Endogenous Retrovirus (HERV-K) sequences in prostate cancer

R. D. Smith[1,2], *B. Khoubehi*[1], *J. D. Beatty*[1], *C. W. Ogden*[2], *M. A. Penny*[1]
[1]*Imperial College of Science, Technology and Medicine; London, United Kingdom;* [2]*Department of Urology, Northwick Park Hospital; Harrow, United Kingdom*
daron.smith@ic.ac.uk

The ability of human endogenous retroviruses to retrotranspose has led to the hypothesis that their expression may have a role in carcinogenesis; 1 person in 8 may carry an endogenous insertional mutation due to a new retrotransposition event. HERV expression has been identified in a number of malignancies including breast and colon cancer. Transcription of HERV-K, the biologically most active HERV family, can be up-regulated by steroid hormones in the breast cancer cell line T47D. Prostate cancer, like breast cancer, is sensitive to its hormonal environment, raising the possibility of similar regulation of HERV-K expression. RNA was extracted from prostate cancer cell lines DU-145, PC3 and LNCaP, 16 prostate cancer and 9 benign prostatic hyperplasia specimens and subjected to RT-PCR. A modified RT-PCR technique was developed to specifically amplify expressed HERV-K without amplifying genomic HERV sequences. Southern hybridisation and DNA sequencing confirmed the results. HERV-K envelope expression was identified in the cell lines. Statistically significant HERV-K expression was detected in prostate cancer [10/16 (63%) cancer specimens compared to 1/9 (11%) benign specimens (p=0.03, 2-tailed Fisher s exact test)]. These results suggest that HERV-K expression is generally silenced in benign tissue but does occur in prostate cancer. Expressed HERVs may act as mobile genetic elements with the potential to disrupt the function of tumour suppressor genes or enhance expression of proto-oncogenes via promoter activity in the Long Terminal Repeats (LTRs) of the viral sequence. Assessment of HERV-K sequences expressed in the presence of testosterone is currently being investigated.

P0003. Cyclin genes expression in human acute leukemia cells.

D. Szczesniak[1], *J. Kocki*[1], *M. Cioch*[2], *A. Dmoszynska*[2], *B. Marzec*[1], *I. Pielecka-Korszen*[1], *J. Wojcierowski*[1]
[1]*Medical Academy, Department of Medical Genetics; Lublin, Poland;* [2]*Medical Academy, Department of Hematology; Lublin, Poland*
szczes@poczta.wp.pl

The cell cycle alteration may cause tumor cells resistance to therapy. Such genetic instability, arising from mutations in cell cycle checkpoint or DNA repair genes, enables the cells uncontrolled proliferation. The past several years research has resulted in defining new agents participating in cell proliferation and contributed to the fact that we have started to understand the cell cycle machinery. Molecules involves in basic pathway of cell division are cyclins, the positive regulators of cell cycle, which overexpression is associated with uncontrolled cell growth and cancer. In our research we examined the level of cyclin genes expression in bone marrow samples from patients with acute leukemia before the therapy. For the detection of cyclin mRNA we used The Multi-Probe RNase Protection Assay System (RiboQuant). We analyzed the expression of cyclin of G0/G1, G1, G1/S cell cycle phases. The results we obtained shows the leukemic cells reveal significantly high levels of cyclin D3 and cyclin I mRNA as well as slightly higher cyclin G1 gene expression than other cyclins. The correlation between the clinical data and a level of expression of different cyclin gene in hematological malignancies may be a prognostic factor the relapse or poor prognosis This research is supported by Polish Committee of Scientific Research — grant No PO5B 134 19.

P0005. A real-time PCR system for the differential quantification of the alpha- and beta-transcript of the CDKN2A gene

F. von Eggeling, *C. Hoppe, U. Claussen, G. Ernst*
Institute of Human Genetics and Anthropology; Jena, Germany
fegg@mti-n.uni-jena.de

Since the tumor suppressor gene p16INK4A (CDKN2A/MTS1) was first described in 1993, it was found to be frequently inactivated in various cancer types and, thus, seems to play a crucial role in carcinogenesis. From CDKN2A two distinct transcripts with common used exon 2 and 3, but a different promotor and exon 1 are described as alpha (p16INK4A)- and beta (p14ARF)-form. P16INK4A interacts over cyclin D/CDK4 with pRB, whereas p14ARF interacts with the p53 regulator MDM2 by blocking p53 degradation. Both transcripts could be independently inactivated by homozygous deletion, methylation of the promotor region or point mutation. Interaction of both transcripts are postulated, but until now the relative expression of both transcripts was not simultaneously determined. Consequently, we investigated this expression ratio by real-time quantitative PCR. Therefore a primer system was established on the LightCycler with two different forward primers (exon 1) and one common reverse primer (exon 3). Isolated RNA was used from three tumor cell lines (Colo 320, Colo 620, Sk-Mel), one normal cell line (G292) and one primary cell culture (AC) as control. In Colo 320 and 620 the alpha-transcript could not be detected, whereas the beta-form was expressed at a normal level. The remaining cell types (Sk-Mel, G292, AC) showed similar expression for the beta-form, but the alpha transcript was equally or minute less expressed. In conclusion this newly established system for the differential quantification of both CDKN2A transcripts offers the opportunity to analyse the inactivation in the progression of tumors in more detail.

P0006. Prognostic significance of p53 mutations and expression of PAX5 and Shb in superficial bladder cancer

J. Mares, *M. Babjuk, M. Trkova, J. Duskova, Z. Sedlacek*
Charles University; Prague, Czech Republic
jaroslav.mares@lfmotol.cuni.cz

Bladder cancer is the most common urologic cancer and 75 % bladder tumours are classified as noninvasive superficial transitional cell carcinomas. Recent research of the prognostic potential of p53 mutations revealed contradictory results. The aim of our study was to define new combinations od markers reflecting biological behaviour of individual tumours in order to identify patients at risk for tumour progression. We investigated 35 patients with the superficial bladder cancer, 18 ones of the invasive type and 20 controls for p53 mutations in exons 5-9 and adjacent intronic sequences by the SSCP and following direct genomic sequencing. Point mutations were detected in 11% (4/36). The correlation of mutation analysis and expression of two genes PAX 5 and Shb, both localized on the 9p21-23, with patient clinical and histopatological data was evaluated. In conclusion, our results indicate that the p53 mutation analysis combined

with expression data might be used as a marker for superficial bladder cancer. Supported by the grant IGA NC/5961-3.

P0007. nm23-H1 and Colon Adenocarcinoma

S. Kapitanovic[1], *T. Cacev*[1], *S. Radosevic*[2], *M. Berkovic*[1], *K. Pavelic*[1], *R. Spaventi*[2]
[1]*Rudjer Boskovic Institute, Division of Molecular Medicine; Zagreb, Croatia;* [2]*PLIVA d.d., Research & Development; Zagreb, Croatia*
kapitan@rudjer.irb.hr

Nm23 gene was originally identified by differential hybridisation of K-1735 melanoma cell line clones of varying metastatic potential. A tumor metastases-suppressor function was implicated by the reduced expression of nm23 in highly metastatic sublines compared with non-metastatic sublines derived from the same K-1735 clone. The purpose of this study was to evaluate whether the expression of the nm23-H1 protein or loss of heterozygosity (LOH) of the nm23-H1 gene is associated with tumor stage and grade of tumor differentiation. We also investigated the correlation of nm23-H1 expression with 5-year survival. Paraffin tissue sections were analyzed immunohistochemically using monoclonal antibody NM301 to human nm23-H1 protein. DNAs (normal and tumor) isolated from microdissections of parafin sections were used for LOH analysis. Of 102 adenocarcinomas that were examined, 41% showed a weak positive immunostaining for nm23-H1 protein. The most nm23-H1 positive tumors were in Dukes B (67%) and in the well differentiated tumors (65%). Statistical analysis showed that there was no statistically significant difference in survival between the patients with nm23-H1 positive tumors and patients with tumors that were not stained for nm23-H1 protein. To analyze LOH at the nm23-H1 gene we used VNTR marker located in untranslated 5 region of the nm23-H1 gene. At this nm23-H1 locus 60% of samples were informative and 33% of them demonstrated LOH. The nm23-H1 LOH was more frequent in tumors that were larger than 5 cm than in smaller ones. Positive correlation was found between the nm23-H1 LOH and histological grade as well as between the nm23-H1 LOH and Dukes stage of tumor samples.

P0008. Inhibition Of The Epidermal Growth Factor Receptor (EGFR) Expression By The Promyelocytic Leukemia Tumor Suppressor

S. Vallian[1], *K. Chang*[2]
[1]*Dept. of Biology, Faculty of Science, Isfahan University; Isfahan, Islamic Republic of Iran;* [2]*University of Texas MD Anderson Cancer Center; Houston, TX United States*
svallian@altavista.com

The promyelocytic leukemia protein, PML, was first identified in the breakpoint region of the t(15q;17q) translocation in acute promyelocytic leukemia (APL). We and others have previously reported that PML could function as a tumor suppressor. Its introduction into a number of cancer cell lines including the breast and prostate, resulted in the inhibition of their growth in vitro, and their tumorigenicity in nude mice. When brought to the promoter by a DNA-binding domain, the PML protein was found to function as transcription inhibitor. A number of natural promoters including the epidermal growth factor receptor(EGFR) gene promoter and the dihydrofolate reuductase (DHFR) promoter, which both are involved in cellular growth, were found to respond to the PMLs inhibitory effects. Here we have analysed the effects of PML on the EGFR gene promoter and expression. Our data show that PMLs repression of the EGFR promoter is caused by inhibition of the Sp1-dependent activity of the promoter. On functional analysis, the repressive effect of PML was mapped to a 150-bp element (relative to the ATG initiation site) of the EGFR promoter. In vitro and in vivo anylsis by means of the glutathione S-transferase (GST) pull-down assay, coimmunoperecipitation and transient transfection assays demonstrated that PML and Sp1 are associated. Furthermore, overexpression of PML in A431 cells, which express high level of EGFR protein, resulted in significant repression of EGFR expression. Together, these data show that PML inhibits the expression of the EGFR by affecting its promoter. Since EGFR is overexpressed in a number of malignancies such as the breast cancer and head and neck carcinomas, PML could be considered as a potential agent in gene therapy of these cancers.

P0009. Shifting toward an angiogenic phenotype after inactivation of suppressor genes in pancreatic cancer

M. Sunamura, *D. G. Duda, T. Furukawa, A. Horii, S. Matsuno*
Tohoku University Hospital; Sendai, Japan
msun-thk@umin.ac.jp

Recent evidence points toward the involvement of genetic alterations, such as p16 or p53 in the switching on of angiogenic phenotypes in the tumor tissue. This study examines the inhibitory effect of BAI1 and SMAD4 genes on the onset of angiogenesis and invasion in pancreatic adenocarcinoma. Using adenoviral mediated transfer, the expression of these genes were restored in pancreatic cancer cells. Moreover, p53 transfection in these cells induced BAI1 expression. While no significant in vitro antiproliferative effect could be achieved, the in vivo growth of these cells in NK-depleted SCID mice was strongly inhibited due to the antiangiogenic properties of these genes, both subcutaneously and in dorsal skin chamber. It was found that the effect was related at least in part to the implication of these genes in the regulation of the proteolytic balance, namely the matrix metalloproteinases expression. The expression of endothelial cell mitogens VEGF and bFGF was not significantly changed by restoring the presence of these genes and is generally low in the pancreatic adenocarcinoma cell lines that were used. It was rather the down-regulation of ETS-1, a transcription factor largely involved in angiogenesis, by the SMAD4 gene that appear to account for the reduced vascularity and invasiveness of the transfected tumor cells. Gaining molecular understanding of the processes associated with growth, invasion, and metastasis in pancreatic malignancies is crucial, as their poor prognosis emphasizes the need for new approaches among which the antiangiogenic therapy is highly promising.

P0010. Prune and NM23-H1 and H2 (NDP-Kinase) Human Protein Interaction; Involvement in Human Cancer.

M. Zollo[1], *A. Forus*[2], *A. D Angelo*[3], *V. Aglio*[3], *S. Olivieri*[3], *G. Merla*[3], *A. Reymond*[3], *F. Sanvito*[4], *G. Arrigoni*[4], *O. Myklebost*[5], *A. Ballabio*[3]
[1]*Telethon Institute of Gentics and Medicine; Naples, Italy;* [2]*Department of Tumour Biology, The Norwegian Radium; Oslo, Norway;* [3]*TIGEM; Naples, Italy;* [4]*Department of Pathology, HSR; Milan, Italy;* [5]*Department of Tumour Biology, The Norwegian Radium Hospital; Oslo, Norway*
zollo@tigem.it

We have isolated a human and murine homolog of the Drosophila prune gene through dbEST searches 1,4. PRUNE protein retains the four characteristic domains of DHH phosphoesterases 2. The homo- or hemizygous prune mutant is lethal in the presence of just a single copy of a gain-of-function mutation in the abnormal wing disc gene (awd), dubbed Killer-of-Prune (awdK-pn) which dies at the third larval instar stage developing melanotic tumors. By using interaction-mating and in vitro and in vivo co-immunoprecipitation experiments, we show the ability of human PRUNE to interact with the human homolog of awd protein (nm23-H1) 3,4 and nm23-H2 and demonstrated that it is impaired with nm-23-H1-S120G mutant, a gain-of-function mutation associated with advanced neuroblastoma. PRUNE is a new phospodiesterase (PDE) specific for cAMP substrate and is amplified (3-6 copies) in 89% of Sarcoma tumors analysed. Furthermore, we have found a dominant negative PRUNE mutation (with a partial loss of function) affecting mice skin hair follicle cellular proliferation. Our working hypothesis is that PRUNE is negative regulating the anti-metastatic function of nm23 protein. Results will be presented.
REFERENCES 1. Banfi, S., et al. Nature Genetics 13, 167-174 (1996). 2. Aravind, L. & Koonin, E.V. Trends in Biochemical Sciences 23, 17-19 (1998). 3. Hartsough, M.T. & Steeg, P.S. Am J Hum Genet 63, 6-10 (1998). 4. Reymond, A., et al. Oncogene 18, 7244-52 (1999).

P0011. Alternative splice products of HMGIC may play a major role in tumorigenesis

S. Hauke, *A. M. Flohr, J. Bullerdiek*
Center for Human Genetics; Bremen, Germany
shauke@uni-bremen.de

Aberrations affecting the gene encoding the high mobility group protein HMGIC have been found in a variety of human tumors as e.g. uterine leiomyomas, lipomas, and pulmonary chondroid hamartomas. These aberrations lead either to fusion genes, transcriptional up-regulation, or aberrant transcripts of HMGIC. In the latter cases truncated transcripts consisting of exon 1 to 3 of HMGIC encoding the three DNA-binding domains and ectopic sequences derived from chromosome 12 were frequently detectable as determined by CASH (chromosome assignment using somatic cell hybrids) analyses. There are several lines of evidence indicating that the biological and tumorigenic features of truncated HMGIC derivatives, i.e., those composed of the DNA-binding domains and a shortened acidic tail clearly differ from those of the normal protein consisting of three DNA-binding domains and one large acidic tail. By sequencing the complete 112 kb third intron of HMGIC we were able to detect several of the ectopic sequences, known as fused to HMGIC. Expression studies revealed co-expression of these transcripts with the normal transcript in tumors with 12q14-15 aberrations as well as in other tumors, and in normal tissues. Therefore, for the most part, truncated HMGIC transcripts previously thought to be aberrant transcripts seem to result from alternative splicing. Due to the loss of the part encoding the acidic tail the expression of the alternatively spliced HMGIC may have more striking effects than the

wild type HMGIC in terms of tumorigenesis.

P0012. Targeted expression of SV40 large T antigen in spermatocytes

S. Tascou[1], *A. Meinhardt*[2], *M. Schweier*[3], *K. Nayernia*[1], *T. Kang*[1], *W. Engel*[1], *P. Burfeind*[1]
[1]*Institute of Human Genetics; G ttingen, Germany;* [2]*Institute of Anatomy and Cell Biology; Marburg, Germany;* [3]*Department of Pathology; G ttingen, Germany*
stascou@gwdg.de

Abstract In an attempt to determine the susceptibility of spermatocytes to malignant transformation by simian virus 40 (SV40) large tumor antigen (TAg), transgenic mice harboring a chimeric gene composed of the SV40 TAg gene fused to the 1.4 kb promoter sequence of the human phosphoglycerate kinase 2 (PGK2) gene were generated. Previously, it was shown that this regulatory sequence is able to direct the expression of a CAT reporter gene in male germ cells with the onset of expression in spermatocytes of transgenic mice. Northern blot analysis of RNA from different tissues indicated a specific transcription of TAg in testis of PGK2-TAg transgenic mice. RT-PCR analysis on RNAs and Western blot analysis on proteins isolated from testis at different stages of development revealed that transcription and translation of the TAg gene starts in 12-day-old testis, which is in accordance with the onset of endogenous Pgk2 expression and coincides with the appearance of preleptotene spermatocytes. PGK2-TAg transgenic mice are fertile and show no tendency toward transformation. However, prepubertal 18-day-old transgenic male mice exhibited a significant increase in spermatocyte number but no differences in number of Sertoli cells as compared to wildtype mice. With proceeding age the increased amount of spermatocytes in prepubertal transgenic mice assimilates to that of wildtype mice. Tunel assays revealed a higher rate of apoptosis in testis of prepubertal transgenic mice compared to that of wildtype mice. This indicates that the excessive spermatocytes in prepubertal transgenic mice undergo apoptosis during spermatogenesis.

P0013. Genetic Determination of Variation in Urinary Bombesin Like Peptide Levels

Y. E. Miller[1,2], *S. M. Aguayo*[3], *E. Pugh*[4], *J. Bailey-Wilson*[4], *A. J. Cohen*[5], *P. Fain*[6]
[1]*University of Colorado Health Sciences Center; Denver, CO United States;* [2]*Denver Veterans Affairs Medical Center; Denver, CO United States;* [3]*Emory University, Atlanta Veterans Affairs Medical Center; Atlanta, GA United States;* [4]*NHGRI; Bethesda, MD United States;* [5]*University of Colorado Health Sciences Center, Denver Veterans Affairs Medical Center; Denver, CO United States;* [6]*Barbara Davis Diabetes Center, University of Colorado Health Sciences Center; Denver, CO United States*
york.miller@uchsc.edu

The bombesin like peptides (BLP) have a variety of properties, including appetite suppression and growth promotion of respiratory epithelial cells and lung cancer, that are relevant to tobacco addiction and carcinogenesis. Several studies in humans and experimental animals have demonstrated that tobacco smoking or nicotine treatment is correlated with increases in levels of BLP. Furthermore, tobacco smoking is associated with expression of one bombesin receptor subtype, the gastrin releasing peptide receptor, especially in females. We have found that longterm smokers with elevated urinary BLP levels are significantly more likely to have either chronic obstructive pulmonary disease or lung cancer than are longterm smokers with low urinary BLP levels. In addition, there is a negative correlation between urinary BLP and body weight. However, the large degree of variation in urinary BLP levels that is found in either smokers or nonsmokers makes correlation with susceptibility to tobacco induced lung disease difficult. In order to determine whether variation in urinary BLP levels is partly genetically determined, we measured urinary BLP (expressed as fmol/mg creatinine) in a population selected for lack of environmental factors, particularly tobacco smoke exposure, that might influence levels. We studied 105 healthy individuals from 80 families, taking no medications, between 18-45 years of age, with less than 100 cigarettes/lifetime, and with no history of parental smoking or exposure to passive smoke at home or in the workplace. Data were analyzed using the S.A.G.E. REGC and NOCOM programs. A trimodal distribution is the best fit for the data, with a bimodal distribution marginally less likely ($p < 0.03$) and a unimodal distribution highly unlikley ($p < 0.00001$). Thus, the data are most consistent with genetic determination of variability in BLP excretion, most likely as a result of variation in a codominant fashion at a single locus. In this two allele model, three subtypes (LL, mean 188 fm/mg creat; HL, mean 383 fm/mg creat; HH mean 635 fm/mg creat) would occur, with gene frequencies of 0.866 for L and 0.134 for H. Observed genotype frequencies agree well with those predicted by the Hardy-Weinberg equation. In 20 individuals, multiple urinary BLP determinations were made; subtype reproducibility was 95%. Analysis of 25 sib pairs demonstrated a heritability of approximately 80%, based on an intraclass correlation of 0.42. This suggests that it should be feasible to map the locus responsible for variation in BLP excretion in humans using standard linkage strategies. Elevated levels of another growth factor, IGF1, have been associated with increased risk for breast, prostate and lung cancer; however, the contribution of genetic variation to IGF1 levels is not known. Genetic variation in urinary BLP excretion may contribute to susceptibility to the tobacco induced lung diseases chronic obstructive pulmonary disease and lung cancer and may affect alterations in body weight associated with tobacco smoking.

P0014. A clinical genetics and cancer registry database with single server, multiple clients, and choice of client interface.

G. K. Suthers
Women s & Children s Hospital; North Adelaide, Australia
suthersg@mail.wch.sa.gov.au

The SA Clinical Genetics Service provides a general genetic counselling service, a Familial Cancer Service, and hosts the Familial Cancer Registry and research studies. We need prompt access to the records of over 30,000 clients and relatives. The overlapping roles within the Service demand that we have a single database, but the diversity of roles dictates that one interface cannot meet the needs of all users. The core of our database is the client-server implementation of Progeny Enterprise, a combined database and pedigree-drawing program. The flexible nature of Progeny allowed us to develop and readily modify the database and the pedigree interface has been invaluable in reviewing families. However, Progeny s flexibility means that the interface is not very user-friendly , and the lack of a tight database structure limits the data export and reporting formats. We have developed a non-graphical interface, KinTrakDB, that accesses a standard sub-set of the Progeny fields. This meets our requirements for clinic and record management. KinTrakDB also supports cancer registry functions. Other database fields can be readily defined and accessed using the Progeny interface. The combination of different client interfaces represents a cost-effective means of providing tailored interfaces to the one database. The principle could be readily applied in other clinical settings.

P0015. New Challenges Face Medical Geneticists in the Tumor Case Conference Setting

R. Young[1], *K. Brooks*[2]
[1]*University of South Carolina School of Medicine; Columbia, SC United States;* [2]*University of South of Carolina School of Medicine; Columbia, SC United States*
byoung@richmed.medpark.sc.edu

As the study of cancer genes, DNA markers, and molecular technologies worked their way from the research laboratories to the clinic many geneticists now find themselves attending tumor conferences. This brings the opportunity and challenges of offering new technologies to help solve long-standing clinical dilemmas. Following are just two examples of many situations. A 40-year old male, heavy smoker with tongue cancer diagnosed two years previously. The aggressive malignancy was seemingly treated successfully with extensive surgery. After two years of remission, a medical examination showed a lung lesion. If the lung lesion was a metastasis, the prognosis would be poor. If the lung lesion was a new primary, a surgical cure would be possible. The geneticist present at the conference suggested that molecular studies might assist this differential. FISH study of HER-2/neu, c-myc, cyclin D, centromeres 8, 11, and 17 showed complete concurrence suggesting a metastasis. A 53-year old women was presented regarding planned lumpectomy to remove a small breast lesion. During the presentation the surgeon casually added that a sister had breast cancer at age 42 and that the patient was Ashkenazi Jewish. The geneticist suggested gene predisposition testing, citing that recent studies favor consideration of less conservative surgery in the presence of BRCA1/2 mutations. Molecular genetics is proving to be of great value in the basic understanding of the etiology, progression, and prognosis of certain cancers. We are now finding that this technology will have an important use in assisting in the surgical and medical management decisions.

P0016. The Carrier Clinic-a new model for oncological genetic practice?

A. T. Ardern-Jones[1], *G. Mitchell*[2], *R. A. Eeles*[2]
[1]*Royal Marsden Hospital, Department of Cancer Genetics; Sutton, United Kingdom;* [2]*Institute of Cancer Research; Sutton, United Kingdom*
Audrey.Ardern-Jones@rmh.nthames.nhs.uk

Patients with germ line mutations in cancer predisposition genes are at markedly increased risk of developing cancer. Furthermore, having devel-

oped cancer once does not preclude the development of further primaries. The early symptoms of cancer may be subtle or masquerade as benign disease. It is essential for nurses caring for such patients to have an understanding of cancer in order to realise the significance of symptoms that may seem non threatening to the patient. This paper describes three different case scenarios of families where urgent referral was expedited following incidental reported symptoms during telephone follow-up calls from the nurse counsellor. Currently with the changing knowledge related to gene carriers and their risks of other primaries, other health professionals are not necessarily aware of the implications for such cases. At the Royal Marsden Hospital in London, UK an annual follow up gene carrier clinic has been set up and this clinic acts as a gate keeping clinic for such families. This is staffed by a multidisciplinary team and both the nursing and medical staff have in-depth oncological as well as genetic training. The clinic has an open-door policy via the clinical nurse specialist who is available by telephone to answer patient s queries. The clinic team liases closely with other practice specialists, tailoring these contacts to the patient s needs. We propose that gene carriers are followed up in such Carrier Clinics to provide up-to-date information and recommendations for management of these high risk individuals.

P0017. The prevalence of familial cancers in our Department of Medical Oncology

I. Sandovici[1], *A. Buiuc*[2], *M. Covic*[1], *E. Bild*[2]
[1]*University of Medicine; Iasi, Romania;* [2] *St. Spiridon Hospital; Iasi, Romania*
isandovici@yahoo.com

The positive familial history is a major risk factor for common cancer. We present preliminary results of the first clinical trial on familial cancer aimed at building a familial cancer register and introducing genetic testing and counseling in the affected families. In 1999 the 2045 new patients with cancer, regardless the localization, received a questionnaire on family history. The family history was considered as positive for the patients with at least one affected first degree relative. The overall prevalence of positive family history was 8.06% (165 patients). Family history was positive especially for the common types of cancers; 12.98% for breast cancer, 18.49% for colorectal cancer, 19.04% for endometrial cancer and 10.76% for ovarian cancer. We found families with clinical criteria suggestive for some hereditary cancer syndromes; 6 families with HNPCC (Amsterdam II criteria), 5 families suggestive for Li-Fraumeni syndrome and 6 families with strong arguments for breast-ovarian cancer syndrome. Also, we found 16 families with strong history but without any pattern suggesting one specific entity. Moreover, we have recorded 7.81% lung cancer patients with positive family history, frequently nonsmokers and 7.98% positive history for cervical cancer patients. In conclusion, the overall prevalence of positive family history we have found is similar with the data in the literature. These high prevalence underlines the necessity of the set up of genetic testing in familial cancer in Romania. Future investigations are necessary for lung and cervical cancer due to the possibility of a special genetic risk factor.

P0018. Involvement of G1, G2/M phase checkpoints gene alterations in tumorigenesis.

M. Aranda, *J. Carrasco, J. Toro*
University of Santiago de Chile; Santiago, Chile
maranda@lauca.usach.cl

Alterations in genes involved in cell cycle regulation are common in many tumor types. Abnormalities in the CDK4 and p53 genes, which regulate transition through G1 phase of the cell cycle, have been implicated in the progression of diverse types of cancer. The general goal of p53 appears to be the prevention of cell propagation if mutations are present. The p53 protein acts as a transcription factor by binding to certain specific genes and regulating their expression. The function of p53 can be suppressed by another gene, MDM2, which is overexpressed in certain tumorigenic cells and binds to p53 protein, thus inhibiting its transcriptional activation function. Since in different tumours MDM2 amplification involves amplicons encompassing flanking genes, such as CDK4, this gene could act as an alternative or additional gene involved in the transformation mechanism. To evaluate the involvement of G1, G2/M alterations in the chemical tumorigenesis, the status of these genes or gene products and cell cycle phases were examined. The genetic alterations were analysed by mutation analysis, fluorescence in situ hybridization (FISH) and single-cell gel electrophoresis (comet assay). Analysis of flow cytometry showed changes in both the G0/G1 phase and G2/M phase checkpoints. We detected G2/M arrest and apoptosis, in Hep-2 cell line in response to the treatment with the mycotoxin FB1. However, the maximal rate of apoptosis was rather small. Single strand DNA breaks were shown and repair diminished when BrdU incorporation was utilized. Furthermore, the loss of p53 gene probe in relation to the 17 centromere probe was detected. In summary, the early events in chemical tumorigenesis by FB1 leads to increased survival, disruption of the cell cycle G1, G2/M restriction point and single strand DNA breaks that produce p53 gene lost. Supported by FONDECYT # 1990212

P0019. Normal tissue radiosensitivity of cancer patients is not commonly associated with a constitutional dysfunction of the p53- or mre11/rad50/nibrin-mediated pathways in DNA double strand break repair

M. Bigalke[1], *R. Bendix*[1], *M. Stumm*[2], *J. Dahm-Daphi*[3], *M. Rave-Fr nk*[4], *D. Schindler*[5], *M. Bremer*[1], *D. Rades*[1], *J. H. Karstens*[1], *T. D rk*[1]
[1]*Medizinische Hochschule; Hannover, Germany;* [2]*Otto-von-Guericke-Universit t; Magdeburg, Germany;* [3]*Universit tsklinikum Eppendorf; Hamburg, Germany;* [4]*Georg-August-Universit t; G ttingen, Germany;* [5]*Biozentrum, Universit t; W rzburg, Germany*
doerk.thilo@mh-hannover.de

Clinical radiosensitivity is manifested as acute or late normal tissue toxicity in about 0.5 - 1 % of cancer patients receiving conventional radiotherapy. Because clinical radiosensitivity and radiation-induced chromosomal instability are associated in patients with ataxia telagiectasia (A-T) and the Nijmegen breakage syndrome (NBS), we have investigated lymphoblast cells lines from 15 radiosensitive cancer patients (10 with breast cancer, 5 with head and neck cancer) for molecular defects in DNA double-strand break repair pathways. Western blotting revealed apparently normal levels of the mre11, rad50 and nibrin proteins, components of the radiation-induced nuclear repair foci. ATM-dependent phosphorylation of p53 at serine-15 appeared to occur normally after irradiation with a single dosis of 6 Gy in all patient lymphoblasts, independent of whether they carried the Arg or Pro variant of p53 at codon 72. Similarly, the phosphorylation of nibrin proceeded normally after irradiation in all investigated cell lines. We also performed an additional mutation scanning of the entire coding regions of the Mre11, Rad50 and DNA-ligase-IV genes. Only in the DNA-ligase-IV gene of one patient a heterozygous sequence alteration was detected, a cytidine deletion three nucleotides downstream of the stop codon, which was also present at low abundance in the general population (allele frequency 0.02) and thus is unlikely to be of functional significance. We conclude that normal tissue radiation toxicity of cancer patients, apart from the classical radiosensitivity syndromes A-T and NBS, is not commonly associated with a constitutional dysfunction of p53- or nibrin-mediated pathways in DNA double strand break repair.

P0020. Dominant Negative Effect Of Novel Mutations On Pyruvate Kinase-m2 Activity In Bloom Syndrome Cells

M. Anitha, *R. Bamezai*
Jawaharlal Nehru University; Delhi, India
anitha52@hotmail.com

The present study reports for the first time natural mutations in Pyruvate kinase (PK)-M2 isozymic form, responsible for the regulation of the enzyme activity. The PK enzyme activity showed down regulation, in the B-lymphoblastoid cells established from two Bloom syndrome patients, BS1, BS3 by; 50% and 90%, respectively; and by 75% in the freshly obtained PHA stimulated lymphocytes of a BS patient diagnosed for the first time in India. An analysis of the critical domains of the PK-M gene in BS cells, resulted in the observation of different mutations in exon-10 which codes for the inter sub-unit contact domain (ISCD) region of the PK-M2 protein. Apart from these mutations, few single nucleotide polymorphisms (SNPs) were observed in this region of the gene both in normal and BS cells which did not affect the enzyme activity. Modelling studies of each of the PK-M2 protein with a mutation was suggestive of a changed interaction between two domains within a sub-unit in BS1, a gross structural change in BS3 and a changed interaction between two sub-units of the tetramer in the BS patient. The presence of mutations in PK-M2 ISCD region and its correlation with the downregulated enzyme activity in the Bloom syndrome cells has opened the possibility of looking at this gene in more details in future in the background of a variety of pleiotropic phenotypic features observed in this syndrome.

P0021. Receptore Tyrosine Kinases Signaling In Spleen Lymphocytes Under The Influence Of X-ray Irradiation In Low Doses.

T. Kirpenko[1], *L. Ostapchenko*[1], *I. Kondrichin*[2]
[1]*Kiev National Taras Shevchenko University; Kiev, Ukraine;* [2]*R.E. Kavetsky Institute of Experemental Oncology, Pathology and Radiobiology; Kiev, Ukraine*
tanyakirpenko@yahoo.com

The Receptor Tyrosine Protein Kinases (RTPK) have been shown to play a crucial role in radio-induced signaling response in rat spleen lympho-

cytes. The 2-fold increase of its tyrosine kinase domain activity under the influence of X-ray irradiation in dose of 1 Gy has been established. However, we have also determined a partial reduction of cytokine stimulating action on RTPK activity in conditions of irradiation. To clarify a reason of RTPK hyper-activation we have carried out partial purification procedures with using the antiphosphotyrosine antibodies and then valued the autophosphorylation rate of this enzyme by autoradiography and kinetic analysis. These data reveal the significant increase of RTPK autophosphorylation degree under the influence of X-ray irradiation in dose of 1 Gy. Furthermore, the studies performed by us earlier testify to disruptions on the level of enzyme-substrate association under the influence of X-ray irradiation. Obviously, the above mentioned disruptions could result in alteration of downstream signaling pathways, which include activation of distinct Protein kinases. As a result of examination of intracellular Protein kinases activities (cAMP-, cGMP-, Ca2+/phoshpolipid- and Calmodulin-dependent Protein kinases) after spleenocytes stimulation by cytokine we have observed a significant increase of cAMP-dependent protein kinase activity in control conditions, while it has been reduced under the influence of X-ray irradiation in 1 Gy. After performed researches we suggest that cytosolic cAMP-dependent Protein kinase participates in radio-induced signal transduction pathways through RTPK in rat spleen lymphocytes and it is accompanied by tyrosine kinase domain hyperautophosphorylation. Thus, RTPK are involved in radiation-induced response of immune competent spleen cells and mediate the effects of cytokines signal transduction in these cells in 12 hours after rat s irradiation in dose of 1 Gy. Our researches suggest that X-ray induced disorders of RTPK functioning are caused by qualitative alteration of this enzyme and in the future need to be proved by checking of RTPK expression level in spleen lymphocytes as well as gene targeting which will show important clues about the function of RTPKs.

P0022. Quantification of the hTR and hTERT telomerase subunits using real-time Polymerase Chain Reaction

***R. Bauer**, U. Claussen, G. Ernst, F. von Eggeling*
Institute of Human Genetics and Anthropology; Jena, Germany
gruenowski@yahoo.com

Telomerase, a ribonucleoprotein complex, plays an important role in the maintenance of telomeres in eucaryotic cells. The telomerase is found in normal somatic cells during the entire embryogenesis but later on only in stem cells, germ cells and activated lymphocytes. An abnormal activation of the enzyme can result in an indefinite proliferation and immortalization of the cell. In more than 80% of malignant tumours telomerase activity has been shown. Till now, the TRAP assay (Telomeric Repeat Amplification Protocol) has been a common tool for activation analysis, but it is not suitable for quantification. This problem can be overcome by quantification of telomerase specific RNAs using real-time PCR. Therefore, we quantified the functional RNA (human telomerase associated RNA, hTR) and the mRNA of the catalytic subunit (human telomerase reverse transkriptase, hTERT) of telomerase using real-time PCR. For wich a new primer system for the detection of both telomerase units was established. Isolated RNA was used from tumour cell lines (COLO 230, SK-MEL), normal cell line (G-292) and blood as control. By comparing hTR and hTERT it was possible to detect differences in the expression between normal and tumour cells. In all the cell lines and the blood the functional RNA of telomerase was constitutively expressed at least on a low level. A high level of hTERT was only detected in COLO320, whereas in SK-MEL and the normal cell line the expression was low. In the blood sample we found no hTERT at all. We conclude that this newly established system for differential quantification of hTR and hTERT offers the opportunity to distinguish between expression profiles of normal and tumour cells and to define a cut off value for cellular malignity.

P0023. Occurence and clinical significance of telomerase activity in tumor tissue and resection margins of head and neck squamous cell carcinomas (HNSCCs)

***G. Ernst**[1], W. Fiedler[2], F. von Eggeling[1], R. Dahse[1], S. Koscielny[3]*
[1]Institute of Human Genetics and Anthropology; Jena, Germany; [2]Molecular Gastroenterological Oncology, MLU Halle-Wittenberg; Halle, Germany; [3]ENT-Department; Jena, Germany
gernst@mti-n.uni-jena.de

Using Telomeric Repeat Amplification Protocol (TRAP) we have analyzed telomerase activity in tissue samples from HNSCCs (n=80) and in the corresponding pathohistological tumor-free resection margins (RMs) (n=69). The results were correlated with the clinical data and the disease course of the patients (median postoperative follow up time=37.3 month) by statistical means. In 60 of 80 (75%) carcinomas and in 38 of 69 (55%) matched RMs telomerase activity was detected. 33 of 69 (48%) cases showed telomerase activity in both, tumor tissue and RMs. In 9 of 69 (13%) cases enzyme activity was found in RMs but not in the corresponding tumor tissues. 17/60 (28%) telomerase-positive tumors showed telomerase-negative RMs. Enzyme activity in the RMs were observed in 75 % of the hypopharyngeal carcinomas, 61% of the laryngeal carcinomas, 54% of the oral carcinomas and 44% of the oropharyngeal carcinomas. The occurence of local recurrences was not influenced by the telomerase acticity of the tumors itself, but patients with telomerase-positive RMs developed fewer (18.4%) local recurrences than patients with telomerase-negative margins (25.8%). In 58% of the tumors with and 45% of the tumors without enzyme activity regional lymph node metastases were found. In 63% of the patients with telomerase activity in the RMs lymph node metastases are present at first diagnosis of the tumor in contrast to 48% of the patients without telomerase activity in the RMs. Regional recurrences occurred in 18% of the patients with telomerase activity in the tumors and in 15% of the patients without telomerase activity. Patients with telomerase activity in the RMs tend to have a higher rate of regional recurrences (27%) than patients without telomerase activity (16%). Telomerase activity in the RMs may have no influence on the tumor-dependent survival of the patients. Although the obtained values achieved no statistical significance we conclude that the detection of telomerase activity in HNSCCs and RMs correlated to the clinical data can be helpful for identification of a subset of patients with higher risk for regional lymph node metastasis and regional recurrences.

P0024. Detection of telomerase activity by PRINS; An alternative to in situ TRAP

***J. Mey**[1], J. Koch[2], S. Michel[1], F. von Eggeling[1], U. Claussen[1], G. Ernst[1]*
[1]Institute of Human Genetics and Anthropology; Jena, Germany; [2]Institute of Human Genetics; Aarhus, Denmark
b6meja@mti-n.uni-jena.de

Over the last years numerous protocols for detecting telomerase activity have been described as modifications and refinements of the PCR-based TRAP assay (Telomeric Repeat Amplification Protocol, Kim et al., 1994). However, only a few reports concern determining telomerase in situ. This could be due to difficulties associated with in situ PCR, e.g. the amplification of non-specific sequences. In this work we tried to circumvent such problems by replacing in situ PCR with a PRINS (Primed In Situ Labeling)-based detection method. For the establishment of this new approach all reactions were performed using the telomerase positive cell-line COLO 320. First, the cells were incubated with a primer (TS) for telomerase-mediated primer extension. Elongated oligonucleotides were then detected with a PRINS reaction including incorporation of indirectly labeled nucleotides. After immunostaining, the cells were assayed under a fluorescence microscope. Control reactions comprised RNase-treatment, heat-treatment before telomerase reaction, and omission of the TS primer. With the PRINS approach we obtained specific signals which seemed to be telomerase-dependent. The advantage of this new method over in situ TRAP mainly relates to the stability of cell structure and specificity gained by eliminating cyclic denaturation and amplification. Moreover, the use of ddGTP at the PRINS-reaction prevents elongation of unspecific genomic sequences. The further development of our method will focus on the reduction of background staining, testing of reproducibility, and the analysis of more cell lines prior to application on tissue sections.

P0025. Expression analysis of telomerase subunits (hTR, hTERT, TP1) in cells from the urine of bladder cancer patients

***A. Bleichert**[1], W. Fiedler[2], J. Mey[1], F. von Eggeling[1], R. Dahse[1], K. Junker[3]*
[1]Institute of Human Genetics and Anthropology, Friedrich-Schiller University; Jena, Germany; [2]KIM 1 - Molecular Gastro. Oncology, Martin-Luther-University; Halle-Wittenberg, Germany; [3]Department of Urology, Friedrich-Schiller University; Jena, Germany
able@mti-n.uni-jena.de

Recent investigations have demonstrated that telomerase, a ribonucleoprotein complex linked to cellular immortalization, is activated in a variety of malignant human tumors. Until now most investigations deal with activity analysis using the telomeric repeat amplification protocol (TRAP). Some reports show that, although the tumor is telomerase negative, the expression of telomerase subunits is of diagnostic significance. Therefore, we combined three existing RT-PCR assays to determine the telomerase subunits (hTR, hTERT and TP1) at the RNA-level in cells from urine samples of patients with bladder cancer and in healthy subjects as control. Of the 50 bladder cell carcinoma specimens almost all cases showed an expression of hTR, whereas hTERT and TP1 were not detectable. In patients with benign bladder disease (n = 10) and healthy subjects (n = 10) hTR was also found to be expressed, but not the hTERT and the TP1 subunits. The

expression pattern of the 3 telomerase subunits seems to be identical irrespective whether the cells were obtained from healthy individuals or from patients with bladder cancer. The results suggest that the expression of the telomerase subunits is not significant higher in cells of urine samples from bladder cancer patients compared to healthy persons. Therefore, the analysis of the telomerase activity in exfoliated urothelial cells from voided urine samples seems not to be a reliable method for differentiation between benign and malignant bladder carcinomas.

P0026. Semi-quantitative evaluation of telomerase activity and expression as a tumour marker in gastric and colon cancer

J. S. Nowak[1], *K. Lewandowski*[1], *K. Nowicka-Kujawska*[1], *M. Pernak*[1], *D. Januszkiewicz*[2]
[1]*Institute of Human Genetics Polish Academy of Sciences; Poznan, Poland;* [2]*Academy of Medical Sciences, Institute of Human Genetics Polish Academy of Sciences; Poznan, Poland*
nowakjs@man.poznan.pl

Reactivation of telomerase is believed to play an important role in immortalization and carcinogenesis. Despite criticism on etiopathological significance of telomerase in carcinogenesis, evaluation of telomerase expression and activity may be considered to be a potentially useful diagnostic marker.Telomerase expression has been studied by RT-PCR using specific primers for telomerase reverse trancriptase. Telomerase repeat amplification protocol-TRAP (qualitative TRAPEZE telomerase detection kit,Intergen and quantitative TeloTAGGG telomerase PCR ELISA, Roche) was used for analysis telomerase activity.In a preliminary study high expression of telomerase in gastric and colon cancer has been found. Lymphocytes stimulated by PHA also showed high telomerase expression. Low telomerase expression has been found only in some cases of non-cancerous mucosa from corresponding patients with gastric and colorectal cancer and peripheral blood lymphocytes. Telomerase activity in 10 000 cells has been observed in all studied samples. 300-cells lysate of all cancer cells, as well as PHA stimulated lymphocytes showed telomerase activity in contrary to normal cells. On the basis of obtained results it can be concluded that all cancer cells tested have higher telomerase expression, as compared to normal cells. Taking into account clear-cut relationship of telomerase activity and cell number, it can be postulated, that quantitative or semiquantitative evaluation of telomerase activity and expression should allow to distinguish cancer from normal tissue.

P0027. Mismatch repair defects and tumour predisposition

R. M. W. Hofstra[1], *Y. Wu*[1], *M. J. W. Berends*[2], *R. G. J. Mensink*[1], *C. Kempinga*[3], *R. H. Sijmons*[4], *A. G. J. van der Zee*[5], *H. Hollema*[3], *J. H. Kleibeuker*[6], *C. H. C. Buys*[1]
[1]*Dept. Medical Genetics; Groningen, The Netherlands;* [2]*Dept. Gastroenterology; Groningen, The Netherlands;* [3]*Dept. Pathology; Groningen, The Netherlands;* [4]*Dept. Medical Genetics; Groningen, The Netherlands;* [5]*Dept. Gynaecology; Groningen, The Netherlands;* [6]*Dept. Gastroenterology; Groningen, The Netherlands*
r.m.w.hofstra@medgen.azg.nl

Defects in mismatch repair (MMR) genes result in an accumulation of mutations, in tumours reflected as microsatellite instability (MSI). Germline mutations in MMR genes predispose to hereditary cancer and have been found first and in particular in families with (non-polyposis) colorectal cancer (HNPCC). Occurrence of MSI is used as a criterion for mutation analysis of MMR genes. The tighter the definition of familial CRC, the higher the probability to find mutations, in particular in MSH2 and MLH1. With a looser definition, there is a relative increase of cases with tumour types other than CRC, mostly endometrial cancer (EC). Different MSI frequencies are observed in CRC and EC, dependent on the type of microsatellite marker analysed, indicating tissue-specificity with respect to the relative amounts of MMR proteins. Because mismatch preferences of MMR proteins are different, we argued that absence of tumour MSI in families with CRC or related types of tumour cannot imply non-involvement of MMR genes. Indeed, MSH6, involved in repairing single nucleotide mismatches that do not show up as MSI, is mutated in a substantial proportion of families. We also identified germline mutations in MLH3 and EXO1. In some cases, different mutant genes may need to occur in combination in order to predispose to tumour development

P0028. HPC2/ELAC2 is a marker for prostate cancer.

D. S. Gerhard, *B. K. Suarez, L. T. Nguyen, N. K. Kesterson, J. Lin, B. Haberer, W. J. Catalona*
Washington University School of Medicine; St. Louis, MO United States
gerhard@genetics.wustl.edu

We report an analysis of two polymorphisms in the HPC2/ELAC2 gene in multiplex prostate cancer (CaP) sibships and matched controls. Two missense variants have been reported to be associated with CaP; S217L and A540T in a sample of 359 CaP cases and 266 matched controls (Rebbeck et al., 2000). The association was due to a significant increase of the 540T variant in the CaP cases compared to controls. The 540T variant was in complete linkage disequilibrium with the 217L variant.
We genotyped these polymorphisms in 257 multiplex CaP sibships and 355 matched controls. The control subjects have been followed for years as part of a large PSA study. All are free of CaP and none have ever registered a PSA level >2.5 ng/ml. All subjects in this study are of European ancestry.
The frequency of the 540T allele in our CaP cases is significantly greater than in the control series (0.051 vs. 0.021; $p < 0.01$). We also observe complete linkage disequilibrium between these two variants. Among the 281 multiplex sibships, 40 were found to contain at least one sibling carrying the 540T allele. All were heterozygous. The Table reports a nonsignificant difference in the observed and expected distribution of affected sib pairs (ASP) and affected trios with respect to 540T.

Sibship configuration	Observed	Expected
ASP; T/A, T/A	10	11.55
T/A, A/A	23	21.45
Trios; T/A, T/A, A/A	3	3.52
T/A, A/A, A/A	4	3.48

Additionally, the 540T carriers did not differ from noncarriers in their mean Gleason score (p=0.84) nor in their mean age-of-onset (p=0.45).

P0029. Genetic variants of the prostate carcinoma tumor antigen-1 (PCTA-1) in hereditary and sporadic prostate cancer

C. Maier[1], *J. Haeussler*[1], *K. Roesch*[1], *S. Bochum*[1], *T. Paiss*[2], *G. Cancel-Tassin*[3], *W. Vogel*[1]
[1]*Abteilung Humangenetik, Universit tsklinikum Ulm; Ulm, Germany;* [2]*Abteilung Urologie, Universit tsklinikum Ulm; Ulm, Germany;* [3]*CeRePP; Evry, France*
christiane.maier@medizin.uni-ulm.de

Prostate cancer is a complex disease with an estimated portion of 10 % familial cases caused by rarely mutated susceptibility genes. Recent linkage analyses have revealed at least five loci expected to harbour high risk alleles. In a candidate gene approach concerning the susceptibility locus PCaP (1q42.2-43) which was identified in a French and German population, we focused on the PCTA-1 gene encoding a member of the tumor associated galectin family. Selected on the base of cosegregation, 36 German and French prostate cancer sibships were examined by Enzymatic Mutation Detection (EMD). In 77 affected men no mutations were found within the coding sequence of the PCTA-1 gene, while in three affected brothers an intronic insertion upstream exon 6 was observed. In vitro investigations have been worked out to address a putative influence on splicing. In addition, the detection of three amino acid alterations (19Y/F, 36C/R and 184S/R) tempted us to evaluate frequent PCTA-1 alleles as putative low penetrance factors to predispose for prostate cancer. In an association analysis of 73 German sporadic cases versus 73 controls a heterozygous state with the haplotype 19F-36R-184S was found significantly overrepresented in sporadics and became even more prominent after stratification according Gleason scores higher than 6 (odds ratio = 5.3; p=0.022). However, in comparable French cases and controls these phenomena have not been recovered. Since general implications of PCTA-1 in malignancy and metastasis have been suggested but not yet assessed in detail, we started to search for interacting proteins using the two hybrid system.

P0030. Molecular genetic analysis of colorectal cancers demands a commitment to service delivery in developing countries.

R. Felix[1], *U. Algar*[2], *P. Goldberg*[2], *R. S. Ramesar*[3]
[1]*University Of Cape Town; Cape Town, South Africa;* [2]*GIT Unit, Groote Schuur Hospital; Cape Town, South Africa;* [3]*Department of Human Genetics, University of Cape Town; Cape Town, South Africa*
rf@anat.uct.ac.za

Hereditary nonpolyposis colorectal cancer (HNPCC) is a syndrome characterised by familial predisposition to colorectal carcinoma and extracolonic cancers. This dominant disorder is caused by germline mutations in one of at least five mismatch repair genes, viz. hMLH1, hMSH2, hPMS1, hPMS2 and hMSH6. Mutations in the hMLH1 and hMSH2 genes account for 80-90% of HNPCC cases. The frequency of HNPCC among South African colorectal cancer subjects has not yet been determined. The purpose of this report is to estimate the frequency of HNPCC amongst individuals diagnosed with generic colorectal cancer, excluding FAP, under the

age of 45 years. In this study the entire coding region of the hMLH1 and hMSH2 genes were screened using single stranded conformational polymorphism analysis and DNA sequencing in 100 subjects. Ten biologically significant mutations were detected. These mutations were not detected in 50 unrelated, unaffected control individuals tested. Genetic tests facilitate the molecular diagnosis of the disorder in sporadic cases and this aids in the identification of other asymptomatic family members. The results obtained in this study will allow us to determine the frequency of colorectal cancer due to HNPCC in South Africa. Ethically there is a commitment to genealogical tracings and investigation of familiality of HNPCC mutation carriers. Ideally, recruitment of family members for genetic testing and counselling, where appropriate, amounts to commitment of vast human and other resources for this purpose. The capacity to do this in a developing country on a sustainable basis may be used as an indicator for establishing other gene-based diagnoses .

P0031. Genetic Analysis of a Nova Scotia Kindred with Essential Thrombocythemia

J. R. Higgs, *I. Sadek, P. E. Neumann, V. W. Ing, E. Z. Zayed, W. L. Greer*
Dalhousie University; Halifax, NS Canada
jillianhiggs@hotmail.com

Essential Thrombocythemia (ET) is a chronic myleoproliferative disorder characterized by increased proliferation of megakaryocytes and elevated platelet counts. While ET usually occurs sporadically, we have identified a rare family with at least 5 affected individuals in two generations. Since platelet production is regulated by thrombopoetin (THPO) through its receptor, MPL, the respective genes were considered likely candidates for the underlying genetic defect segregating in this family. The pedigree was consistent with linkage to THPO; but MPL was excluded by linkage analysis. Elevated levels of THPO in affected individuals strongly supported the possibility of a mutation in the THPO gene that resulted in increased expression. Three similar families have been reported to have mutations in the 5 untranslated region of the full length THPO mRNA that were associated with loss of translational inhibition of THPO mRNA. Therefore, we are sequencing the corresponding genomic region in affected family members.

P0032. Cancer Risk in NBS Heterozygotes

E. Seemanova[1], J. Pelz[2], R. Varon[2], P. Jarolim[2], ***K. Sperling***[2]
[1]Institute of Biology & Medical Genetics, Charles University; Prague, Czech Republic; [2]Institute of Human Genetics, Humboldt University; Berlin, Germany
karl.sperling@charite.de

Nijmegen Breakage Syndrome (NBS) is an autosomal recessive disorder associated with high susceptibility to lymphoid malignancies. More than 90% of all patients known so far are of slavic origin and carry the same founder mutation. Its population frequency was estimated at 1;130 in the Czech Republic. The aim of the present study was to calculate the potential cancer risk of NBS heterozygotes. In a cohort study histories were taken of 181 persons from 18 pedigrees in a personal interview using a structured questionnaire covering reproductive history, X-ray history, working place history and current and former health status as well as possible confounding factors of life style. For 176 participants the genotype has been determined (85 heterozygotes and 91 normal homozygotes). Among the homozygous probands 2 cases of cancer were found, while 8 carcinomas were detected in the heterozygous (all 8 were of different type, average age of onset 62 years). This elevated risk of malignancies in the heterozygotes is also obvious in the pedigrees for those members, for which the probability of heterozygosity has been estimated by conventional calculations only. Already in 1990 an increased incidence of cancer amongst relatives of NBS patients has been noted (E. Seemanova, Mutat Res. 238, 321). Here, we present the first direct evidence for this assumption.

P0033. Association of E-Cadherin germline alterations with prostate and gastric cancer

T. Ikonen[1], *M. Matikainen[1], N. Mononen[1], E. Hyytinen[2], H. Helin[3], S. Tommola[3], T. Tammela[1], E. Pukkala[4], J. Schleutker[1], O. Kallioniemi[5], P. Koivisto[2], P. Koivisto[2]*
[1]Laboratory of Cancer Genetics, Institute of Medical Technology, University of Tampere and Tampere University Hospital; Tampere, Finland; [2]Department of Clinical Genetics, Tampere University Hospital; Tampere, Finland; [3]Department of Pathology, Tampere University Hospital; Tampere, Finland; [4]Finnish Cancer Registry; Helsinki, Finland; [5]Cancer Genetics Branch, NHGRI, NIH, Bethesda,; Maryland 20892, United States
lotaik@uta.fi

In our recent cancer registry-based study, the incidence of gastric carcinoma was increased up to 5-fold in male relatives of early-onset prostate cancer patients. This association may reflect the influence of genetic factors predisposing to both tumor types. Germline mutations of the CDH1 gene at 16q have recently been associated with familial gastric cancer. Furthermore, two genome-wide linkage studies of prostate cancer recently reported positivity at 16q. We therefore identified families and individual patients with both gastric and prostate cancer and investigated, whether the CDH1 gene mutations would be involved in cancer predisposition in these cases. Fifteen of the 180 Finnish hereditary prostate cancer families (8.3%) had one or more gastric cancer cases. No truncating or splice-site CDH1 mutations were identified by PCR-SSCP in these families, or in eight individual patients who had both prostate and gastric cancer. However, a novel S270A missense mutation in exon 6 of the CDH1 gene was seen in a single family with 4 prostate and 2 gastric cancers. A large-scale population-based survey indicated a higher prevalence of S270A among both familial prostate cancer cases (3.3%, n=120, p=0.01) as well as in unselected prostate cancer patients (1.5%, n=472, p=0.12) as compared with blood donors serving as population controls (0.5%, n=923). We conclude that individual rare mutations and polymorphisms in the CDH1 gene, such as the S270A, may contribute to the onset of prostate cancer and warrant further investigations in other populations. CDH1 gene does not, however, appear to explain the link between prostate and gastric cancer.

P0034. Mutational Analyses Of The Ret Proto-oncogene In Slovenian Men2 Families

B. Korosec, *S. Caserman, D. Bergant, M. Ravnik-Glavac, D. Glavac*
Medical faculty; Ljubljana, Slovenia
LMG@mf.uni-lj.si

Multiple endocrine neoplasia type 2 (MEN2) is an autosomal dominantly inherited cancer syndrome that appears in different subtypes MEN2A, MEN2B and FMTC. MEN2A is characterized by the combined occurrence of medullary thyroid carcinoma (MTC), pheochromocytoma (PCC) and parathyroid hyperplasia (PH). In FMTC the only manifestations of the syndrome are MTCs which usually develop at more advanced age. The MEN2B syndrome is the most severe form of the MEN2 phenotypes, characterized by an early onset of MTC and PCC, rapid progression to the metastatic MTC. The loci for MEN2 have been mapped to the centromeric region of chromosome 10q11.2. This region encompasses the RET proto-oncogene, a receptor-type tyrosine kinase. DNA was isolated from blood. Exons 10, 11, 12, 13, 14, 15 and 16 of RET proto-oncogene were amplified in PCR and were analysed by single stranded conformational analysis (SSCA) on polyacrylamide gels. Mutations were determined by the direct sequencing method. To confirm the mutations restriction enzyme Cfo I, Fnu4H1, Rsa I, and Fok I were used. In MEN2A families C634R, C643S, C634Y, C611R and L790F mutations were detected. In MEN2B families only mutation C918R mutation was identified. The mutation in FMTC family was C618R. Molecular genetic analysis of RET point mutations is a reliable method to detect asymptomatic gene carriers of MEN2. The early identification of gene carriers among members of MEN2 families will help to improve the planning of clinical management of affected individuals and to define the optimal point for preventative thyroidectxomy to reduce the risk of metastatic MTC.

P0035. Genetic analysis of ret proto-oncogene in 124 Spanish patients with at least Medullary Thyroid Carcinoma (MTC) or only pheochromocytoma.

A. Cebri n, *S. Ru z-Llorente, M. Urioste, J. J. D ez, A. Barroso, M. Robledo*
Centro Nacional de Investigaciones Oncol gicas; Madrid, Spain
acebrian@cnio.es

Multiple endocrine neoplasia type 2A (MEN 2A) is a syndrome characterized by medullary thyroid carcinoma (MTC) and pheochromocytoma, sometimes combined with parathyroid adenomas. Penetrance is almost complete, but gene expression is variable. We have available DNA from 124 unselected, consecutively patientes submitted to reject or confirm the familial character of the disease. These patients were divided in two groups. The first, included 105 patients with al least MTC, with or without familiar history. In the second group we included 19 patients with only phaeochromocytoma. By sequencing analysis, we studied, in all patients, the 6 mainly involved exons of the ret protooncogen, independently of age of onset. From 105 patients with at least MTC, 30 had familial history of the disease, and 93.3% of them presented germline mutations in ret. Among the 75 non-familial cases, only 7% showed germline mutations. In patients with only pheochromocytoma, 21% of cases presented germline mutations either in ret or in vhl gene. We were looking in both groups for a correlation between mutation, the patient s age and the clinical variables. With regard to patients who presented germline mutations in ret, the most striking result was the wide age distribution of non-symptomatic carriers of non-

634 mutation, versus the clustering noted within the 634 mutation carriers. On the other hand, in patients with only pheochromocytoma, there was no clear association between the presence or absence of germline mutation and the factors that we considered (bilateral or unilateral character, and familial history of the disease).

P0036. p53, hCHK1, and hCHK2 genes in Finnish families with Li-Fraumeni syndrome; further evidence of hCHK2 in inherited cancer predisposition

P. Vahteristo[1], *A. Tamminen*[1], *P. Karvinen*[1], *H. Eerola*[2,1], *C. Blomqvist*[2], *K. Aittom ki*[3], *H. Nevanlinna*[1]

[1]*Department of Obstetrics and Gynaecology, Helsinki University Central Hospital; Helsinki, Finland;* [2]*Department of Oncology, Helsinki University Central Hospital; Helsinki, Finland;* [3]*Department of Clinical Genetics, Helsinki University Central Hospital; Helsinki, Finland*

pia.vahteristo@helsinki.fi

Germline mutations in the p53 gene account for 70% of Li-Fraumeni families and 30% of families with Li-Fraumeni-like (LFL) cancer syndrome. The cell cycle check point kinases Chk1 and Chk2 act upstream of p53 in DNA damage responses. Recently, a germline mutation in hCHK2 gene was reported in a LFS family (Bell et al, Science 286;2528, 1999). We have analyzed p53, hCHK1 and hCHK2 genes for mutations in 45 Finnish families with LFS, LFL or phenotypically suggestive of LFS with conformation sensitive gel electrophoresis (CSGE). Genomic sequence comparisons were carried out to ensure specificity in the analysis of hCHK2 exons 10-14 with multiple homologous sequencies in the genome. Five different disease causing mutations were observed in seven families (15.6%); five in the p53 (11.1%) and two in the hCHK2 gene (4.4%). No mutations in the hCHK1 gene were identified. The p53 mutations (Pro151Thr, Arg196Stop, Arg 213Stop, Arg248Gln in two families) were found in the conserved, mutation rich exons 5-7. Two p53-families had a LFS phenotype (total 3 LFS families) and two LFL phenotype, and one was a family history-negative patient with both childhood sarcoma and early onset breast cancer. In the hCHK2 gene, a frameshift mutation in exon ten (1100delC) was found in two families, final analysis of two other sequence variants is currently underway. The cancer phenotype in the1100delC-families was not typically LFS or LFL, and may indicate variable phenotypic expression in the rare families with hCHK2 mutations. Still other genes may account for the remaining LFS/LFL families.

P0037. Characterisation of a neurofibromatosis NF1 mutation which results in the skipping of both exons 11 and 12a.

J. Thirion[1], *L. Fang*[1], *M. Simard*[1], *D. Vidaud*[2], *B. Assouline*[2], *B. Chabot*[1], *M. Vidaud*[2]

[1]*Facult de M decine; Sherbrooke, PQ Canada;* [2]*Univ. Paris V; Paris, France*

jpthir@courrier.usherb.ca

Neurofibromatosis type1 (NF1) is an autosomal dominant genetic disorder affecting neural crest-cell derivatives and resulting in cutaneous manifestations of caf -au-lait patches and neurofibromas. It is characterised by a wide variety of phenotypes, even in the same family. In order to correlate the genotypes with their phenotypes and to find the functions of neurofibromin, we have characterised a number of mutations from NF1 patients by Southern blots and a protein truncation test. A new mutation consisting of a G to A transition at position +1 of the 5 splice site of exon 12a causes the loss of both exons 11 and 12a without affecting the reading frame of the mutant mRNA. Transfection of HeLa cells with portion of the mutant and the wild type genes cloned in DUP4-1 vector derivatives and in vitro splicing assays using chimeric mutant or wild type adenovirus/NF1 pre-mRNAs indicate the mutation prevents the definition of exon 12a, a process that is normally required to activate the weak 3 splice site of exon 12a. Because the mutation affects exon 12a definition, exon11/exon 12a splicing is also compromised leading to the exclusion of both exons 11 and 12a. Thus these results provide strong support for the 1995 model of exon definition as proposed by Berget and suggest an important role for exons 11 and 12a in the activity of neurofibromin.

P0038. Mutation spectrum and genotype-phenotype analyses in Peutz-Jeghers syndrome

H. Mehenni[1], *C. Rossier*[2], *R. Chergui*[3], *K. F. Richards*[4], *L. Mota-Vieira*[5], *A. S. Leal*[6], *A. Lerner*[7], *S. E. Antonarakis*[8]

[1]*University of Geneva; Geneva, Switzerland;* [2]*Division of Medical Genetics, University of Geneva Medical School,; Geneva, Switzerland;* [3]*Service de Gastro-ent rologie, Clinique Rahmouni Djilali, West University Hospital Center; Algiers, Algeria;* [4]*LDS Hospital and University of Utah Medical Center; Salt Lake City, UT United States;* [5]*Unidade de Gen tica e Patologia Moleculares, Hospital De Ponta Delgada; Ponta Delgada,A ores, Portugal;* [6]*Surgery Department, University of Porto; Porto, Portugal;* [7]*Dept. of Pediatrics, Haifa Hospital; Haifa, Israel;* [8]*Division of Medical Genetics, University of Geneva Medical School; Geneva, Switzerland*

mehenni@medecine.unige.ch

Peutz-Jeghers syndrome is an autosomal dominant disorder characterized by mucocunateous pigmentation, hamartomatous polyps and an increased risk of associated malignancies. Colon and breast cancers are the most frequent malignancies, but also genital, and small intestine cancers are found. The LKB1/STK11 serine/threonine kinase is mutated in Peutz-Jeghers syndrome and functions as a tumour suppressor. There are, however, many families with PJS without mutations in LKB1, suggesting locus heterogeneity in this disorder. We have studied the molecular etiology in 20 PJS families, and identified 10 independent LKB1 mutations (a different one in each family). The spectrum of mutation includes missense and nonsense amino acid substitutions, deletions, insertions, a complex deletion/insertion and splice site mutations. Most of the LKB1 mutations result in truncated protein and/or disrupt the protein kinase catalytic domain leading to inactivation or a dramatic decrease of the kinase activity. The clinical features of affected individuals (N=105) of the 10 PJS families without a LKB1 mutations do not differ from PJS patients (N=204) with such mutations. Affected individuals from 5 out of 10 families with LKB1 mutations had developed cancers. Similarly, affecteds from 4 out of 10 PJS families without recognizable LKB1 mutations developed cancers. Finally, there was no significant correlation between the different mutations in LKB1 and the presence or absence of cancer, although the sample size is small for meaningful conclusions.

P0039. STK11/LKB1 gene; identification of alternative transcripts and new germline mutations

N. Resta[1], *A. Stella*[1], *F. Susca*[1], *M. Di Giacomo*[1], *M. Genuardi*[2], *W. Lattanzi*[1], *C. Simone*[1], *F. P. Rossini*[3], *G. Guanti*[1]

[1]*DIMIMP-Sezione di Genetica Medica-Universit di Bari; Bari, Italy;* [2]*Istituto di Genetica Medica-Universit Cattolica; Rome, Italy;* [3]*Servizio di Gastroenterologia —Endoscopia Ospedale San Giovanni; Torino, Italy*

resusc@medgene.uniba.it

STK11/LKB1 codifies for a ser-thr kinase whose function is still unknown. Germline STK11/LKB1 mutations can be found in about 50% of Peutz-Jeghers(PJ) patients. Somatic STK11/LKB1 mutations have be found in a small number of sporadic tumors of lung, ovary, skin, colon. The wild-type STK11/LKB1 protein shows a nuclear as well as cytoplasmic localization and an autocatalitic activity. Linkage studies in PJ families seem indicate the existence of an other locus on the long arm of the chromosome 19. In the last three years we collected 18 cases of PJS. At least another affected member was present in the family of 6 patients, the remaining 12 cases were sporadic. Germline mutations of STK11/LKB1 gene were found in 4 sporadic and 4 familial cases; in addition several different exonic an intronic polymorphisms were identified. Of relevant interest are a mutation(IVS2+1A >G) involving the non canonic consensus sequence lying in the splicing junction of the exon 2 and a change (IVS5+5A >G) in the intron 5 . The splicing effects of these two changes have been evaluated in COS-7 cells transfected with a pSPL3 plasmid in which the exonic sequences and the flanking intronic regions were cloned. In addition we have identified several mRNA isoforms, products of alternative splicing. The sequencing of two of these isoforms revealed the presence of two different out of frame deletions from the exon 2 to 7. This work was partially supported by AIRC and MURST-COFIN 98.

P0040. Exclusion of several genes mapped in 19q13.3-13.4 as candidates for a second locus in Peutz-Jeghers syndrome

K. Buchet, *H. Mehenni, C. Rossier, S. E. Antonarakis*

Division of Medical Genetics, Geneva University Medical School; Geneva, Switzerland

buchet@cmu.unige.ch

Peutz-Jegher s syndrome (PJS; OMIM 175200) is an autosomal dominant disease characterized by hamartomatous polyps and mucocutaneous pigmentation. There is an increased risk for developing various neoplasms with predisposition to benign and malignant tumors of the gastrointestinal tract, breast, ovary, uterine, cervix, and testis. Mutations in the serine/threonine kinase STK11 / LKB1 cause Peutz-Jeghers syndrome in most of affected individuals. However, a number of PJS-patients show no mutations in STK11 / LKB1 suggesting genetic heterogeneity. One large PJS-family has previously been described with significant evidence for linkage to a second potential PJS disease locus on 19q13.3-13.4. Mutation analysis of STK11 / LKB1 in the affected members of this family revealed no mutations, which supported the likelihood for the existence of a yet not identified second PJS gene in this region. In the current study we used a gene candidate approach in which we investigated the genomic region

between markers D19S180 and D19S254 for which multipoint linkage analysis yielded the maximal LOD score (3.9) in this particular family. Gene mapping and mutation search analysis revealed no pathologic mutations in several genes mapped in this region including STK13 and NES1, even if a number of polymorphic variants have been identified. Further analysis of candidate genes in this region is in progress.

P0041. Novel mutations in the SDHD gene in two cases of familial paraganglioma with associated pheochromocytoma

O. A. Haas[1], *A. Weinh usel*[1], *P. Waldner*[1], *C. Neumayer*[2], *B. Niederle*[2]
[1]*CCRI, St. Anna Children s Hospital; Vienna, Austria;* [2]*Surgical Univ. Clinic; Vienna, Austria*
o.a.haas@magnet.at

Familial paraganglioma (PGL1; OMIM 168000) is a rare tumor predisposition syndrome that is inherited in an autosomal dominant fashion, but exclusively through the male germ line. This pattern of inheritance is consistent with genomic imprinting. Affected individuals develop slow growing tumors of the head and neck region that affect most commonly the carotid body. Recently, the responsible gene, the succinate-ubiquinone oxidoreductase subunit D (SDHD) was identified (Science 287;848;2000). It encodes the small subunit of cytochrome b in mitochondrial complex II. Heterogeneous mutations were detected in five PGL1 families. Based on these findings, we studied two patients with hereditary paragangliomas and concomitant pheochromocytoma. Sequencing of the coding region revealed two novel mutations. In the first case, a 36-year-old man, we found a mutation in the start codon (ATG>AAG, met>lys) together with a 3 bp deletion in intron 1. Analyses of four siblings and two children revealed that the start codon mutation segregated with individuals affected by paragangliomas, whereas the deletion did not. This suggests that the latter most likely represents a polymorphism on the second allele. This deletion was not found in 100 normal control chromosomes. The second case, a 29-year-old woman, had a 12 bp deletions in exon 3, codons 91-94. Her potentially affected relatives are currently under investigation. The identification of asymptomatic gene carriers in PGL1 families is particularly relevant, because regular screening programs can be offered that allow the early recognition and treatment of tumors even before they become clinically apparent.

P0042. Hereditary paraganglioma with pheochromocytoma;genetic and functional study of a new mutation of SDHD gene

A. Gimenez-Roqueplo[1], *J. Favier*[2], *J. J. Mourad*[3], *P. F. Plouin*[4], *J. M. Gasc*[2], *X. Jeunemaitre*[1]
[1]*D partement de G n tique Mol culaire, H pital Europ en Georges Pompidou; Paris, France;* [2]*INSERM U36, Coll ge de France; Paris, France;* [3]*Service de M decine Interne et de Chirurgie Vasculaire, H pital Saint-Michel; Paris, France;* [4]*R seau PHRC AOM 95201 COMETE ; Paris, France*
Anne-Paule.GIMENEZ@hop.egp.ap-hop-paris.fr

Background; The paragangliomas are highly vasculars tumors arising from neural crest cells. They are commonly located at the carotid body (chemodectoma) and could be secreting (pheochromocytoma). Familial paragangliomas are commonly multiple, bilateral and present at an earlier age. Transmission of familial tumors occurs through autosomal dominant inheritance via the paternal line. Three loci were described PGL1, PGL2 and PGL3. Recently, the PGL1 and PGL3 loci have been identified as corresponding to the SDHD and SDHC genes, respectively, which encode for two proteins involved in the mitochondrial complex II. Patients; We report a French family with three affected men. The father and his first son exhibited bilateral chemodectomas. The second son developed a left chemodectoma and an ectopic cardiac pheochromocytoma. Genetic study; Polymorphic markers were selected from the PGL1 and PGL2 chromosome 11q region. The transmission of the paternal allele was demonstrated in affected children. Direct sequencing of SDHD revealed a new nonsense mutation at codon 22 in all affected individuals. Functional analysis; As mitochondria play a critical role in oxygen sensing, we have analyzed the expression of angiogenic markers by in situ hybridization and immunohistochemistry in chemodectomas and pheochromocytoma. A high expression of VEGF and his receptor VEGF-R1, EPAS and HIF1 were detected, suggesting that these markers induced by hypoxia could contribute to tumorigenesis and tumor vascularity. Others markers are under current investigation. Conclusion; As the VHL gene, SDHD is a hypoxia-response gene that might stimulate hyperplasia and tumorigenesis through the expression of angiogenic factors.

P0043. Frequency and parental origin of de novo APC mutations in familial adenomatous polyposis

W. Friedl[1], *R. Caspari*[2], *M. Sengteller*[1], *S. Uhlhaas*[1], *E. Mangold*[1], *P. Propping*[1]
[1]*Institute of Human Genetics, University of Bonn; Bonn, Germany;* [2]*Department of Internal Medicine, University of Bonn; Bonn, Germany*
friedlw@meb.uni-bonn.de

A predominance of de novo mutations in the paternal germline has been reported for several disorders (for review see Crow, Nature Reviews, Genetics 1, Oct. 2000). Parental origin of APC mutations in familial adenomatous polyposis (FAP) has not been systematically examined so far. FAP is a precancerous condition characterized by the occurrence of hundreds to thousands of colorectal adenomas and extracolonic manifestations. FAP is caused by germline mutations in the tumour suppressor gene APC. We examined 680 unrelated FAP families for germline mutations. In 53 out of 411 families with known family history (13%) both parents of the index patient had not developed colorectal adenomas suggesting a de novo mutation. A germline mutation was detected in 41 of these 53 patients; in 27 cases the mutation was excluded in both parents. Overall, the 5 bp deletion at codon 1309 was found in 47/680 patients (7%). This mutation was overrepresented in the group of patients with de novo mutations (17/53 = 32%), thus demonstrating that the high frequency of this mutation is not due to a founder effect but rather due to de novo mutation events. Parental origin of de novo mutations could be traced in 8 families. Five mutations were of paternal, and 3 of maternal origin. Interestingly, the 1309 de novo mutation was observed in the maternal germline, only. Mutations in the paternal germline included one large deletion of about 10Mb, two base exchanges (R564X; S1201X) and twice the 5bp deletion at codon 1061. In the maternal germline the 5bp deletion at codon 1309 was found twice, in addition to the mutation c.3556delGA at codon 1186. In conclusion, in our sample de novo APC germline mutations show a slightly higher frequency in the paternal germline. Further families have to be studied to see whether the observed difference in parental origin and mutation spectrum are reproducible. Acknowledgement; This study was supported by the Deutsche Krebshilfe.

P0044. Molecular and Clinical Profiles of Singapore Familial Adenomatous Polyposis Patients

P. Y. Cheah, *X. Cao, K. W. Eu, F. Seow-Choen*
Singapore General Hospital; Singapore, Singapore
gcscpy@sgh.com.sg

Familial adenomatous polyposis (FAP) is a heritable form of colon cancer caused by autosomal dominant inheritance of the mutated adenomatous polyposis coli (APC) gene. We investigated the APC mutation and phenotypic spectrum of 180 members of 39 Singapore FAP families. The protein truncation test (PTT) and DNA sequencing were used to screen the entire APC coding region for germline mutations. APC mutations were found in 31 families (79 %). 72 patients tested positive while 64 non-affected members tested negative. The correlation of PTT to clinical diagnosis is therefore 100%, suggesting that PTT is a highly specific presymptomatic test for FAP. Twenty two different APC mutations, including two genomic deletions, were identified. Thirteen mutations were novel. All mutations, except one, resulted in the classical colonic phenotype. Nine families have the same (AAAGA) deletion at codon 1309, indicating that like the Western families, codon 1309 is also the mutation hot spot for Singapore families. Interestingly, mutation at codon 332 resulted in attenuated FAP with left-sided predominance of polyps rather than the right. For the eight families without APC mutations, we screened for beta-catenin mutation which was shown to substitute for APC mutation in sporadic colorectal cancer. No germline beta-catenin mutation was found. Further analysis reveals atypical clinical features such as the co-existence of adenomatous and hyperplastic polyps and other non-FAP associated cancers in these patients. Our results suggest the involvement of other genes and possibly new variants for the polyposis syndrome.

P0045. Study Of Pedigree Families With Familial Adenomatous Polyposis

W. Lubinga
Medical University of Southern Africa; Pietersburg, South Africa
wlubinga@worldonline.co.za

BACKGROUND; This study was done at Donald Fraser hospital in rural remote South Africa. There were 18 pedigree families descended from a chief with 5 wives and 17 children. To date, this family has ~465 pedigree members excluding ~135 spouses. Of this pedigree, 15 persons died from colon cancer and 9 members have colorectal adenomatous polyps. Four persons in their late teens have had proctocolectomy. No colorectal cancer-screening program exists for these families. So far no genetic studies

have been done. OBJECTIVE; To screen pedigree members suspected with FAP for colorectal polyps by colonoscopy. METHODS; Selections were based on risk of having colon polyps from family history and on satisfying inclusion and exclusion criteria for colonoscopy. RESULTS; Seventy-seven persons were selected. Thirty-three (37%) persons responded and had colonoscopy. A mother had four and her daughter had eight colon adenomatous polyps. In the latter, polyps showed severe dysplasia with no malignancy. Both declined further interventions despite counselling for potential benefits of proctocolectomy. CONCLUSIONS; The study established a need for regular continuous colon cancer screening for the pedigree and provides information of use in setting up a hereditary colon cancer registry. These families need education and counselling about FAP, to improve compliance to screening programmes and surgical treatment. Chemotherapy for inducing polyp regression may be considered as an alternative treatment for objectors to colorectal surgery. Genetic testing identifies persons with the genetic trait and further research in this respect is recommended.

P0046. APC-gene mosaicism in sporadic attenuated FAP

J. Meuller[1], *J. Bj rk*[2], *Y. Engwall*[1], *H. Gronberg*[3], *E. Holmberg*[4], *M. Nordling*[1]

[1]*Clinical Genetics, G teborg University; G teborg, Sweden;* [2]*Dep. Gastroenterology & Hepatology, Karolinska Hosptital; Stockholm, Sweden;* [3]*Dept of Oncology, Ume University Hospital; Ume , Sweden;* [4]*Dept of Clinical Genetics, Ume University Hospital; Ume , Sweden*

johan.meuller@pediat.gu.se

Familial adenomatous polyposis (FAP {MIM175100}) is an autosomal dominantly inherited disease caused by germ line mutations in the APC gene. It predisposes to the development of hundreds to thousands of colorectal polyps, of which some will progress to cancer. Additionally, FAP-patients often develop extracolonic manifestation, such as desmoids, osteomas and retinal lesions. There is a high new-mutation rate with estimates of up to 25% of all cases being sporadic. Sporadic FAP-patients from the Swedish population are analysed with respect to APC-gene mutations to investigate whether mutational mosaicism could account for some of the new cases. We have to date found one case of low level mutational mosaicism. The patient had an attenuated form of FAP (few polyps) but mutational screening with direct sequencing or protein truncation tests on blood-derived DNA indicated a normal APC-gene. However, using single-stranded conformational polymorphism (SSCP) low level mutational mosaicism was demonstrated. Sequencing of the corresponding DNA-band, excised from a silverstained polyacrylamide gel, confirmed the presence of a mutation in the APC-gene. Mutation screening of various tissue from the affected patient and from the parents will be used to investigate the origin of the mutation. Our findings demonstrate the importance of using mutation detection methods which can detect mutated APC-alleles present at only low levels, especially for sporadic cases of FAP.

P0047. MALDI-TOF-MS based molecular diagnosis of familial adenomatous polyposis

A. Humeny[1], *T. Bonk*[1], *C. Sutter*[2], *J. Gebert*[2], *M. von Knebel-D beritz*[2], *C. Becker*[1]

[1]*Institut f r Biochemie, Emil-Fischer-Zentrum, Friedrich-Alexander-Universit t Erlangen-N rnberg; Erlangen, Germany;* [2]*Sektion f r Molekulare Diagnostik und Therapie, Chirurgische Klinik, Universit t Heidelberg; Heidelberg, Germany*

andreas.humeny@biochem.uni-erlangen.de

Familial adenomatous polyposis (FAP) is an autosomal dominant disorder which typically progresses from extensive adenomatous polyp formation of the colon to colorectal cancer in early adult life. FAP is associated with mutations in the adenomatous polyposis coli gene (APC) located on chr 5q21 which acts as a tumor suppressor gene. The most frequent mutation in APC is a 5 bp deletion starting in codon 1309 which predicts a translational frame shift leading to a truncated gene product. In general, the application of MALDI-MS (MALDI-TOF-MS, matrix assisted laser desorption ionization - time of flight - mass spectrometry) to DNA analysis provides a powerful tool for an efficient analysis of genetic polymorphisms and mutations. In comparison to gel-based or chromatographic techniques, MALDI-TOF-MS offers high accuracy and fast analysis in parallel allowing for high-throughput qualities. Here, we present a primer extension assay optimized for the analysis of the 5 bp APC deletion. The specific extension primer is elongated to different length depending on the presence or absence of the deletion. This results in defined molecular masses of the products generated, which are reliably distinguished by MALDI-TOF-MS. In addition, a multiplex assay to screen for other known mutations associated with APC is currently being developed.

P0048. Analysis Of Apc Gene Mutations In Familial Adenomatous Polyposis By Primer Extension Preamplification Of Microsamples Of Fixed Paraffin-embedded Tissue

K. Husnjak, *S. Kapitanovic, K. Pavelic*

Rudjer Boskovic Institute; Zagreb, Croatia

husnjak@rudjer.irb.hr

The purpose of our study was to determine the usefulness of primer extension preamplification (PEP) method in analysis of APC gene mutations in familial adenomatous polyposis (FAP) patients. We analysed polyps and peripheral blood from two patients with FAP. They had the germline 5 bp deletion at the codon 1309 of the APC gene. We assumed that at least some parts of polyps in two patients had the second hit at APC gene and other early genetic changes. The inability to detect them in DNA isolated from the whole polyp could be explained by presence of normal tissue within polyps or heterogenous genetic changes within the epithelium of a polyp. We analysed microdissections of deparaffinized tissue for mutations and loss of heterozygosity at the APC gene. In order to increase the quantity of target DNA available from small samples, each microdissection was first preamplifed by using PEP. Aliquouts of PEP reactions were then amplifed by using three pairs of APC primers, which could amplify three different parts of exon 15 of the APC gene. All samples were also analysed for K-ras mutation. PCR products were analysed by restriction fragment length polymorphism (RFLP) method and by electrophoresis on Spreadex EL 300 gels. By using microdissection and PEP-PCR we were able to prove germline, second hit mutation and loss of heterozygosity (LOH) of the APC gene. In order to confirm the accuracy of PEP method, three different pairs of primers for exon 15 of the APC gene were used - the same results were obtained. Our study showed that PEP method provided more accurate insight into different genetic changes of FAP.

P0049. Novel germ line mutations in Czech FAP families

M. Kohoutova[1], *J. Stekrova*[2], *V. Jirasek*[3], *J. Kotlas*[1], *J. Kapras*[1]

[1]*Institute of Biology and Medical Genetics, 1st Faculty of Medicine, Charles University; Prague, Czech Republic;* [2]*General Teaching Hospital of the Faculty, Charles University; Prague, Czech Republic;* [3]*1st Medical Department, 1st Faculty of Medicine, Charles University; Prague, Czech Republic*

mkoho@lf1.cuni.cz

Familial Adenomatous Polyposis (FAP) is an autosomal dominantly inherited disorder caused by germline mutation in the adenomatous polyposis coli (APC) gene. It is characterised by early onset of polyposis coli and development of colorectal carcinoma as a consequence of mutations in a number of tumor suppressor genes and oncogenes. The APC gene mutations were investigated in 46 Czech unrelated FAP families and 9 suspected FAP families to cover the entire of exons 1 - 15 to the codon 1773. Molecular studies involve DGGE and sequence analysis. The 25 germline APC mutations were found in total of 55 cases. Of the identified mutations eleven mutations have not yet been described and are presumed to be novel;1)seven frameshift mutations 1225delC in exon 9, 2733-2734insA, 3049-3052delAATG, 3332-3335delAAAC, 3751-3752insT, 3818-3822delGATGT and 3872-3873insA, all in exon 15 2) two nonsense mutations, 1297C>T in exon 9 and 1411G>T in exon 11. Both frameshift and nonsense mutations resulted in the classical form of FAP but the deletion C in exon 9 caused attenuated FAP. 3) two splicing mutations, IVS11+1G>A and IVS11+1G>T in intron 11. Family with this type of mutation showed variable individual phenotypes including an occurrence of CHRPE. The study continues using the combination of PTT and DGGE methods. Supported by the grant projects IGA MZ CR NC/6009-3 and GAUK 29/00

P0050. Identification of modifiers of the disease expression in FAP patients with a known APC mutation.

M. Plasilova[1], *N. B sch*[2], *H. M ller*[2], *Z. Dobbie*[2]

[1]*University Clinics Basel; Basel, Switzerland;* [2]*University Clinics; Basel, Switzerland*

martina.plasilova@unibas.ch

Familial adenomatous polyposis (FAP) is an autosomal dominantly inherited colorectal cancer predisposition syndrome, that is caused in the majority of cases by germline mutation within the adenomatous polyposis coli (APC) gene. However, based on the mouse model studies and a strong phenotypic variability observed both within and among affected families, FAP has been considered as a complex condition where environmental as well as genetic factors play a role in the disease expression. So far, such additional genetic factors remain unknown. A large FAP kinderd which harbours an adenine deletion at codon 1982 of the APC gene, has been previously reported by our group. Though carrying the same mutation, the affected subjects (45) present with variable colonic and extracolonic manifestations which are in several branches transmitted through the genera-

tions. To evaluate its correlation with the occurrence of extracolonic disease we initially performed linkage analysis of the 1p35-36 region, which revealed lod score of 2.08 for marker D1S211. Furthermore, a detailed mutation analysis of the candidate sPLA2, NAT2 and COX2 genes excluded them as potential modifiers of FAP phenotype. Recent evaluation of the potential of this pedigree to detect a modifier gene using simulation studies revealed very promising results that led us to continue the linkage mapping on the genome-wide level. The results of simulation studies, a consequent linkage analysis of further candidate regions and eventually results on the genome wide screening for a modifier gene in FAP condition will be presented.

P0051. Mutations of the APC gene in Polish patients with familial adenomatous polyposis coli.

A. Plawski[1], *T. Banasiewicz*[2], *M. Drews*[2], *P. Krokowicz*[2], *R. Slomski*[1,3]
[1]*Institute of Human Genetics; Poznan, Poland;* [2]*Regional Hospital; Poznan, Poland;* [3]*Department of Biochemistry and Biotechnology; Poznan, Poland*
slomski@au.poznan.pl

Analysis was performed on DNA isolated from 85 FAP patients, 165 FAP family members and 50 healthy controls. From all FAP patients 8,5% had features of Gardner syndrome. In 4 other cases the attenuated adenomatous polyposis of colon (AAPC) symptoms were observed. In analysis of APC gene in Polish population exons 10, 11, 12, 13, 14 and the 5 region of exon 15 were scanned for mutations using PCR-HD, PCR-SSCP and PTT. All differential SSCP patterns and heteroduplex bands were analyzed by direct sequencing of PCR product. Premature termination of translation (PTT) was found in 2 families (total 17 families were studied for segment 3 of exon 15). Mutations of APC gene in Polish population are heterozygous, but most of them (70%) were observed between codons 1040-1309. The frequency the common 5 bp deletions at codon 1309 are the same as that reported by the other groups (9.5%). Results from scanning for mutation were subsequently used for carrier detection in FAP families. In 9 families comprising 36 persons at mutation risk but without clinical symptoms we found 11 persons with APC gene mutation. Thus our study excluded 25 person from standard clinical treatment used for group at risk. Direct analysis of mutation heredity was the most effective but indirect methods of carrier detection are also useful, but genetic markers selected for this analysis should be characterized by high PIC (polymorphic information content) and heterozygosity. For indirect detection of carrier status we used three highly polymorphic (CA)n markers closely linked to APC gene. One hundred chromosomes from unrelated individuals and 128 chromosomes from unrelated FAP patients were subjected to analysis of allele frequencies at loci D5S299, D5S346 and D5S82. In our studies significantly higher frequency of allele A4 of D5S299 was observed among FAP patients. We also observed new alleles; in locus D5S299 allele A 6.1 (158 bp), in locus D5S346 allele A14 (94 bp) and in locus D5S82 allele C6.1 (167 bp). In four cases polymorphic molecular markers were used for presymptomatic diagnostics. Our strategy, permitted us to diagnose 49 persons of group at risk in 11 Polish FAP families using both direct and indirect methods. Supported by National Committee for Research 4 P05A 004 16.

P0052. Genetic Predisposition To Prostate Cancer - Results From The CRC/BPG UK Familial Prostate Cancer Study and the ACTANE Consortium

R. A. Eeles[1,2], *S. Edwards*[1], *Z. Kote-Jarai*[1], *D. F. Easton*[3], *D. Teare*[3], *D. P. Dearnaley*[1,2], *A. Ardern-Jones*[2], *A. Murkin*[2]
[1]*Institute of Cancer Research; Sutton, United Kingdom;* [2]*Royal Marsden NHS Trust; Sutton, United Kingdom;* [3]*CRC Genetic Epidemiology Unit; Cambridge, United Kingdom*
ros@icr.ac.uk

There is evidence for genetic predisposition to prostate cancer; about 5-10% of patients have a family history of the disease. The CRC/BPG UK Familial Prostate Cancer Study has a multifaceted approach to try to identify the genes involved. There is evidence for both highly penetrant and lower penetrance genes and so several different DNA collections have been targeted. These are; a collection of blood DNA from 285 single young cases with prostate cancer diagnosed at <55 years; a systematic series of over 1000 prostate cancer patients treated at one centre and for high risk genes, and an international collaboration (the ACTANE consortium) has 194 families with three or more cases of prostate cancer to perform linkage studies. Several linkage studies by other groups have suggested multiple locations for high risk prostate cancer genes, the first in 1996 at chromosome 1q24 (called HPC1), followed by other loci at 1q42, 1p36 (associated particularly with primary brain tumour), and Xq. A recent locus has also been suggested at 20q and other weaker suggestions of locations have been described. To date, none of these have been cloned. In October, a gene located at 17p was cloned (HPC2) which has sequence variants that may be associated with a 2.7-fold prostate cancer risk. Within ACTANE, we have shown that HPC1 is more likely to contribute to larger families with >4 cases and a meta analysis of 772 families from a worldwide international consortium has shown that only about 6% of prostate cancer families are likely to be due to HPC1. Overall, we have evidence against linkage for Xq, 1p36 and 1q42 loci and have reported that the Xq gene is more likely to be present in smaller clusters and 1p36 is associated with earlier onset disease but not with any other cancers, unlike the first report. Our studies of candidate genes have shown that maybe 5% of prostate cancer clusters may be due to germline mutations in the breast cancer-predisposition gene, BRCA2 which has implications for screening of female relatives in such prostate cancer clusters. We are currently investigating what percentage of young onset cases are due to BRCA2. Our studies of low penetrance genes have shown that longer repeat lengths in the androgen receptor gene (>16 GGC repeats) are associated with a halving of disease-free survival. We have identified a genotype at increased risk (GST T/M null & GST P val/val) that is associated with a 1.8-fold risk. Since selenium has been reported to be protective, we are currently analysing data to assess if there is an association of genotypes of the selenium dependent glutathione enzyme GPX with prostate cancer risk. Such results may be useful for identifying individuals at increased risk of developing prostate cancer so that they can be offered targeted screening and prevention. These studies are funded by the Cancer Research Campaign and The Prostate Cancer Charitable Trust.

P0053. Analysis of the R72P polymorphism at the p53 gene in individuals with Barrett s esophagus and control population

S. Leistner[1], *F. Segal*[2], *A. P. B. Kaspary*[3]
[1]*Hospital de Cl nicas de Porto Alegre; Porto Alegre - RS, Brazil;* [2]*Hospital Moinhos de Vento; Porto Alegre, RS, Brazil;* [3]*Hospital de Cl nicas de Porto Alegre; Porto Alegre, RS, Brazil*
segal@myway.com.br

The p53 is a tumour suppressor gene which codes for a nuclear phosphoprotein of 53KDa related to the regulation of cellular proliferation through mechanisms of blockage of the celular cycle and apoptosis, mainly after damage caused to DNA. Most mutations in the p53 gene result in a non-functional protein which present an increase half life, accumulating in the nucleus of tumoral cells. Generally, this mutations are involved in the development or progression of a series of neoplasias, including oesophagus and cardic adenocarcinomas. Some reports demonstrate iots presence also in intestinal metaplasia and displasic lesions. Various polimorphisms were also described at the p53 gene, and one of the most frequent is the R72P present in the exon 4 which can be related to an increased suscetibility to various cancers. Loss of heterozygosity its also a common and early event in the majority of cancers and is used to describe loss of chromossomic material which, as a consequence, leaves the cell with only an allele of a mutant gene.

The aims of the present work were a)to analyse the frequency of the R72P polimorphism in controls individuals representing the population of Porto Alegre and in individuals with Barrett s Esophagous (a pre-malignant epithelial alteration leading to adenocarcinoma in 10% of those subjects); b) to verify the presence of an association between the frequencies observed for controls and Barrett s; and c) to verify loss of heterozygosity when analysing DNA extracted from blood and affected tissue from individuals with Barrett s.

The method used was PCR of exon 4 of the p53 gene followed by restriction analysis with the enzyme *Acc*II from 80 controls and 23 Barrett s.

Our results showed that the proportion of homozygous for A72, homozygous for P72 and heterozygous for the two alleles was 38.75%, 15% and 46,25% respectively for the control group and 39.1%, 8.7% and 52.2% in Barrett s individuals. No statistically significant differences were found regarding the genotype distribution amongst the two groups.

We have found one LOH amongst the informative individuals (8.33%) and surprisingly, 4 cases of genomic instability (22,2%) suggesting an association of these events and development of adenocarcinoma.

P0054. Combination of GSTM1 null genotype and GSTP1 105 Val allele leads to increased risk of bladder cancer in the Turkish population

G. A. Toruner[1], *C. Akyerli*[1], *A. U ar*[1], *T. Aki*[2], *N. Atsu*[2], *H. zen*[2], *M. Tez*[3], *T. Ozcelik*[1]
[1]*Dept. of Molecular Biology and Genetics, Bilkent University; Ankara, Turkey;* [2]*Dept. of Urology, Hacettepe University Faculty of Medicine; Ankara, Turkey;* [3]*Ataturk Chest Disease Research Hospital; Ankara, Turkey*
toruner@fen.bilkent.edu.tr

Gluathione S- transferases (GSTs) are phase II enzymes, which introduce a glutathione for conjugation of electrophilic metabolites. The polymorphisms of GSTs are demonstrated to be associated with bladder cancer in some populations. No data was available for the Turkish population. GSTM1 null, GSTP1 105 Ile/Val and GSTT1 null genotype polymorphisms were determined in 100 age-sex matched controls, 98 random controls, and 121 bladder cancer patients in the Turkish population. The relative risk of GSTM1 null genotype was 1.91 (95% CI 1.11- 3.28). This risk is male specific (OR; 2.21, 95 % CI 1.18-4.22). The combination of GSTM1 null genotype and GSTP1 105 Val allele results in a 3.66 fold risk (95% CI 1.68-7.84). GSTP1 105 Val allele could not be shown to be associated with the invasiveness of tumor either alone, (OR 2.06, 95% 0.91-4.63) or in combination with GSTM1 null genotype (OR 3.42, 95%CI 0.96-12.12). In this study a slight male-specific risk of bladder cancer due to GSTM1 null genotype, and a substantial risk for combination of risky GSTM1 null and GSTP1 105 Val genotypes was observed in the Turkish population.

P0055. Study of the relationship between TNF-alpha gene polymorphisms and retinoblastoma

I. Imbert, *F. Pellestor*
Institut de G n tique Humaine, CNRS, UPR 1142; Montpellier, France
Isabelle.Imbert@igh.cnrs.fr

Retinoblastoma is a retinal neoplasm occuring in early childhood in both heritable and sporadic forms. Current knowledge indicates that mutations of both alleles of the RB-1 gene localized on chromosome 13 are prerequisite for the development of this tumor. Previous investigations on retinoblastoma patient samples with abnormalities on chromosome 6p have suggested that some genes on 6p could be important for tumor progression. We suggested in a recent study on Y-79 retinoblastoma cell line that the tumor necrosis factor-alpha (TNF-alpha) could be a probable candidate gene on chromosome 6p involved in the tumoral development or progression pathway. In this present study focused on the molecular analysis of the TNF-alpha gene in 70 retinoblastoma patient samples, we described a single nucleotide polymorphism in the 5 UTR regulatory region of the TNF-alpha gene. This single nucleotide change found in retinoblastoma patients could be associated with the disease susceptibility or severity. Further in this study, the possible consequences of this mutation in the retinal tumor development or progression and the action of TNF-alpha on cell cycle regulated proteins will be discussed.

P0056. NAT2 polymorphisms among Macedonian lung cancer patients

A. Anevska[1], *A. J. Dimovski*[1], *A. M. Stefanovska*[1], *E. Kovkarova*[2], *G. D. Efremov*[1]
[1]*Macedonian Academy of Sciences and Arts; Skopje, The former Yugoslav Republic of Macedonia;* [2]*Pulmology Clinics, Faculty of Medicine; Skopje, The former Yugoslav Republic of Macedonia*
aleks@manu.edu.mk

N-acetyltransferase (NAT) is one of the major liver enzymes that is involved in biotransformation of drugs and other exogenous substances. More than 20 different NAT1 and NAT2 allelic variants have been detected so far, most of which result from single nucleotide substitutions and/or small deletion/insertions. Mutant alleles of NAT genes are associated with either a slow or a rapid acetylator phenotype. Recent studies have suggested that the frequency of certain NAT2 allels is statistically different in lung cancer (LC) patients compared to that of normal control population, which suggests that they are important genetic modifiers of individual susceptibility for LC development. The aim of this study was to determine allele frequency distribution of the most common NAT2 polymorphisms in LC patients and normal control population from Macedonia. The study was performed on DNA samples isolated from peripheral blood lymphocytes of 50 LC patients and a matching number of newborns. The methodology for the detection of NAT2 polymorphisms included the combination of PCR/RLFP and allele specific PCR amplification. The frequencies of NAT2 homozygous rapid, heterozygous and homozygous slow genotypes were 5%, 42.5% and 52.5% in LC patients and 8%, 32% and 60% in control individuals, respectively (p>0.05). These data suggest that NAT2 acetylation polymorphisms are not LC sussceptibility factors among Macedonian population.

P0057. CYP1A1 and GSTM1 polymorphic genotypes in benign prostatic hyperplasia and prostate cancer patients

D. Aktas, *M. Hascicek, S. Sozen, H. Ozen, E. Tuncbilek*
Hacettepe University School of Medicine; Ankara, Turkey
dilekaktas6@hotmail.com

The role of CYP1A1 and GSTM1 genotype in benign prostatic hyperplasia and prostate cancer risk was investigated in Turkish populations through a case control study. Using allele-specific polymerase chain reaction (PCR)-based method; the CYP1A1*3 polymorphism, in exon 7 of the gene, was analyzed in 46 benign prostate hyperplasia (BPH) patients, 63 prostate cancer (PCa) patients and 155 control subjects. The CYP1A1 Ile/Val genotype was significantly increased the risk for patients with BPH (OR; 2.8, 95% CI; 1.38-5.91). However, there were no statistical differences in the distribution of CYP1A1 Val/Val genotype among BPH individuals (OR; 1.5, 95% CI; 0.29-8.05). In the PCa populations, patients with CYP1A1 Val/Val revealed a 10.4-fold higher risk of having prostate cancer than those with [Ile/Ile] (OR; 10.47, 95% CI; 3.49-31.39). In other words, the presence of Val/Val genotype significantly increased the risk of prostate cancer. Furthermore, the frequency of Ile/Val genotype was found to be statistically different with an OR of 6.10 (95% CI; 3.07-12.13), suggesting that patients carrying this genotype were at increased risk for developing prostate cancer. We also analyzed the influence of GSTM1 polymorphism in patients and 202 control individuals. The GSTM1 null genotype was observed in 34.7% of the control subjects. No significant differences were observed in the frequency of the nulled individuals in BPH and PCa patients when compared with control populations. To determine if any combined association of BPH and PCa development, we analyzed the combined effect of the GSTM1 and CYP1A1 genoype. Among the BPH patients, the combination of GSTM1 null genotype and CYP1A1 Ile/Ile was found to be statistically different (OR; 2.461, 95%CI; 1.028-5.850). Furthermore, CYP1A1 Ile/Val genotype when combined with GSTM1 +/+ and 0/0 genotypes were also statistically significant (OR; 3.346, 95%CI; 1.293-8.658 and OR; 5.086, 95%CI; 1.715-15.083, respectively). However, BPH patients having Val/Val genotype with GSTM1 +/+ or 0/0 was not different than those with Ile/Ile and GSTM +/+. Among the PCa populations, patients with Val allele revealed higher risk of having prostate cancer when combined GSTM1 genotypes.

P0058. CYP1A2 And NAT2; Susceptibility Genes For Bladder Cancer

S. Horn[1], *E. Schnakenberg*[1,2], *R. Werdin*[3], *C. Ehlers*[4], *K. Dreikorn*[3], *W. Schloot*[1]
[1]*Center for Human Genetics and Genetic Counselling, University of Bremen; Bremen, Germany;* [2]*AdnaGen AG; Hannover-Langenhagen, Germany;* [3]*Urological Division, St. J rgen Hospital; Bremen, Germany;* [4]*Gemeinschaftspraxis Prager & Junge; Dresden, Germany*
es@adnagen.com

The acetylation polymorphism plays an important part in the detoxification of numerous exogenous substances. The metabolism of aromatic amines via phase I enzymes (cytochrome P450; CYP1A2) and phase II enzymes e.g. N-acetyltransferase 2 (NAT2) have been shown to be individually different. Therefore they can lead to a different predisposition of genotoxic and carcinogenic effects. In our study we analysed mutations of CYP1A2 and NAT2 genes in 194 patients suffering from primary bladder cancer and 209 control individuals. Slow acetylators of NAT2 have been said to be more affected by bladder cancer in contrast to rapid acetylators which detoxify toxic metabolites in a faster way. We identified slow acetylators to be significantly more prone to primary bladder cancer (odds ratio; 1.88; confidence interval; 1.25-2.82) especially male persons with the frequent genotype NAT2*5/*6 (OR; 2.68; CI; 1.51-4.75). The CYP1A2 gene is characterized by mutation within the promotor region. This mutation is associated with an increased transcription rate leading to an elevated level of highly reactive metabolites. We have identified this mutation significantly more frequent in patients with bladder cancer (OR; 1.54; CI; 1.04-2.28). In addition, for the combination of these NAT2 and CYP1A2 mutations we calculated a significantly potentiated risk for the development of bladder cancer (OR; 4.88; CI; 2.08-11.42). These results indicate that polymorphic genes of phase I and phase II enzymes contribute to an individual susceptibility for the development of cancer f.i. bladder cancer. In addition, the altered detoxification capacity can release also genotoxic effects within tumor suppressor genes.

P0059. Frequency of Type I Transforming Growth Factor -b receptor (6A) polymorphism in colorectal cancer patients from Macedonia

A. M. Stefanovska[1], *A. J. Dimovski*[1], *M. Panovski*[2], *G. Zografski*[3], *J. Dingis*[3], *G. D. Efremov*[1]
[1]*Macedonian Academy of Sciences and Arts; Skopje, The former Yugoslav Republic of Macedonia;* [2]*Department of Surgery, Faculty of Medicine; Skopje, The former Yugoslav Republic of Macedonia;* [3]*Institute of Oncology, Faculty of Medicine; Skopje, The former Yugoslav Republic of Macedonia*
kliment@mt.net.mk

Transforming growth factor b (TGF-b) is a tumor suppressor molecule that

acts by inhibition of cellular proliferation or by promotion of cellular differentiation and apoptosis. Its effects are exerted through binding to three high affinity cell surface receptors. Type I TGFb receptor shows a common polymorphism which is a deletion of 3 out of 9 alanine stretch. In recent studies, homozygotes, and higher frequency of heterozygotes, for this polymorphic allele TbRI(6A) were only found in cancer cases. It was suggested that TbRI(6A) receptor has an impaired antiproliferative capacity and acts as a tumor susceptibility allele. Here we present our data on the frequency of the TbRI(6A) allele in colorectal cancer (CRC) patients and a matching number of normal controls (NC) from Macedonia. DNA was isolated from 100 paraffin embedded tissue samples of CRC patients and from peripheral blood leukocytes of 100 newborns. The presence of the TbRI(6A) allele was evaluated by PAG electrophoresis of BssSI digested 118 bp PCR fragment from exon 1 of the TbRI gene. Only one homozygote for TbRI(6A) allele was present in CRC patients while in NC homozygosity for this allele was not detected. (p>0.05). Six and nine heterozygotes were detected in CRC patients and in NC, respectively (p>0.05). The allele frequency of TbRI(6A) was 0.08 and 0.09 in CRC patients and in NC, respectively (p>0.05). These data excludes the TbRI(6A) allele as a tumor susceptibility allele for the development of CRC in our population.

P0060. Genetic Predisposition to Familial and Hereditary Colon Cancer Attributable to DNA repair (MSH2, MLH1, MSH6) and other Genes (TGFBR2, CTNNB1)

A. E. Lagarde[1], *D. Dewar*[1], *E. Bauman*[1], *H. Perras*[2], *S. Viertelhausen*[3], *H. Stern*[3]

[1]*Ottawa Hospital Loeb Health Research Institute; Ottawa, ON Canada;* [2]*CHEO Genetics; Ottawa, ON Canada;* [3]*Ottawa Regional Cancer Center; Ottawa, ON Canada*

alagarde@lri.ca

We addressed the role of known and presumed genetic factors in colon cancer predisposition, most particularly in the context of familial cancers that are not recognized clinically as classical HNPCC, which may represent up to 20% of the cases in the population. One study group (n=87) was selected from a hospital-based series of newly diagnosed colorectal patients. It included patients diagnosed under the age of 50 (n=20); 31 sporadic cases and 56 patients with a family cancer history. Among these, twelve were HNPCC or suspected-HNPCC patients; 18 had one first-degree relative affected with colon cancer, and 26 had more distant relatives affected with colon or other cancer types. Most tumors in this familial group were stable at microsatellite markers (41/56, 73% MSS), while 13% were highly unstable (MSI). Another group of 82 individuals representing 63 families were referred to the genetic clinics in Ottawa, and were identified through the Ontario Familial Colon Cancer Registry. PCR-SSCP and more recently total DNA sequencing were applied to analyze the entire coding regions of the MSH2, MLH1 and MSH6 genes. Pathogenic MSH2 and MLH1 germline mutations were identified in a minority of newly diagnosed cases (1.1%), unselected for MSI, family history or age of onset. A similar incidence was reported in population-based studies from Finland and Slovania. MSH2 and MLH1 mutations accounted for 10 % of all colon cancer families examined (n~100), unstratified by complexity or MSI status. However, MSH2/MLH1 mutations were more prevalent among families meeting Amsterdam criteria I (6/21, 29%) than among those meeting neither criteria I nor II (4/54, 7%). The A(IVS5, +3)T splicing MSH2 mutation was recurrent, being present in 5% of all familial cases, 19% of criteria I-positive and 2% of criteria I,II-negative families. Thirty familial cases (4 MSI high, 6 low, 20 MSS) were selected for MSH6 analysis, a study which is still in progress. Exon-4 mutations were detected in 2 cases (7%). After sequencing the entire TGFBR2 gene (TGF-beta type II receptor), a single pathogenic mutation was identified among 25 MSS familial cases, and none in 8 MSI cases. No germline mutation, nor deletions, in exon 3 of the beta-catenin gene (CTNNB1) were detected among 40 MSS familial cases. A relatively important proportion of HNPCC (70%) and of suspected-HNPCC families (>90%), and all cases of common familial colon cancer remain unaccounted for at the genetic level. The limited success of the candidate gene approach suggests that linkage or affected sib-pair analyses have a greater potential to elucidate the mechanisms predisposing to MSS hereditary and familial cancers.

P0061. ATM variants in breast cancer

G. Chenevix-Trench[1], *A. Spurdle*[1], *G. Mann*[2], *J. Hopper*[3], *K. Khanna*[1], *M. Taylor*[4]

[1]*Queensland Institute of Medical Research; Herston, Australia;* [2]*Millenium Institute; Sydney, Australia;* [3]*University of Melbourne; Melbourne, Australia;* [4]*University of Birmingham; Birmingham, United Kingdom*

georgiaT@qirm.edu.au

The role of the ATM gene in breast cancer has been controversial since it was first suggested in 1986. Several studies have shown that obligate ATM carriers have an elevated breast cancer risk but most analyses have not demonstrated an increased incidence of protein truncating ATM mutations in women with breast cancer However, Gatti et al have proposed that missense mutations may be responsible for the elevated breast cancer risk observed in ATM carriers. To test this hypothesis we are analysing 83 non-BRCA1/2 breast cancer families ascertained by kConFaB, a research consortium established to coordinate the collection of data on large numbers of Australasian families with severe histories of breast cancer. So far one family has been detected with an ATM mutation (T7271G) present in 5/5 affected family members (P= 0.03), as well in three unaffected carriers. Pathological review and LOH analysis of these five breast tumours are underway, as are mutation and LOH analyses of two gastric tumours from the same family. Full-length ATM cDNAs with and without this mutation will be expressed in ATM-null cells to study the interaction between wt and mutant ATM protein and to determine whether the mutation acts in a dominant negative fashion. In addition we are conducting a case-control study to analyse the relationship between ATM polymorphisms and breast cancer. 1353 cases and 688 controls have been genotyped for the T2119C polymorphism, previously associated with an elevated breast cancer risk. No significant difference was detected in the frequency of the rare C allele in cases (2.8%) compared to controls (2.6%) (P = 0.8), even when the analysis was restricted to individuals under the age of 40 with a history of breast cancer in first or second degree relatives. These data do not support the hypothesis that the T2119C ATM polymorphism plays a role in breast cancer susceptibility but an increased sensitivity to radiation remains a possibility.

P0062. Search for mutations predisposing to breast cancer in St.Petersburg, Russia.

C. Lamber

Institute for Experimental Medicine; St.-Petersburg, Russian Federation

CatLamber@aport.ru

Inherited mutations in the BRCA genes are common in patients with familial breast and ovarian cancer. Ashkenazi Jewish population is characterized by three common mutations, namely 185delAG and 5382insC in BRCA1 gene and 6174delT in BRCA2 gene. 5382insC BRCA1 mutation contributes significantly to ovarian cancer in Moscow. All the mutations increase 8-10 fold risk of breast tumour development.We have performed screening for the common BRCA mutations in the cohort of 26 Jewish and 38 Slavic patients with familial or sporadic breast cancer and 38 Ashkenazi controls. PCR followed by heteroduplex analysis and DNA sequencing allowed us to find carriers of these mutations. We have found one carrier of 6174delT mutation among patients unselected in respect of breast cancer. It was the first report of BRCA2 gene mutation from Russia. No cases of 185delAG and 6174delT mutation in BRCA1 gene were found in our patient sample. However, 3 unrelated cases of 5382insC mutations were found in Slavic breast cancer patients and none in control group. New mutation g71741ins12nt in BRCA1 gene was described. This mutation is due to duplication of 12 nucleotides and results in formation of two direct repeats in intron 20 of BRCA1 gene. Our results suggest the importance of screening the St.Petersburg Slavic patients for 5382insC BRCA1 gene mutation and the low impact of 185delAG BRCA1 mutation and 6174delT BRCA2 mutation on breast cancer development in St.Petersburg. This reseach was supported by grant RFBR ?98-04-49869.

P0063. BRCA1/2 mutation analysis in 72 families with breast cancer

P. Derakhshandeh-Peykar[1,2], *O. Brandau*[3], *A. Meindl*[3], *E. Holinski-Feder*[3], *J. Murken*[3]

[1]*Dep. of Human Genetics, School of Public Health, Tehran Univ. of Medical Sciences; Tehran, Islamic Republic of Iran;* [2]*Genetic Clinic, Valie-Assr Sq. 16, Keshavarz blvd.; Tehran, Islamic Republic of Iran;;* [3]*Abt. Paed. Genetik, Kinderpoliklinik der LMU; Munich, Germany*

pupak-peykar@yahoo.com

To determine the pattern and incidence of BRCA1/2-mutation in the German population, the Deutsche-Kerbshilfe is funding 10 groups, including the munich study group. 72 families were divided in riskgroups according to the number of affected patients and age of diseaseonset. Mutaion screening of the BRCA1/2-gene by SSCP-analysis, PTT (Western blot), Southern blot and sequencing reveald 4 BRCA1-mutations and 4 BRCA2-mutation in 37 families with at least 2-3 familial cases of isolated breast cancer. At least 1-2 below 50 y.o.a; 2 BRCA1-mutations in 15 families. With breast and ovarian cancer. One mutation in 27 pationts from families with les than three affected members or multitumor-families. The 5382insC and the 4650delCA mutations were found twice. Suggesting perscreening for these mutations in the German population. Screening of 20 these families

for BRCA2-mutation revealed 4 mutations. These results are in ocordance with recent findings; (i) a high incidence of BRCA1-mutations in large breast cancer families or families with breast and ovarial cancer, (ii) low incidence in families with less than three affected females, (iii) preponderance of putative founder mutations.

P0064. BRCA1 and BRCA2 Mutation Analysis of Early-onset and Familial Breast Cancer Cases in Mexico

H. A. Barrera-Salda a[1], *P. Ruiz-Flores*[1], *O. M. Sinilnikova*[2], *M. Badzioch*[2], *A. L. Calderon-Garcidue as*[3], *J. F. Gonz lez-Guerrero*[4], *S. Chopin*[2], *C. Szabo*[2], *G. Lenoir*[2], *D. Goldgar*[2]
[1]*Medicine Faculty, Universidad Autonoma de Nuevo Leon; Monterrey, Mexico;* [2]*International Agency for Research on Cancer; Lyon, France;* [3]*Clinica 25 del Seguro Social; Monterrey, Mexico;* [4]*Centro Universitario Contra el Cancer UANL; Monterrey, Mexico*
HBARRERA@FM.UANL.MX

The entire coding regions of BRCA1 and BRCA2 were screened for mutations by heteroduplex analysis in 51 Mexican breast cancer patients. One BRCA1 and one BRCA2 truncating mutations were identified in the group of 32 (6%) early-onset breast cancer patients (£ 35 years). Besides these two likely deleterious mutations, nine rare variants of unknown significance, mostly in the BRCA2 gene, were detected in seven of 32 (22%) early-onset breast cancer cases and in four of 17 (24%) site-specific breast cancer families, one containing a male breast cancer case. No mutations or rare sequence variants have been identified in two additional families including each an early-onset breast cancer case and an ovarian cancer patient. The two truncating mutations and six of the rare variants have never been reported before and may be of country specific origin. The majority of the alterations appeared to be distinct, with only two of them being observed in more than one family. Analysis of a larger series of Mexican breast cancer patients for mutations identified in this study may reveal these to be founder mutations in the Mexican population.

P0065. High Rate of Germline Mutations in BRCA1 and BRCA2 genes in the Czech Hereditary Forms of Breast / Ovarian Cancer

E. Machackova[1], *M. Navratilova*[1], *H. Pavlu*[1], *D. Valik*[1], *L. Messiaen*[2], *K. Claes*[2], *L. Foretova*[1]
[1]*Masaryk Memorial Cancer Institute; Brno, Czech Republic;* [2]*University of Gent; Gent, Belgium*
emachack@mou.cz

ABSTRACT Background; Estimated 5-10% of all breast and ovarian cancers can be of hereditary origin. Germline mutations in highly penetrant susceptibility BRCA1 and BRCA2 genes could cause predisposition to these cancers. Material and Methods; Molecular genetic testing of BRCA1 and BRCA2 genes have been performed in 69 high-risk breast and breast/ovarian cancer families and in 16 early-onset sporadic breast or ovarian cancer in women diagnosed below 40 years at Masaryk Memorial Cancer Institute in Brno, Czech Republic. The mutation analysis was carried out on genomic DNA isolated from blood samples of affected individuals. Protein truncation test and heteroduplex analysis followed by direct sequencing were used. The genetic counseling and preventive clinical follow-up of gene carriers is part of the genetic program. Results; A germline disease causing mutation was found in 34 screened high-risk families (49%), 21 mutations (7 different) in BRCA1 gene and 13 mutations (9 different) in BRCA2 gene. One frame shift mutation detected in BRCA1 and two frame shift mutations detected in BRCA2 gene were novel mutations. In the group of 16 woman diagnosed with sporadic early-onset breast/ovarian cancer no disease-causing mutation was found. Conclusion; A mutation was identified in either gene in 49% of high-risk families proving that germline mutations in these breast cancer susceptibility genes might be responsible for an important fraction of inherited breast and ovarian cancer cases in the Czech Republic. Spectrum of mutations found in both genes is variable.

P0066. Haplotype analyses and age estimation of a west Swedish founder mutation of the BRCA1 gene.

A. Bergman[1], *U. Olofsson*[2], *Z. Einbeigi*[3], *Z. Taib*[2], *A. Wallgren*[3], *P. Karlsson*[3], *J. Wahlstr m*[1], *T. Martinsson*[1], *M. Nordling*[1]
[1]*Dept. of Clinical Genetics, Sahlgrenska University hospital/East; Gothenburg, Sweden;* [2]*Dept. of Mathematical Statistics, Chalmers University of Technology; Gothenburg, Sweden;* [3]*Dept. of Oncology, Sahlgrenska University hospital; Gothenburg, Sweden*
annika.bergman@clingen.gu.se

The cloning of BRCA1 and BRCA2 genes has led to the identification of several hundreds germline mutations in families with a history of hereditary breast cancer. Some of the mutations in BRCA1 and BRCA2 are recurrently identified in distinct geographic and ethnic populations and are believed to have spread from a single ancestor. It is important to characterize these founder mutations and determine their geographic distribution in order to be able to design efficient mutation screening. The most recurrent mutation in Sweden is the 3171ins5 mutation in BRCA1. In the western part of Sweden this mutation accounts for as much as 77% of all identified mutations in this region. Our aim was to in detail analyze the haplotype and founder effects of the 3171ins5 mutation and furthermore attempt to estimate the time since the first appearance of the mutation. In the study we included eighteen families with hereditary breast and/or ovarian cancer. At least one individual in each family had previously tested positive for the 3171ins5 mutation. We used polymorphic microsatellite markers for the haplotype analyses. The markers were located within or flanking the BRCA1 gene spanning a region of 17,3 cM. We found several different haplotypes as well on the disease allele as on the normal allele. We observed however a conserved haplotype in the 3171ins5 carriers for three markers within or very close to the BRCA1 gene. As this haplotype only was found once in the normal alleles it is highly likely that this is a mutation identical by descent i.e. a true founder. The results from the haplotype analyses were used to estimate the age of the mutation. Using the method of moments, based on the theory of Galton-Watson branching processes we estimate that the mutation first appeared sometime around the 11th century, approximately 50 generations ago.

P0067. First German Breast Cancer Patient with Germline Mutations in Both BRCA1 and BRCA2 Genes

B. Fischer[1], *U. Schwarz-Boeger*[2], *B. Albacht*[3], *M. Du*[4], *D. Niederacher*[4], *W. Grote*[1], *W. Gerber*[3], *W. Jonat*[2], *M. Kiechle*[2], *R. Siebert*[1], *N. Arnold*[2]
[1]*Institute of Human Genetics; Kiel, Germany;* [2]*Department of Gynecology and Obstetrics; Kiel, Germany;* [3]*Institute of Medical Psychology; Kiel, Germany;* [4]*Department of Gynecology and Obstetrics; D sseldorf, Germany*
fischer@medgen.uni-kiel.de

Very few individuals with a germline mutation in both breast/ovarian cancer susceptibility genes, BRCA1 and BRCA2, have been described so far. The majority of them are of Ashkenazi-Jewish descendent. We report the first German case with heterozygous germline mutation in both BRCA1 and BRCA2 genes. The female patient was diagnosed with breast cancer at age 32. Her paternal family branch contained four relatives with breast cancer as well as cases of testicular, prostate and pancreatic cancer. The mother of the propositus deceased from ovarian cancer and the maternal grandmother from early-onset breast cancer. Mutation screening of the index patient revealed heterozygosity of the mutations 185delAG and 5950delCT in the BRCA1 and BRCA2 genes, respectively. A paternal sister with breast cancer and the unaffected father were found to carry the BRCA2 mutation 5950delCT but not the BRCA1 mutation 185delAG. As to the family history segregation of this BRCA1 mutation from the maternal family branch seems likely. Nevertheless, a novel mutation cannot be ruled out as no members of the maternal branch have been tested so far. Neither family history nor haplotype analyses of BRCA1 and BRCA2 linked markers provided evidence for an Ashkenazi-Jewish origin of the mutations. To the best of our knowledge we present the first non-Ashkenazy European case heterozygous for both breast cancer susceptibility genes. As to the implications of double-heterozygosity on genetic counselling we recommend complete screening of both genes BRCA1 and BRCA2 in families suspicious for heredity of breast and/or ovarian cancer in both paternal and maternal branch. This work was supported by the Deutsche Krebshilfe.

P0068. Mutations of the BRCA1 and BRCA2 genes in Saudi Arabia

E. A. El-Harith[1,2], *M. S. Abdel-Hadi*[2], *D. Steinmann*[1], *T. D rk*[1]
[1]*Medizinische Hochschule; Hannover, Germany;* [2]*King Faisal University; Al-Khobar, Saudi Arabia*
doerk.thilo@mh-hannover.de

Breast cancer is the most common cancer among the female population of Saudi Arabia and is characterised by low age at onset and diagnosis at late stages of malignancy. We have scanned the major segments of the BRCA1 and BRCA2 genes for pathogenic mutations in genomic DNA samples of 40 Arab and Asian women from Saudi Arabia who had unilateral breast cancer. One truncating mutation, the novel frameshift deletion 2482delGACT, was found in exon 11 of the BRCA2 gene in one Arab patient of Palestinian descent. The potentially disease-associated missense substitution R841W in exon 11 of BRCA1 was identified in a second Arab patient. Further unclassified variants included the double mutant BRCA1 allele F486L-N550H and the BRCA2 substitution D1420Y, each identified in single breast cancer patients. Allele frequencies of common polymorphisms did not differ significantly from those observed in Cau-

casian populations. Our findings indicate that BRCA1 and BRCA2 mutations exist at low levels and may be responsible for some proportion of breast cancer cases in the ethnically heterogeneous population of Saudi Arabia.

P0069. Molecular genetics of bilateral breast cancer

D. Steinmann, *A. Hector, M. Bremer, D. Rades, J. H. Karstens, T. D rk*
Medizinische Hochschule; Hannover, Germany
doerk.thilo@mh-hannover.de

Bilateral breast cancer accounts for about 5-10 % of breast cancer patients in Germany. In order to test the hypothesis that mutations in breast cancer genes are more frequent in patients with bilateral breast cancer, we are investigating a hospital-based series of 75 consecutive patients with bilateral breast cancer and a comparison group of 75 patients with unilateral breast cancer for mutations in the BRCA1, BRCA2, ATM and p53 genes. Five frameshift deletions (517delGT in BRCA1, 5946delCT, 6174delT, 6772delA and 8138del5 in BRCA2) were identified in patients with bilateral disease, whereas three pathogenic BRCA1 mutations (T300G, 3814del5, 5382insC) were identified in the group of patients with unilateral breast cancer. Furthermore, one truncating ATM gene mutation (3802delG) was found among the patients with bilateral cancer but none in the comparison group. Trends towards an increased prevalence of certain missense substitutions (e.g. Q356R of BRCA1, S707P of ATM and R72P of p53) in the bilateral cancer cohort were noted, with differences being most pronounced in case of the S707P substitution. We suggest that germline alterations in all four investigated genes contribute to the development of contralateral breast cancer in a subset of patients, though their prevalence and relative risks appear to be lower than previously indicated by family-based studies.

P0070. Genetic Testing For Brca Gene Mutations

Z. Rudzki[1], *F. Firgaira*[2], *G. Suthers*[3]
[1]*Institute of Medical & Veterinary Science; Adelaide, Australia;* [2]*Flinders Medical Centre; Adelaide, Australia;* [3]*SA Clinical Genetics Service; Adelaide, Australia*
barney.rudzki@imvs.sa.gov.au

Background & purpose; Mutation analysis of the BRCA1 and BRCA2 genes is expensive and frequently fails to identify any pathogenic abnormality. We have reviewed the outcome of genetic testing of patients tested through the SA Familial Cancer Service and divided them into categories with a view of increasing the efficiency of mutation testing. Methods; DNA samples were collected from patients who fulfilled the Australian National Breast Cancer Centre (NBCC) high genetic risk criteria (ie three close relatives with breast or ovarian cancer, or two with high-risk features), or had breast cancer under the age of 30, or ovarian cancer under the age of 40, or were males with breast cancer. Patients were divided into categorises according to the clinical setting. Both genes were fully screened by CCM/direct sequencing or PTT/DGGE/direct sequencing unless a pathogenic mutation was found. Results; Overall, 30% of 244 affected individuals had a mutation in either gene; this represented 26% of the families tested. The frequency of mutations in each clinical category varied from 0% to 80%. In most categories, BRCA1 and BRCA2 mutations were equally likely. However, early-onset ovarian cancer was associated with BRCA1 mutations, and late-onset breast cancer and male breast cancer were associated with BRCA2 mutations. Conclusions; Clinical categories can provide some clues regarding the chance of identifying a mutation in BRCA1 or BRCA2, but the overall detection rate remains low. Other selection criteria (perhaps histological indices or immuno-histochemistry) are required to improve the efficiency of genetic testing in familial breast cancer.

P0071. Frequency of BRCA1 & BRCA2 occurring mutations in Greece

D. Yannoukakos[1,2], *A. Ladopoulou*[3], *I. Konstantopoulou*[3], *A. Pantazidis*[3], *C. Kroupis*[4], *E. Lianidou*[4], *C. Tsionou*[3], *G. Nasioulas*[5], *G. Fountzilas*[6], *N. Pandis*[7]
[1]*National Center For Scientific Research; Athens, Greece;* [2]*Hellenic Cooperative Oncology Group; Athens, Greece;* [3]*National Center for Scientific Research Demokritos ; Athens, Greece;* [4]*Dept. of Chemistry, University of Athens; Athens, Greece;* [5]*Molecular Biology Dept., Hygeia Hospital; Athens, Greece;* [6]*AHEPA Hospital, Hellenic Cooperative Oncology Group; Thessaloniki, Greece;* [7]*Dept. of Genetics, Saint Savas Hospital; Athens, Greece*
yannouka@mail.demokritos.gr

The mutational spectrum of breast cancer susceptibility genes BRCA1 and BRCA2 exhibits considerable ethnic and geographical diversity. Both genes have a broad spectrum of putative pathologic mutations and both have variant alleles found at high frequency in the population but not associated with an apparent increase in cancer risk. We have screened for mutations extensively the coding sequence and intron- exon boundaries of BRCA1 gene and in a less extend of BRCA2 gene in 100 Greek unrelated patients with a family history of at least one relative with breast cancer under the age 50 yrs and ovarian cancer at any age. With respect to the known founder mutations in the European population 185delAG, C61G and 5382insC only the third one was found in five patients (5%) and their affected relatives. Several other unique deleterious mutations were identified in exon 11 of BRCA1. Interestingly five rare missense mutations were identified in the exons 16-24 corresponding to probably functionally important residues into the BRCT domains. For one of them (G1738R) has been shown by Hayes et al., 60, p2411 Canc Res 2000 that G1738E results in loss of function in vitro. The in vitro function of these missense mutations is under investigation in our laboratory. Regarding to BRCA2 although the study is still going there are mainly unique mutations. Overall according to our data 5382insC is the only proven deleterious mutation occurring in a relatively high frequency in the Greek population. The majority of the other mutations seems to be unique mutations

P0072. Characterization of a potential BRCA2 splice site variant

P. J. Bridge[1], *B. Fraser*[1], *L. Dimnik*[1], *G. Graham*[1], *K. Fedosoff*[1], *R. Hughes*[2], *J. Parboosingh*[1]
[1]*Alberta Children s Hospital; Calgary, AB Canada;* [2]*Tom Baker Cancer Centre; Calgary, AB Canada*
peter.bridge@crha-health.ab.ca

BRCA mutation screening is being offered to families with a strong history of breast cancer. Over 800 sequence variants have been identified in each gene world wide, most being reported only once. The significance of approximately 5% of the variants identified in the breast cancer information core database (BIC) is unknown. We have identified a previously unreported BRCA2 intron 2 missense variation, IVS2-7T>A in a 70 year old woman with breast cancer at 58 years of age. Three of her five sisters have also had breast cancer (57-76 years of age) and one niece (early 40 s). Colon and uterine cancers are also present in the family. The normal intronic sequence leading up to the splice acceptor site is T(10)A(3)TAG; the IVS2-7T>A mutation results in T(9)A(4)TAG. The IVS2-7T nucleotide has been shown to cause a splicing error deletion when deleted (IVS2-7delT) resulting in a splice acceptor sequence of T(9)A(3)TAG. Sequence analysis of mRNA showed that the variant causes a deletion of exon 3 similar to that seen in the IVS-7delT mutation. The presence of mutations in intronic sequences downstream of the 3 splice site has implications for sequence-based and SSCP-based screening strategies. Five to ten percent of BRCA1 and 2 sequence variants in BIC are splice mutations. This may be an underestimate given current sequence and SSCP screening strategies.

P0073. The Occurrence of Breast or Ovarian Cancer in Families under Inherited Predisposition is Associated with Single Nucleotide Polymorphisms of BRCA1 Gene.

A. V. Karpukhin[1], *N. I. Pospekhova*[1], *L. N. Lubchenko*[2], *A. N. Loginova*[1], *E. V. Khomich*[1], *A. V. Budilov*[3], *V. M. Zakharyev*[3], *R. F. Garkavtseva*[2], *E. K. Ginter*[1]
[1]*Research Centre For Medical Genetics, Russian Academy of Medical Sciences; Moscow, Russian Federation;* [2]*Cancer Research Centre, Russian Academy of Medical Sciences; Moscow, Russian Federation;* [3]*Engelgardt Institute of Molecular Biology, Russian Academy of Sciences; Moscow, Russian Federation*
socrates@orc.ru

Inherited mutation of BRCA1/2 gene results in breast or ovarian cancer. However, genome peculiarities that have an influence on occurrence of breast or ovarian cancer in family are not clear for the present.

In present study occurrence of breast or ovarian cancer among predisposed families was analyzed in connection with single nucleotide polymorphism (SNP) of BRCA1 gene.

The entire coding region of BRCA1 gene in a sample of 44 patients and 13 members of proband families was screened by conformation sensitive gel electrophoresis (CSGE). All structural variants of amplified DNA fragments found by CSGE were sequenced on both strands.

BRCA1 mutations and a frequently occurred set of 8 SNPs were found. Mutations were predominantly identified in breast/ovarian families. Analysis of more than one affected individual in available families was performed. It was shown that the set of SNPs is inherited as a whole. Both the mutation and SNP set were on the same chromosome when these gene alterations were investigated in individual.

Patients were classified in accordance with the BRCA1 alterations as hav-

ing a mutation, mutation and the SNP set, SNP set only, none alterations. It was found that the frequencies of breast and ovarian cancer are not uniformly distributed in this series. This phenomenon was revealed when affected individuals in families were analyzed (P<0.001). Under BRCA1 mutation the ratio of breast to ovarian cancer in familieswas higher when the gene had SNP set also (P < 0,01).
The presence of the SNP set among patients with and without mutations was different (29% and 72%, respectively; P < 0,01). An inheritance of breast cancer was found in 68% of families if proband had the SNP set whereas only 25% of probands had these variations if ovarian cancer occurred in families (P < 0,01).
In summary, the occurrence of ovarian or breast cancer under inherited predisposition is in dependence on the SNPs found. These findings may have predictive and diagnostics significance.

P0074. Mutations and Sequence Variations of BRCA1 Gene in Breast/Ovarian Cancer Families from Russia.

A. N. Loginova[1], *N. I. Pospekhova*[1], *L. N. Lubchenko*[2], *E. V. Khomich*[1], *A. V. Budilov*[3], *V. M. Zakharyev*[3], *E. K. Ginter*[1], *R. F. Garkavtseva*[2], *A. V. Karpukhin*[1]
[1]*Research Centre For Medical Genetics, Russian Academy of Medical Sciences; Moscow, Russian Federation;* [2]*Cancer Research Centre, Russian Academy of Medical Sciences; Moscow, Russian Federation;* [3]*Engelgardt Institute of Molecular Biology, Russian Academy of Sciences; Moscow, Russian Federation*
socrates@orc.ru
Distribution of BRCA1 gene mutations appears to be population specific. Extending information about sequence variations in different geographical regions is essential for more understanding of mutation spreading and for medical diagnostics.
In present study the entire coding region of BRCA1 gene in a sample of 44 patients, 13 members of proband families and 28 individuals of control group (predominantly Moscow region) was screened by conformation sensitive gel electrophoresis (CSGE). All structural variants of amplified DNA fragments found by CSGE were sequenced on both strands.
There were 17 mutations among patients (38.6%). These include five different mutations with predominance of 5382insC (76,4% of all mutations). Such high frequency (the most of known one) may suggest on geographic region of the mutation origin. Germ-line variations of the BRCA1 gene were found as a frequently occurred set of predominantly 8 polymorphisms that is inherited as a whole. Three of these - IVS1-103T/C, IVS1-115T/C and IVS14-63C/G - are described for the first time. This set of polymorphisms was observed in approximately 50% of both patients and control individuals investigated. Frequency of the genes having single variations was 7% only. Analysis of more than one affected individual in available families was performed and alterations found were confirmed in all cases. Haplotypes described by single nucleotide polymorphism of the BRCA1 gene were determined for all patients.
Detailed characterization of the BRCA1 gene permits investigation of phenotype/genotype correlation and is necessary for medical diagnostics.

P0075. Mutation analysis of the 5 untranslated region of BRCA1 gene

W. Hofmann, *R. Frege, E. Cla§en, S. Scherneck*
Max Delbr ck Center for Molecular Medicine; Berlin, Germany
whofmann@mdc-berlin.de
We screened 92 families with a strong history of breast and/or ovarian cancer for mutations in BRCA1 and BRCA2 genes. To date, the routine diagnostic of these two breast cancer susceptibility genes comprises analysis for potential mutations within the genomic sequence of the protein coding regions including all exons and exon flanking regions of the introns. Mutations within the non-coding regions of the BRCA1 gene are however likely to exist. To evaluate, if such mutations occur and subsequently affect the transcription of the BRCA1 gene, we analysed the sequences of the 5 UTR of BRCA1 from the bi-directional promoter to the ATG start codon (nucleotide position 3140-4638, GenBank accession no. L78833) in our breast and/or ovarian cancer families. Two deletions could identified in a CAAAAn repeat at the 3 of exon1b; 3985del5bp and 3979del10bp. Both, sequences carrying the deletions and the wildtype BRCA1 promoter sequence respectively were subcloned to drive the luciferase gene. The constructs were tested by transient transfection into various cell lines following analysis of luciferase activity. Data as to the activity of deletions-containing constructs relative to the construct contains the wildtype BRCA1 promoter will be presented and discussed with respect to their relevance in BRCA1 diagnostic.

P0076. Sequence Variations of BRCA2 Gene in Breast/Ovarian Cancer Families from Russia.

N. Pospekhova[1], *E. V. Khomich*[1], *L. N. Lubchenko*[2], *A. N. Loginova*[1], *A. V. Budilov*[3], *V. M. Zakharyev*[3], *E. K. Ginter*[1], *R. F. Garkavtseva*[2], *A. V. Karpukhin*[1]
[1]*Research Centre For Medical Genetics, Russian Academy of Medical Sciences; Moscow, Russian Federation;* [2]*Cancer Research Centre, Russian Academy of Medical Sciences; Moscow, Russian Federation;* [3]*Engelgardt Institute of Molecular Biology, Russian Academy of Sciences; Moscow, Russian Federation*
socrates@orc.ru
Significant part of inherited breast/ovarian cancer is connected with BRCA2 gene mutation. Information about sequence variations of the gene is necessary for more understanding of mutation spreading, genome polymorphism and medical diagnostics.
In present study BRCA2 gene sequence variations among breast/ovarian cancer families in Russia (Moscow region) were analyzed. The coding region of BRCA2 gene in a sample of 25 patients was screened by conformation sensitive gel electrophoresis (CSGE). All structural variants of amplified DNA fragments found by CSGE were sequenced on both strands.
There were 8 mutations (32% of 25), 2 of which and two single nucleotide polymorphism (SNP) are revealed for the first time. Two mutations were recurrent (in two families each). High frequency of some variances was shown; H372N (25%) and IVS11+80del4 (40%). The latter is seldom in other populations. Analysis of more than one affected individual in available families was performed and alterations were confirmed in all cases.
SNP haplotypes were determined for all the patients. So frequent and extensive set of SNPs that was typical for BRCA1 gene in Russia was not found for BRCA2. At the same time, the sets of 3-5 SNPs were found in the patients. It is interesting that 7 of 8 mutations of BRCA2 are correlative to SNP set of BRCA1 gene. This may suggest on association between BRCA2 mutation and SNP set of BRCA1.
Spectrum of BRCA2 mutations and variations in Russia is described for the first time.
At present the SNPs are analyzed on association with clinical phenotype.

P0077. LOH In Tumoral Samples From Patients With Familiar Breast Cancer Studied For The Brca1 And Brca2 Genes.

A. Osorio[1], *R. Rodr guez*[1], *A. Mart nez-Ram rez*[1], *A. Cazorla*[2], *M. de la Hoya*[3], *T. CaId s*[3], *J. Ben tez*[1]
[1]*Centro Nacional de Investigaciones Oncol gicas; Madrid, Spain;* [2]*Fundaci n Jim nez D az; Madrid, Spain;* [3]*Hospital Cl nico San Carlos; Madrid, Spain*
aosorio@cnio.es
BRCA1 and 2 are susceptibility genes associated with familiar breast cancer. In these genes the first mutation is germinal while the most frequent somatic inactivation is a loss of heterozygosity (LOH). We selected 30 patients belonging to high risk families with variants in BRCA1 and 2 genes; ten cases presented with a truncated mutation, eleven a polymorphism, and 9 cases a variant with unknown significance. We analyzed LOH comparing normal and tumoral tissue from paraphynes. a) In all cases with a truncated mutation LOH was found. b) We have not found LOH in the 11 cases with polymorphisms. c) Six of the 9 cases presented different unknown variants. In one case, there was LOH; an analysis of segregation in different affected members of the family was compatible with a mutation. Four cases presented with the same variation in the BRCA1 gene and absence of LOH. In one of these cases, we could demonstrate the existence of a true mutation in the other gene, BRCA2. Finally we did not find LOH in the other 4 cases with unknown variants . Our results suggest that: A) LOH is the most frequent mechanism of inactivation in patients with true mutations in BRCA genes. B) LOH could represent a good mechanism to differentiate mutations versus unknown variants. B) The absence of LOH in patients with polymorphisms could suggest a different role of BRCA genes in familiar versus sporadic breast tumors where LOH of BRCAs is present in about 25% of cases.

P0078. Genome-wide scanning for linkage in Finnish breast cancer families

P. Huusko[1], *P. Vahteristo*[2], *L. Sarantaus*[2], *S. Juo*[3], *E. Gillanders*[1], *T. Kainu*[1], *K. Rapakko*[4], *M. Jones*[1], *C. Markey*[1], *H. Eerola*[2], *M. Allinen*[4], *P. Vehmanen*[2], *J. Leisti*[4], *G. Blanco*[5], *C. Blomqvist*[6], *J. Trent*[1], *J. Bailey-Wilson*[3], *R. Winqvist*[4], *H. Nevanlinna*[2], *O. Kallioniemi*[1]
[1]*Cancer Genetics Branch/NHGRI/NIH; Bethesda, MD United States;* [2]*Department of Gynecology and Obstetrics/Helsinki University Central Hospital; Helsinki, Finland;* [3]*Inherited Disease Research Branch/NHGRI/NIH; Bethesda, MD United States;* [4]*Department of Clinical*

Genetics/Oulu University Hospital/University of Oulu; Oulu, Finland; [5]Department of Oncology/Oulu University Hospital; Oulu, Finland; [6]Department of Oncology; Helsinki, Finland
phuusko@nhgri.nih.gov
Mutations in the BRCA1 and BRCA2 genes account for a smaller proportion of hereditary breast cancer cases than previously suspected. Less than 20% of Finnish families with 3 or more breast cancers showed BRCA1 or BRCA2 mutations (Vehmanen et al. AJHG 60;1050, 1997; Huusko et al. AJHG 62;1544, 1998). We recently reported a possible third breast cancer susceptibility locus at 13q21-q22 by linkage analysis in 77 Finnish, Swedish and Icelandic breast cancer families (Kainu et al. PNAS 97;9603). This new locus explains only a proportion (estimated at <30%) of the remaining families suggesting presence of additional susceptibility loci. Here, we undertook an exploratory genome-wide search focusing on a sample of 14 linkage-informative, multiplex breast cancer families (93 DNA specimens) from the genetically homogeneous Finnish population. These families tested negative for BRCA1 and BRCA2 and showed no linkage to the distal 13q21-q22 region. Simulations generated by the FASTSLINK program assuming genetic homogeneity predicted an expected maximum LOD score of 1.25 (52% of replications with LOD>1, 21%>2 and 3%>3). Genome-wide linkage analysis was performed with 398 dinucleotide repeat markers on ABI 377 DNA sequencers. Based on a modified CASH model which assumes dominant transmission and age dependent penetrances, a peak two-point LOD score of 2.04 (q=0.0) was seen at 2q. In addition, one other chromosomal region showed a two-point LOD score >1.0. Analysis of more markers from the chromosomal regions of interest, more individuals from the same families, as well as additional families is in progress to follow-up these leads

P0079. Characterisation of a new BRCA1 mutation in two Aboriginal Manitoban women with family history of breast and ovarian cancer

R. Ray[1], *E. Grudeski*[1], *S. Kim*[2], *A. E. Chudley*[1], *B. N. Chodirker*[1], *E. L. Spriggs*[1]
[1]*Health Sciences Centre; Winnipeg, MB Canada;* [2]*WRHA Breast Health Centre; Winnipeg, MB Canada*
rray@hsc.mb.ca
Mutations in cancer susceptibility genes BRCA1 and BRCA2 account for approximately 3-10% of breast cancer in the general population and a much higher proportion in those with strong family history of breast and ovarian cancer. Germline mutations in these genes have been identified in individuals of all races and ethnic backgrounds. As part of an ongoing breast cancer-screening program in Manitoba, a novel mutation has been characterised in two unrelated women of Ojibway background. Both were diagnosed with breast cancer under the age of 42 and have a strong family history of early onset breast and ovarian cancer. The protein truncation test (PTT) is routinely used to screen for BRCA1 mutations in genomic DNA and RNA extracted from peripheral blood. By PTT, the exon 11 wild type protein fragment (amino acids 224-841) and truncated fragment were detected at ~76.5kDa and ~35kDa, respectively, in both women. DNA sequence analysis revealed two alterations occurring on the same BRCA1 allele; a transition, 1506A>G resulting in Lys463Glu and an insertion, 1509-1510insG, leading to a stop codon following residue 478. Although the women studied share the same ethnic background, they live in separate Ojibway communities and their family history spanning two generations does not identify a common relative to suggest the families are closely related. We believe that we have identified a new ethnic specific mutation that may allow for better breast cancer-screening in women of Aboriginal descent. Further studies will help to determine whether this particular mutation is unique to the Manitoba Ojibway population.

P0080. Molecular epidemiology of BRCA1 and BRCA2 mutations in high risk French Canadian breast / ovarian cancer families

J. Simard[1], *M. Dumont*[1], *S. D los*[1], *A. Moisan*[1], *C. Samson*[1], *L. Larouche*[1], *A. MacMillan*[1], *T. Babineau*[1], *H. Malouin*[1], *M. Tranchant*[1], *H. V zina*[2], *M. Dorval*[3], *P. Bessette*[4], *P. Voyer*[5], *J. L pine*[6], *R. Pichette*[7], *J. Chiquette*[8], *M. Plante*[1], *R. Laframboise*[1], *P. Bridge*[9], *D. Easton*[10], *F. Durocher*[10]
[1]*Centre Hospitalier Universitaire de Qu bec and Laval University; Quebec City, PQ Canada;* [2]*Universit du Qu bec Chicoutimi; Chicoutimi, PQ Canada;* [3]*Laval University; Quebec, PQ Canada;* [4]*Centre Hospitalier Universitaire de Sherbrooke; Sherbrooke, PQ Canada;* [5]*Carrefour de la Sant et des Services Sociaux; Jonqui re, PQ Canada;* [6]*Centre Hospitalier R gional de Rimouski; Rimouski, PQ Canada;* [7]*H pital du Sacr Coeur; Montr al, PQ Canada;* [8]*H pital du St-Sacrement; Quebec City, PQ Canada;* [9]*Alberta Children s Hospital, University of Calgary; Calgary, AB Canada;* [10]*CRC Genetic Epidemiology Unit, University of Cambridge; Cambridge, United Kingdom*
Jacques.Simard@crchul.ulaval.ca
Our study was designed to estimate the frequency and penetrance of mutations in BRCA1 and BRCA2, as well as the role of the demographic history of the population in the origin and diffusion of these mutated alleles in high risk French Canadian breast/ovarian cancer families. 527 individuals from 96 families, which included ‡ 3 cases of breast/ovarian cancer, were first tested for all BRCA1 and BRCA2 deleterious mutations reported among French Canadians. Our analysis led to the detection of the R1443X (BRCA1) allele in 10 families, the 8765delAG (BRCA2) allele in 16 families and the 2816insA (BRCA2) allele in one family. Thereafter, the complete sequence of all exons and splicing sites of BRCA1 and BRCA2 was done in 69 affected individuals or obligate carriers from 61 families, leading to the detection of 6 novel frameshift mutations and 15 sequence variants. Additional analyses were carried out to test for regulatory mutations and for the presence of large duplication or deletion in both BRCA1 and BRCA2 in 63 individuals. The proportion of BRCA1/2-positive families among those showing <4 (n=8), 4 or 5 (n=50) and ‡6 (n=31) breast cancer cases of first or second degree, was 0%, 32% and 48%, whereas a mutation was found in 65% of families with at least one ovarian cancer case (n=20). The case for pursuing the search for new susceptibility loci is compelling, given the fact that approximately two-thirds of high risk French Canadian families undergoing BRCA1/2 testing receive an inconclusive results. In this perspective, this founder cohort will allow us to create subgroups of non-BRCA1/2 families based on their genealogical characteristics and thus contributing to sampling design in linkage disequilibrium mapping studies.

P0081. Spectrum and frequency of BRCA1 and BRCA2 polymorphisms in patients with a familiy history of breast and/or ovarian cancer in Southern Saxonia

A. Kujat, *S. Strenge, U. Froster*
University of Leipzig; Leipzig, Germany
frou@medizin.uni-leipzig.de
Carcinoma of the breast are one of the most common cancer in women before menopause. Nearly 5% of breast cancer cases are hereditary. In 1994 two cancer related genes were identified following linkage analysis in families with breast and ovarian cancer on chromosomes 13 and 17, respectively. In the following years a wide spectrum of mutations and sequence alterations in both genes were listed in the Breast Information Core (BIC). Most studies show a strong correlation between typical sequence variants and ethnical origin. Thus it appears important to delineate mutation spectrums for specific regional areas. As one of the 12 German Breast Cancer Consortium centers we have undertaken genetic counselling in over 200 patients with breast and ovarian cancer during the last 3 years. We perform mutation screening for the index patients in BRCA1- and BRCA2-Gene by complete direct sequencing of 90% of the coding region including the exon-intron boundaries. 40/55 index patients agreed for molecular testing. In 18 patients we found 19 different mutations, 9 mutations in the BRCA1-gene, 10 mutations in the BRCA2-gene. One patient showed a cancer related sequence alteration both in BRCA1 as well as in BRCA2. In addition we identified a wide spectrum of unclassified variants and polymorphisms.

P0082. Different allele distribution of BRCA1 haplotypes in breast cancer patients and controls

S. Preisler-Adams[1], *N. Acar*[2], *R. Diallo*[3], *C. Jackisch*[4], *A. Rody*[5], *B. Dworniczak*[2], *J. Horst*[2]
[1]*Westf lische Wilhelms-Universit t; M nster, Germany;* [2]*Institute of Human Genetics; M nster, Germany;* [3]*Gerhard-Domagk-Institute of Pathology; M nster, Germany;* [4]*Department of Obstetrics; M nster, Germany;* [5]*Department of Ostetrics; M nster, Germany*
adams@uni-muenster.de
BRCA1 encodes a tumor suppressor that is mutated in a substantial proportion of familial breast cancers. Unexpectedly, only few somatic BRCA1 mutations have been reported in sporadic cancers. However, frequent loss of heterozygosity (LOH) at the BRCA1 locus, reduction of BRCA1 mRNA levels in tumors and hypermethylation of the BRCA1 promoter region suggest a pathogenic role of BRCA1 in the development of sporadic breast tumors as well. The majority of tumor predisposing mutations in the BRCA1 gene is attributed to frame shift or nonsense mutations and only few of the identified missense mutations have been verified as pathogenic by segregation analysis. Most of the rare sequence variants causing amino aciod changes remain unclassified variants . Among the known polymorphisms of the BRCA1 gene there are five common, amino acid exchange causing polymorphisms, which form two distinct haplotypes; either L871-E1038-K1183-S1431-S1613 (haplotype A) or P871-G1038-R1183-P1431-G1613 (haplotype B), with an unequal distribution in favor of A .

To investigate a possible functional difference between these two BRCA1 isoforms we analysed the allele distribution in three different groups of patients; 1) 30 patients with sporadic breast cancer, 2) 118 patients with a familiy history of breast cancer and 3) 198 individuals not selected for breast cancer. Our results show a significant higher proportion of B alleles in both groups of cancer patients as compared to the control group. This finding suggests a disadvantage for carrying the B allele and therefore supports the hypothesis that some of the sequence variants of both BRCA genes, so far rated as unclassified , are indeed deleterious for BRCA1/BRCA2 protein function. This work was supported by a grant from the Deutsche Krebshilfe.

P0083. BRCA1 and BRCA2 mutations in 382 chilean healthy women with family history of breast cancer

***L. Jara**, S. Ampuero, L. Seccia, M. Bustamante, J. M. Ojeda*
Institute of Biomedical Science, Faculty of Medicine, University of Chile; Santiago, Chile
ljara@machi.med.uchile.cl

Breast cancer is the most common malignancy among women. Chilean studies reveal that this cancer present the second mortality rate. A family history of breast cancer is one of the main risk factor for the development of the disease. BRCA1 and BRCA2 are the two major hereditary breast cancer susceptibility genes. Mutations in these genes are major causes of inherited breast cancer, 664 predisposing mutations have been described, but in specific populations only some of them were found to be associated with susceptibility. The purpose of this study was to establish the frequency of BRCA1 and BRCA2 germline mutations in 382 chilean healthy women with two relatives affected with breast cancer. We have determined the frequency of 185delAG and 5382insC mutations in BRCA1 and 6174delT mutation in BRCA2 using mismatch PCR assay. Only the PCR product of the normal allele acquired a restriction site, this was confirmed by specific restriction endonucleases digestion. The frequency of the 185delAG was 0.0026 (1/382). The other two mutations were not detected in studied women group. This genetic study is part of a breast cancer screening program that includes also annual mammography and clinical breast examination during five years. Strategies to reduce morbility and mortality associated with breast cancer lie in the early detection of women with genetic risk. Supported by CONAC-Chile (National Corporation of Cancer)

P0084. Haplotype analysis in german families with recurrent BRCA1 and 2 mutations

B. Wappenschmidt
Rheinische Friedrich-Wilhelms Universitaet Bonn; Bonn, Germany
Barbara.Wappenschmidt@meb.uni-bonn.de

Introduction; In the german population 15 frequent BRCA1 mutations and 8 frequent BRCA2 mutations represent 67% and 41% of all mutations found in families with hereditary breast and ovarian cancer (German breast cancer consortium). The aim of this study is the identification of founder mutations and hot spot mutations specific for the german population. Association analysis was performed for 19 different BRCA mutations (BRCA1; 14, BRCA2; 5) which were detected at least 3 times. Methods/Material; Patients were genotyped for three intragenic markers D17S855, D17S1322 and D17S1323 in the BRCA1 gene and for closely flanking markers D13S1698, D13S171 and D13S267 in the BRCA2 gene. Allele frequencies were established in a german control group. The observed frequencies were compared to the frequencies expected from the control allele frequences, assuming Hardy-Weinberg equilibrium. Statistical analysis was performed with an exact test of goodness of fit (M ller et al., 1991). Results; BRCA1; For the 3 most frequent mutations (5382insC, T300G and 3819del5) we could demonstrate a statistical significant association with a specific allele for all three markers. Other frequent mutations showed a statistical significance for at least one marker. Patients with the 3. most frequent mutation 4184del4bp had no association with any markers. BRCA2; Statistical significant association with one or all analysed markers could be demonstrated for patients with mutations 2034insA and 9317insA. In case of mutation C5910G a common haplotype was defined, which was not significant because of high allele frequencies of the shared alleles. In patients with mutation 3034del4 or 4702del4 we could not identify a common haplotype. Conclusions; The genotype data for the three markers analysed each in the BRCA1 and BRCA2 gene are in concordance with the presence of specific haplotypes. Therefore, most of the frequent mutations detected are likely to be founder mutations. Suprisingly, four C to T transitions in the BRCA1 gene, which had been expected to result from independent mutational events, are probably also founder mutations. In contrast, the 4bp deletion in the BRCA1 gene (4184del4bp) and the most frequent mutation 3034delA in BRCA2 are recurrent mutations, for which no significant associations with specific founder alleles could be shown. To define the specific haplotype, further informative family members are collected to confirm the data.

P0085. Caveolin-1 gene expression is controlled by CpG methylation in the promotor region

***J. Haeusler**, P. Geyer, S. Bochum, W. Vogel, J. Haeussler*
University of Ulm, Department of Human Genetics; Ulm, Germany
juergen.haeusler@medizin.uni-ulm.de

Caveolin-1 (Cav-1) is an integral membran protein and the principal component of small vesicular invaginations of the plasma membran, the so-called caveolae. A number of studies suggest that caveolin is involved in cell signaling and could function as a tumor suppressor. It has also been shown that Cav-1 is located in close proximity to the microsatellite marker D7S522 on chromosome 7q31, a region which frequently attracted attention in prostate cancer LOH studies. The absence of caveolin-1 mRNA and protein expression in the human prostate cancer cell line LNCaP and the observation that a 400 bp fragment of the Cav-1 promotor is methylated at least in the breast cancer cell line MCF7 prompted us to analyze the methylation status of the Cav-1 promotor in prostate cancer cell lines. Sequencing of bisulfite modified genomic DNA revealed frequent methylation of four out of seven CpGs in the region tested. In order to evaluate the functional relevance of this promotor methylation we prepared chimeric luciferase reporter constructs bearing a SssI-methylated as well as an unmethylated 356 bp Cav-1 promoter fragment. After transient expression in a prostate cancer cells line we observed a significant decrease in promoter activity of the SssI-methylated construct compared to the reporter gene driven by the unmethylated Cav-1 promotor fragment. These results suggest that regulation of caveolin-1 gene expression may be controlled, at least in part, by methylation of only a few CpG dinucleotids in the Cav-1 promotor region.

P0086. Methylation Status of E-Cadherin in Colitis Ulcerosa Patients

***P. Azarschab**[1], R. Porschen[2], N. Blin[1], K. Holzmann[2]*
[1]Molecular Genetics, Univ. T bingen; T bingen, Germany; [2]Internal Medicine, Univ.-Hospital; T bingen, Germany
piaaza@yahoo.de

E-cadherin belongs to the cadherin family of calcium dependent cell adhesion molecules. The cadherins mediate cell adhesion, differentiation, migration and intercellular cell signalling. Given these essential roles it has been postulated that changes in E-cadherin expression may account for changes in cell-cell interaction concerning inflammatory, dysplastic and, finally, neoplastic conditions. In long-standing colitis ulcerosa, a precancerous inflammation likely predisposing to the development of colorectal cancer hypermethylation of the p16 promotor has been observed as an early occuring event in neoplastic progression. Presently, we analyzed the hypermethylation status of the E-cadherin promotor (-94 to -210) in a collection of 70 ulcerative colitis samples and 70 controls. 80% of dysplastic samples showed E-cadherin hypermethylation while this status was noted in only 20% of the controls. This finding suggests that E-cadherin, considered a tumor suppressor gene in gastric cancer, may play a comparable role in additional, pre-neoplastic stages of the digestive tract.

P0087. Hypermethylation of the human homeobox gene, Alx3, correlates with expression repression in neuroblastoma

***K. Wimmer**[1], X. Zhu[2], B. Lamb[2], R. Kuick[2], M. Eckart[1], P. Ambros[3], A. Weinh usl[3], C. Fonatsch[1], S. Hanash[2]*
[1]Institut f r Medizinische Biologie, Universit t Wien; Wien, Austria; [2]Department of Pediatrics, University of Michigan; Ann Arbor, MI United States; [3]Childrens Cancer Research Institute,St. Anna Kinderspital; Wien, Austria
katharina.wimmer@univie.ac.at

Aberrant CpG island hypermethylation has been shown to be associated with gene inactivation in cancer. Recent reports demonstrate that the patterns of CpG island methylation in different neoplasias are non-random and tumour-type specific. We have investigated the extent of DNA methylation in neuroblastomas using methylation sensitive Restriction Landmark Genome Scanning (RLGS). A novel NotI-EcoRV fragment containing a CpG island was found to be methylated in neuroblastoma cell lines. This fragment was mapped to chromosome band 1p13 and the corresponding gene identified as the human orthologue of the murine homeobox gene, Alx3. Extensive methylation sensitive sequencing analysis of the promoter region of Alx3 in four neuroblastoma cell lines indicated methylation-dependent reduced expression of the Alx3 gene in these cell lines. Furthermore, treatment with the methylation inhibitor 5-aza 2«deoxycytidine induced expression of Alx3 in four neuroblastoma cell lines in which the

Alx3 gene was hypermethylated and silenced. We have demonstrated using methylation sensitive PCR that hypermethylation of the putative promoter region of the Alx3 gene occurred primarily in advanced-stage but rarely in limited-stage primary neuroblastoma tumours. Our data indicate that in neuroblastoma tumours hypermethylation of the human homeobox gene Alx3, correlated with disease progression, and in neuroblastoma cell lines hypermethylation of Alx3 correlated with reduced expression. Thus, hypermethylation represents an epigenetic mechanism which may substitute for deletion in altering expression of cancer related genes in neuroblastoma.

P0088. Promotor analysis of the DICE1 gene; a candidate tumor suppressor gene

***A. R pke**, I. Wieland, P. F. Wieacker*
Institute for Human Genetics, Otto-von-Guericke-University; Magdeburg, Germany
albrecht.roepke@medizin.uni-magdeburg.de

DICE1 is located in a LOH critical region on chromosome 13q14 and is a candidate for a tumor suppressor gene. The gene DICE1 is expressed in human heart, lung, liver, brain, kidney and skeletal muscle. It has been found that DICE1 is downregulated in lung and prostate tumors underlining that this gene is a tumor suppressor gene. To explain this downregulation the DICE1 upstream sequence was analysed for methylation in various non-small cell lung and prostate tumor cell lines. This sequence shows a high G/C-level and is rich in CpG islands. Computational analysis indicated two promoter-like domains, one of them with a TATA box. In these promoter regions various binding sites for transcription factors like SP1, AP1, NF-1, GATA, ETF, Oct and c-Myc were found. An accumulation of binding sites is observed 100-400 bp upstream of the cDNA start. Both promoter regions show several restrictions sites for methylation sensitive enzymes. By analysis of lung tumors with methylation sensitive enzymes a methylation pattern corresponding to the expression level was found. The tumor cell line SW900 shows high expression and is hypomethylated in contrast to the low expressing Calu-3 and SK-Mes1, which are hypermethylated at all investigated restriction sites. No expression and hypermethylation was found in prostate tumor cell lines LNCaP and Du145. These results suggest that; 1) DICE1, located in a LOH critical region on chromosome 13q14, is downregulated in tumor cells; 2) methylation may be the 2nd event in tumor progression. This work was supported by Dr.Mildred Scheel Stiftung.

P0089. The use of real-time quantitative PCR to detect hypermethylation of the CpG islands in the promoter region flanking the GSTP1 gene for diagnosis of prostate carcinoma

***D. Chu**[1], G. Fu[1], C. Chuang[2], H. Huang[2], J. Chia[2], C. Sun[2]*
[1]Chang Gung University; Tao-Yuan, Taiwan Republic of China; [2]Chang Gung memorial hospital; Lin-Kou, Taiwan Republic of China
dcchu@mail.cgu.edu.tw

Prostate cancer is one of the most devastating male neoplastic diseases in the developed countries. Digital rectal examination (DRE) and serum prostate-specific antigen (PSA) levels are currently used for the detection of prostate cancer. However, the former relies on the examiner s subjective judgement and the sensitivity of the latter is still sub-optimal. A novel method with higher detection ability is warranted. It has been reported that hypermethylation of the CpG islands in the promoter region upstream of GSTP1 gene is common in prostate cancer tissues and this genomic alteration could be used as diagnostic index for diagnosis for prostate cancer. In this study we developed a protocol incorporating methylation-sensitive restriction enzyme digestion and the innovated real-time PCR strategy to evaluate whether hypermethylation of this region could be used as a molecular diagnostic index for prostate cancer. Our data showed that hypermethylation of the promoter region of the GSTP1 gene was observed in all 21 prostate cancer tissues analyzed, yet not in 24 of 25 (96%) samples from subjects with benign prostate hyperplasia (BPH). Based on the current results it was concluded that hypermethylation of the 5 promoter region flanking the GSTP1 gene could be a candidate molecular index for prostate cancer diagnosis.

P0090. Association of hMLH1 Promoter Methylation with MSI Phenotype and Presence of Target Gene Mutations in Sporadic Gastric Carcinoma

M. Pinto[1], R. Mensink[2], L. Cirnes[3], F. Carneiro[3], R. Hofstra[2], R. Seruca[3], M. Sobrinho-Sim es[4]
[1]Institute of Molecular Pathology and Immunology of the University of Porto; Porto, Portugal; Department of Medical Genetics, University of Groningen; Groningen, The Netherlands; [2]Department of Medical Genetics, University of Groningen; Groningen, The Netherlands; [3]Institute of Molecular Pathology and Immunology of the University of Porto; Porto, Portugal; [4]Institute of Molecular Pathology and Immunology of the University of Porto; Porto, Portugal; Department of Pathology, Medical Faculty of Porto, Hospital S. Jo o; Porto, Portugal
mafaldap@hotmail.com

Literature suggests that in sporadic gastric carcinoma microsatellite instability (MSI) phenotype is strongly correlated with hMLH1 promoter hypermethylation. Fifty-seven sporadic gastric carcinomas, classified as MSI-H (n=28), MSI-L (n=12) and MSS (n=17), were analysed for hMLH1 promoter methylation status in order to verify this association. Also, the presence of mutations in the repetitive sequences of some target genes, TGFb RII, IGFII R, BAX and TCF4, was analysed. Possible correlation between hypermethylation of hMHL1 and mutations in these target genes were examined. A statistically significant difference in hMLH1 methylation status was observed between MSI-H cases (75% hypermethylated) and MSI-L cases (50% hypermethylated), in contrast to 0% hypermethylation in MSS cases (P=0.0001). A significant association was also found between the presence of mutations in target genes and hypermethylation of hMLH1 (P=0.0001). Mutations in the target genes were found in 21 cases (TGFb RII), 11 cases (BAX), 8 cases (IGFII R) and 4 cases (TCF4). Methylation of hMLH1 in these tumours was seen in 86%, 91%, 88% and 75%, respectively. MSI-H tumours had mutations in the repetitive sequences of target genes in 85% of the cases. MSI-L tumours were indistinguishable, regardless of the methylation status, from MSS tumours concerning (absence of) target gene mutations.

P0091. T-RNA-based probe design for microarray analysis of defined tumor areas

***R. Dahse**[1], A. Berndt[2], H. Kosmehl[2], U. Claussen[1]*
[1]Institute of Human Genetics and Anthropology; Jena, Germany; [2]Institute of Pathology; Jena, Germany
rdah@mti-n.uni-jena.de

DNA microarray technology is one of the most important recent breakthroughs in experimental molecular biology. The aim of our project is to use this novel technology for the identification of differentially expressed genes from areas at the tumor-host interface in order to search for signalling pathways critical for tumor invasion. Efficient expression analysis at the cellular level using microarrays requires the development and successful implementation of a variety of laboratory protocols and strategies. Major problems that have to be overcome are; to efficiently obtain high purity and quality RNA from limited amounts of neoplastic cells separated from contaminating normal cells and to perform linear and reproducible RNA amplification. Currently, about 5 g of total RNA (T-RNA) is used for a radioactive labelled cDNA probe - this equals about 10000-200000 cells. To overcome these limitations, we developed a concise guide to a T-RNA-based probe design for microarray analysis involving protocols for i) HE staining and laser microdissecting tumor cells and thereby maintaining the RNA integrity ii) isolating DNA-free RNA from as few as 2000 cells by simply using heat lysis accompanied by DNase and proteinase K treatment, iii) generating amplified RNA (aRNA) by RNA in vitro transcription with a template-switching primer. The procedures described here have been tested and refined from many recently published technologies. Our Cy3- and Cy5-labeled probes have been tested successfully in hybridisation of control microarrays (ArrayLink-control-slides, GeneScan Europe AG, Freiburg, Germany). Our guidelines should be applicable to all experimental conditions in which starting tumor material is the limiting factor as in small biopsies, fine-needle aspirates or microdissected tissue areas.

P0092. Genomic Profiling in Cancer

S. Shah
Spectral Genomics, Inc.; Houston, TX United States
sshah@spectralgenomics.com

Drug discovery and disease diagnostics is being transformed by the introduction of new technologies such as microarrays and bioinformatic solutions. Utilization of a microarray format for analyzing copy number changes with a resolution dependent on the map position of the DNA within the genome will allow for analysis of somatically acquired variations. The BAC microarrays available from Spectral Genomics, Inc. provide a set of unique clones giving a 3Mb coverage over the genome. Each of the BACs contain a mapped sequence tag, and most of them have been FISH mapped to confirm the locations and uniqueness. The array provides a format for high sensitivity analysis where the variation between normal genomic DNA samples is less than 5%. Further, comparing the normal genomic DNA from a male and female sample provides a consistent 1;2 copy number change with a variation of less than 5%. Utility of the BAC microarrays as applicable for genomic profiling in cancer will be shown based on results obtained from breast tumor samples. Known cytogenetic variations are

confirmed based on an entire genome profile while identifying novel regions of amplifications and deletions. As little as 1ug of total genomic DNA labeled with distinct fluorochromes is used and hybridized with the array for 4 hours. The sensitivity of the array will be shown through a series of titration experiments along with comparisons of experimental procedures to reduce non-specific binding and background noise. Automated analysis of the arrays based on SpectralWare software quantitatively determines the levels of amplifications and deletions affecting individual arrays elements.

P0093. Gene expression profile of a human cancer cell line; effects of Ginkgo biloba extract

J. Fuchs[1], *M. Podda*[1], *K. Gohil*[2], *J. J. Maguire*[3], *L. Packer*[2]
[1]*Department of Dermatology, Medical School, University of Frankfurt; Frankfurt, Germany;* [2]*Department of Molecular and Cell Biology; Berkeley, CA United States;* [3]*Environmental Energy Technologies Division, Lawrence Berkeley National Laboratory, University of California; Berkeley, CA United States*
juergenfuchs@hotmail.com

DNA microarrays and oligonucleotide array Gene Chips offer new opportunities to monitor the effects of pharmaceutical compounds on human cells. Gene Chips permit the simultaneous monitoring of the activities of thousands of human genes and this technology has been particularly useful for analyzing gene expression profiles in cell and tissue cultures. We have applied Affymetrix Gene Chips to define the transcriptional response of human epithelial T-24 cells to Ginkgo biloba extract.Ginkgo biloba extract treated cells showed increased levels of reduced glutathione and decreased synthesis of DNA. Quantitative analysis of hybridization signals from Gene Chips revealed net induction of transcription in the T-24 cells over a period of 72 hours in the presence of the extract. Functional classification of affected transcripts revealed changes in the expression of genes encoding antioxidant proteins, vesicular membrane proteins and transport, DNA synthesis-repair, cell cycle and transcription. The gene expression profile may define, for the first time, the effects of Ginkgo biloba extract on gene expression of a human cell line and may explain some of the pharmacological properties of the extract.

P0094. Identification of differentially expressed genes in colon carcinoma by pairwise hybridization of normal and tumor tissue on global human cDNA arrays

A. Schroth[1], *B. Sipos*[2], *W. Huber*[1], *D. G ssow*[3], *G. Kl ppel*[4], *B. Kremer*[5], *B. Hensch*[6], *M. Grell*[6], *A. Poustka*[1], *H. Kalthoff*[5], *S. Matzku*[6], *B. Korn*[7]
[1]*German Cancer Research Center; Heidelberg, Germany;* [2]*Institute for Pathology,University of Kiel; Kiel, Germany;* [3]*Merck KGaA Preclinical Research, GBT; Darmstadt, Germany;* [4]*Institute for Pathology, University of Kiel; Kiel, Germany;* [5]*Clinic for General Surgery and Thoracic Surgery; Kiel, Germany;* [6]*Merck KGaA Preclinical Research, Oncology; Darmstadt, Germany;* [7]*Resource Center Primary Database; Heidelberg, Germany*
schroth@dkfz.de

Colorectal cancer is the second leading cause of death from cancer in the United States. Development and progression of cancer is accompanied by complex changes in patterns of gene expression. The development of colorectal tumors is a multistep process that is known to depend on the deregulation or mutation of certain critical genes. Therefore colorectal tumors provide an excellent system for the research of different gene expression patterns because clinical and histopathological data suggest, that most malignant colorectal tumors(carcinomas) arise from preexisting benign tumors (adenomas). Complementary DNA arrays provide a powerful tool for studying these complex phenomena. To measure variations in gene expression between different stages of colon carcinomas, metastasis and normal colon tissue we used cDNA arrays carrying a global human cDNA set of 75.000 non-redundant genes. Poly (A)+ RNA from tumor, metastasis and normal colon tissue was reversed transcribed into radioactive labelled first strand cDNA and hybridized to two filters each. Array expression data for colon or metastasis versus normal colon tissue confirmed overexpression of several genes known to be upregulated e.g. lamr1 (laminin receptor 1 a 40s ribosomal protein) and downregulated e.g. ceacam7 (carcinoembryonic antigen-related cell adhesion molecule) in colon carcinomas. Other known genes with interesting functions, that have not previously been descriped to be implicated in colon cancer were found up- or downregulated in the tumors and the metastasis. . In addition many ESTs with unknown names and functions have been found up- and downregulated. Knowledge of gene expression patterns typical for certain types and stages of tumors will give insight into tumorgenetics. Molecular changes involved in tumor development and progression will provide molecular markers for tumor diagnosis, and clinical prognosis. Finally differently expressed gene products offer a promising stategy for testing new targets in colon cancer therapy.

P0095. Comparative Genomic Hybridization Studies on Cholangiocarcinoma in Korea

C. Lee, *B. Choi, M. Kim, J. Kyhm, H. Choi, Y. Cho*
College of Medicine, Hanyang University; Seoul, Republic of Korea
chhlee@email.hanyang.ac.kr

The elucidation of the genetic changes of cholangiocarcinoma is very important for understanding the molecular mechanism of carcinogenesis and progression of cholangiocarcinoma. In order to identify the gains or losses in the copy number of DNA sequence in cholangiocarcinoma, we used comparative genomic hybridization to study 33 cases of cholangiocarcinoma. The whole DNAs from each tumor tissue were labeled with different fluorochromes and then simultaneously hybridized to normal metaphase spread chromosomes. An image acquisition system was used to quantitate the signal intensities contributed by tumor and reference DNA along the entire length of each chromosome. Regions of amplification and deletion were demonstrated as quantitative alterations. The losses were prevalent on chromosome regions 19p, 19q and 22q, and the gains frequently occurred on 8q and 4q. The minimal regions of overlap for deletions were assigned to 1p36(TNFR2) and 17p13.1(p53) and 9p13-22(p15, p16). Minimal overlapping amplified site could be seen at 13q14.1-q34(RB1, RAP2A), 12q12-q14(CDK2, CDK4, MDM2) and 4q21-q25(FGF5, EGF). This study has provided a detailed comparative genomic hybridization-map of cholangiocarcinoma documenting new genetic change and also genetic divergence has been revealed in this poorly understood group of cancer.

P0096. cDNA Array Analysis of Gene Expression in Basal Cell Carcinoma

F. Aberger[1], *G. Neill*[2], *G. Regl*[1], *T. Eichberger*[1], *K. Biedermann*[1], *J. Koller*[3], *H. Hintner*[3], *A. Quinn*[2], *A. Frischauf*[1]
[1]*University of Salzburg; Salzburg, Austria;* [2]*Imperial Cancer Research Fund; London, United Kingdom;* [3]*LKA Salzburg; Salzburg, Austria*
fritz.aberger@sbg.ac.at

Basal Cell Carcinoma (BCC) of the skin is the commonest human malignancy . Although genetic studies have established that activation of the sonic hedgehog signalling pathway (SHH) plays a key role in BCC development, very little is known about the identity of SHH regulated target genes.

Using cDNA array technology we have established mRNA expression profiles of BCC samples and in human keratinocytes in the presence and absence of recombinant SHH.

For the identification of SHH target genes two types of cDNA arrays were used; i) High-density human UniGene cDNA arrays for global expression profiling from RZPD (www.rzpd.de). These filter arrays represent about 32 000 different EST clones selected from the UniGene EST database . ii) We produced filter arrays containing about 100 selected candidate SHH target genes.

Differential hybridisation of both filter array types with cDNA from BCC and normal skin, and SHH-treated and untreated keratinocyte samples led to the identification of a number of genes either responisve to SHH signalling or differentially expressed in BCC versus skin samples. Selected results were verified by Northern blots and/or real-time PCR analysis.

P0097. Genomic imbalances in drug-resistant and -sensitive T-cell acute lymphoblastic leukemia (T-ALL) cell lines

E. R. E. Gebhart[1], *A. Sauerbrey*[2], *H. Miyachi*[3], *C. R. Chitambar*[4], *H. G. Drexler*[5], *T. Efferth*[6]
[1]*Institute of Human Genetics; Erlangen, Germany;* [2]*Department of Pediatrics, University of Jena; Jena, Germany;* [3]*Department of Clinical Pathology, Tokai University School of Medicine; Bohseidai, Isehara, Japan;* [4]*Division of Hematology/Oncology, Medical College of Wisconsin; Milwaukee, WI United States;* [5]*Deutsche Sammlung von Mikroorganismen u. Zellkulturen; Braunschweig, Germany;* [6]*Virtual Campus Rheinland-Pfalz; Mainz, Germany*
egebhart@humgenet.uni-erlangen.de

Seven CCRF-CEM cell lines selected by step-wise drug exposure for resistance towards doxorubicin, vincristine, methotrexate, or hydroxyurea, resp., have been analyzed using comparative genomic hybridization (CGH). These cell lines were compared to parental, drug-sensitive CCRF-CEM as well as to other 15 T-ALL lines not selected for drug resistance. The resistant lines revealed distinct patterns of genomic imbalances. Loss of distal bands of chromosome 9p was shared by all sensitive and resistant CCRF-CEM lines, as was gain of chromosome 20. Gain of distal bands of 21q was seen in four resistant sublines. Other imbalances were common in two or three sublines or seen as single differences as compared to

parental CCRF-CEM. Among those were amplification of 5q12-q14 in methotrexate resistant cells and of 7q21 in vincristine resistant ones. These chromosomal regions harbor the drug resistance genes DHFR amd MDR1, resp. Other differences of resistant CCR-CEM lines were also seen as compared to other T-ALL lines. Our future efforts aim at those imbalances with still unknown candidate genes conferring drug resistance. Work supported by grants of Johannes and Frieda Marohn Stiftung.

P0098. Low-Grade Fibromyxoid Sarcoma Reveals Recurrent DNA Copy Number Losses at 13q21-q22 by Comparative Genomic Hybridization

S. Kiuru-Kuhlefelt[1,2], *S. Knuutila*[1,2], *M. Miettinen*[3]
[1]Haartman Institute; Helsinki, Finland; [2]University Central Hospital; Helsinki, Finland; [3]Armed Forces Institute of Pathology; Washington, DC, WA United States
sonja.kiuru-kuhlefelt@helsinki.fi

Since the description of low-grade fibromyxoid sarcoma (LGFMS) in 1987, the histologic and cytologic features of this tumor type are now well established, and LGFMS has been accepted as its own entity. LGFMS is a rare soft tissue tumor, histologically deceptively benign, yet carrying a metastatic potential. Knowledge of the molecular cytogenetic background of LGFMS is restricted to one published case with a ring chromosome. We applied comparative genomic hybridization to evaluate DNA copy number changes in eleven LGFMS cases. Nine cases were found to exhibit DNA copy number changes. The most prevalent change was loss of chromosome 13q21-q22, seen in six cases. Loss of 13q21-q22, although not pathognomonic of LGFMS, indicates a tumor suppressor gene(s) in this genomic region to be involved in the pathogenesis of LGFMS.

P0099. Chromosomal imbalances assessed by Comparative Genomic Hybridization in 97 renal tumors

D. Reutzel[1], *W. Brenner*[2], *B. Zabel*[3], *J. Decker*[1]
[1]Bioscientia Institute, and University Mainz; Ingelheim - Mainz, Germany; [2]University Mainz - Dep Urology; Mainz, Germany; [3]University Mainz - Children s Hospital; Mainz, Germany
decker.jochen@bioscientia.de

Ninety-seven primary renal tumors of different subtypes have been analysed applying Comparative Genomic Hybridization (CGH). Samples included clear cell (n=47), papillary (n=15), chromophobic (n=13) and oncocytic subtype (n=16). In addition to known subtype specific changes, new imbalances not reported yet in significant frequencies were found. Within the group of clear cell RCCs we found a high proportion of tumors showing gains of chromosome 1q (28%), and 8q (19%), loss of chromosome 9 (30%), and 4 (21%). Within the group of papillary RCCs, we found additional gain of copy number of chromosome 3 (33%). The genetic findings were correlated with pathological and clinical data (grading / staging / tumor size / survival / smoker/non-smoker, characteristics of the tumor, and occurence of metastasis). Breakpoint regions assessed by CGH that are potentially involved in tumorigenesis or tumor progression will be discussed.

P0100. Detection of DNA gains and losses in ganglioneuroblastoma by comparative genomic hybridization

A. D. Toraman[1], *I. Keser*[2], *G. Luleci*[2], *N. Tunali*[3], *T. Gelen*[4]
[1]Akdeniz University, Faculty of Medicine; Antalya, Turkey; [2]Akdeniz University, Faculty of Medicine, Department of Medical Biology and Genetics; Antalya, Turkey; [3]Cukurova University, Faculty of Medicine, Department of Pathology; Adana, Turkey; [4]Akdeniz University, Faculty of Medicine, Department of Pathology; Antalya, Turkey
toraman@hipokrat.med.akdeniz.edu.tr

The genetic changes in ganglioneuroblastoma, which represents an intermediary stage between neuroblastoma and ganglioneuroma, are not known precisely. We have defined the genomic gains and losses as well as their chromosomal locations, of the ganglioneuroblastoma samples belonging to 5 cases (3 male, 2 female) in between ages of 3-4, by comparative genomic hybridization and two-step degenerate oligonucleotide primed polymerase chain reaction (DOP-PCR). Gains in chromosomes 2p5.1-pter, 5p15.1-p15.3, +7, 13q22-q31, +22 were detected in 3 of our patients as minimal common regions, while gains in chromosomes 1p35, 4p15.1, 10q11.2-q21.2, 12q24.32-qter, +13, 17p12-p13 ve 18p11.35-pter were displayed in 2 of our patients. No oncogenes were reported to be located in the chromosomal region of 13q21-q31 previously. This region may harbor a novel oncogene which contributes to the initiation and progression of ganglioneuroblastoma. Furthermore, whole chromosome amplification of chromosome 22, which was detected in 3 of our patients, and which has not been reported in previous studies on neuronal tumors, may play a spesific role in the progression of ganglioneuroblastoma, with the YESP, SIS, PDGB, NRALS2 oncogenes it harbors.

P0101. Comparative genomic hybridization (CGH) Analysis of Radiation and Non-Radiation induced Meningiomas

E. Friedman[1], *S. Reinstein*[2], *D. Loven*[3], *O. Israeli*[1], *A. Goldring-Aviram*[2]
[1]Oncogenetics Unit; Tel-Hashomer, Israel; [2]Institute of Genetics; Tel-Hashomer, Israel; [3]Institute of Oncology; Petach Tiqvah, Israel
eitan211@netvision.net.il

Ionizing irradiation to the skull is a known risk factor for meningioma development. Skull irradiation for treatment of tinea capitis was applied from 1948-1960 in Israel to about 20,000 new immigrants, mostly children, and a subset of irradiated individuals subsequently developed meningiomas. To gain insight into the genetic factors that determine susceptibility to developing radiation-induced meningiona (RIM), we characterized the somatic genetic alterations in 16 RIMs by employing comparative genomic hybridization (CGH) and compared the pattern of alterations with 17 sporadic-occurring, non -radiation-induced meningiomas (non-RIM). Most of the tumors (29/33 - 87.9%) displayed at least one DNA copy number alteration, and 11/33 (33%) revealed 4 or more changes. The mean number of DNA copy number changes was 2.4+/-1.9 in RIMs and 2.5+/-1.9 in non-RIMs, a statistically insignificant difference. The most common deletions were noted in chromosome 22 (9/16 — 56.2% in RIM and 8/17- 47% in non-RIM) and chromosome 1 (6/16 - 37.5% in RIM and 6/17 — 35.3% in non-RIM), with no significant differences between the two groups. Noteworthy, a gain in DNA copy number of chromosomes 8 and 12 were noted in RIM tumors only. In conclusion, no significant differences were noted between radiation-induced and non-radiation-induced meningiomas regarding the mean number of genetic changes, the extent and frequency of chromosomes 22 and 1 deletions. It seems that the tumorgeneic pathways of meningioma formation are similar, regardless of previous skull irradiation. However, the apparent preferential involvement of chromosomes 8 and 12 in RIM is intri guing, and requires further investigation.

P0102. Testing different techniques for detection of microsatelite instability in Bulgarian patients with Colorectal Cancer

T. K. Petrova[1], *D. Ivanova*[1], *G. Dineva*[1], *M. Tzancheva*[2], *R. Kaneva*[1], *M. Marinov*[2], *H. Kadyan*[2], *P. Pentchev*[2], *D. Nedin*[2], *A. Alexandrova*[2], *V. Draganov*[2], *J. Ilieva*[2], *D. Damyanov*[2], *I. Kremensky*[1]
[1]Laboratory of Molecular Pathology; Sofia, Bulgaria; [2]Queen Jovanna Hospital; Sofia, Bulgaria
dipl1@ns.medfac.acad.bg

Hereditary nonpolyposis colorectal cancer (HNPCC) is the most common type of familial CRC. Germline mutations in one of DNA mismatch repair (MMR) genes are known to be responsible for the HNPCC phenotype. Their mutations induce microsatelite instability (MSI). Approximately 12-17% of the unselected tumors also show MSI. The aim of this study was to test two possible methods for detecting the microsatelite instability in our group of patients- silver staining of denaturing polyacrylamide gels and automated fluorescence detection (ALF, Pharmacia). A total of 91 patients with colorectal cancer have been included in the current study, subgrouped into six main categories- HNPCC, suspected HNPCC, family cases, juvenile cases, sporadic cases and patients with Family Adenomatous Polyposis. Highly efficient set of five markers- D2S123, BAT26, D5S346, BAT25 and D18S35 have been selected for detecting the MSI. The markers D2S123 (CA repeat) and BAT26 (polyA repeat) were chosen to be tested in the current study. The repeat markers were amplified from both normal and tumor DNA samples and were resolved by denaturing polyacrylamide gel electrophoresis and detected by the two different methods. We found 10 microsatelite unstable tumors with markers D2S123 and BAT26. The results from the two detection protocols were compared for reproducibility and accur cy. We recommend these two highly applicable methods in the detection of MSI as two step protocol.

P0103. Genetic Diagnosis Of Hnpcc; Results From Austria

B. Wolf, *D. Ilencikova, S. Henglm ller, C. Ludwig, E. Roth, J. Karner-Hanusch*
University of Vienna Medical School, Department of Surgery; Vienna, Austria
brigitte.wolf@akh-wien.ac.at

Defects in a subclass of mismatch repair proteins result in an absent or reduced ability to correct DNA replication errors, a fact that ultimately leads to the formation of tumors in a syndrome called Hereditary Non-Polyposis Colon Cancer (HNPCC). The aim of our study was to establish a presymptomatic diagnosis for HNPCC, based on the analysis of microsatelliteinstability (MIN) followed by sequencing of the two most frequently affected genes, hMLH1 and hMSH2. Thirty-three families (16x Amsterdam criteria,

17x Bethesda criteria) were included in the study. DNA was isolated from peripheral blood leukocytes and fresh-frozen or paraffinized tumor-tissue. Microsatellite instability was investigated at 5 to 10 loci in matched normal and tumor tissue. Sequence analysis was done on an ABI PRISM“ 310 Genetic Analyzer using the BigDye Terminator Cycle Sequencing Kit (Fa. ABI). Out of 29 families 35 tumors were available for microsatellite analysis; 21 tumors showed high and 2 low instability. Sequence analysis revealed 15 mutations (10x hMLH1 and 5x hMSH2), 3 somatic in two tumors and 12 germline in 14 families. Twelve at the time asymptomatic carriers of a mutation were identified and offered an appropriate screening procedure. Twelve relatives without an inherited mutation could be released from the preventive checkups. We were able to show that the inclusion of genetic data can improve the diagnosis and screening modalities of HNPCC. Following strict criteria for the selection of patients and applying a prescreening method (MIN) can facilitate the laborious diagnostic in this multigene disease characterized by various symptoms.

P0104. Clonal origin of recurrent bladder cancer as determined by microsatellite analysis

K. Junker, *M. Wolf, M. Utting, W. Werner, J. Schubert*
Department of Urology; Jena, Germany
kerstin.junker@med.uni-jena.de

In order to select the optimum therapy for patients at risk for recurrent bladder cancer it is necessary to know the pathway of recurrence development. However, the origin of recurrent bladder cancer is controversely discussed. Therefore, the aim of our study is to define the clonal origin of recurrent tumors of the bladder using molecular genetic markers. Nineteen cases with recurrent bladder cancer (1-4 recurrences per case) were investigated by microsatellite analysis using 4 markers for chromosome 9; D9S747, D9S162, D9S171, D9S1198. PCR was performed according to standard protocols followed by gele electrophoresis and automated analysis using an automated DNA-Sequencer (LI-QOR). In 14 out of 19 cases losses of heterozygosity (LOH) occured at least in one tumor. Identical LOH was detected in 10 cases. In all these cases the same allele was affected. Different LOH pattern was found in 4 cases showing LOH only in one tumor. LOH of different alleles did not occured. In 5 cases, no alterations were detected using these markers. Our results show that the majority of bladder cancer recurrences is characterized by monoclonal origin. These data indicate that recurrence is caused by cell dissemination from the original tumor. For that reason, an early therapy should be performed after transurethral resection.

P0105. Microsatellit instability in sporadic colorectal tumors

B. B. T. Tunca[1], *M. Pedroni*[2], *M. Menigatti*[2], *G. Cecener*[1], *A. Scarselli*[2], *E. Sala*[2], *F. Borghi*[2], *P. Benatti*[2], *U. Egeli*[2], *A. Zorluoglu*[3], *T. Yilmazlar*[3], *S. Gunduz*[2], *M. Ponz de Leon*[2]
[1]*Uludag University, Medical Faculty, Department of Medical Biology; Bursa, Turkey;* [2]*Dipartimento di Medicina Interna, Universita di Modena; Modena, Italy;* [3]*Uludag University, Medical Faculty, Department of General Surgery; Bursa, Turkey*
emre@uludag.edu.tr

Mutations of DNA mismatch repair (MMR) genes induce a generalized instability of DNA particularly evident at microsatellite loci. Because of their repetitive structure, microsatellites are particularly prone to replication errors and microsatellite instability (MSI) is thus a sing of MMR deficiency. We studied 15 patients with rectum cancer and 14 patients with colon cancer. DNA was extracted from normal and cancerous tissue with using fenol-chloroform extraction and proteinase K incubation. It was analyzed for microsatellite instability (MSI) using 5 markers (BAT25, BAT26, BAT40, D2123, and D18S57) by radioactive method. Tumors showing MSI in at least 3 of 5 microsatellite loci were defined as MSI(+). We determined MSI in 4 patients with colorectal cancer (13,8%). Three tumors are localized in colon and one tumor is localized in rectum. Furthermore, we studied methilation pattern of MLH1 promoter in four patients who are determined MSI positive. We observed hypermethylation in two of them (50%). The present report supports the role of hypermethylation is major mechanism of MLH1 gene inactivation in MSI positive colerectal tumors. Consequently, hypermethylation mechanism is an alternative to mutational inactivation of MMR genes.

P0106. Phenotypic characteristics of HNPCC families subdivided according to their mutation status

R. J. Scott[1], *A. D. Spigelman*[2], *C. J. Meldrum*[1], *M. McPhillips*[1], *L. Thomas*[1]
[1]*Hunter Area Pathology Service; New Lambton, Australia;* [2]*University of Newcastle; Newcastle, Australia*
rscott@doh.health.nsw.gov.au

Hereditary non polyposis colorectal cancer (HNPCC) describes a diverse group of families that share a common predisposition to colorectal cancer in the absence of any premalignant phenotype. The genetic basis of this disease has been linked to mutations in genes associated with DNA mismatch repair. A large proportion of families, harbour changes in one of two genes, hMSH1 and hMSH2. Approximately 35% of families that adhere to the Amsterdam Criteria do not appear to harbour mutations in these DNA mismatch repair genes. In this report we present data from a large series of HNPCC families and indicate that there are subtle differences and similarities between families that harbour germline changes in hMSH2 compared to those harbouring hMSH1 mutations. Furthermore, there are differences between the mutation positive group (hMSH2 and hMSH1 combined) and the mutation negative group of families. The major findings identified in this study focused primarily on the extra-colonic disease profile observed between the mutation positive families and the mutation negative families. Breast cancer was not over-represented in the mutation negative groups. There was no difference in the age of the colorectal cancer diagnosis between the hMSH2 and hMSH1 mutation groups but there was a significant difference between these two groups and the mutation negative group.

P0107. Screening for HNPCC mutations through genomic instability and immunohistochemistry

J. L. Blouin[1], *M. A. Brundler*[1], *J. F. Egger*[1], *P. Hutter*[2], *C. Soravia*[1], *C. D. DeLozier-Blanchet*[1]
[1]*University Hospital of Geneva; Geneva, Switzerland;* [2]*Institut Central des H pitaux Valaisans; Sion, Switzerland*
jean-louis.blouin@medecine.unige.ch

Dominantly-inherited mutations are identified in 5-10% of patients with colorectal cancers. Unlike in polyposis coli (APC), mutation search in mismatch-repair (MMR) genes in HNPCC (Hereditary non-polyposic colon cancer, Lynch syndrome) is time-consuming and costly due to locus and allele diversity. Microsatellite instability (MSI) and immunohistochemical analysis (IHC) in patients meeting the Bethesda criteria facilitate the identification of constitutional MMR mutations which may have direct implications for treatment and prognosis. To evaluate MSI and IHC testing for the identification of HNPCC, we analysed 21 unrelated patients meeting the Amsterdam/Bethesda criteria. The MSI test identifies genomic instability by comparing constitutional and tumor DNA with a standard battery of 5 microsatellite markers. IHC determines presence/absence of expression of the hMLH1 and hMSH2 proteins in tumor. Seven individuals (33 %) had abnormal results. In all four Amsterdam+ families both tests were pathological and led to subsequent identification of mutations in hMLH1 (3) or hMSH2 (1). IHC and MSI results were concordant in all cases, and compatible with the underlying molecular defect in the four Amsterdam+ families. Three patients meeting only the Bethesda criteria were positive on MSI and IHC; mutation identification has been initiated. MSI and IHC testing allows more rapid para-clinical diagnosis and renders molecular investigation time and budget-efficient, by targeting specific families for germline mutation research. This multi-step approach is well perceived by both patients and clinicians, and fosters the pluridisciplinary approach needed in these families. (supported by subsidy of Geneva University Hospital, PRD-00-11)

P0108. Immunohistochemistry in HNPCC Tumour Tissue detects most but not all Carriers of Mutations in DNA Mismatch Repair Genes MSH2 and MLH1

E. Mangold[1], *M. Mathiak*[2], *W. Friedl*[1], *C. Lamberti*[3], *M. Jungck*[3], *M. Steinhoff*[1], *M. Ohlendorf*[1], *M. Sengteller*[1], *H. Fischer*[2], *P. Propping*[1]
[1]*Institute of Human Genetics; Bonn, Germany;* [2]*Department of Pathology; Bonn, Germany;* [3]*Department of Internal Medicine; Bonn, Germany*
e.mangold@uni-bonn.de

Identification of germline mutations in HNPCC patients is of major importance for predictive testing in family members at risk. Examination of tumour tissue for microsatellite instability is useful to preselect patients suspected of HNPCC for germline mutation screening in DNA mismatch repair genes. Immunohistochemistry for expression of MLH1 and MSH2 proteins in tumour tissue is a fast and simple method and has been proposed as an alternative preselection procedure. In order to examine the sensitivity of this method we examined the correlation between immunohistochemistry and microsatellite analysis in tumour tissue from HNPCC patients with pathogenic germline mutations in either MSH2 or MLH1. In a large sample of families suspected of HNPCC we had identified pathogenic germline mutations in 65 unrelated patients. A high microsatellite instability (MSI-H) was detected in all tumour samples. In 23 patients with mutations in MSH2 and 25 patients with MLH1 mutations both microsatellite analysis and immunohistochemistry were performed. All patients with MSH2 germline mutations showed loss of MSH2 expression. Loss of

MLH1 expression was observed in tumours from 17 patients with MLH1 mutations, while in 8 cases reduced staining of MLH1 protein was detected. We conclude that immunohistochemistry is a useful method for selecting patients at high risk for HNPCC. As its results are pointing towards the affected gene, it allows straightforward search for the underlying germline mutation. However, within the tumours due to MLH1 mutations there are cases showing MSI-H in tumour tissue, but no complete loss of MLH1 protein expression. Such cases might be misinterpreted when only immunohistochemistry is applied for prescreening. Therefore in cases with normal or reduced mismatch repair protein expression microsatellite analysis should be performed as well. Acknowledgement; This study was supported by the Deutsche Krebshilfe

P0109. Investigation Of Replication Error Phenotype In AML And MDS Patients.

M. H. Sheikhha, *K. Tobal, J. A. Liu Yin*
University Department of Haematology, Manchester Royal Infirmary; Manchester, United Kingdom
sheikhha@yahoo.com

Microsatellite instability (MSI) is associated with defects in the DNA Mismatch repair (MMR) system. MSI was detected in several forms of human cancer including haematological malignancies especially in therapy related leukaemia. We have investigated the presence of MSI in 66 patients including 57 AML and 9 MDS (18 therapy related, 8 secondary, 10 old and 30 denovo). Twelve separate microsatellite loci including three mononucleotide repeat markers (BAT-25, BAT-26 and BAT-40) were used. Tumours were considered as RER+ when they showed instability in at least three loci or one of the mononucleotides. Buccal mucosa was used to obtain the corresponding germline DNA for 53 patient. In 13 therapy-related patients there was no normal DNA available and MSI was tested only in the BAT markers. Microsatellite loci were amplified by PCR. The products were separated by polyacrylamide gel electrophoresis, followed by silver staining. Genetic instability was detected in 24 (36.4%) patients, 21 of the 57 AML (36.8%) and 3 of the 9 MDS (33.3%). The rate of RER+ was significantly higher in the therapy related and secondary patients compared with the de novo and old ones. Five out of 8 (62.5%) secondary and 9 out of 18 (50%) therapy related showed RER+ in comparison with 3 out of 10 (30%) old and 7 out of 30 (23.3%) de novo. Our data suggest that genetic instability is an important event in the development of the AML and MDS especially therapy related and secondary malignancies.

P0110. Analysis on the GAP-related Domain of the NF1 Gene in Neurofibromatosis Type 1 (NF1) Patients with Tumours

S. Ayter, *S. Oguzkan, M. Cinbis, B. Anlar, S. Aysun*
Hacettepe University, School of Medicine; Ankara, Turkey
sayter@gen.hun.edu.tr

Neurofibromatosis type1 (NF1) is a common autosomal dominant disorder caused by mutations of the NF1 gene. It is characterized by multiple neurofibromas, pigmentation anomalies, and a variety of other possible complications, including an increased risk of malignant neoplasias. The NF1 gene product neurofibromin that is a large protein, a member of the negative growth regulatory proteins called tumor supressors. Neurofibromin has a GTPase activating protein (GAP) domain which has shown significant homology between the catalytic region of mammalian Ras-GAP. It is also called GAP-related domain (GRD) that interacts with the Ras protein, to be crucial in regulating the mechanisms of signal transduction and cell proliferation/differentiation. GRD is encoded from exons 21-27a on NF1 mRNA. To date most of the reported NF1 mutations are predicted to result in protein truncation but very few studies have correlated the causative NF1 mutations with tumor formation in NF1 patients. Therefore we analyzed the exon 21-27a DNA from 10 NF1 tumours by using SSCP technology. During the analysis of NF1-GRD, electrophoretically abnormal fragments were detected. Direct sequencing of these fragments allowed us to identify the presence of variations and we used these data for further search. We also plan to compare tumor and nontumor developed NF1 patient s DNAs for the same exons in order to determine to extend of variability present in their NF1 gene.

P0111. Cytogenetic and molecular analysis of early onset renal cell carcinomas in a family with a t(2;3)(q35;q21)

N. de Leeuw, *D. Bodmer, M. J. Eleveld, M. Ligtenberg, A. Geurts van Kessel*
University Medical Centre Nijmegen; Nijmegen, The Netherlands
N.deLeeuw@antrg.azn.nl

Previously, we described a family with renal cell cancer and a constitutional balanced t(2;3)(q35;q21). Based on loss of heterozygosity (LOH) and Von Hippel Lindau (VHL) gene mutation analyses in five tumour biopsies from three patients in this family, we proposed a multistep model for RCC development in which (nondisjunctional) loss of the translocation derived chromosome 3 (der(3)) may act as a primary oncogenic event and somatic mutation of the VHL gene as a secondary event related to tumour progression. Here we describe the cytogenetic and molecular analysis of three novel tumours at early stages of development in two members of this family. Again, loss of the der(3) chromosome was found in two of these tumours and a VHL mutation in one of them. In the third tumour, however, none of these abnormalities could be detected. These results suggest that yet another (epi) genetic event may precede the observed der(3) loss and VHL gene mutation in the development of this type of familial renal cell cancer.

P0112. Alterations Of Patched May Cause Developmental Malformations And Cancer c

S. Levanat[1], *I. Crnic*[1], *S. Oreskovic*[2]
[1]*Rudjer Boskovic Institute; Zagreb, Croatia;* [2]*Clinical hospital-Petrova,University of Zagreb; Zagreb, Croatia*
levanat@rudjer.irb.hr

Constitutional hemizygous inactivation of PTCH, the Shh/Ptch signaling pathway gene, which moderates Shh signalling, manifests itself as Nevoid Basal Cell Carcinoma Syndrome (NBCCS) or Gorlin syndrome, a condition variably characterized by a number of developmental disorders and malformations, and by predisposition to certain malignancies where the most frequent are basal cell carcinomas, medulloblastomas and ovarian fibromas. The PTCH gene, a human homologue of the Drosophila segment polarity gene patched, maps to chromosome 9q22.3 and loss of heterozygosity (LOH) at this site in both sporadic and hereditary basal cell carcinomas and meduloblastomas suggests that it functions as a tumor suppressor. Our studies of LOH in sporadic ovarian fibromas for the same region and aberrant SSCP pattern in sporadic ovarian fibromas contribute to the ptch role in their genesis as well. In our studies we used DNA from fresh tissues and blood leukocytes, which were typed for several short tandem repeat polymorphism s spanning chromosome 9q21-q31 and by SSCP analysis we have been analyzed variability in PTCH exons. LOH for the PTCH region has been found in odontogenic keratocysts, the cyst type with highly increased incidence in NBCCS. Suggestive temporal distribution of Shh signalling, recently observed during tooth development, lead us to investigate its association with dentigerous cysts, the other major non-inflammatory cyst of odontogenic origin. We report here that PTCH is inactivated in dentigerous cysts, the implication being the same gene for their genesis as well. More generally, PTCH alterations may prove to be a necessary, and perhaps the initiating event, in formation and growth of various noninflammatory cysts, especially with our observations of incomplete heterozygosity which we interpreted as LOH in this region for ovarian dermoid cysts. This would be consistent with our view that local PTCH inactivation can, under predisposing circumstances, lead to persistent though not by itself truly aggressive cell proliferation.

P0113. Mutation Analysis of the Nijmegen Breakage Syndrome Gene (NBS1) in Acute Myeloid Leukemia (AML) with Complex Karyotypes

R. Varon[1], *C. Schoch*[2], *A. Reis*[3], *W. Hiddeman*[2], *K. Sperling*[1], *S. Schnittger*[2]
[1]*Institute of Human Genetics, Humboldt University; Berlin, Germany;* [2]*Medizinische Klinik III, Ludwig Maximilians University, Klinikum Gro§hadern; M nchen, Germany;* [3]*Institute of Human Genetics, Friedrich-Alexander-University; Erlangen, Germany*
raymonda.varon-mateeva@charit .de

The chromosomal instability disorder Nijmegen Breakage Syndrome (NBS), is associated with immune deficiency, cellular hypersensitivity to ionizing radiation, and extreme susceptibility to lymphoid malignancies due to a defect in DNA double strand break repair. In this study, we analyzed 19 tumour DNA samples from patients with acute myeloid leukemia (AML) showing complex chromosomal aberrations, indicative of chromosomal instability, for mutations in the NBS1 gene. The samples were analyzed by dHPLC analysis and all shifts were directly sequenced. Of the 19 samples analyzed we found one to be heterozygous for a novel 5 bp deletion in intron 12 (IVS12-53del5). By RT-PCR analysis the expected transcript and an additional faint product with skipped exon 13 was observed, indicative of aberrant splicing. This exon codes for the binding site of nibrin to MRE11. However, we also found that all analyzed controls showed this phenomenon. Thus, the IVS12-53del5 is not responsible for the skipping of exon 13 and most probably represents a rare polymorphism which has not been detected among 250 control chromosomes so far. We found no further NBS1 mutations among the AML samples. Although the number of the analyzed samples is not representative, our study indicates that NBS1

mutations are not common in AML with complex karyotype.

P0114. TP53 mutations, p53 protein expression and EGFR expression in pediatric malignant astrocytomas and glioblastomas.

E. van den Berg[1], *R. W. Hofstra*[1], *A. Dam*[2], *J. M. Fock*[3], *W. M. Molenaar*[2]
[1]*Dept. of Medical Genetics, University of Groningen; Groningen, The Netherlands;* [2]*Dept. of Pathology, University of Groningen; Groningen, The Netherlands;* [3]*Dept. of Neurology, University Hospital of Groningen; Groningen, The Netherlands*
e.van.den.berg-de.ruiter@medgen.azg.nl

Background. TP53 gene mutations play a significant role in the development and progression of astrocytomas. De novo glioblastomas often show overexpression of the epidermal growth factor receptor (EGFR) protein. A previous study of low grade pediatric astrocytomas showed a high frequency of TP53 gene mutations, but not in residual tumors that showed progression. Material and methods. TP53 gene mutation analysis was performed in 3 pediatric malignant astrocytomas (MA) and 4 glioblastomas (GBM) using denaturing gradient gel electrophoresis mutation detection assay. P53 protein expression and EGFR expression were assessed immunohistologically using commercial antibodies. Results. TP 53 mutations were observed in 2 GBMs, which showed limited and extensive p53 protein expression, respectively. One of them also strongly expressed EGFR. One MA showed strong EGFR expression, in combination with intermediate p53 protein expression, without gene mutations. Intermediate p53 protein expression without gene mutations and without EGFR expression was found in 1 MA and 1 GBM. Discussion. Both TP53mutations that were found are possibly causative, as they result in a non-conservative amino acid substitutions. In one case the mutation involves the replacement of the last base at the 3 end of exon 5, probably affecting correct splicing of the mRNA transcript. The mutation at codon 248 in the other case, is a known mutational hot spot in many tumors and codes for a region of the p53 protein involved directly in contacting DNA. The same mutation has been reported several times in glioblastomas of adults. TP53 mutations and p53 protein expression did not parallel each other. Moreover, EGFR expression occurred together with p53 expression and a p53 mutation. This suggests that the pathways in children may be different from those in adults.

P0115. Somatic Mutations And Deletions Of Ptch Gene In Ovarian Fibromas

V. Musani[1], *I. Crnic*[1], *S. Oreskovic*[2], *S. Levanat*[1]
[1]*Rudjer Boskovic Institute; Zagreb, Croatia;* [2]*Gynecological Clinics-Petrova, University School of Medicine, University of Zagreb; Zagreb, Croatia*
vesna.musani@zg.hinet.hr

We have been analyzing molecular genetic alterations of the Gorlin syndrome or Nevoid Basal Cell Carcinoma Syndrome (NBCCS) region, chromosome 9q22.3 in ovarian fibromas, the trird tumor by incidence in the syndrome. Gorlin syndrome or Nevoid Basal Cell Carcinoma Syndrome (NBCCS) is an autosomal dominant disorder that predisposes to basal cell carcinomas of the skin, medulloblastomas and ovarian fibromas, and widespread developmental defects. Syndrome related disorders have been attributed to alterations of PTCH gene, a human homologue of the Drosophila segment polarity gene patched, which maps to chromosome 9q22.3. Loss of heterozygosity (LOH) at this site in both sporadic and hereditary basal cell carcinomas and medulloblastomas suggests that it functions as a tumor suppressor. The aim is to confirm the tumor suppressor role of the ptch in genesis of ovarian fibromas even when they are not syndrome related. DNA was isolated from fresh tissues and blood leukocytes. The DNA samples were typed for several short tandem repeat polymorphism s spanning chromosome 9q21-q31 in tumors (ovarian fibroma) and matched constitutional tissues. Polymorphic markers D9S127, D9S287, D9S180 and D9S196 and 20 PTCH exons were used. We screened for allelic loss and by SSCP analysis we have been analyzing variability in PTCH exons. PCR reactions were performed and products were fractionated on 6-12% polyacrilamide gel. We found LOH in 30% of sporadic ovarian fibromas. In about 70% of ovarian fibromas variability in SSCP pattern was detected. LOH for the ptch region and aberrant SSCP pattern confirmed the expectations that the gene patched has a decisive role in the genesis of ovarian fibromas when they are not syndrome related.

P0116. Beta-catenin and AXIN2 Mutations Contribute to Carcinogenesis in the Pallila of Vater

W. Liu, *K. Taniguchi, S. Krishnadath, C. Qian, L. Burgart, P. Roche*
Mayo Clinic; Rochester, MN United States
liu.wanguo@mayo.edu

Adenomatous polyposis coli (APC) mutations have recently been reported in Ampullary carcinoma indicating that the Wnt signaling pathway is involved in the development of this tumor. However, b-catenin and AXIN2, the two key players in this pathway, have not been investigated in this tumor. Here, we show that 4 of 40 carcinomas of the papilla of Vater harbor b-catenin mutations and 4 others contain AXIN2 mutations. All the b-catenin mutations affect the conserved GSK-3b phosphorylation sites in exon 3, while three out of the four AXIN2 mutations were frameshift mutations resulting in deletion of the DIX domain. The remaining one was a 12 bp deletion removing four amino acids in the PP2A binding domain of AXIN2. Both AXIN2 and b-catenin mutations were found not to co-exist in the same tumor indicating that either one of the mutations is sufficient for tumorigenesis in the papilla of Vater. Immunohistochemical staining of b-catenin showed focal nuclear or cytoplastic accumulation of b-catenin in tumor tissues with b-catenin or AXIN2 mutations. Taken together, this is the first study to demonstrate that b-catenin or AXIN2 is mutated in Ampullary carcinoma and suggest that mutations int these two genes response for tumorigenesis in 20% Ampullary carcinomas through the elevation of b-catenin in tumor cells. The biological impact and the mechanisms of these mutations in the carcinogenesis of the papilla of Vater remain to be elucidated.

P0117. Mutations of the N-ras Gene in Childhood Myelodysplastic Syndromes

B. Jekic[1], *V. Bunjevacki*[1], *M. Kuzmanovic*[2], *J. Milasin*[3], *I. Novakovic*[1], *L. Lukovic*[1]
[1]*Institute of Biology and Human Genetics, School of Medicine; Belgrade, Yugoslavia;* [2]*Institute for Mother and Child Health; Belgrade, Yugoslavia;* [3]*Institute of Biology and Human Genetics, Faculty of Stomatology; Belgrade, Yugoslavia*
llukovic@eunet.yu

The primary myelodysplastic syndromes (MDS) are heterogeneous group of disorders characterized by a failure of the hematopoietic progenitors to differentiate normally, resulting in ineffective and dysplastic hematopoiesis of one or more cell lines. MDS is considered to be preleukemic state and in this context, it provides an opportunity to study molecular mechanisms that precede the development of leukemia. Molecular lesions underlying the evolution of MDS are largely unknown. Mutations of RAS genes have been found in variety of human hematologic malignancies including 5 % to 30% of patients with MDS. The Ras gene family consists of three genes, H-,Ki- and N-Ras genes, which encode p21 ras proteins.They function as GTP buinding proteins with intrinsic GTP-ase activity. Usually, oncogenic activation of Ras arises through missense point mutations in codons 12,13(exon 1) and 61(exon 2). It is yet not clear is the role of Ras genes mutations in childhood and adult MDS (leukemogenesis) is the same. We have studied the frequency of N-Ras point mutations in 20 pediatric patients with MDS. Archival bone-marrow samples were screened by DNA amplification followed by single-strand conformation polymorphism (SSCP) at both, exon 1and exon 2 of N-Ras. . One patient has showed abnormal band shift for exon 1, and two for exon 2,suggesting that role of N-Ras mutations in childhood MDS may be significant.

P0118. Identification of RB1 Gene Mutations and pRB Expression in Mexicans Patients with Retinoblastoma

M. Rodriguez[1], *M. Gonzales*[1], *M. Salcedo*[2], *F. Salamanca*[1], *R. Coral*[1], *D. Arenas*[1]
[1]*Human Genetic Unit, Pediatric Hospital, CMN SXXI IMSS; Mexico City, Mexico;* [2]*Oncology Research Unit, CMN SXXI IMSS; Mexico City, Mexico*
plumita@eudoramail.com

Retinoblastoma, is a chilhood tumor of the eye that affects about 1/13,500-1/25,000 newborns and occurs as sporadic or hereditary form. This cancer result from succesive loss or inactivation of the RB1 gene located in 13q14.1. This gene encodes for a 110Kd nuclear phosphoprotein (pRB), which plays a major role in cell proliferation control. Different types of mutations in the RB1 gene have been reported, but point mutations are the most common. Even though retinoblastoma is common disease in Mexico (about one case/per month, there are not molecular studies about RB1 gene mutations and expression in Mexican children. In this study, we studied 19 patients with bilateral or unilateral retinoblastoma. Genetic and cytogenetic studies were carried out. In addition, detection of RB1 gene and its expression were done. By using SSCP, (single strand conformational polymorphism) assay several polymorphisms were identified in different exons. In all cases, the sequence of the polymorphisms showed mutations that produced a frameshift on the open reading frame. In just one tumor we did no detect the pRB protein. This kind of work is important because the identification of mutations may contribute to basic knowledge of this neoplasia and furthermore the possibility to offer genetic counseling to relatives in risk.

P0119. Promoter Analysis of Human Rad51 Gene

L. Hasselbach[1], *Y. Yuan*[2], *S. Miska*[2], *W. Henning*[2], *H. W. Stuerzbecher*[2]
[1]*Department of Clinical Chemistry, Medical University of Luebeck; Luebeck, Germany;* [2]*Institute of Human Genetics, Medical University of Luebeck; Ratzeburger Allee 160, D-23538 Lubeck, Germany*
liu.hasselbach@medinf.mu-luebeck.de

Human Rad51 is know as a DNA repair protein, which plays crucial roles in both mitotic and meiotic recombination, and also in repair of double strand breaks. Rad51 protein is essential for cell survival in vertebrates and for embryonic development. It appears to associate either directly or indirectly with three tumor suppressor genes, P53, BRCA1 and BRCA2. Altered expression and overexpression of Rad51 protein have been reported in different human cancers like pancreatic carcinoma and invasive ductal breast cancer (Maacke et al, Int J Cancer. 2000 Dec 15;88(6);907-913). To study the expression regulation of this gene, a PAC clone includes the human Rad51 gene was identified and subcloned. A 8.1 kb DNA fragment of 5«-region of this gene was sequenced and submitted to Genbank Database (Accession No. AF203691). This fragment contains intact sequences of exon 1, intron1, exon 2, intron 2, exon 3 and partial sequences of intron 3. The translation start codon ATG is located immediately at the beginning of exon 2. The predicted promoter region has been pointed on 5.4kb upstram of ATG. No TATA-like or initiator sequences were detected, implicating this gene could belong to the TATA less GC rich house-keeping gene family. Three overlapping fragments (F II, F III and F IV) of this predicted promoter region were cloned into a luciferase reporter vector named pGL3. A pair of cell lines, UiRad5-2 and UiLacZ, were delibrately selected for this experiment. UiRad5-2 cell line could express high level of Rad51 protein when induced by Ponasterone A, while UiLacZ cell line express beta-galactosidase. A high luciferase activity was detected in both non-induced UiRad5-2 and UiLacZ cell lines transfected with the fragment II (pGL3-F II, sequence from -4529 to -2135bp to ATG), and no luciferase activity was showed in the other two constructs of pGL3-F III and pGL3-F IV. Nested deletions were made in plasmid pGL3-F II to minimize the predicted promoter region into about 300bp. Further study is now going on to reveal the precise function of this region.

P0120. The spectrum of RB1 gene anomalies in different forms of retinoblastoma.

O. V. Babenko[1], *S. Saakyn*[2], *V. Kozlova*[3], *M. Nemtsova*[1], *D. Zaletayev*[1]
[1]*Research Centre for Medical Genetics; Moscow, Russian Federation;* [2]*Moscow Institute for eye desease; Moscow, Russian Federation;* [3]*National Scientific Oncology Centre; Moscow, Russian Federation*
vstrel@iname.com

Retinoblastoma (Rb) is a childhood tumor of retina with an incidence of 1;13000 live births. All forms of Rb result from inactivation of both alleles of the tumor-suppressor gene RB1. In 5% of cases Rb is found together with visible chromosome abnormalities - deletions in chromosome 13q14.1, and in other cases - with submicroscopic anomalies, small mutations (75-80%) and with hypermethylation (10-15%) of the promoter region of RB1. Our aim was investigation of RB1 molecular anomalies causing retinoblastoma. SSCP and heteroduplex analysis were used for mutation screening, microsatellite analysis (introns 2 & 20, D13S262, D13S284) - for loss of heterozygosity detection, methylation analysis of RB1 promoter region - for functional mutations detection.We have studied 60 families with Rb, 50 of which were sporadic (35 unilateral and 15 bilateral) and 10 were hereditary or familial cases of Rb (4 unilateral and 6 bilateral). In all cases a high-resolution chromosome analysis had been carried out and no chromosomal anomalies were found. Analysis of PCR products mobility by SSCP and heteroduplex analyses revealed 40 possible mutations in exons 3 to 23. Only five mutations were missense (exons 10, 18 and 20). Others were nonsense mutations, frame shift and splice site mutations. We detected germinal mutations in all familial and isolated bilateral cases of Rb. Loss of heterozygosity was found in 32 (70%) tumor specimens of 50 analyzed tumors. Methylation pattern anomalies of the RB1 gene promoter region were found in 7 (15%) of 50 tumors and in 1 case in blood lymphocytes, that allowing us to suppose hypermethylation may represent the first allele inactivation event in carcinogenesis.

P0121. The role of P-glycoprotein (MDR1) polymorphisms and mutations in colorectal cancer

U. Potocnik[1], *M. Ravnik-Glavac*[1], *R. Golouh*[2], *D. Glavac*[1]
[1]*Medical Faculty, Institute of Pathology, Laboratory of Molecular Genetics; Ljubljana, Slovenia;* [2]*Institute of Oncology; Ljubljana, Slovenia*
upotocnik@ibmi.mf.uni-lj.si

Multidrug resistance, the main problem in efficient cancer chemotherapy, is mainly caused by increased expression and acquired mutations in P-glycoprotein (P-gp), encoded by MDR1 gene. P-gp is highly expressed on the apical surface of gastrointestinal epithelium, suggesting its role in protection from xenobiotics. Additional physiological functions for P-gp and its role in cancer beyond multidrug resistance in chemotherapy are poorly understood. We have therefore analyzed tumor and corresponding normal tissue from 400 patients with previously non treated colorectal cancer for germline and somatic alterations in MDR1 gene using PCR-Conformational analysis and direct sequencing. We have identified 12 different germline alterations. There were 4 missense, 3 samesense, 4 intronic alterations close to exon/intron boundary and 1 alteration in promoter region. We observed significant correlation of at least two germline polymorphisms with increased lymphoid infiltration in tumors. Two unique germline alterations, identified in normal and corresponding tumor tissue and all somatic mutations identified only in tumor tissue, were associated with colorectal cancers with high microsatellite instability (MSI-H). There were missense, frameshift and mutations in the promoter region. Interestedly, we found that in all MSI-H tumors with MDR1 mutations, both, the coding and promoter regions were mutated. To our best knowledge this is the first report of naturally occurring mutations in MDR1 gene. These results suggest that MDR1 plays an important role in development of at least a proportion of MSI-H cancers and that P-gp is involved in immune response in colorectal cancer suggesting its physiological function in immunology.

P0122. Frequent LOH and absence of mutations in the coding region of DNA repair genes in ovarian cancer

I. I. Arzimanoglou[1], *L. Hansen*[2], *D. Chong*[1], *M. Psaroudi*[3], *C. Dimitrakakis*[4], *S. Michalas*[4], *F. Gilbert*[1], *F. Chervenak*[1], *H. Barber*[1]
[1]*Cornell University/ Weill Medical College; New York, NY United States;* [2]*University of Aarhus/ Institution of Human Genetics; Aarhus, Denmark;* [3]*National Hellenic Research Foundation; Athens, Greece;* [4]*University of Athens/Medical School; Athens, Greece*
Arziman@lycosmail.com

The aim of our study was to determine loss of heterozygosity (LOH), coding instability and single base sequence alterations specific for ovarian cancer (OC). To accomplish this, a combination of high resolution GeneScanTM software analysis followed by automated DNA sequencing, was used. After optimization, the sensitivity of our GeneScanTM system was brought down to a single base level, as always confirmed by sequencing. In case of long repeats, a cloning step using the PCR-Script TM Amp SK(+) system was required to precede sequencing in order to successfully read through them. Selected DNA repair sequences of ovarian tumor DNA were tested against the matched blood counterparts (DNA pairs). Negligible coding instability was found at hMSH3/ex7 (1/62 DNA pairs), and at hMSH6/ex5 (0/32 DNA pairs). Unlike coding instability, LOH confined to the tumor was found in 41% (17/37 DNA pairs) of the informative OC cases at hMLH1/exon12. In addition, preliminary evidence indicated that such a trend of frequent LOH in OC is likely to extend to other DNA repair sequences as well. Therefore, our findings support a critical role for LOH as a mechanism involved in the development of ovarian cancer. On the other hand, the occurrence of somatic changes in the coding region of genes, presumably caused by DNA instability is not likely to account for gene inactivation in OC.

P0123. DPC4 tumor suppressor gene in human sporadic colon cancer; loss of heterozygosity

M. H. Popovic Hadzija[1], *S. Kapitanovic*[1], *S. Radosevic*[2], *T. Cacev*[1], *R. Spaventi*[2], *M. Hadzija*[1], *K. Pavelic*[1]
[1]*Rudjer Boskovic; Zagreb, Croatia;* [2]*Pliva d.d.; Zagreb, Croatia*
mhadzija@rudjer.irb.hr

The purpose of this study was to examine the prevalence of DPC4 loss of heterozygosity (LOH) in sporadic colorectal cancer. For this purpose we analyzed 36 cases of human sporadic colon carcinoma and corresponding normal tissue samples. We used polymerase chain reaction (PCR), variable nucleotide tandem repeat (VNTR) analysis and restriction fragment polymorphism (RFLP). Each sample was tested for LOH for each of three VNTR flanking markers (D18S474, D18S363, D18S46) onto 10% non-denaturing polyacrylamide gel LOH was defined by a visible change in allele; allele ration in tumors compared with matching normal tissue. We also used BspHI, MaeI and MnlI RFLP sites for detection mutation in exons 8, 10 and 11. Informativity (heterozygosity) was observed in 35 (97%) from 36 analyzed samples. LOH at the DPC4 locus was detected in 18 (51%) of informative tumor DNAs; 13 cases of LOH found by D18S474, 9 cases of LOH found by D18S363 and 4 cases found by D18S46 markers, respectively. At the DPC4 exon 11 locus only one patient had mutation; in exons 8 and 10 did not find mutation. Statistical analysis did not show correlation between DPC4 LOH and age or sex of patients with informative tumor samples. The DPC4 LOH was more frequent in tumors smaller than 5 cm in diameter than in larger ones. Our results support the view that malignant

progression is a consequence of more than one of genetic change and suggest that inactivation of DPC4 gene play a role in a multistep process of colon tumor progression.

P0124. High Frequency Of Allelic Imbalance At Regions Of Chromosome Arm 8p In Ovarian Carcinoma

I. Pribill[1], *A. Pfeffer*[2], *R. Zeillinger*[2], *P. Speiser*[2], *M. Krainer*[2]
[1]*General Hospital Vienna,; Vienna, Austria;* [2]*General Hospital Vienna; Vienna, Austria*
ingrid_pribill@akh-wien.ac.at

Progressive genetic changes such as the inactivation of tumor suppressor genes (TSG) are thought to play an important role in the initiation and progression of ovarian cancer. Frequent non-random allelic imbalance (AI) at 8p11-p21 and 8p22-pter suggests the existence of TSGs that may be involved in the carcinogenesis of several human malignancies. We investigated 70 ovarian tumors with 11 highly polymorphic markers spanning 8p12-p21 and 8p22-pter to produce an AI map of 8p in epithelial ovarian cancer. AI was demonstrated in 54 tumors (77%), most frequently occurring at D8S136 (54%) and at D8S1992 (55%). Poorly differentiated and advanced stage cancers were more often affected by AI (G1+G2 vs. G3; 20% vs. 66%; stage I+II vs. III+IV, 36% vs. 54%, p<0.001; Kruskal-Wallis test) than well differentiated and early stage tumors. There was no relationship between histological subtype and AI. Smallest regions of overlap (SRO) were delineated by analyzing 38 tumors with partial AI. One of these regions is characterized by loss of microsatellite markers D8S1992 and D8S261 and is mainly found in early tumors. This area has also been implicated in tumorigenesis of various other cancers, in particular prostate cancer, where a homozygous loss in this region was described previously. By focusing on this area through datamining and reprobing our previous patient population with additional microsatellite markers we integrated this minimal region of loss into the Genemap99 and the G3 Stanford Radiation Hybrid Map, thus narrowing it down to roughly 900 kilobases. We are currently using probes from this region to isolate bacterial artificial chromosomes (BACs) to achieve genomic coverage of the minimal deletion. These BACs will also be used for in situ hybridization experiments to search for potential homozygous deletions in ovarian carcinomas in this area. Coding sequences are isolated by exon amplification and - together with Expressed Sequence Tags (ESTs) and genes deposited in the public databases - evaluated as potential candidate genes by mutational analysis in cell lines, tumor samples and germ line DNA of ovarian cancer patients. This work is supported by the Austrian Fonds zur F rderung der wissenschaftlichen Forschung Project Nr. P14138-Gen

P0125. Coincident LOH Regions In Mouse Thymic Lymphomas And In Human Lymphomas

B. Melendez[1], *B. Mart nez-Delgado*[1], *M. Herranz*[2], *J. C. Cigudosa*[1], *C. Rivas*[3], *J. Fern ndez-Piqueras*[2], *J. Ben tez*[1]
[1]*Centro Nacional de Investigaciones Oncol gicas; Madrid, Spain;* [2]*Universidad Autonoma de Madrid; Madrid, Spain;* [3]*Fundaci n Jim nez D az; Madrid, Spain*
bmelendez@cnio.es

Using an animal model for the study of T-cell lymphomas, frequent loss of heterozygosity (LOH) was revealed in seven chromosomal regions located on mouse chromosomes 4, 12 and 19. These regions, named TLSR1-7 for Thymic Lymphoma Suppressor Region 1 to 7, present their corresponding orthologous regions on human chromosomes 1 (TLSR2, 3), 9 (TLSR1, 4, 5), 10 (TLSR6) and 14 (TLSR7). In order to know if the mouse LOH regions were maintained in human lymphomas, we analysed a set of 44 non-Hodgkin lymphomas of the B and T-cell lineage for loss of heterozygosity on the corresponding orthologous regions, by using molecular microsatellite markers mapped on each of these regions. All molecular markers analysed showed LOH in a range between 2 and 11% of the cases, thus confirming all the LOH regions found in mouse thymic lymphomas. We also found instability at least in one marker of each region. Three of the regions, 1p32-p36, 9p21-p22 and 10q23-q24, contain candidate genes p73, p16INK4a and PTEN respectively, which are known tumour suppressors. Mutation analyses of p16INK4a and PTEN genes revealed one tumour with a mutation on p16INK4a, whereas no cases with mutations on PTEN were found. This could indicate the existence of other/s suppressor/s genes near PTEN. In addition, the other LOH regions could contain unknown novel tumour suppressor genes.

P0126. LOH-Analysis of chromosome 7, 11 and 16 in Wilms tumors, and comparison to histology

S. K. Sonner[1], *V. Schumacher*[1], *A. Weirich*[2], *B. Royer-Pokora*[1]
[1]*Inst. fuer Humangenetik, Heinrich-Heine-Universitaet; Duesseldorf, Germany;* [2]*Kinderklinik der Universitaet Heidelberg; Heidelberg, Germany*
Sandra.Sonner@gmx.de

Several regions of LOH in Wilms tumor are known on chromosome 7, 11 and 16 but until recently no correlation analysis was done between loss at these loci and tumor histology to characterize the genetic defects leading to a specific histopathological subtype. For this reason we investigated 19 cases of Wilms tumor (4 blastemal, 9 stromal, 3 triphasic, one epithelial and 2 tumors of unspecified histology) with a series of markers, and compared the results to the histology of the studied tumors. We found LOH of chromosome 11 and 16 only in stromal tumors with a rate of 30% and 18%, respectively. LOH of chromosome 7 was found in one blastemal and one stromal tumor. No LOH was found in triphasic tumors, possibly because of the variety of cell types found in that kind of tumor. There seems to be a correlation between LOH of chromosome 11 and 16 and stromal tumors. The next step will be the microdissection of the different cell types to look for LOH in each of them, to compare the results with the histology of the different cell types.

P0127. Identification of genetic alterations on chromosome 4 in the early phase of HPV-induced tumorprogression

C. Backsch[1], *N. Wagenbach*[2], *B. Rudolph*[1], *B. Meyer*[1], *K. Beer*[1], *E. Stanbridge*[3], *A. Schneider*[1], *M. D rst*[1]
[1]*Gyn kologische Molekularbiologie, Abteilung Frauenheilkunde, Frauenklinik der FSU; Jena, Germany;* [2]*Angewandte Tumorvirologie, Deutsches Krebsforschungszentrum; Heidelberg, Germany;* [3]*Department of Microbiology and Molecular Genetics, College of Medicine; Irvine, United States*
Claudia.Backsch@med.uni-jena.de

Human papillomavirus (HPV) types 16 or 18 are involved in the multistep-process of cervical carcinogenesis. Transfection of human primary keratinocytes (HPK) with HPV 16 or 18 DNA generally gives rise immortal cells. This may be explained by the loss of senescence genes as a consequence of HPV-induced genetic instability. Complementation of these gene defects by the reintroduction of specific chromosomes results in the reversion of the immortal phenotype. Comparative genomic hybridization (CGH) has shown that chromosome 4 is consistently underrepresented early during the immortalising process. The aim of our study is to determine a region on chromosome 4 which is associated with senescence in HPV-induced tumorigenesis. Microcell mediated- chromosome transfers (MMCT) with an entire chromosome 4 and different derivated chromosome 4 (der (4)) were performed. As recipients served two HPV positive cell lines (HeLa, HPK II). Successful microcell fusion was confirmed by microsatellite-PCR and fluorescence in situ hybridization using coatasome 4 probe. The introduction of a normal copy of chromosome 4 induced senescence in 80-100% of the resulting HPK II- and HeLa- hybrids. In order to localise potential senescence genes on chromosome 4 we have established 10 new donor cell lines with different derivated chromosome 4. We have now started to examine these new donor cells with regard to their ability to induce senescence in HPK II, HeLa recipient cells.

P0128. 1p/19q-deleted Oligodendroglioma Is a Distinct Histological Tumour

C. Godfraind[1], *E. Rousseau*[2], *C. Raftopoulos*[1], *M. Ruchoux*[3], *F. Scaravilli*[4], *M. Vikkula*[2]
[1]*Universit catholique de Louvain; Brussels, Belgium;* [2]*Christian de Duve Institute of Cellular Pathology; Brussels, Belgium;* [3]*H pital R. Salingro; Lille, France;* [4]*Institute of Neurology; London, United Kingdom*
catherine.godfraind@anpg.ucl.ac.be

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

P0129. Allelic imbalance in chromosome 22q13 in Eastern finnish breast cancer patients

H. Kujala[1], *M. Anttila*[2], *J. Hartikainen*[1], *V. Kataja*[3], *M. Eskelinen*[4], *V. Kosma*[5], *A. Mannermaa*[1]
[1]*Kuopio University Hospital, Department of Clinical Genetics; Kuopio, Finland;* [2]*University of Kuopio, Department of Obstetrics and Gynecology; Kuopio, Finland;* [3]*University of Kuopio, Department of Oncology; Kuopio, Finland;* [4]*University of Kuopio, Department of Surgery; Kuopio, Finland;* [5]*University of Kuopio, Department of Pathology; Kuopio, Finland*
Hanna.Kujala@kuh.fi

Chromosome 22q has been suggested as a possible location for a tumour suppressor gene in several studies. Loss of heterozygosity (LOH) or allelic imbalance (AI) has been observed throughout chromosome 22q in different malignant tumour tissues, e.g. breast, ovary and colon. We studied chromosome 22 to find further evidence for a breast cancer susceptibility/risk gene and screened it for AI using 13 polymorphic microsatellite markers spanning 6.7 Mb along the q-arm with average distance from each other of 0.5 Mb.

We used 45 breast cancer patients who did not have evident family history of cancer from the late-settlement area of Eastern Finland. Pairs of paraffin-embedded carefully microdissected tumour and matched normal specimens were extracted by standard proteinase-K — phenol —cloroform method, PCR amplified with fluorescent-labelled microsatellite marker primers and analyzed with Abi Prism 310 genetic analyzer. The AI-value was calculated useing formula AI=(T2 x N1)/(T1 x N2), where T is a tumour allele and N is a normal tissue allele. Values under 0.6 or greater than 1.67 were assessed AI.

AI was found in 37 of 45 tumours (82 %) and five microsatellite markers showed AI over 30 %, with one marker reaching 55 %. These results confirm the occurrence of a breast cancer tumour suppressor gene in a 1.1 Mb wide region in chromosome 22q13. The highest AI frequency was however found somewhat more centromeric than previously reported.

P0130. Genetic Alterations In Cervical Carcinomas; Loss of Heterozygosity and Microsatellite Instability

D. Popovic-Kuzmanovic[1], *I. Novakovic*[2], *D. Lukovic*[2], *K. Kacar*[2], *J. Milasin*[3], *L. Lukovic*[2], *N. Ostojic*[4], *M. Krajinovic*[2]
[1] *Bezanijska Kosa , University Medical Center; Belgrade, Yugoslavia;* [2]*Institute of Biology and Human Genetics, School of Medicine; Belgrade, Yugoslavia;* [3]*Institute of Biology and Human Genetics, School of Stomatology; Belgrade, Yugoslavia;* [4]*Institute for Obstetrics and Gynecology; Belgrade, Yugoslavia*
bkosa@bkosa.edu.yu

It is well known that cervical cancer is multigenetic disease. As the genetic alterations are the cause of malignant transformation, it is likely that specific genetic alterations lead to specific clinical behavior. The aim of this study was to localize chromosome arms that harbor likely tumor - suppressor genes, by analyzing loss of heterozygosity (LOH). Also we analyzed presence of microsatellite instability (MI) as a result of replication errors in malignant cells. To define the regions of interest we studied the presence of LOH and MI at chromosomes 3p, 6p, 11p/q and 18q in a series of 20 cervical carcinomas. All the tumors were squamous cell carcinomas with various degrees of differentiation. LOH was observed at regions 6p14-24, 18q21.1-qter, 3p13-26, 11p12-15.2, and 11q13-23 with the frequency of 50%, 30%, 25%, 25%, and 20%, respectively. MI was detected in only one case on chromosome 3p (3p26) and in 3 cases on 6p (6p21.3 and 6p23). Majority of the tumors with LOH and MI were histologic grade II, but had heterogeneous clinical stages. Losses of analyzed chromosomal regions in cervical carcinomas suggest that a tumor suppressor genes in these regions may be important in the prognosis of the tumor. Low frequency of MI in our study suggests that this type of genetic alteration be not very often in cervical carcinomas.

P0131. Genomic analysis of 18q suggests an existence of a novel tumor suppressor gene that is associated with pancreatic ductal carcinogenesis.

A. Horii, *H. Inoue, T. Furukawa, M. Sunamura, T. Yatsuoka, T. Yokoyama, K. Takeda, S. Fukushige, S. Matsuno*
Tohoku University School of Medicine; Sendai, Japan
horii@mail.cc.tohoku.ac.jp

Human pancreatic cancer is one of the malignant diseases with the poorest prognosis, mainly due to difficulty of diagnosis at the early stage for curative operation and lacking of curative therapies other than operations. To invent more effective methods for better diagnostic and treatment methods for pancreatic cancer patients, it is necessary to understand its molecular mechanisms. We previously identified that LOH of 18q at the SMAD4 locus, along with LOHs on 17p and 12q, positively associated with poor prognosis of pancreatic cancer patients. We also found that loss of 18q is an early genetic alteration in human pancreatic ductal carcinogenesis. However, introduction of the SMAD4 gene did not suppress the growth of pancreatic cancer cells that harbor homozygous deletion of this gene. IPMT (intraductal papillary-mucinous tumor) is thought to be one of the premalignant lesions of the pancreas, which would transform into carcinomas. A total of 20 IPMTs were analyzed, and we observed frequent LOH (7/14, 50%) at the SMAD4 locus. However, SMAD4 protein was observed in tumor cells immunohistochemically, and no mutations of the SMAD4 gene were observed in any of the tumors. Introduction of the normal copy of chromosome 18 into pancreatic cancer cells with and without defective SMAD4 gene is now under way, and our preliminary results suggested the existence of a gene(s), other than SMAD4, that suppresses cell growth. Identification and characterization of the gene(s) that is a candidate for tumor suppressor will also be discussed.

P0132. Loss of heterozygosity of Adenomatous polyposis coli and E-cadherin genes in renal cell carcinoma

N. Pecina-Slaus[1], *K. Gall-Troselj*[2], *K. Radic*[3], *J. Pavelic*[2], *K. Pavelic*[2]
[1]*Department of Biology, Medical School University of Zagreb; Zagreb, Croatia;* [2]*Division of Molecular Medicine, Rudjer Boskovic Institute; Zagreb, Croatia;* [3]*Medical School University of Zagreb; Zagreb, Croatia*
nina@mef.hr

In our study the roles of two tumor suppressor genes (adenomatous polyposis coli, APC and E-cadherin, CDH1) were investigated in renal cell carcinoma (RCC). Both gene products are components of adherens junction, where E-cadherin is bound to beta-catenin which in turn binds to the central part of the APC protein. Twenty eight human renal cell carcinomas together with autologous peripheral blood samples were tested for gene instability. Using specific oligonucleotide primers for exons 11 and 15 of the APC gene, instability was followed by polymerase chain reaction/loss of heterozygosity (LOH) using RFLP method. E-cadherin gene was analyzed by PCR amplification of tetranucleotide repeat polymorphic marker (D16S752) and the alleles were visualized by PAGE/silver staining. Our results demonstrate that from 28 tumor samples 15 (53,6 %) were informative for Rsa I/exon 11 polymorphic site. From the noninformative sample, 2 were heterozygous for MspI/exon 15 polymorphism. The overall proportion of LOH cases of the APC gene was 47,1 % (8/17). The polymorphic marker for E-cadherin gene was highly informative 26/28 (92,9 %), while only 2 of these (7,7 %) demonstrated LOH. Interestingly, in another three RCC samples we detected another type of genomic instability; replication error (RER). Both samples showing LOH of the E-cadherin had also LOH of the APC gene, while only one RER+ sample of the E-cadherin gene showed LOH at the APC locus. Pathohistological diagnosis showed no correlation with molecular data. However, multivariate analysis indicated strong positive correlation of age and TNM stage with the presence of LOH of the APC gene. Our results suggest that alterations both in APC and E-cadherin genes are responsible for evolution and progression of RCC. Genetic changes of the E-cadherin gene are less frequent and are associated with the APC gene alterations.

P0133. Genetic Markers for Prostate Cancer Progression

L. Chu, *P. Troncoso, J. Liang*
M.D.Anderson Cancer Center; Houston, TX United States
janliang@yahoo.com

Prostate cancer (PC) is the second leading cause of cancer death among men in the United States. Because PC is currently detected at an early stage and only a portion of them will progress to become life threatening, there is a great need for understanding the genetic basis for PC progression. Such an understanding will aid in the development of tests that can be used to predict the clinical behavior of each tumor for which optimal treatment strategies can be designed. For the purpose of identifying genetic markers that distinguish low from high-stage PC, we analyzed 24 organ-confined (T2NoMo) and 21 locally advanced (T3NoMo) tumors by the comparative genomic hybridization (CGH) method. Smears from fine needle biopsies were used for study. Our results identified six chromosomal regions that were significantly different in organ-confined and locally advanced tumors. Locally advanced tumors exhibited a significant gain of 3p13-q13, and showed significant losses of 6p21, 6q24-27, 8cen-p23, and 10q25-26. In addition, low stage tumors showed a significant loss of 18q11-12. Our study confirmed the loss of 8p and 10q as the two most frequent regions of loss in PC, as seen in published LOH studies, and identified several regions of gain or loss not previously reported. Further investigation of these regions may reveal genes that are important for the progression of PC.

P0134. Identification of Novel Tumor-Associated Genes in 11q12-q23

***R. A. Kuner**, E. Dahl, C. Pilarsky, T. Specht, A. O. Schmitt, B. Hinzmann, B. Weber, I. Klaman, E. Staub, E. Klopocki, A. Bu§, B. Petschke, A. Rosenthal*
metaGen; Berlin, Germany
ruprecht.kuner@metagen.de

Tumor development is characterized by chromosomal disorders which may lead to activation of oncogenes or loss of tumor suppressor genes. In gynecological tumors, genetic information within chromosomal region 11q12-q23 is often amplified or lost. Assuming that chromosomal rearrangements in tumors cause changes in gene expression, four million ESTs available in public and proprietary EST databases were subjected to in silico expression analysis using assembled cDNA sequences (Schmitt et al., 1999). In this first approach 600 candidate genes were identified which are associated with sporadic forms of gynecological tumors (Dahl et al., in preparation). Chromosomal assignment of these 600 genes localized 15 genes within 11q12-q23. To achieve an even higher coverage of tumor-associated genes for this critical chromosomal region we analyzed 968 STS-markers between RH13699 (11q12) and RH27416 (11q23) for expression analysis as aforementioned. Using this strategy we identified a total of 69 genes which are significantly differentially expressed in gynecological tumors. 47 of these genes have been described previously, whereas 22 are novel. Verifying these in silico data by Northern blot technique, RNA in situ hybridization and Taqman analysis, we identified one tumor suppressor gene candidate for breast cancer in 11q14.1 .

P0135. Identification of A Cell Senescence Gene at 6q16.3 for Ovarian Cancer

***R. Athwal**, S. Gauthami, V. K. Zhawar, N. Rane, A. K. Sandhu, G. P. Kaur*
Fels Institute for Cancer Research; Philadelphia, PA United States
rathwal@unix.temple.edu

Applying a Functional Positional approach, we have identified a genomic clone that carries a cell senescence gene, SEN6A, for ovarian tumor cells. SEN6A locus was previously identified at 6q14-21. The introduction of chromosome 6 or 6q into human (SKOV.3) and rat (ROSE199 and ROV12) ovarian tumor cell lines led to the restoration of normal cell growth phenotypes and senescence. Analysis of spontaneous revertant clones, which occurred due to deletions in the donor chromosome, allowed us to map the location of SEN6A locus within a 1cM genetic interval at 6q16.3. Precise localization of the deletions allowed us to identify three overlapping Yeast Artificial Chromosome (YAC) clones, corresponding to the smallest deletion, shared among independent revertant clones. Functional testing of candidate YAC clones identified a YAC that restored normal cell growth and senescence in ovarian tumor cells. Following the library screen, we assembled a contig of 17 BAC clones corresponding to the complementing YAC. Candidate BAC clones were tested for function by introduction into ovarian tumor cells. In these experiments two BAC clones were identified to carry SEN6A gene. To further define the smallest genomic region, carrying SEN6A, three overlapping BACs were subcloned to generate cosmid clones, each carrying a 35-50 Kb segment. Cosmid clones have been assembled in a contig across SEN6A locus. Experiments are currently underway to test cosmid clones for senescence activity in tumor cell lines. These studies not only identify a genomic clone carrying the senescence gene but also demonstrate the utility of the functional approach to identify cell senescence and/ or tumor suppressor genes.

P0136. A PAC Contig Covering the Chromosome 16q22.1 Breast Cancer Loss of Heterozygosity Region

***E. Frengen**[1], P. Rocca-Serra[2], S. Shaposhnikov[1], L. Taine[3], J. Thorsen[1], A. A. El Mon im[3], M. Longy[2], H. Prydz[1], F. Dorion-Bonnet[2]*
[1]The Biotechnology Centre of Oslo; Oslo, Norway; [2]Institut Bergoni ; Bordeaux, France; [3]Centre Hospitalier Universitaire Pellegrin; Bordeaux, France
efrengen@biotek.uio.no

Loss of heterozygosity (LOH) on human chromosome 16q is frequently observed both in ductal and lobular invasive breast carcinomas. We have generated a 2.8 Mb PAC contig covering the smallest region of LOH overlap on 16q22.1 (SRO2). Two-color FISH established the contig orientation, and long-range mapping verified that the contig faithfully represents the SRO2 region. 68 transcripts have been identified in the map based on EST screening and CpG island subcloning. One of the genes residing within SRO2 is the E-cadherin gene, CDH1. This gene is known to be mutated in lobular breast carcinomas, resulting in altered E-cadherin expression. However, E-cadherin shows normal expression in most cases of ductal carcinoma, the major mammary cancer type. Thus, other genes within 16q22.1 are expected to be involved in the development of this tumor subtype. A minimal-tiling path of the contig presented consists of PAC clones, which have the potential of being transferred to mammalian cells as stably replicating episomes. This feature might facilitate the use of a functional strategy by introducing PACs into tumor cells for the identification, verification and characterization of the tumor suppressor gene expected to be present within SRO2. [The present work was supported by The Norwegian Cancer Society and The Research Council of Norway (EF and HP), the Association pour la Recherche contre le Cancer and the European Molecular Biology Organization (Grant STF#8970)].

P0137. Characterization of a novel trp-gene from the BWS-WT2 critical region on chromosome 11p15.5

***L. R. Brixel**[1], T. Enklaar[1], M. Thesker[1], C. Spangenberg[1], S. Fees[1], M. Higgins[2], B. U. Zabel[1], D. Prawitt[1]*
[1]Universitaetskinderklinik; Mainz, Germany; [2]Roswell Park Cancer Center; Buffalo, NY United States
brixel@kinder.klinik.uni-mainz.de

Positional cloning strategies combined with comparative genome analysis of human chromosomal region 11p15.5 led to the isolation of a new gene of the transient receptor potential protein (TRP) gene family. Members of this family encode calcium channels, that can be devided into three subgroups according to their structural features. Comparison of the new transcript with the known trp genes displayed highest homologies to human TRPC7 and MLSN1, thus classifying it as a member of the l-TRP subgroup. The novel gene was therefore named MTR1 (= MLSN1 and TRP related gene). While activation of o-TRPs and s-TRPs results in currents due to ion influx into cells after agonist stimulation (e.g. via IGF1), little is known about the function of l-TRPs. However, the expression of MLSN1 correlates with the metastatic process in melanomas, and initial studies in C.elegans (ced-11) point to an involvement of l-TRPs in cell growth, death and differentiation. As MTR1 maps to a genomic region that is linked with Beckwith-Wiedemann syndrome (BWS) and a variety of mostly embryonal neoplasias (e.g. Wilms tumors, rhabdoid tumors) we currently study the role of the gene in tumorigenesis. We found that increased amounts of MTR1 mRNA are present in a significant proportion of Wilms tumors. We are currently looking for mutations in BWS- and tumor-DNA. Further functional studies include the search for interacting proteins with a yeast two-hybrid assay to elucidate the role of MTR1 in cellular physiology and pathogenetic processes.

P0138. Fine mapping and molecular characterization of the breakpoint cluster in thyroid adenomas with 19q13.4 aberrations

***M. Meiboom**, V. Rippe, G. Belge, N. Drieschner, J. Bullerdiek*
Center for Human Genetics; Bremen, Germany
meiboom@uni-bremen.de

Chromosomal aberrations involving 19q13.4 are the most frequent structural alterations in follicular thyroid adenomas and hyperplasias and constitute one of the most frequent specific chromosomal abnormalities in epithelial tumors at all. Recently, we had been able to establish the cDNA sequence and genomic structure of a KRAB zinc finger gene lying in close vicinity to the breakpoint region. This gene we have tentatively designated as RITA (Rearranged in Thyroid Adenomas). The location, type and the different expression pattern of RITA in normal tissues (including thyroid) and in thyroid cell lines with 19q13 aberrations make it a candidate target gene for specific 19q13 alteration. While we had been able to identify part of the genomic structure of part of the gene probably including its complete open reading frame the 5 part of the gene and its promoter still remain unknown. To further complete the genomic structure of RITA we have performed PCR experiments and sequencing. RACE experiments indicate that there might be at least two different transcription start sites. The results also revealed the existence of two nearly identical untranslated exons which apparently are evolved by exon duplication. We have also improved a 470 kbp high-resolution metric EcoRI restriction cosmid/BAC contig around and within RITA and the breakpoint cluster region by sequencing and STS mapping which enabled us to restrict the breakpoint region to a region of about 150 kbp in four primary tumors and to an interval of about 25 kbp in two previously described cell lines, respectively.

P0139. Amplification and overexpression of PI3KC2B, a candidate oncogene in 1q32, in a case with low-grade glioma.

***M. H. Abdel-Rahman**, Z. Dai, T. Prior, A. J. Yates, C. Plass, R. Krahe*
The Ohio State University; Columbus, OH United States
abdel-rahman.4@osu.edu

DNA amplification in 1q32 has been reported in a wide variety of human tumors, including high-grade gliomas. Two candidate oncogenes have been suggested; GAC1 and MDM4. Using comparative genomic hybridiza-

tion, we identified a DNA amplification in the 1q32.1 region as the sole cytogenetic abnormality in a 5-year old male with low-grade glioma. By restriction landmark genomic scanning (RLGS) with AscI-EcoRV-HinfI as the restriction enzyme combination, we identified a highly enhanced RLGS fragment. Sequence and BLAST analyses identified the clone as PI3KC2B. Amplification of PI3KC2B was confirmed by Southern blot analysis; three-fold mRNA overexpression relative to a normal control pool was determined by real-time PCR. In addition, amplification and overexpression of MDM4 and GAC1, the closest telomeric flanking genes, was detected. None of an additional 21 low-grade gliomas (seven pilocytic astrocytomas, seven astrocytomas and seven oligodendrogliomas) showed a gain in 1q32, amplification or overexpression of PI3KCB, MDM4 and/or GAC1. Expression profiling of the low-grade glioma against cerebrum of a normal control male with a cDNA microarray of 4,000 known genes identified several genes VIM, ADD3 and ACVR2 with more than 3-fold increases in expression. However, more genes showed greater than 3-fold decreases in expression, including BAF60B, TEB4, CYSC, MBP, GAPDH and synaptopodin. PI3KC2B acts downstream of EGFR, but until now has not been implicated as an oncogene. EGFR-PTEN-AKT pathway is the most commonly affected pathway in gliomas. Based on our data we suggest PI3KC2B as an additional target in this pathway and a candidate oncogene in 1q32.1 amplification.

P0140. Deletion of part of the HMGIC locus in a lipoma with a translocation t(3;12)(q27-28;q14-15) leads to a new fusion gene

I. Lemke, *P. Rogalla, J. Bullerdiek*
Center for Human Genetics; Bremen, Germany
ilemke@uni-bremen.de

The high mobility group protein gene HMGIC has been found to be rearranged in a variety of human benign solid tumors. Often, these rearrangements lead to fusion genes of which that between HMGIC and LPP underlying the translocation t(3;12)(q27-28;q14-15) is by far the most common. The expression of the HMGIC-LPP transcript in all tumors with the t(3;12)(q27-28;q14-15) and the absence of the reciprocal LPP-HMGIC fusion transcript has lead to the conclusion that only the former fusion is of functional significance in terms of tumor development. In this study we have analysed in more detail a lipoma with a t(3;12)(q27-28;q14-15). RT-PCR confirmed the expression of the HMGIC-LPP fusion transcript. FISH experiments using different probes derived from the HMGIC gene and its 3« vicinity showed the absence of FISH signals on the derivative chromosome 3 indicating that the t(3;12)(q27-28;q14-15) was accompanied by a large genomic deletion of about 170 kb. 3«-RACE-PCR experiments revealed the presence of a new fusion gene composed of exons 1-6 of LPP and the last two exons of an as yet unknown gene located in 12q15. To the best of our knowledge, this is the first study showing that the simple translocation t(3;12)(q27-28;q14-15) results in a large deletion of DNA which may explain the lack of LPP-HMGIC fusion transcripts in most lipomas with a t(3;12) analysed so far. In addition, the detection of a new fusion transcript beside the HMGIC-LPP fusion transcript indicate that fusions involving the part of LPP involving exons 1-6 may play an as yet underestimated role in tumors with the t(3;12)(q27-28;q14-15).

P0141. Discovery Of The Bcl11a Gene Family By Its Involvement In The Pathogenesis Of Malignant Lymphoma

R. Siebert[1], *T. Sonoki*[2], *E. Satterwhite*[3], *T. G. Willis*[2], *R. Nowak*[2], *E. L. Arriola*[2], *H. Liu*[3], *L. Harder*[1], *S. Gesk*[1], *D. Steinemann*[1], *H. P. Price*[2], *D. G. Oscier*[4], *B. Schlegelberger*[1], *P. W. Tucker*[3], *M. J. S. Dyer*[2]
[1]*Institute of Human Genetics, University of Kiel; Kiel, Germany;* [2]*Institute of Cancer Research; Sutton, Surrey, United Kingdom;* [3]*Molecular Genetics and Microbiology, University of Texas; Austin, TX United States;* [4]*Royal Bournemouth Hospital; Bournemouth, Hampshire, United Kingdom*
rsiebert@medgen.uni-kiel.de

Mature B-cell malignancies are characterized by chromosomal translocations involving the immunoglobulin heavy chain locus on chromosome 14q32.3, resulting in deregulated expression of different oncogenes. Chromosomal translocation t(2;14)(p13;q32.3) is a rare but recurrent event in B-cell malignancies, whereas gains and amplifications of chromosome 2p13 are seen not only in mediastinal and about 20% of extranodal B-cell non-Hodgkin s lymphomas (B-NHL) but also in up to 50% of Hodgkin s disease (HD). We have identified a zinc finger gene (BCL11A) through molecular analysis of 4 cases of clinically aggressive CLL with t(2;14)(p13;q32.3) using long-distance inverse PCR methods. This gene is also commonly targeted in B-NHL and HD with 2p13 amplifications. BCL11A was identical within its zinc finger motifs to another gene (BCL11B) on chromosome 14q32.1 and was highly conserved, being 95% identical to mouse, chicken and Xenopus homologues. BCL11A is the human homologue of mouse evi-9, which is deregulated in murine myeloid leukemias following proviral integration. Like evi-9, there were three major isoforms of BCL11A, which differed in the number of carboxy-terminal zinc fingers. Two BCL11A isoforms interacted with BCL6, another highly conserved zinc finger gene implicated in the formation of normal germinal centers and the pathogenesis of B-NHL. All three isoforms of BCL11A, like BCL6, repressed transcription. Deregulated expression of BCL11A is thus commonly involved in various lymphoid malignancies through either chromosomal translocation or amplification and may transform B-cells through transcriptional repression of the same set of genes involved in the control of B-cell differentiation and proliferation as BCL6.

P0142. A Gene at a familial RCC-associated t(2;3)(q33;q21) chromosome translocation

T. Druck[1], *J. Podolski*[2], *T. Byrski*[3], *S. Zajaczek*[3], *J. Lubinski*[3], *K. Huebner*[1]
[1]*Thomas Jefferson University; Philadelphia, PA United States;* [2]*University of Szczecin; Szczecin, Poland;* [3]*Pomeranian Academy of Medicine; Szczecin, Poland*
huebner@lac.jci.tju.edu

We have studied a balanced constitutional chromosome translocation, t(2;3)(q33;q21), observed in lymphocytes of a Polish family in which multifocal clear cell renal carcinoma segregates with the translocation. YAC and BAC contigs were constructed for the 2q and 3q breakpoint regions and all known STSs and ESTs in these regions were mapped relative to the contigs. The two BACs crossing the breakpoints were partially sequenced and a consensus sequence assembled using our sequence data, as well as Human Genome Project submissions. The chromosome 2 assembled sequence contained an EST, which was extended by 5 and 3 RACE. The resulting ~1.5 kb cDNA contains two exons that are separated by the translocation. By RTPCR, low level expression was detected in ovary, fetal kidney, fetal thymus and fetal skeletal muscle. An ~11 kDa protein, as predicted by the amino acid sequence, was detected by in vitro transcription and translation. Bacterial and mammalian expression vectors have been constructed and transfections of mammalian cells will be described.

P0143. Analysis of the human chromosome 13 region q14.3 often deleted in human malignancies; unusual distribution of the repeats and new expressed sequences.

T. V. Tyazelova[1], *A. V. Baranova*[1], *D. V. Ivanov*[1], *B. I. Kapanadze*[2], *T. A. Borodina*[1], *M. Y. Skoblov*[1], *A. A. Pestova*[1], *S. Einhorn*[2], *D. Grander*[2], *O. Sangfeldt*[2], *N. K. Yankovsky*[1]
[1]*Vavilov Institute of General Genetics; Moscow, Russian Federation;* [2]*Karolinska Institutet and Hospital; Stockholm, Sweden*
baranova@vigg.ru

Human chromosome 13q14.3 region is often rearranged in a spectrum of human malignancies particulary B-cell chronic lymphocytic leukemias (BCLL), non-Hodgkin s lymphomas, multiple myelomas, prostatic carcinomas. Detailed delineation of the minimally deleted regions (MDRs) in BCLL patients were conducted by many research groups including our own. No clear overlaps between MDRs found by different researchers were found, although all deleteion are clustreed between D13S1168 and D13S25 STS-markers. Two candidate genes DLEU1 and DLEU2 were cloned from the 10-kb MDR found by us, but no point mutations in them were detected in more than 150 BCLL patients. We have build a 620-kb cosmid contig that overlaps D13S1168-D13S25 region, map 8 STS-markers on this contig and sequence 17-kb area encompassing 10-kb MDR. We have searched GenBank with STS-markers and revealed several BAC sequences that are partially cover the region of interest. Sequence analysis reveal that Alu-repeats and putative gene fragments are distributed unequally; centromeric flank is rich in Alu (24,8%) and in putative candidate genes (WI-9598, RFP2, DLEU1, DLEU2 and others), telomeric flank centered around STS-marker GCT16C05 is extremely Alu-poor (only 4,1%) and contains only two expressed sequences. One of them consists of three exons divided by introns 19366 and 96661 nt in legth. This exons can represent a putative extremely large gene rearranged or interstitially deleted in BCLL. Another interesting feature of the region is unusual abundance of the Alu subfamily Y - the youngest Alu, that is still capable to propagation.

P0144. A transcript map of the human chromosome 12p12.3 tumor suppressor locus

D. Sinnett, *A. Montpetit, N. Trudel*
H pital Sainte-Justine; Montreal, PQ Canada
sinnettd@ere.umontreal.ca

Allellic loss of the chromosome 12p is a frequent event in childhood acute lymphoblastic leukemia. This region was also found deleted in several other hematological malignancies as well as a variety of solid tumors suggesting the presence of a tumor suppressor gene. The chromosomal

region containing this suppressor locus was narrowed down to a ~750kb interval delimited by D12S89 and D12S358. Since no known candidate gene was found, we initiated the construction of a detailed transcription map focusing on a contig of 4 overlapping BACs. Towards this goal we applied a strategy integrating several complementary approaches; (1) Computer-based data mining tools applied to the existing genomic sequence (750 kb) derived form the BAC contig; (2) Exon amplification and EST resources to identify putative cDNAs; (3) Determination of general expression pattern by RT-PCR and Northern blotting; (4) Comparative genomic analysis with distant vertebrate species such as Fugu rubripes and Tetraodon nigroviridis. This transcript mapping strategy has identified 42 potential transcription units, including 2 known genes, 5 new genes and 35 Unigene entries. The region contains also 6 pseudogenes. This map should facilitate subsequent effort to characterize the candidate genes. This study is a good illustration of how the integration of genome-based approaches facilitates the identification of genes in large interval.

P0145. Cloning of A Cell Senescence Gene at 16q24.3 That Reverses the Immortal Tumor Phenotype

G. P. Kaur, *R. Athwal*
Fels Institute for Cancer Research; Philadelphia, PA United States
pkaur001@unix.temple.edu

Escape from senescence has been postulated to be a prerequisite for progressive tumor growth. Cells cultured from many tumors can proliferate indefinitely, while normal diploid cells become senescent after defined number of generations. Cellular senescence is expressed as a dominant phenotype over immortal cell growth. We have applied a functional-positional approach to map and isolate human genes that induce senescence in immortal tumor cell lines. A cell senescence gene, SEN16, identified on chromosome 16 by microcell mediated chromosome transfer into immortal mammary tumor cells, has been mapped within a genetic interval of 3-7cM at 16q24.3 (Reddy et al.1999, Oncogene 18;5100-5107). Six yeast artificial clones corresponding to candidate locus were identified and tested for functional complementation of immortal phenotype by introducing into mammary tumor cells. These studies led to the identification of a 360 kb YAC (Reddy et al. 2000, Oncogene 19;217-222) In order to construct a high-resolution map of the candidate region carrying the senescence gene, a human BAC library was screened with DNA markers to obtain relevant BAC clones, which were assembled in a contig based upon PCR analysis. Some selected BAC clones were retrofitted with a selectable marker and introduced into human and rat mammary tumor cells for functional complementation. These results identified a 85 kb BAC clone that carries the senescence gene. In following experiments, we isolated expressed sequences from the complementing BAC clone by exon trapping. Twelve exons were identified and their sequence comparison with databases has identified eight partial cDNA clones. Experiments are in progress to determine the expression of these clones in tumor cell lines. In conclusion, we have mapped a senescence gene to a 85kb genomic clone and identified expressed sequences from this clone.

P0146. Isolation of a novel candidate oncogene within a frequently amplified region at 3q26 in ovarian cancer

X. Guan[1], *J. Sham*[1], *T. Tang*[1], *Y. Fang*[2], *K. Huo*[3], *J. Yang*[4]
[1]*The University of Hong Kong; Hong Kong, Hong Kong Special Administrative Region of China;* [2]*Sun Yet-Sen University of Medical Sciences; Guangzhou, China;* [3]*Fudan University; Shanghai, China;* [4]*The University of Arizona; Tucson, AZ United States*
xyguan@hkucc.hku.hk

Amplification of 3q25-q26 is one of the most frequent chromosomal alterations in human ovarian carcinoma. A chromosome microdissection—hybrid selection method was applied to isolate transcribed sequences from a primary ovarian cancer containing high-copy-number amplification of 3q25-q26 using 3q26 band-specific DNAs generated by chromosome microdissection. Using this method, we have isolated a novel candidate oncogene eIF-5A2 (eukaryotic initiation factor 5A2). EIF-5A2 shares 82% identity of amino acid sequence with eIF-5A including the minimum domain needed for eIF-5A maturation by hypusine modification at lysine-50 residue. Amplification and overexpression of eIF-5A2 was frequently detected in primary ovarian cancers and ovarian cancer cell lines. The proliferation-related function of eIF-5A supports that eIF-5A2 is a candidate oncogene related to the development of ovarian cancer.

P0147. Isolating genes from the interstitial SRO on the long arm of chromosome 6 in gastric carcinoma by means of suppression subtractive hybridisation

B. Carvalho[1], *R. Seruca*[2], *C. H. C. M. Buys*[3], *K. Kok*[3]
[1]*Instituto de Patologia e Imunologia Molecular da Universidade do Porto; Porto, Portugal; Dept. Medical Genetics, University of Groningen; Groningen, The Netherlands;* [2]*Instituto de Patologia e Imunologia Molecular da Universidade do Porto; Porto, Portugal;* [3]*Dept. Medical Genetics, University of Groningen; Groningen, The Netherlands*
beatriz.carvalho@ipatimup.pt

Deletions on chromosome 6 have been reported in different types of cancer. In gastric cancer two smallest regions of overlap (SRO) have been identified — one interstitial (6q16.3-q23.1) and one distal (6q26-q27). Recently we delimited the interstitial SRO to a region of 2 cM. To facilitate a search for genes in this region we assembled a YAC contig that covered the SRO. We applied a new strategy to analyse the gene content of the region, based on the assumption that many of the human genes present on the YACs may be expressed in the yeast strain. Thus, in a cDNA subtraction protocol using two yeast strains containing different YACs it can be expected that the resulting pool of differentially expressed cDNAs will be enriched for the genes encoded by the human inserts of the YACs. By subtracting different YACs from the 2-cM region, we isolated 12 human specific cDNA clones, of which 8 could be confirmed to originate from 6q16.3-q23 based on sequence analysis. The remaining 4 clones specifically hybridised to the YAC from which they originated. Northern analyses have shown so far, that 4 out of the 12 cDNA clones have expression in different tissues, including stomach.

P0148. Use of Genetic Models in Antimutagenicity Assay

M. Afzal, *G. Shadab, M. S. Ahmad, A. Huda, M. E. Ahmad*
Section of Genetics, Department of Zoology, AMU; Aligarh, India
hep_amu@usa.net

Genotoxicity assay of a number of synthetic as well as natural products of human use are increasingly being taken to screen their qualitative as well as quantitative effect on various test systems. This has resulted into searching antimutagenicity and anticarcinogenicity principles to be used as protective measures against harmful effects of the essential chemicals. The list includes antioxidants, scarvengers of free oxygen radicals and MRO species generated in the cellular metabolism. Recent genetic models for antimutagenicity assay include both prokaryotic and eukaryotic systems and cell lines, especially with human tissue culture technique and use of recombinant DNA technology. Taking some steroidal pharmaceutics as mutagens, we have studied synergistic as well as antagonistic action of Vitamins of B complex, ascorbic acid, super oxide dismutase and certain phenolics of plant origin. Metabolic activation system (S9 mix) was used to enhance mutagenicity. Clastogeny, SEC and cell-cycle kinetics profiles suggest amelioration of genotoxic damage in human lymphocytes and in mice bone marrow cells. Findings will be discussed.

P0149. Research About Heritability Of Lung Cancer

D. C. T. D. Tatiana
National Institute V.Babes for Pathology and Biomedical Research; Bucharest, United States
gentica@vbabes.ro

In the framework of a larger research progect concering the Risck Factor s in Lung Cancer we obtained data about family history of the subjects.There has been interviewed 132 patients with lung cancer and 200 without cancer constituting the control group.We obtaind information about 905 relatives of probands and about 930 relatives from the controls group.Using the Falconer s formula we calculated the heritability wich has a value 14%+_ 1.98. This value indicated reduced implication of heredofamilial factor s in lung cancer ethiopathogeny being in concordance with data form the literature.The calculation of the heritability proves to be proper instrument for quantification in genetic factor s in the common desease.

P0150. Polymorphism of glutathione S-transferase M1 gene in woman with mastopathy and breast cancer from Bashkortostan.

N. Eliseeva, *Z. Soultanaeva, O. Grinchuk, T. Victorova, E. Khusnutdinova*
Institute of Biochemistry and Genetics; Ufa, Russian Federation
ekkh@anrb.ru

The mastopathy and breast cancer are disgormonal hyperplastic process in lactic gland. A defining reason of progressing of a mastopathy and breast cancer are neuro-humoral factor stipulated by a stress, depression, neurosis and environmental factors. As a rule, mastopathy and breast cancer suffer the women of age from 25 till 45 years. A number of molecular-genetic studies have been conducted to evaluate associations between GSTM1 polymorphism and diseases. Polymorphisms at the glutathione S-transferase M1 (GSTM1) gene locus have attracted much interest because the homozygous condition on GSTM1-deletion (GSTM1 0/0) seems to modify the risk for different types of cancer, allergy and other diseases. By PCR-method polimorphism of GSTM1 gene in 15 patients with mastopa-

thy and 17 with breast cancer was carried out. As control group 45 healthy woman from Bashkortostan were analysed. It was established, that the frequency of GSTM1(0/0) genotype for mastopathy patients was 0,80 and 0,58 for brest cancer patients, whereas in control group it has constituted 0,49. The differences between womans with mastopathy and control group were significant (X2 =20,99; p<0,001).

P0151. The Effect of Glutathione S-Transferase M1 (GSTM1) and Glutathione S-Transferase T1 (GSTT1) Polymorphisms on Leukemia

N. Ozten[1], *H. G. Karabulut*[2], *A. Sunguroglu*[1], *I. Bokesoy*[2]
[1]*Ankara University School of Medicine, Department of Medical Biology; Ankara, Turkey;* [2]*Ankara University School of Medicine, Department of Medical Genetics; Ankara, Turkey*
karabul@dialup.ankara.edu.tr

GST (glutathione S-transferase) genes (GSTs) are defined as a gene superfamily including four gene families, , ?, p and ? namely. It is thought that carcinogenesis is associated with the absence of functional GSTM1 and GSTT1 which leads to a deficit in detoxification of carcinogens. In this study, the effect of GST polymorphism on leukemia was investigated. Patients diagnosed with Acute Lymphoblastic Leukemia (ALL), Acute Myeloid Leukemia (AML) and Chronic Myeloid Leukemia (CML) between 18 - 70 years of age consisted of 52 males (55.3%) and 42 females (47.2%) were included in the study. In control group healty 42 males (47.2%) and 47 females (52.8%) at the same age were studied. PCR analysis were performed with GSTT1 and GSTM1 specific primers on genomic DNA obtained from peripheral blood samples from both patient and control group after obtaining their informed consent. GSTT1 and GSTM1 genotypes were determined by agarose gel electrophoresis of PCR products. GSTM1 null genotype was seen in 13 cases of 38 AML patients (34.2%), 11 cases of 22 ALL patients (50%), 10 cases of 23 CML patients (42.5%) compared to 37 cases in control group. There was no association for GSTM1 null genotype between AML, ALL, CML and control groups. GSTT1 null genotype was seen in 11 cases of 38 AML patients (28.9%), 7 cases of 22 ALL patients (31.8%), 6 cases of 23 CML patients (26.1%) compared to control group in which no GSTT1 null genotype was observed. A significantly higher incidance of GSTT1 null genotype was seen in both total patient group and ALL, AML, CML subgroups when compared to control. On the other hand, no significant correlation were observed in the incidence of GSTM1 and GSTT1 null genotype with smoking, family history, sex and age parameters in patient group. To our knowledge, this is the first study in which GSTM1 and GSTT1 polymorphisms were investigated in CML patients and we found an association between GSTT1 null genotype and leukemia whereas no association could be obtained for GSTM1 null genotype.

P0152. Heterochromatin variants and their association with leukemia

A. Movaffagh
Department of Human Genetics, Shahid Beheshti Medical University; Tehran, Islamic Republic of Iran
ms_sheikhan@hotmail.com

A study of hromatin regins in chromosome 1, 9, and 16 was performed on peripheral blood and heteroc bone marrow frome 10 normal individuals and 16 patients with chronic myeloid leukemia (CML) and actue nonlymphocytic leukemia (ANLL) was studied. The results showed that there was significant differences in chromosome 1 and 9 between the CML patients $p < 0.01$ and the nomal individuals employed as controls . Analysis of the inversion in chromosomes 1 , 9 and 16 of CML patients and control groups in the present study found significant increases in partial and total inversions in CML patients . the results did not show increases in C — band heterochromatin variants in chromosomes 1,9 and 16 ANLL patients compared to controls. However, frequency of complete inversion was noticed to be significantly higher $p < 0.05$ in ANLL patients compared with controls.

P0153. Detection of hyperdiploidy in childhood acute lymphoblastic leukemia (ALL) by FISH methods

L. Sindelarova[1], *Z. Zemanova*[1], *J. Brezinova*[2], *S. Kurkova*[2], *P. Smisek*[3], *J. Stary*[3], *K. Michalova*[1]
[1]*3rd Medical Department, General Faculty Hospital and 1st Medical Faculty of Charles University; Prague, Czech Republic;* [2]*Institute of Hematology and Blood Transfusion; Prague, Czech Republic;* [3]*2nd Pediatric Department, Faculty Hospital Motol; Prague, Czech Republic*
lsindel@vfn.cz

Early detection of hyperdiploid clones is of great significance in diagnostic and prognostic evaluation of children with ALL. However, conventional cytogenetic analyses are often hampered by a low yield of dividing leukemic cells and/or poor quality of chromosomal preparations. Therefore we use for assessment of hyperdiploidy double-color interphase FISH (I-FISH) with different satellite and/or locus specific probes (VYSIS) for 10 chromosomes most commonly gained in hyperdiploid cells. According to molecular-cytogenetics results we divided patients into several subgroups with different prognosis. During the last 3 years we examined by I-FISH prospectively or retrospectively 69 children (46 boys, 23 girls; mean age 8,4 years) with ALL. Among them we found 29 (42%) with high hyperdiploidy (>50 chromosomes), 11 (16%) with low hyperdiploidy (47-50 chromosomes), 2 (2,9%) with near triploidy/tetraploidy and 4 (5,8%) with unspecified hyperdiploidy. In 23 (33,3%) of patients hyperdiploidy was not ascertained. In all patients we analysed at least 200 interphase nuclei and the extent of hyperdiploid clones was 2,5 — 95%. (Cut-off level 2,5% was tested on controls). Small clones under 10% were detected in 17 (24,6%) of patients. In 4 cases where the presence of chromosomal rearrangements was suspected by G-banding mFISH with probes and isis software from METASYSTEMS was performed. Significance of small side clones with hidden hyperdiploidy detected by I-FISH and presence of chromosomal rearrangements for patient s prognosis will be discussed. This work was supported by grant IGA MZ CR 4744-3 and GACR 302-98-0071

P0154. A Novel Translocation , t(11;17)(p15;q21), in a Putative Case of Acute Promyelocytic Leukemia

A. A. Al Saadi[1], *I. Jaiyesimi*[2], *J. C. Mattson*[3]
[1]*Div. of Cytogenetics, Dept. of Anatomic Pathology, William Beaumont Hospital; Royal Oak, MI United States;* [2]*Div. of Hematology/Oncology, William Beaumont Hospital; Royal Oak, MI United States;* [3]*Div. of Hematopathology, Dept. of Clinical Pathology, William Beaumont Hospital; Royal Oak, MI United States*
alsaadi@beaumont.edu

Acute promyelogenous leukemia (APL) is a clinical subtype of acute myelogenous leukemia (AML) characterized by a translocation involving the RARa gene on 17q12-21, and five other genes, reported to date; PML at 15q21, RARa gene; PML at 15q21, PMZL at 11q23, NuMA at 11q13, NPM at 5q35, and STAT5b. We report an unusual case of AML in a 48 year old woman who had ecchymoses, a high white count (29.8 bil/L) with 55% myeloblasts, anemia (Hb 8.9 gm/dL), and thrombocytopenia (65 bil/L). With no coagulopathy at presentation. Marrow examination revealed replacement of normal marrow by leukemic blasts with reniform nuclei and cytoplasmic granulation, Auer bodies, but without increased hypergranular promyelocytes. A variant APL (M3v) was suspected but interphase FISH, using a PML/RARa probe, failed to reveal the classic t(15;17). The patient received induction chemotherapy with Idarubicin and Ara C achieving a complete remission after two courses. She was then treated with high-dose Ara C for 4 cycles as consolidation followed by maintenance therapy with Daunorubicin and Ara C. She did well for 21 months, then presented with neutropenia and thrombocytopenia. A repeat marrow showed relapsed leukemia. She was reinduced with Mitoxantrone, Ara C, and Etoposide. Allogenic transplantation is planned after complete remission. At presentation and at relapse the patient had a unique t(11;17)(p15;q21). Further FISH studies using RARa dual color rearrangement probe documented that the entire RARa gene was translocated to 11p15 in metaphases. This novel translocation, t(11;17)(p15;q21), has not been previously reported in either classic or variant APL.

P0155. Deletion of the Long Arm of Chromosome 15 with an Extra Copy of the Deleted Chromosome. A Rare Abnormality In Myelodysplastic Syndrome Transforming to AML.

K. J. Saunders, *B. Czepulkowski, K. Williams, M. Aldouri, N. Telford*
Rayne Institute; London, United Kingdom
karensaundrs@hotmail.com

We report on two patients with MDS transforming to AML which, after chromosome analysis were found to have an interstitial deletion of the long arm of one chromosome 15 with an extra copy of the deleted chromosome. Deletion of chromosome 15 is an unusual abnormality in haematological malignancies and after a thorough search of the literature we were only able to find one other reported case. A search of the Chromosome Abnormality Database, Oxford UK (CAD) listed a second case. However, both of these cases have only one copy of the deleted chromosome 15. This is, therefore, the first report of the duplication of a deleted chromosome 15. We compare our cases with the other two recorded cases in order to determine whether a trend exists which may associate this chromosome abnormality with a particular disease type and prognosis.

P0156. A highly unusual case of Acute Myleloid Leukemia (AML) M5a; Atypical clinical and cytogenetic findings.

H. C. Rossbach[1], *L. Glaskova*[1], *D. P. Dumont*[2], *W. Chamizo*[2], *J. Barbosa*[2],

M. J. Sutcliffe[3]
[1]All Children s Hospital; St. Petersburg, FL United States; [2]All Children s Hospital; ST PETERSBURG, FL United States; [3]All Children s Hospital/Univ. of South Florida; St. Petersburg, FL United States
rossbach@allkids.org

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

P0157. Cytogenetic, molecular cytogenetic and molecular genetic investigations in 17 patients with AML or MDS and amplification of the MLL gene

***B. Streubel**[1], C. Schoch[2], H. Karlic[3], G. Mittterbauer[4], K. Lechner[5], T. B chner[6], C. Fonatsch[1]*
[1]Institut fuer Medizinische Biologie; Vienna, Austria; [2]Medizinische Klinik III, Klinikum Grosshadern, Ludwig Maximilians University; Munich, Germany; [3]3. Medizinische Abteilung des Hanusch-Krankenhauses; Vienna, Austria; [4]Medizinische und Chemische Labordiagnostik; Vienna, Austria; [5]Department of Internal Medicine I; Vienna, Austria; [6]Abteilung f r Innere Medizin A, Westf lische-Wilhelms-Universit t; Muenster, Germany
berthold.streubel@univie.ac.at

Chromosome aberrations involving the MLL gene located in 11q23 are frequently observed in acute leukemias. The aberrations mainly consist of translocations with a variety of partner chromosomes. The amplification of MLL has been described recently only in a few patients. We report 17 patients with MLL amplification suspected by G-banding and confirmed by FISH analysis. Twelve patients had the diagnosis de novo acute myeloid leukemia (AML), one patient had a de novo myelodysplastic syndrome (MDS), one a secondary AML after MDS, and three patients suffered from secondary AML/MDS after genotoxic treatment. Eight patients presented MLL amplification on long uniformly stained regions, 6 patients within ring chromosomes, two patients on double-minute chromosomes, and one patient in form of long uniformly stained regions as well as in ring chromosomes. In all patients FISH analysis with two-colour probes for the 5 region and the 3 region of MLL showed a cluster consisting of multiple hybridization spots of both probes. Southern blot analysis of four patients with a probe spanning the breakpoint cluster region of MLL did not reveal any rearrangements. To explore the expression of MLL we performed semi-quantitative RT-PCR and real-time RT-PCR in 11 of our 17 patients. In comparsion to the relative levels of MLL expression of bone marrow cells from healthy volunteers and AML patients without 11q23 rearrangement the 11 patients wih MLL amplification did not show an overexpression of MLL. Our based on Southern bolt analysis using only a probe spanning the breakpoint cluster region of MLL suggest that the amplification of 11q23 does not involve a rearranged copy of the MLL gene. As our RT-PCR results did not show high level expression of MLL it could be speculated that the MLL promoter and enhancer sequences - which have yet to be identified and characterized - may not be amplified with the coding sequence.

P0158. Spectral Karyotyping (SKY) in Acute Myeloid Leukemia.

***B. Preiss**, G. Kerndrup*
Department of Pathology - Chromosome Laboratory, Odense University Hospital; 5000 Odense C, Denmark
birgitte.preiss@ouh.dk

Chromosomal abnormalities are often difficult to characterize completely in hematologic neoplasia. In acute myeloid leukemia (AML) we find that 37% of the patients with clonal chromosome aberrations show an incomplete karyotype by G-banding. As clonal chromosome aberrations are of independent prognostic significance, and karyotype analysis in AML is used as an up-front investigation for treatment stratification in our institution, it was important to obtain a complete karyotype. By single or oligotarget FISH it will be impossible to characterize but a few cases, money and amount of material being the limiting factor. With a success rate (obtainment of mitoses) of nearly 100% in AML, multitarget FISH becomes an attractive solution as an adjuvant diagnostic tool. From November 1, 1997 till November 1, 2000 we investigated 95 cases of AML. M/F ratio 0.78, median age 66 years (16-88 years). By SKY the abnormal clones were detected in all cases but 4 (few abnormal G-band mitoses). The SKY extended or confirmed the G-band findings in 96%. All marker chromosomes, except 3, were characterized. Cryptic translocations (translocations not suspected from the G-band karyotype) were found in 26 cases. The chromosomes most often involved in G-band and SKY aberrations, respectively, were; #5, #21, #7, #8, #17 and #5, #8, #7, #17, #21 in descending order. We find SKY a powerful adjuvant diagnostic tool that, in contrast to molecular biological techniques, does not compromise one of the advantages of karyotyping techniques, the analysis of the entire genome in one investigation. Data to be presented will be updated.

P0159. Prognostic impact of cytogenetics in untreated AML patients aged 16-60 years; preliminary results of the randomized AML-10 phase III trial of the EORTC and GIMEMA Leukemia Groups

***A. Hagemeijer**[1], S. Suciu[2], G. Solbu[2], S. Bhola[3], M. Mancini[4], A. Bernheim[5], P. Rodts[2], P. Fazi[4], R. Willemze[6], F. Mandelli[4], R. Zittoun[7], T. de Witte[8]*
[1]Center for Human Genetics; Leuven, Belgium; [2]EORTC Data Center; Brussels, Belgium; [3]Department of Clinical Cytogenetics; Leiden, The Netherlands; [4]Department of Human Biopathology and Hematology, University la Sapienze; Rome, Italy; [5]Cytogenetic Laboratory; Villejuif, France; [6]Department of Hematology; Leiden, The Netherlands; [7]H tel-Dieu; Paris, France; [8]Department of Hematology; Nijmegen, The Netherlands
Anne.Hagemeijer@med.kuleuven.ac;be

The AML10 protocol is a randomized study of induction and intensive consolidation followed by stem cell transplantation, allogeneic or autologous. All FAB types except M3, were eligible. A total of 2,157 pts were included, 915 by EORTC (E) and 1,242 by GIMEMA (G) members. Cytogenetics at diagnosis was peer-reviewed. Excluding unknowns (43 E, 338 G), not done (37 E, 148 G), failure or insufficient mitoses (150 E, 353 G), valid data were obtained for 1088 patients (pts) (685 E (75%) and 403 G (32%)). 1,038 pts were evaluable for response; 402 (39%) had a normal karyotype (>= 20 mitoses) and 636 (61%) an abnormal one. Among abnormal cases, the following changes were found; t(8;21) (15 %), inv(16) (15%), translocation 11q23/MLL (7%), t(9;11) (3%), +8 (15%), -7 (8%), -5 (4%), 7q- (5%), 5q- (4%), abnormalities of 3q (7%), t(9;22) (3%), del(12p) (2.5 %). A total of 111 (17.5%) pts had a complex karyotypes (> 3 aberrations), half of them with aberrations of chromosomes 5 and 7. Overall survival (OS) rate at 3 yrs was 40%. The Cox s model showed that the cytogenetic findings were the strongest independent prognostic factor, followed by age and WBC. Pts with t(8;21) and inv(16) had the best outcome; OS rate of 60%. The worst outcome was related to -7, -5, 12p- and t(9;22), followed by t(11q23) and 7q-. Additional analyses identified cytogenetic subgroups and allowed refined assessment of reason for treatment failure. In conclusion, karyotype appears as the strongest independent prognostic factor for outcome.

P0160. Analysis of Men1 Gene in AML by Application of FISH Technique

***H. Acar**[1], M. Kaynak[1], S. Yildirim[1], T. Cora[1], D. A. Largaespada[2]*
[1]Department of Medical Genetics, Selcuk University, Medical Faculty; Konya, Turkey; [2]Department of Genetics, Cell Biology and Development, Cancer Center, University of Minnesota; Minneapolis, MN United States
yaseminsena@hotmail.com

Acute myeloid leukemia (AML) is a malignant disease which effects immature or undifferentiated myeloid cells and is characterised by their accumulation in bone marrow and pheripherial blood. It has different genetic abnormalities involving numarical and structural anomalies of chromosome 11. Recently, a tumor suppressor gene, the multiple endocrine neoplasia type 1 gene (Men1) was cloned and mapped to chromosome 11q13. Mutations of the Men1 tumor suppressor gene cause the multiple endocrine neoplasia type 1 (Men1) syndrome in humans, and they are involved in a

variety of sporadic human tumors. To assess the potential involvement of this gene in the patogenesis of myloid leukemia, we analysed the Men1 deletions in myeloid leukemias. This was achieved by using fluorescence in situ hybridization with Men1 cosmid gene probe in interphase nuclei.With this technique reliably detected the Men1 gene in interphase nuclei of myeloid leukemias. *This study was supported by Selcuk University Research Fund

P0161. Specific FISH screening assays for MLL/11q23 translocations

S. Strehl[1], *M. K nig*[1], *M. Reichel*[2], *R. Marschalek*[3], *O. A. Haas*[1,4]
[1]*CCRI, Children«s Cancer Research Institute; Vienna, Austria;* [2]*Department of Genetics; University of Erlangen, Germany;* [3]*Institute of Pharmaceutical Biology; University of Frankfurt/Main, Germany;* [4]*LBICD, Ludwig Boltzmann-Institute for Cytogenetic Diagnosis; Vienna, Austria*
strehl@ccri.univie.ac.at

Rearrangements of the MLL gene on chromosome 11q23 account for approximately 5-10% of acquired karyotype abnormalities encountered in children and adults with acute lymphoblastic, acute myeloblastic, poorly differentiated or biphenotypic leukemias and myelodysplastic syndromes. To date, at least 40 different 11q23 translocations have been determined cytogenetically and at least 25 MLL fusion partner genes have been cloned. Rapid detection of MLL gene translocations at diagnosis is of clinical significance and can be easily accomplished by a recently developed dual-color FISH method that allows the detection of MLL/11q23 rearrangements in metaphase and interphase nuclei even in cases with 3« MLL deletions. This assay is appropriate for the analysis of MLL involvement, however, detection of the involved fusion partner gene is limited to samples where metaphases are available, and impossible in cases with 3« MLL deletions. We have adapted this FISH assay for the detection of specific translocations involving different MLL partners. Using various gene locus-specific BAC clones in combination with the MLL probes we have already established FISH assays for the detection of translocation partners AF4(4q21), AF6(6q27), AF17(17q21), and MSF(17q25), and the development of further assays for AF9(9p22), AF10(10p12) and other MLL partners is under way. In general, the FISH probes are set up in a way that specific translocations result in two fusion signals and in one fusion signal in case of a 3« MLL deletion in metaphase and interphase nuclei. These assays will facilitate studies of specific translocations by interphase FISH.

P0162. Interphase Fish Assay For The Analysis Of Chromosome Breakpoints In 3q21 In Myeloid Leukemia

R. Wieser[1], *U. Schreiner*[1], *M. Aytekin*[1], *H. Pirc-Danoewinata*[2], *H. Rieder*[3], *C. Fonatsch*[1]
[1]*Institut fuer Medizinische Biologie; Wien, Austria;* [2]*CCRI, St. Anna Kinderspital; Wien, Austria;* [3]*Institut f r Klinische Genetik, Klinikum der Philipps-Universit t; Marburg, Germany*
rotraud.wieser@univie.ac.at

Chromosome aberrations affecting band 3q21, namely, the inv(3)(q21q26), the t(3;3)(q21;q26), and the t(1;3)(p36;q21), are associated with megakaryocytic dysplasia, normal or elevated platelet counts, and a particularly unfavourable prognosis for the affected patients due to poor responsiveness to chemotherapy. Their diagnosis is therefore of great clinical importance. This prompted us to develop a molecular assay that can be used to confirm or exclude 3q aberrations suspected on the basis of classical cytogenetic analysis, and to monitor disease progression. Since no fusion genes are consistently associated with any of the 3q21 rearrangements, and overexpression of the EVI-1 gene in 3q26 is also not strictly correlated with the presence of the inv(3) or t(3;3), RT-PCR assays are not suitable to confirm and/or track these chromosome aberrations. We therefore developed an interphase fluorescence in situ hybridization (FISH) assay, in which separation of red and green fluorescent probes that colocalize in normal interphases indicates the presence of a 3q21 rearrangement. This assay was validated on three cell lines and 25 primary samples from different leukemias with and without 3q21 aberrations, and was shown to be highly sensitive and specific.

P0163. Chromosomal instability of deleted long arms of chromosome 5 in myeloid malignancies.

J. Brezinova[1], *Z. Zemanova*[2], *S. Kurkova*[1], *L. Sindelarova*[2], *I. Chudoba*[3], *K. Michalova*[2]
[1]*Institute of Hematology and Blood Transfusion; Prague, Czech Republic;* [2]*3rd Medical Department, General Faculty Hospital and 1st Medical Faculty of Charles University; Prague, Czech Republic;* [3]*MetaSystems GmbH; Altlussheim, Germany*
brez@uhkt.cz

Deletions of the long arms of chromosome 5 are common findings in hematological malignancies, in particular in myelodysplastic syndromes (MDS) and acute myeloid leukemias (AML). The size and position of deleted segment vary among patients but band 5q31 is commonly deleted in most of them. Although del(5q) sometimes occurs as a sole chromosomal abnormality, more often it is present with additional chromosomal changes. On the contrary, translocations of 5q were found to be rather sporadic events in MDS. In our study we followed 39 patients (29 men, 10 women, average age 60,6 years) with MDS/AML with deletion 5q and complex chromosomal rearrangements by FISH methods. In the first group of patients (9) whole chromosomal painting probes (WCP) were used to determine the chromosomal partners in translocations. In the second group (30 patients) FISH with 5q31 locus specific probe confirmed deletion of this chromosomal region in all cases except one and we suspect 5q31 as a primary cytogenetic lesion. We were able to detect chromosomal instability of deleted chromosome 5q by FISH with WCP probes and multicolor FISH (mFISH) using multicolor probes specific for all chromosomes. mFISH revealed cryptic aberrations; reciprocal and terminal translocations and small insertions to various chromosomal partners (chromosome 7 as the most frequent), fragmentations of deleted long arms of chromosome 5 was also frequently found. In all patients complex rearrangements of 5q were connected with poor clinical outcome. This work was supported by grant GA CR 302/98/0071 and CEZ J 13 98 111100004 M? MT CR.

P0164. Complex translocation between the two homologue chromosomes 5 in CML - characterization of the aberration by multicolor banding (MCB)

K. Mrasek[1], *A. Heller*[1], *H. Starke*[1], *V. Trifonov*[2], *N. Rubtsov*[2], *U. Wedding*[3], *I. F. Loncarevic*[1], *C. Bleck*[1], *U. Claussen*[1], *T. Liehr*[1]
[1]*Institute of Human Genetics and Anthropology; Jena, Germany;* [2]*Institute of Cytology and Genetics; Novosibirsk, Russian Federation;* [3]*Clinic of Internal Medicine; Jena, Germany*
i8lith@mti-n.mti.uni-jena.de

We report on a patient with a clinically diagnosed Philadelphia negative chronic myelogenous leukemia (CML) with a so far unrecorded complex translocation event between the two homologue chromosomes 5. At the GTG-band level the karyotype was normal, apart from an enlarged chromosome 5 and an extremely shortened second chromosome 5. Both derivative chromosomes 5 consisted exclusively of #5 derived material as proven by 24-color FISH. To characterize the complex aberration in more detail the multicolor banding (MCB) technique using a chromosome 5 specific probe set was applied. Using this DNA based high resolution banding procedure, the karyotype could be described as 46,XX,del(5)(pter->q12;;q33->qter),ins(5)(pter->q15;;q12->q21;;q21->qter) [see Figure]. In consequence, the aberration leads to a partial deletion of the long arm of chromosome 5; del(5)(q21q33), which would not have been identified using conventional banding techniques or 24-color FISH. Even though 5q- is a frequent finding in cases with MDS, 25% of MDS cases have a deletion in the long arm of chromosome 5, up to now 5q deletions are described in only 2 cases with Ph-positive CML. In summary, our results together with the data from the literature indicate, that the long arm of chromosome 5 - especially the region 5q33 - is not only frequently involved in rearrangements in MDS, but are also of importance for a small subset of CML patients. Acknowledgments; This work was supported by the Herbert Quandt Stiftung der VARTA AG, the Madeleine Schickedanz-Kinderkrebs-Stiftung, the DAAD, the DFG and the Wilhelm Sander-Stiftung.

P0165. Characterization of a Rare Variant Philadelphia Translocation; t(9;10;22)(q34;q22;q11) in Chronic Myeloid Leukemia by Fluorescence in situ Hybridization

W. Emberger, *A. Behmel, M. Tschernigg, E. Petek, P. M. Kroisel, H. Zierler, K. Wagner*
Institute of Medical Biology and Human Genetics, University of Graz; Graz, Austria
werner.emberger@kfunigraz.ac.at

We report on a currently 59 year old male chronic myeloid leukemia patient with a rare variant Philadelphia translocation t(9;10;22)(q34;q22;q11). Fluorescence in situ hybridization with whole chromosome paints was used to confirm the cytogenetic findings. With a BCR/ABL specific probe the known rearrangement on the derivative chromosome 22 could be found. Variant Philadelphia translocations may result from a two step process with primary formation of the classical rearrangement and a second aberration event, or a one step complex chromosomal rearrangement based on simultaneous chromosomal break events. Our results showed no evidence for a second break event of one of the involved chromosomes but a two step process can not be ruled out completely. Variant Philadelphia translocations involving chromosome 10 are rare and the majority of chromosome 10 breakpoints of variant Ph translocations cluster to chromosomal sub-

band 10q22 and 10q26. The prognostic implications as well as the relevance of the additional breakpoint region 10q22 are discussed.

P0166. Does ABL-BCR RNA negativity indicate a deletion in chromosome der(9)t(9;22) in CML?

J. R mer[1], *C. Bleck*[1], *U. Claussen*[1], *A. Heller*[1], *H. G. Sayer*[2], *H. Starke*[1], *M. Ziegler*[1], *I. F. Loncarevic*[1]
[1]*Institute of Human Genetics and Anthropology, FSU; Jena, Germany;* [2]*Clinic for Internal Medicine II, FSU; Jena, Germany*
ilon@mti-n.uni-jena.de

ABL-BCR expression is only found in about 60-70% in BCR-ABL positive chronic myeloid leukemia (CML). ABL-BCR transcription is suggested not to correlate with the response to interferon alpha therapy, although, a deletion in der(9) chromosome, where the ABL-BCR gene resides, seems to be associated with a worth prognosis. To investigate whether ABL-BCR RNA negativity indicates (1) a deletion in der(9)t(9;22)(q34;q11) and (2) correlates with a worth prognosis in CML we have analyzed 18 CML patients for BCR-ABL and ABL-BCR RNA and DNA by RT-PCR and FISH, receptively, so far. BCR-ABL expression serves as positive control for RNA origin and integrity. In 5 of the 18 patients we detected no ABL-BCR transcription. Fluorescence in situ hybridization (FISH) with a commercial probe, that comprises all the ABL and BCR genes, confirmed the suspected deletion of the ABL-BCR fusion gene in one, and elucidates a complex translocation in a second patient, who lack the ABL-BCR fusion gene due to a translocation t(8;9;22). FISH could not confirm the deletion in the remaining 3 patients, which have to be investigated in more detail to resolve whether small deletions are present and not detected by FISH. We speculate, that patients lacking the ABL-BCR transcript belong to the same clinical group as those with a deletion in the der(9)t(9;22) chromosome.

P0167. Chromosome instability in patients with chronic myeloid leukemia (CML) treated with Interferon.

B. Porto[1], *M. Cunha*[2], *I. Malheiro*[1]
[1]*Laborat rio Citogen tica, Instituto Ci ncias Biom dicas Abel Salazar; Porto, Portugal;* [2]*Servi o de Hematologia, Hospital St… Ant nio; Porto, Portugal*
vaspor@mail.telepac.pt

The response of patients with chronic myeloid leukemia (CML) to the treatment with Interferon can be evaluated by cytogenetic analysis, using the number of Philadelphia chromosome positive (Ph1+) and negative (Ph1-) cells to classify the cytogenetic response as complete (no Ph1+ cells), major (less than 35% Ph1+ cells), minor (equal or more than 35% Ph1+ cells), or no response (equal or more than 95% Ph1+ cells). We have performed a cytogenetic study in 24h non-stimulated cultures of bone marrow cells from 18 patients with CML at diagnosis, during and after treatment with Interferon. 9 patients presented cytogenetic response and 9 patients had no response to treatment. In this study we evaluated the presence of chromosome instability (CI), i. e., the presence of chromosome and chromatid breaks and rearrangements. For the 18 patients studied, the results showed that; 1. CI is observed during and after treatment with Interferon and not at diagnosis; 2. the presence of cells with CI was higher in the group of patients that had cytogenetic response (6/9) than in the group of patients that had no response (3/9); 3. considering all the studies together, the presence of cells with CI was higher in occasions of complete cytogenetic response (7/8) than in occasions of minor (3/10) and no response (1/6); for major response, we observe the presence of CI in 7 out of 14 occasions. This results suggest that the presence of chromosome instability in CML patients treated with Interferon may be related to good cytogenetic responses.

P0168. Atypical Chronic Myelogenous Leukemia; a 7 and 9 Translocation in a Filipino Child.

F. F. Natividad[1,2], *M. L. Enriquez*[1,3], *F. G. Hernandez*[4], *M. N. Chua*[4], *C. T. Tahimic*[2], *A. G. Eduria*[1], *C. P. Urrete*[5]
[1]*Research and Biotechnology Division, St. Luke s Medical Center; Quezon City, Philippines;* [2]*College of Science, University of the Philippines, Diliman; Quezon City, Philippines;* [3]*Physics Department, College of Science, De La Salle University; Manila, Philippines;* [4]*Department of Pediatrics, UST Hospital; Manila, Philippines;* [5]*Medical Research Laboratory, Philippine General Hospital; Manila, Philippines*
mampi@stluke.com.ph

A case of an atypical chronic myelogenous leukemia in a Filipino child is presented. At diagnosis the seven year-old boy had mild anemia, hyperleucocystosis with granulocytes in all stages of maturation, eosinophil of 16% and normal megakaryocytes. Leucocyte alkaline phosphatase was zero. Using the GTG-banding technique, the absence of Philadelphia chromosome was noted in all cells analyzed. The involvement of 7q31 and 9q34 was observed. Using the fluorescence in situ hybridization whole chromosome probes, it was established that the segment of chromosome 7(q22-qter) translocated to chromosome 9 at or near 9q34. No translocation of the broken segment of chromosome 9 has been observed to 7 or to any other chromosome. The involvement of 7q31 and 9q34 in this translocation suggests a novel gene fusion in chronic leukemogenesis.

P0169. Fluorescence In Situ Hybridization (FISH) signal patterns in Chronic Myeloid Leukaemia(CML) and Acute Lymphoblastic Leukaemia (ALL) patients in Singapore using ES-FISH BCR-ABL Probe

S. L. Tien[1], *C. Siwaswaren*[2], *P. Lim*[2], *E. Lim*[2]
[1]*Singapore General Hospital; Singapore 169608, Singapore;* [2]*Singapore General Hospital; Singapore, Singapore*
ghetsl@sgh.com.sg

We used Vysis LSI bcr/abl extra signal (ES) translocation probe to study FISH signal patterns in CML and ALL patients in Singapore General Hospital. From 1999 to 2000 we studied 51 consecutive CML cases and found 12 cases (23.5%) with deletion of ASS region giving 1R1G1F signal, 1 of which had small deletion of ASS region giving 1R1dimR1G1F signal. Two other cases (3.9%) with 1R1G1F signal were found to have masked Philadelphia chromosomes due to insertion of BCR into ABL region. The remaining 37 cases showed the usual 2R1G1F fusion with no deletion of ASS region. Deletion of ASS region in Philadelphia chromosomes appeared to confer a poorer prognosis for CML patients. In ALL patients, using the similar ES-FISH probe, we found 4 cases of t(9;22) which gave double fusion signals, 1R1G2F and polymerase chain reaction (PCR) study showed that these cases were due to minor-bcr p190 e1a2/e1a3. Two ALL cases gave 2R1G1F signals and PCR showed that they had major-bcr p210 b2a2/b3a2. We concluded that using the ES-FISH signal probe in interphase nuclei and metaphase spreads the incidence of ASS deletion in CML patients was found to be 23.5% in Singapore and its signal patterns could apparently differentiate the major-bcr from minor-bcr in t(9;22) ALL.

P0170. Cytogenetic alterations in hematological malignancies

S. A. C. T. Castillo Taucher, A. M. S. Fuentes, A. A. M. Paulos, T. *T. C. Anguita*
Secci n Citogen tica, Laboratorio Cl nica Alemana; Santiago, Chile
scastill@ns.hospital.uchile.cl

Among 518 cytogenetic studies done in hematological malignancies, we had 52 failures (10%). Normal results represented 290 cases (56%), expected when controlling remissions or in uncertain diagnosis. Different aberrations were found in 176 cases (34%). The total number of studied patients is 386, many of them have repeated controls, the pediatric population is 78 (20,2%). The most frequent reference diagnosis were acute myeloid leukemia (15,2%), chronic myeloid leukemia (12,3%), acute lymphoid leukemia (11,6%), myelodysplasia (11,2%) and multiple myeloma (7,6%). The most common cytogenetic finding was t(9;22)(q34;q11) in 76 studies (43%), either isolated or with additional aberrations. We also performed FISH studies in 57 patients; in 25 cases with X and Y probes for control of grafting of bone marrow transplantation with a non-sex matched relative; in 15 cases in search of t(9;22)(q34;q11) with 13% positive results; in 14 cases looking at del13q14 in multiple myeloma with 57% positive results and in 3 cases for t(15;17)(q22;q21) with 1 positive result. We intend to clarify some complex cases with many marker chromosomes with multiple FISH or comparative genomic hybridisation.

P0171. Characterization of complex aberrant leukemia cases by means of multicolor banding (MCB)

A. Heller[1], *N. Rubtsov*[2], *V. Trifonov*[2], *H. Starke*[1], *I. F. Loncarevic*[1], *U. Claussen*[1], *T. Liehr*[1]
[1]*Institute of Human Genetics and Anthropology; Jena, Germany;* [2]*Institute of Cytology and Genetics; Novosibirsk, Russian Federation*
ahel@mti-n.mti.uni-jena.de

In tumor cytogenetics complex aberrant karyotypes are frequently observed. GTG-banding and 24-color-FISH approaches using whole chromosome paints as probes often cannot resolve the karyotypes of such cases. The multicolor-banding (MCB) technique, allowing the differentiation of chromosome region specific areas at the band and sub-band level, is the technique of choice to enlighten the chromosomal aberrations, regions and exact breakpoints involved. MCB is based on regionspecific libraries producing changing fluorescence intensity ratios along the chromosomes, which are used to assign different pseudocolors to specific chromosomal regions. Three leukemia cases with highly aberrant chromosomal constitution, each, have been studied by GTG-banding and 24-color FISH, the latter was applied to clarify which chromosomes contributed

material to which marker. The exact breakpoints and the orientation of chromosomal regions involved was subsequently determined by MCB. Case 1 was a 72 year old patient suffering from plasmocytoma. In conventional cytogenetic analysis (GTG-banding) a highly complex karyotype with 51 to 53 chromosomes and 2 different subclones could be identified. Trisomy 3, 5, 7, 14, 15 and 19 could be observed by GTG-banding and confirmed by 24-color FISH. MCB mixes for #1, #5 revealed moreover an inv(1)(p13.3;q21.1) and a defined the exact breakpoint for chromosome 5 in a t(5;11;1) to 5q14. Case 2 was a 14 year old boy suffering from a secondary MDS due to a methotrexat and cyclophosphamid treatment against juvenile rheumatoid arthritis for several years. Complex changes including a der(7)t(19;7;19;7;6;19;6) and translocations of chromosomes #6 and #7 and #12 and #13 have been characterized by 24-color-FISH and in more detail by MCB. Case 3 was an AML patient presenting e.g. the following aberrations; dic(5;18), der(20)t(1;20;18) and der(18)t(8;18;5;2;20). The exact breakpoints and the orientation of the involved chromosomal regions were determined by MCB. In summary, the power of the MCB technique to determine breakpoints and orientation of chromosomal parts within even highly complex marker chromosomes with only one hybridization is demonstrated. Acknowledgments; This work was supported by the Herbert Quandt Stiftung der VARTA AG, the Madeleine Schickedanz-Kinderkrebs-Stiftung, the DAAD, the DFG and the Wilhelm Sander-Stiftung.

P0172. Chromosome rearrangements in leukemia under the first year of age.

S. Acevedo[1], *M. Cuello*[1], *A. Picon*[2], *E. Dibar*[3], *I. Larripa*[1]
[1]*Instituto de Investigaciones Hematologicas, Academia Nacional de Medicina; Buenos Aires, Argentina;* [2]*IHospital Alejandro Posadas; Buenos Aires, Argentina;* [3]*Hospital Italiano; Buenos Aires, Argentina*
shacev@intramed.net.ar

Leukemia in infants has been defined as a rare event arising in stem cells of early hematopoietic differentiation, up to their first year of life. We present a series of eight patients with acute leukemia, including three cases of neonatal leukemia,< a month of age. We excluded two Down Syndrome with somatic mosaisism of low proportion (12% in PB) associated cases diagnosed as biphenotypic and lymphoblastic leukemia . These patients showed a 100% +21 in the bone marrow aspirate, while the leukemogeneous reaction was present. Bone marrow aspirates and/or peripheral blood were cultured in RPMI 1640, supplemented with fetal calf serum and without mitogen stimulation. Cases were GTG banding analyzed and karyotypes were established according to the ISCN. These were characterized by a higher incidence of structural aberrations such as; t(4;11)(three cases) and del(7)(q21-qter)(two cases), add(2q) (one case). The remaining two cases correspond to a normal karyotype and to a numerical alteration (+5,+22). Sex ratio was 5;3 showing a little preference of affected girls. Six patients were diagnosed as AML while the other two were ALL and Biphenotypic leukemia. We conclude that cytogenetic studies are very important to differentiate those Down related cases with leukemogeneous reaction which will spontaneously remit from those acute leukemias with chromosome rearrangements of poor prognosis. Determination of the neoplastic cell genotype is a relevant parameter to establish the correct therapy.

P0173. Novel FISH assays to detect immunoglobulin light chain loci rearrangements in B-cell malignancies

J. I. Martin-Subero[1], *L. Harder*[1], *S. Gesk*[1], *F. J. Novo*[2], *M. J. Calasanz*[2], *W. Grote*[1], *B. Schlegelberger*[1], *R. Siebert*[1]
[1]*Institute of Human Genetics, University Hospital Kiel; Kiel, Germany;* [2]*Department of Genetics, University of Navarra; Pamplona, Spain*
imartin@unav.es

The majority of B-cell malignancies bear chromosomal translocations between the immunoglobulin (IG) genes and oncogenes leading to deregulation of the latter. IGH at 14q32 is most frequently involved, whereas translocations of IG kappa (IGK) at 2p12 and IG lambda (IGL) at 22q11 have been described in 5-10% of the cases. The most reliable method to detect translocations affecting a promiscuous locus is Fluorescence In Situ Hybridization (FISH) with differentially labeled probes flanking the breakpoint. As no break apart probes have been available for IG light chain loci, we designed two novel FISH assays to detect translocations involving IGK and IGL. BAC clones flanking each breakpoint were differentially labeled. False-positive ratios were calculated from experiments on healthy donors, being 0.3% for IGK and 1.4% for IGL. We tested twenty-five cases of B-cell malignancies with cytogenetically detectable chromosomal abnormalities at 2p11-14 (n=13) and 22q11-12 (n=12). FISH revealed IGK involvement in six of 13 cases (46%) with cytogenetically detectable 2p11-14 break. Translocation partners were chromosome bands 7q21, 8q24 (three cases), 10q24 and 16q24 containing the B-cell associated oncogenes CDK6, c-MYC, NFKB2 and c-MAF, respectively. In 6 of 12 cases (50%) with cytogenetically detectable 22q11-12 alteration FISH indicated a breakpoint in the IGL locus. Translocation partners were 3q27 (BCL6) and 8q24 (c-MYC) as well as chromosomal regions 2p13, 2p16, 4q13 and 16p12 (oncogenes unknown). The new FISH assays provide a flexible, simple and reliable tool to detect IGK and IGL translocations, providing an important means for the clinical-management of lymphoma patients as well as for the identification of new B-cell associated oncogenes. Supported by the Deutsche Krebshilfe and IZKF (Kiel).

P0174. Suptyping Blastic Peripheral B-cell Lymphomas According To Chromosomal Aberrations

L. Harder[1], *B. Schlegelberger*[1], *T. Springer*[1], *S. Gesk*[1], *H. Nowotny*[2], *T. Zwingers*[3], *W. Grote*[1], *R. Siebert*[1]
[1]*Institute of Human Genetics, University of Kiel; Kiel, Germany;* [2]*Ludwig-Boltzmann Institut f r Leuk mieforschung; Wien, Austria;* [3]*estimate GmbH; Augsburg, Germany*
sharder@medgen.uni-kiel.de

Blastic peripheral B-cell lymphoma (BBCL) is a biologically and clinically heterogenous group of lymphoid neoplasms. No reliable criteria for subclassification of BBCL for clinical practice are currently available. We thus investigated whether a subclassification of BBCL is possible on the basis of recurrent genetic aberrations detected by chromosome analysis and FISH. There was a significant difference in the frequency of changes affecting the IgH locus in 14q32 in the morphologic variants of BBCL. Nearly half of the lymphomas of centroblastic (cb) subtype but only 25% of the lymphomas of immunoblastic (ib) subtype had a breakpoint in 14q32 ($p<0.04$). The translocation t(11;14) occured in all blastoid mantle cell lymphomas (bmcl) and was restricted to this subtype. In ib lymphomas, t(8;14), t(11;14) and t(14;18) were present significantly less frequently than in Burkitt s lymphomas, bmcl and cb lymphomas, respectively. Moreover, cytogenetic and FISH analyses revealed the IgH translocations in ib lymphomas to be predominantly those characteristic for B-CLL, like t(2;14)(p13;q32) affecting the BCL11A gene or t(14;19)(q32;p13) involving the BCL3 locus. There were also significant differences in the frequency of changes affecting other chromosomal regions; ib lymphomas contained predominantly deletions in 8q and 14q, changes of 4q and losses of chromosome 10; cb lymphomas showing gains of chromosoms X, 7 and 18, losses of chromosomes 13 and 15, changes of 1p36, 1q, 3q27, 8q and 11q; bmcl showing losses in 1p21~31 and 9p, gains of 3q21~24 or complete chromosome 3. Multivariate analysis revealed deletions in 1q42~qter, duplications in 1q23~32, trisomy 5 and changes of 15q as independent prognostic factors in cb lymphomas. In ib lymphomas, changes of 7q and 8q showed an adverse effect with even stronger impact on survival than the International Index. These findings underline that genetic aberrations might help to define biologically and clinically distinct entities of BBCL. We acknowledge the collegues from the Kiel-Wien-Lymphoma Study Group and the support of the Deutsche Krebshilfe grant 10-1556-Schl4

P0175. Three different types of Robertsonian Translocations cause a similar Myelodysplastic syndrome

S. Kokkinou[1], *I. Papadimitriou*[2], *K. Pavlou*[3], *E. Meidanis*[1]
[1]*Cytogenetic Unit, Sismanoglio General Hospital; Athens, Greece;* [2]*Biochemistry Dept., Sismanoglio General Hospital; Athens, Greece;* [3]*Immunology Dept., Sismanoglio General Hospital; Athens, Greece*
dimitris0@otenet.gr

The occurrence of Robertsonian translocations is rarely encountered in hematopoietic malignancies. We identified five patients carrying a Robertsonian translocation suffering from persistent leukopenia, with a low value of vitamin B12 in their serum, progressing to anemia and thrombocytopenia.

The bone marrow and the trephine biospy specimene revealed a myelodysplastic syndrome, not fitted to any type of FAB classification.

Standard PHA-stimulated cultures of peripheral blood showed the translocation in 100% of metaphase cells with a modal number of 45 chromosomes, including X and Y. Chromosomes were G banded and 40 metaphases analysed. C-banding was also performed. The three types of Robertsonian translocation were t(13;14)(p11; q11) in three out of five (3/5) and t(14;21)(p11;q11) and t(13;13) (p11;p11) in the other two patients.

We suggest that these patients had two oncogenic advantages;

1. The Robertsonian translocation itself, is associated with genetic instability.
2. The low value of vitamin B12, since it is known that it can interfere to double strand DNA break.

In conclusion, we suggest that carriers of Robertsonian translocation have an increased risk of aquiring a hematological malignancy.

P0176. Molecular Cytogenetic Analysis of the Monoblastic Cell Line U937

J. Lee, Y. Cho, C. Lee, S. Shim, H. Seo, J. Khym
College of Medicine, Hanyang University; Seoul, Republic of Korea
yhcho@email.hanyang.ac.kr

The cell line U937 was derived from the pleural fluid of a patient with diffuse histocytic lymphoma, and the cells phenotypically resemble blast cells of the monocytic lineage. Previous analysis of the cell line U937 has shown that several chromosomal rearrangements and markers. The U937 karyotype however, has remained incomplete, and marker structures have never been fully described. The origins of the marker chromosomes of the U937 were identified by conventional G-banding analysis and molecular cytogeneric techniques. Fluorescence in situ hybridization(FISH) using whole chromosome painting probe confirmed the identities of markers which were partially identified by G-banding as t(1;5), t(6;12), t(10;11), der(2)t(2;6), der(3)t(1;3), der(6)t(2;6), der(13)t(1;13), der(16)t(4;16), ider(13q), der(16), dup(6p) and partial deletions of 2p, 3q, 15q. By comparative genomic hybridization(CGH), the genomic abnormalities detected were overexpressions of chromosomes 1, 2, 3, 6, 13, 15, 2o, 21. These overexpressions nearly consist with chromosomal abnormalities.

P0177. Frequency and Significance of Cytogenetic Aberrations in B-NHL

E. Kr mer[1], H. Gr ner[1], H. Nowotny[1], H. T chler[1], G. Hopfinger[2], R. Heinz[1]
[1]LBI Leukemia Research and Hematology; Wien/Vienna, Austria; [2]3rd Medical Department Hanusch-Hospital; Wien/Vienna, Austria
kroemel@gmx.at

At our department cytogenetic analysis of NHL-patients has been done for more than one decade. Using the REAL classification system the cytogenetic data-bank of patients with B-NHL was searched for the most frequent chromosoal aberrations. The analysis was focused on the four largest diagnosis-groups of B-NHL; CLL, DLBL, follicular lymphoma (FOLL) and MCL. Klonal cytogenetic aberrations were found in 97 of 111 CLL, 87 of 109 DLBL, 66 of 76 FOLL and 31 of 43 MCL. Only cases with chromosome changes (281 in total) were taken into account for analysis. Frequencies of primary and secondary aberrations were determined for the different NHL-subgroups, and in addition the incidence of non-random associations between primary (f.e. t(11;14), t(14;18)) and secondary chromosomal changes was looked for. According to the new WHO classification system there are now only 2 grading subgroups (based on morphological criteria) remaining in follicular lymphoma (1+2 and 3 of the REAL classification). Cytogenetic data seems to not entirely support this change.

P0178. Prognostic Importance of Trisomy 3 in Gastrointestinal MALT Lymphomas - FISH Analysis of 26 Cases

J. Krugmann[1], S. Dirnhofer[2], A. Tzankov[1], H. C. Duba[3], M. Erdel[3]
[1]Institut f r Pathologische Anatomie der Universit t; Innsbruck, Austria; [2]Institut f r Anatomie der Universit t; Basel, Switzerland; [3]Institut f r Medizinische Biologie und Humangenetik der Universit t; Innsbruck, Austria
jens.krugmann@uibk.ac.at

Trisomy 3 was found to be a recurrent numerical chromosomal change in lymphomas of mucosa associated lymphoid tissue (MALT) type. Different frequencies, however, have been reported and the pathogenetic role of trisomy 3 remains unknown. In the present study we examined archival paraffin embedded tissues from 26 well documented cases of surgically resected primary gastrointestinal MALT lymphomas for the occurrence of trisomy 3 and 12 by bicolor fluorescence in situ hybridization on isolated interphase nuclei and tried to compare the results with the clinical outcome. The collective includes 14 men and 12 women with a mean age of 60 years (male 41-83, female 21-85). 12 cases were classical low grade marginal zone MALT lymphoma, 1 case showed a low and high grade lymphoma component (mixed type) and 13 cases were diffuse large B-cell lymphoma of MALT type (high grade). The lymphoma were staged according to the modified Ann Arbor system by Musshoff. Six patients presented with stage EI1, 8 with EI2, 10 with EII and one patient with stage III and IV. According to control experiments on nuclei from reactive tissues the cutoff level for trisomies was set at 15% nuclei with three signals. No trisomy 12 was found and trisomy 3 was detected only in 4 of 26 (15%) MALT lymphomas including 2 low grade and 2 high grade lymphoma. Two patients had a lymphoma recurrence and died during the follow-up period. We conclude that in our series trisomy 3 showed low occurrence and was found in both low grade and high grade MALT lymphoma. We speculate that trisomy 3 might correlate with increased recurrence rate in patients with gastrointestinal MALT lymphomas, but this needs further investigations.

P0179. Cytogenetic studies in familial Waldenstr m s macroglobulinemia.

M. De Braekeleer[1], F. Morel[1], A. Herry[1], M. J. Le Bris[2], G. Le Calvez[2], V. Marion[2], C. Berthou[1], J. F. Abgrall[1]
[1]CHU Morvan & Universit de Bretagne Occidentale; Brest, France; [2]CHU Morvan; Brest, France
marc.debraekeleer@univ-brest.fr

Waldenstr m s macroglobulinemia (WM) is a rare disease, occurring mainly in the 6th and 7th decades of life. It is characterized by an excessive proliferation of an IgM-producing cell clone. The serum protein electrophoresis shows a monoclonal spike due to the secretion of large amounts of IgM. Familial occurrence of Waldenstr m macroglobulinemia has been only seldomly reported and no cytogenetic analyses performed. We have followed three brothers with WM for the last 10 years. Two of them, diagnosed at 65 and 61 years old, and now aged 72 and 69 respectively, are still in a stable condition, with no clinical symptoms, although one of them has hyperlymphocytosis. Cytogenetic analyses on bone marrow short and long term cultures showed no chromosomal abnormalities. The third brother was diagnosed at age 57; he remained stable during two years, at which time he developed neuropathy of the lower limbs. He was then treated with chlorambucil for 34 months. Two years later, he developed chronic myelomonocytic leukemia (CMML) that evolved a year later in refractory anemia with excess of blasts (RAEB), of which he eventually died. Cytogenetic analyses on a bone marrow sample obtained while he was in transformation showed complex rearrangements. An abnormal clone, with minor variations, was found in all 20 metaphases analysed; 44,XY, t(1;21)(p36;q11), del (2)(p23), add(3)(p13), +der(3) (3qter->3p12;;?;;14q21->14qter), del(4)(q32), der(5) t(5;13)(q10;q10), -6, -13, del(14)(q21), hsr(20)(q11), -21. Some of these chromosomal aberrations, such as trisomy 3q and partial deletion of 6q, have been previously described during the evolution of WM or multiple myeloma whereas 5q- is often observed in secondary myelodysplastic syndromes, mainly following chemotherapy. Homogeneously staining regions (hsr) have rarely been reported in leukemia and, to the best of our knowledge, only once in CMML.

P0180. Clonal chromosome aberrations in bone marrow cells of Fanconi anemia patients - conventional cytogenetic and molecular cytogenetic studies

H. T nnies[1], W. Ebell[2], H. Neitzel[1]
[1]Charit , Campus Virchow-Klinikum, Institute of Human Genetics; Berlin, Germany; [2]Charit , Campus Virchow-Klinikum; Berlin, Germany
Holger.Toennies@charite.de

Fanconi anemia (FA) is an autosomal resessive chromosome instability disorder characterized by a variety of congenital anomalies and a high incidence of bone marrow failure (aplastic anemia), as well as increased rate of malignancies, including leukemias and solid tumors. FA patients are considered preleukemic and the disorder presents a model for the study of the etiology of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). During bone marrow failure in FA-patients, clonal chromosomal aberrations are detectable in bone marrow cells of the patients. Today the prognostic value of the variety of this genomic imbalances is not known, however it seems that they differ to these found in non-FA patients with aplastic anemia, MDS, and AML. In our institute more than 50 FA-patients were diagnosed for FA. Most of these patients take part on a long term follow up including yearly conventional cytogenetic (CC) analysis of the bone marrow. In most of the cases, clonal numerical and structural chromosomal aberrations could be detected. Chromosome aberrations are further characterized in detail by molecular-cytogenetic techniques as fluorescence in situ hybridization (FISH), microdissection and comparative genomic hybridization (CGH). Additionally, for some patients, we were able to collect further data on karyotype evolution of different clones due to several bone marrow analyses. Main objective of our study is to uncover the origin of unknown additional chromosomal material and the detection of partial losses of chromosomal segments by different molecular cytogenetic strategies. The data collected gives us the opportunity to find possible links between chromosomal imbalances, bone marrow failure, and MDS/AML in FA patients.

P0181. In Situ Analysis of Telomeres in Haematological Malignancies

M. Sklenickova[1], K. Krejci[2], J. Malaska[3], J. Fajkus[4]
[1]Department of Analysis of Biologically Important Molecular Complexes, Masaryk University Brno; Brno, Czech Republic; [2]Institute of Biophysics, Academy of Sciences of the Czech Republic; Brno, Czech Republic; [3]Faculty Hospital Brno; Brno, Czech Republic; [4]Institute of Biophysics, Academy of Sciences of the Czech Republic, Department of Analysis of Biologi-

cally Important Molecular Complexes, Masaryk University; Brno, Czech Republic
sklemar@sci.muni.cz
Analysis of telomere length is performed in malignant cells, in which telomeres usually gradually shorten and are stabilized only upon activation of telomerase. In rare cases an extreme expansion or complete loss of individual telomere occurs. These individual differences cannot be detected using the most common technique, TRF (Southern hybridization of terminal restriction fragments), which is able to show only an average size of telomeres. Therefore an in situ labeling technique (dideoxy-PRINS) was used for detailed analysis of telomeres in childhood leukemia and multiple myeloma samples. PRINS utilizes an unlabelled probe (in this case Telo2; (CCCTAA)7), which hybridizes to its target seguence and serves as a primer for chain elongation using Tth DNA-polymerase to incorporate labeled nucleotides. Dideoxy PRINS is more specific for labeling sequences which lack one or more of the four bases of DNA; the base(s) can be added in the form of dideoxynucleotide(s). For evaluation of telomere dynamics, samples were also assayed for telomerase activity using TRAP assay (telomere repeat amplification protocol) and for expression of telomerase reverse transcriptase and RNA subunits using RT-PCR. In our presentation, we show examples of our recent results, e.g. observation of a single-telomere expansion (about 9-fold elongation with respect to the average length of all the other telomeres on a given metaphase) on chromosome 4, arm q in one of childhood leukemia samples. Besides, current results of telomere analysis of childhood leukemia and multiple myeloma are shown and discussed in relation to possible regulatory mechanisms of telomere maintenance.

P0182. Telomere Shortening In Patients With Chronic Lymphocytic Leukemia

S. Palanduz[1], *N. Serakinci*[2], *K. Cefle*[1], *M. Aktan*[3], *G. Tutkan*[1], *S. Ozturk*[1], *G. Dincol*[3], *Y. Pekcelen*[3], *J. Koch*[4]
[1]*Istanbul University, Istanbul Medical Faculty, Department of Internal Medicine, Division of Medical Genetics; Istanbul, Turkey;* [2]*Danish Cancer Society, Dept. Cytogenetics; Aarhus, Denmark;* [3]*Istanbul University, Istanbul Medical Faculty, Department of Internal Medicine, Division of Hematology; Istanbul, Turkey;* [4]*Danish Cancer Society; Aarhus, Denmark*
spalanduz@istanbul.edu.tr
Telomeric region of the chromosomes are involved in the maintenance of genomic integrity. Telomeres compensate for the chromosomal shortening as it happens after each round of cell division. Because of the dynamics of telomeric DNA replication, telomeres are thought to influence the progress of cellular senescence and cancer progression. Not unexpectedly, it has been reported that telomeres are shortened in acute leukemias where the cell turnover is high. B-cell chronic lymphocytic leukemia (CLL) is a particularly interesting haematological malignancy in regard to telomere dynamics because most of the malignant cells in CLL are mitotically inactive. In this study we aimed to evaluate the length of telomeric sequences in patients in B-cell CLL by the ddPRINS technique. Twenty patients with CLL (female/male; 5/15, mean age; 62.05–8.48) and four healthy donors lyomphocytes as our control group (female/male;1/2, mean age;60.03–12.05) were included. We found short telomers and no detectable telomeric repeats at the sites of chromosome fusion. In the patient group, we observed less intense telomeric signals than the control group. We are in the opinion that reduced telomeres in CLL reflects the dominance of malignant cells with an abnormally long life span. These cells may have encountered many antigenic stimulants in the past and hence underwent multipl clonal expansions. Our findings imply that shortened telomeres in CLL may be reflecting the history of the disease and serve as an independent prognostic factor.

P0183. Fine mapping of the episomal Epstein-Barr virus (EBV) on DNA fibers of the Burkitt s lymphoma cell line Daudi

J. Reisinger, *S. Rumpler, P. F. Ambros*
CCRI, St. Anna Kinderspital; Vienna, Austria
ambros@ccri.univie.ac.at
Background; Intracellular Epstein-Barr virus (EBV) exists as either stable integrated into the genome or as covalently closed episome circularized via the terminal repeats (TR). The integration of DNA viruses into the host genome is frequently associated with immortalisation and malignant transformation of human cells whereas episomal copies are not linked to tumor development.
Aim; As the two forms of viral persistence are associated with different biological features we wanted to work out a simple technique to discriminate both forms. We applied a DNA spreading technique to enable deeper insights into the organization of the virus to be able to clearly discriminate both forms.
Methods; DNA from the cell line Daudi was prepared according to the procedure of dynamic molecular combing. Double target FISH was applied using the W-fragment and different cosmid probes (kindly provided by Dr. G. W. Bornkamm) covering the whole genome of the virus. The cosmid covering both terminal repeats (TR) was double labeled with biotin and digoxygenin, thus allowing a simple identification among the rest of the viral genome. Results; We constantly observed a non interrupted hybridisation pattern of the double labeled cosmid spanning both TRs. In case of an integration of the viral genome into the host we would expect to see a separation of the hybridization signals. Thus, we interpret this picture as fusion of the two TR as it is the case in the episomal form.
Conclusion; The method applied allows a visualization and unambiguous identification of the episomal form of the EBV.

P0184. Rapid FISH analysis on native smears in haematology

B. Klein, *M. Gerusel-Bleck, M. F hrer, C. Bender-G tze, J. U. Walther*
Kinderklinik und Kinderpoliklinik der Universit t M nchen; M nchen, Germany
JU.Walther@kk-i.med.uni-muenchen.de
Minimal residual disease is an essential parameter for monitoring childhood leukaemia under therapy especially after stem cell transplantation (SCT). Detection of chromosomal markers by metaphase analysis fails in low grade mosaicism. Therefore we adopted a simple and fast FISH approach to detect numerical and structural aberrations in smears from peripheral blood, bone marrow and other body fluids. Clonality was interpreted according to a cut-off value specific for each category of anomalies established using healthy donors. In ALL, t(12;21) and classical hyperdiploidy had disappeared by day 33 in all three and four patients investigated, respectively.After HLA identical SCT for Ph-positive CML in two patients, the marker disappeared after 30 days in one, in the other it persisted for three years and disappeared only after immunotherapy (DLI). Monosomy 7 was found in five patients with AML/ MDS; three were successfully grafted from identical family donors with quick clearing of the monosomy; in one the clone finally disappeared after 2nd SCT. His slides were successfully hybridised and analysed after nine years of storage. The fifth was grafted from the haploidentical mother and only transiently lost the marker for 28 days. One infant with AUL and trisomy 8 grafted from his identical mother has remained free from trisomy for one year after SCT. In addition we detected small clones (<15%) with monosomy 7 (n=6) and trisomy 8 (n=1) in seven patients with acquired aplastic anaemia. Five of them progressed to overt MDS. In conclusion, FISH can be used for rapid monitoring of clonal markers - even in stored material and small clones - in childhood leukaemia and MDS.

P0185. Genetic and Functional Profiling of Disseminated Tumor Cells by a fully Automated Microscopical Device

P. F. Ambros[1], *G. Mehes*[1], *A. Luegmayr*[1], *A. Witt*[2], *E. Kubista*[2], *T. L rch*[3], *C. Hattinger*[1], *R. Ladenstein*[1], *H. Gadner*[1]
[1]*CCRI, St. Anna Kinderspital; Vienna, Austria;* [2]*Dept. of Special Gynecology, University of Vienna; Vienna, Austria;* [3]*MetaSystems; Altlussheim, Germany*
ambros@ccri.univie.ac.at
The detection, quantification as well as the genetic and functional characterization of disseminated tumor cells (DTC) present in the bone marrow (BM), peripheral blood (PB) and apheresis products (AP) are becoming increasingly significant in the treatment of patients with solid tumors both at diagnosis and during the course of cytotoxic treatment (MRD detection). To overcome limitations due to the current techniques and to enable an exact quantification, we have fully automated the screening and relocation procedure of immunological stained samples (RCDetect, MetaSystems). This device allows the simultaneous search for up to three fluorescence signals. The automatic relocation of immunolabeled cells enable sequential genetic (FISH) and also functional analyses of positive cells. With this approach, tumor-typical genetic aberrations such as translocations, gene amplifications or simple gains or losses can be identified allowing the unambiguous proof of the malignant nature of the questionable cell. The sensitivity of the system is only limited by the number of cells available for analysis. Data on quantitative and qualitative analysis of tumor cells carried out in over 700 clinical samples (BM, PB and AP) from neuroblastoma and breast carcinoma patients will be presented. In addition, functional analyses, e.g. proliferation capacity, and apoptotic rate was monitored in a large series of bone marrow samples with disseminated tumor cells. With the help of this computer assisted microscopic system the automatic search, quantification, as well as genetic and functional analysis of low tumor cell infiltrates in routine bone marrow preparations can be carried out efficiently and reliably.

P0186. Deficiency in a double strand break repair subpathway in ataxia telangiectasia during S phase promotes multiple chromosome exchanges; probands and obligate heterozygotes

J. C. Leonard, J. Schmidt, A. M. Mullinger, R. T. Johnson
Coriell Institute for Medical Research; Camden, NJ United States
leonard@umdnj.edu

A-T cells are chromosomally unstable, and are hypersensitive to ionizing radiation and other agents causing DNA double strand breaks (DSBs). Chromosome studies have suggested a defect in DSB repair, but direct measurements are less clear, and the underlying defect and basis for cell killing are uncertain. Here we use the topoisomerase I inhibitor camptothecin (CPT), which induces DSBs predominantly in replication forks, and show that A-T cells are defective in the repair of this subclass of DSBs. After CPT treatment, A-T cells pass to G2, where they remain; normal cells repair most of their DSBs and arrest briefly in G2. The frequency of CPT-induced chromosome aberrations in each of a wide variety of A-T cells examined, including different ATM gene mutations, is abnormally high; aberrations are S-phase-derived and many are multiple illegitimate chromatid exchanges. In normal cells the aberrations are mostly chromatid breaks. Data also suggest that obligate A-T heterozygotes show more aberrations than normal controls. Whole chromosome painting reveals the complexity and type of exchange aberrations. The data suggest that the A-T protein recognises DSBs in active replicons and targets homologous DSB repair there, thus helping to suppress illegitimate S-phase recombination.

P0187. CGH analysis of radiation induced meningioma

E. Rajcan-Separovic[1], J. Maguire[2], T. Loukianova[1], M. Nisha[1], D. Kalousek[1]
[1]BC Children s Hospital, Department of Pathology; Vancouver, Canada; [2]Vancouver General Hospital,Department of Anatomical Pathology; Vancouver, Canada
eseparovic@cw.bc.ca

Epidemiological studies of patients previously exposed to ionizing radiation suggest an increased risk for developing meningiomas. Genetic events underlying such tumorigenesis are unknown. Cytogenetic studies of radiation induced meningiomas (RIMs) are rare and so far discordant; one case was reported without, and one case with loss of chromosome 22, a change frequently associated with spontaneous meningiomas (SMs). Molecular genetic analysis using markers from chromosome 22q showed no LOH in the 15 RIMs studied. There are no comparative genomic hybridization (CGH) studies of RIMs so far. We used CGH to investigate the genetic basis of 7 RIMs. CGH analysis was done on paraffin embedded tissues in a blinded manner, including SMs and normal brain tissue. CGH analysis of three RIMs identified common losses of 1p, 7p and 18q. Losses of 2p, 3p, 6q, 10q,11q and 11p were present in at least one of the RIMs. Interestingly, 7p loss is infrequently observed in SMs and yet it was present in all of the 3 RIMs studied so far(analysis of the other 4 RIMs is in progress). CGH analysis of one SM demonstrated monosomy 22, loss of 1p, 6q,14,18q and gain of 1q. These changes are in accordance with common genetic changes previously identified by CGH in sporadic meningomas. The identification of RIM-specific genetic changes is of clinical importance since they often show more aggressive behavior with a high recurrence rate following therapy. The observation of distinct genetic characteristics in RIMs may also provide new insight into the mechanism of tumorigenesis.

P0188. Detection Of A Common Chromosomal Rearrangement In Seven Cases Of Radiation-induced Meningiomas

P. Roll[1], H. Zattara-Cannoni[1], M. Krahn[1], H. Dufour[2], G. Lena[2], A. M. Vagner-Capodano[1]
[1]Cytogenetic Oncology Laboratory, CHU Timone; Marseille, France; [2]Department of Neurosurgery, CHU Timone; Marseille, France
proll@wanadoo.fr

Exposure to ionizing radiations is known to increase cancer risk. Radiotherapy may also induce solid tumors. We report the cytogenetic study of 7 cases of radiation-induced meningiomas ; 2 cases following irradiation of children with tinea capitis, 1 case following radiotherapy of a glioma in a 6 months-old child, 1 case after radiotherapy of an astrocytoma WHO grade II at age 14 and finally 1 case of temporal angioma, 1 case of cranial pharyngioma and 1 case of medulloblastoma irradiated during childhood. The cytogenetic findings included complex karyotypes with numerical and structural abnormalities. All metaphases showed a der(1) and monosomy 22. Molecular cytogenetics using CGH and SKY technique permitted us to refine the karyotype findings ; del(1)(p11-pter) and del(22)(q12-qter) using CGH and translocation of a part of chromosome 22 on the 1p13-pcen region of chromosome 1 using SKY technique, der(1)(qter-1p11 ;; 22q12->22pter). The existence of complex chromosomal abnormalities stresses the atypic aspect and the radio-induced origin of these tumors. Deletion of the short arm of chromosome 1 is frequently observed in meningiomas, but with different breakpoints. The 1p11 region could be implicated in radiation induced meningiomas in particular. Further molecular investigations of this region would be of great interest.

P0189. Comparative genomic hybridization of 5 new medulloblastoma cell lines.

S. Dub [1,2], M. Muleris[3], R. Molist[3], F. Fortin[1], Y. Th or t[2], F. L veill [1,2], N. Lemieux[1,2,4]
[1]D partement de Pathologie et de biologie cellulaire, Universit de Montr al; Montr al, PQ Canada; [2]Centre de recherche de l H pital Sainte-Justine; Montreal, PQ Canada; [3]Institut Curie; Paris, France; [4]D partement de Pathologie de l H pital Sainte-Justine; Montr al, PQ Canada
sopdub@hotmail.com

Medulloblastomas, a primitive neuroectodermal tumor of the cerebellum, represents approximately 25% of all intracranial tumors in children. Several studies demonstrated numerous non-randomly chromosomal abnormalities. However, the specific oncogenic events involved in the development and progression of this tumor remain unknow. In this study, molecular cytogenetic approaches were performed to allow a more comprehensive cytogenetic evaluation of 5 new MB cell lines that we have previously established. Using comparative genomic hybridization (CGH), we detected changes in copy number (gains and losses) in our cell lines. The most recurrent chromosomal abnormalities found were gains of 3q, 4q, 7q, 8q, 15q, 17q and losses of 11q and 17p. Also, karyotypes and fluorescence in situ hybridization (FISH) were done and show multiple chromosomal rearrangements and polyploidy in accordance with the CGH results. These results reveal many clonal imbalances that had previously been reported and some novel changes such as 3q, 4q and 15q. We also show that these new cell lines are cytogenetically representative of the PNET/MB tumors and should be helpful for further understanding of the biology of these tumors. This study confirms many of the previously reported cytogenetic data such as losses of 11q and 17p and gains of 7q, 8q and 17q, and also reveals potentially important novel regions that could be implicated in the tumorigenic process of MBs. Additional studies are needed to furnish more insight on the roles played by the changes observed. This project is partially supported by the Centre de recherche de l H pital Sainte-Justine.

P0190. Molecular and Conventional Cytogenetics in Yugoslav Neuroblastoma Patients

M. M. Djurisic, M. Guc-Scekic, D. Radivojevic, T. Lalic, D. Djokic
Mother and Child Health Institute; Belgrade, Yugoslavia
makid@ptt.yu

Neuroblastoma (NB) is the most common extracranial solid tumor in children occurring at a rate of about 1 in 10.000 live births. This tumor is characterised by its unpredictable behavior; some neuroblastomas mature and regress spontaneously whereas others progress rapidly and agressively. Genetic alterations found in NB cells, beside clinical parameters and the age of patient, has a great significance in development and clinical course of the disease. Molecular and cytogenetic analyses of genetic determinants in NB cells have an object of establishing precise and prompt diagnosis of NB. Double coloured FISH were performed on tumor imprints with the probes for centromeric and telomeric region of chromosome 1. Also, markers D1S80 and D1S76 were analysed using PCR. Routine cytogenetic analysis were done on G banded chromosomes. Simultaneous combining of the molecular genetic methods such as FISH and PCR and classical cytogenetics will provide making quick, precise and prompt diagnosis of neuroblastoma.

P0191. Analysis of five different prognostic markers in neuroblastic tumors

C. Stock, I. M. Ambros, S. Rumpler, A. Luegmayr, R. Ladenstein, P. F. Ambros
CCRI, St. Anna Kinderspital; Vienna, Austria
ambros@ccri.univie.ac.at

Objectives; Established genetic factors of aggressive tumor growth in neuroblastomas are; MYCN amplification (NMA), deletions at 1p36.3, and a near diploid or near tetraploid DNA content. Recently, gain of 17q21-qter was described to be the most frequent prognostic marker. Another study, however, suggests that gain of 1q21-23 define a subset of NB with adverse outcome. We investigated all these prognostic markers in an attempt to correlate specific genetic changes and outcome.
Patients and methods; The tumor collection encompassed 14 primary tumors of all stages and three samples from patients under cytotoxic treatment. We analyzed the status of 1p36.3, 1q21-23, MYCN, and 17qter by

FISH using D1Z1, D1Z2, 955E11 (1q21), 910C8 (1q21), and 918H5 (1q23) (M. Rocchi, Italy), MYCN, D2Z, TP53, and 17q25 (probe 946e12, T. Haaf, MPIMG; Berlin, Germany).
Results; Among the 10 patients with localized/regional (stage 1, 2A, 2B, and 3) or 4s disease, all in complete remission, we observed none of the chromosomal anomalies mentioned above except two cases exhibiting gain of 17q (totally or sub-totally resected tumors). All stage 4 tumors (7 patients) displayed at least one of the prognostic markers; two tumors from patients with fatal outcome exhibited gain of 17q as sole genetic aberration, three tumors (patients in partial remission) displayed NMA, 1p36 deletion, and 17q gain, and two tumors from patients with fatal outcome were positive for all analyzed prognostic markers.
Conclusion; Preliminary results suggest that the simultaneous presence of all genetic aberrations define a subset of NB with extremely unfavorable prognosis.

P0192. Cytogenetic Studies In Human Carcinoma Esophagus

S. M. A. N. Gupta
University of Jammu; Jammu, INdia, India
lalitsen@yahoo.com

Cytogenetic finding in 30 carcinoma eso pts. Carcinoma esphagus , the cancer of food pipe forms an important class of cancers. It ranks sixth in ts ncidence in the world and makes up about 10% of G.I.T. cancers. Besides other clinical symptoms, dysphagia and epigastric pain are two main symptoms that makes endoscopic study a must. A total of 40 pts complaining of dysphagia with or without epigastric pain were subjected to endoscopy. 10 patients were found to have normal esophagus while 30 patients showed a growth in their food pipe. Biopsis form of growths in all 30 cases were collected for histopathology and cytogenetic study. Majority of these growths were histopathologically confirned to have cancerous growth. Cytogenetic study carried out in the biopsis collected for this purpose contained different kinds chromosomal changes. Centromere spreading, centromere stretching fragmentation, ring formation and sickness were the main structural changes. Most of the aneuploid cells contained a Marker chromosome. Polyploid cells had circular arrangement of the chromosomes with a hollow space in the centre. Attempts have been made to find the etiology of cancerous esophagus by studying the socio-economic conditions and other related environmental factors.

P0193. Characterization of the colon cell line HT29 clone 19A by means of GTG-banding, 24-color FISH and multicolor banding (MCB)

A. K chler[1], *S. Michel*[1], *B. Pool-Zobel*[2], *A. Sch ferhenrich*[2], *A. Heller*[1], *H. Starke*[1], *V. Trifonov*[3], *N. Rubtsov*[3], *U. Claussen*[1], *T. Liehr*[1]
[1]*Institute of Human Genetics and Anthropology; Jena, Germany;* [2]*Department of Nutritional Toxicology, Institute of Nutrition; Jena, Germany;* [3]*Insitute of Cytology and Genetics; Novosibirsk, Russian Federation*
almu@mti-n.uni-jena.de

The human colorectal adenocarcinoma cell line HT29 was established in 1964 and its subclone 19A was terminally differentiated with 5mM sodium butyrate in 1984. However, it was never karyotyped in detail before, even though used in a variety of mutagenesis experiments. GTG-banding revealed that the cell line was hyperdiploid (66-68 chromosomes) and a number of marker chromosomes were present. For further characterization of the chromosomal rearrangements 24-color flourescence in situ hybridization (FISH) experiments have been performed. A composite karyotype was determined as follows;
64~69,XX,+del(Xp),+1,+der(1)t(1;11;16),+2,+der(2)t(1;2),+der(3)ins(3;12),+der(4)t(2;4),+5,+del(5q),+7,+7,8,+dup(8),+del(9),+der(9)t(6;9;X;9),+10,+11,+11,+del(11p),+del(11q),+12,13,13,+i(13q),+i(13q),+der(13)t(5;i(13q)),+15,+16,+17,+del(18),-19,+del(19),+der(19)t(5;19),+der(19)t(17;19),+20,+20,+22,+22 [cp10].
Moreover, 32 non-clonal aberrations have been observed in the 10 analyzed metaphase spreads. Multicolor banding studies on selected chromosomes (e.g. #8, #13) are in progress. In the present case HT29 clone 19A was described earlier as terminally differentiated due to the shape of the cells in culture, implicating an at least almost normal karyotype, which could not be confirmed. Thus, to clarify if a cell line is suited for molecular genetic approaches cytogenetics should be performed before. Acknowledgments; Supported by the Herbert Quandt Stiftung der VARTA AG, the Madeleine Schickedanz-Kinderkrebs-Stiftung, the DAAD, the DFG and the Wilhelm Sander-Stiftung.

P0194. Spectral karyotyping of the human colorectal carcinoma cell lines HT-29 and CX-1

S. Koehler[1], *H. Luehrs*[2], *T. Menzel*[2], *W. Scheppach*[2], *C. Steinlein*[1], *M. Schmid*[1], *R. Melcher*[2]
[1]*Institute of human genetics; Wuerzburg, Germany;* [2]*Department of Medicine; Wuerzburg, Germany*
sigridkoehler@web.de

Knowledge of the specific genetic changes in colorectal cancer could be important for prognosis and therapy. HT-29 and CX-1 are commonly used cell lines for in-vitro studies of colorectal cancer. In the present study the chromosomal alterations of HT-29 and CX-1 were analysed by spectral karyotyping (SKY) in order to clarify the complex translocations, that could not been detected by standard banding techniques. Spectral karyotyping (SKY) is a new sensitive method to detect chromosomal aberations by classifying each chromosome in different colors. The application of SKY allowed detailed analysis of marker chromosomes in the colorectal cells lines HT-29 and CX-1. We identified common gains (chromosomes 7, 11, 15 , and 20) that were amplified about four times or more in both cell lines. By comparing the two cell lines five similar structural alterations were found; del(4)(q31), i(8q), i(13q), der(17)t(17;19)(p10;q10), and del (18)(q21). Because of this similarity a fingerprint analysis was performed and revealed no differences in the banding pattern between both cell lines. Most of the structural and numerical aberration found in HT-29 and CX-1 were identical. A fingerprint analysis revealed a common genetic origin. Obviously both cell lines were derived from the same patient. This results have important implications for past and present studies on these colorectal cell lines, because to date these cell lines were thought to be of different genetic origin.

P0195. Mutagen-induced Chromosome Lesions in Peripheral Blood of Filipino Jeepney Drivers; Evaluation of Cancer Susceptibility

M. L. D. Enriquez[1,2], *I. Q. Postor*[3], *C. R. Cheng*[2], *G. L. Hamoy*[2], *D. R. Santos*[1], *F. F. Natividad*[1,3]
[1]*Research and Biotechnology Division, St. Luke s Medical Center; Quezon City, Philippines;* [2]*Physics Dept., College of Science, De La Salle University; Manila, Philippines;* [3]*College of Science , University of the Philippines; Quezon City, Philippines*
mldenriquez@stluke.com.ph

Drivers of public utility vehicles particularly jeepneys which are not air- conditioned are constantly exposed to the hazards of pollution. In this study, jeepney drivers were chosen to provide preliminary data on the possible effects of this exposure on the sensitivity of chromosomes. Preliminary data on the mutagen sensitivity of chromosomes from peripheral blood lymphocytes of thirty five (35) Filipino jeepney drivers are presented. The peripheral blood samples were cultured following the routine 72-hour microculture technique. Five hours before harvest the cells were exposed to bleomycin, a radiomimetic agent. Responses to the clastogenic effects of the mutagen expressed as average number of chromatid lesions or breaks per cell (b/c) were noted. The mean b/c in the drivers group is 1.46 while that of the control group is 0.75. Results also show that 77.14% of the drivers showed a b/c value higher than 1.0 which is established as the borderline for mutagen sensitivity. Higher than this value is taken as hypersensitive to the effects of bleomycin. In the control group, only 22.8 % have a b/c value higher than 1.0. If sensitivity to mutagens is considered an indirect measure of DNA repair capacity, these drivers of jeepneys are at high risk of acquiring environment-induced cancer.

P0196. Changes in the sex chromosomes in male breast cancer patients

C. Hanson[1], *T. Gerdes*[2], *M. Kirchhoff*[2], *I. K pf*[3], *H. Rose*[2], *B. Weijdeg rd*[1], *A. Weimarck*[3], *Y. Wettergren*[4], *R. Hultborn*[3]
[1]*Department of Obstetrics and Gynecology, Sahlgrenska University Hospital; G teborg, Sweden;* [2]*Chromosome Laboratory, Department of Clinical Genetics, Juliane Marie Center, Rigshospitalet; Copenhagen, Denmark;* [3]*Department of Oncology, Sahlgrenska University Hospital; G teborg, Sweden;* [4]*Department of Surgery, Oncolog lab, Sahlgrens University Hospital/ S; G teborg, Sweden*
charles.hanson@obgyn.gu.se

Following a previous study of male breast cancer (MBC) (Hultborn et al, 1997) performed on archival paraffin-embedded tissue, we started an investigation applying the high resolution comparative genomic hybridization (CGH) technique on fresh material (lymphocytes) from MBC patients. Here we report results from the study of eight men being under current follow-up at the Department of Oncology, Sahlgrenska University Hospital, G teborg, Sweden. The aim of the study was to disclose suspected minor genomic gains and losses, specifically in X- and Y-chromosomes, in male

breast cancer patients. One patient turned out to be a Klinefelter (47, XXY) male. Finding one XXY male among such a small group of MBC patients supports our finding of a closer association between MBC and Klinefelter s syndrome (Hultborn et al, 1997). The aberrations in the Y chromosome of patients Nos 2, 7 and 8 seemed to be deletions in the same Y q-arm region. Further, two patients (Nos 3 and 5) seemed to have amplifications in this same Y q-arm region as well as in the Y p-arm. We hypothesise that changes in the Y chromosome may be a risk factor for male breast cancer. Result of CGH-analysis of the X and Y chromosome arms in males with breast cancer

Patient No	Chromosome X (+ amplif, -del)	Chromosome Y (+ amplif, -del)
1	---	---
2	---	p-, q-
3	---	p+, q+
4	---	---
5	---	p+, q+
6	---	---
7	XX	p+, q-
8	p+, q+	q-

P0197. Constitutional chromosomal rearrangements of 9p23-24 in BRCA2 mutation carriers

***L. Savelyeva*[1]**, *A. Claas*[1], *I. Matzner*[1], *P. Schlag*[2], *W. Hofmann*[3], *S. Scherneck*[3], *B. Weber*[4], *M. Schwab*[1]

[1]*German Cancer Research Center; Heidelberg, Germany;* [2]*Robert-R ssle Klinik; Berlin-Buch, Germany;* [3]*Max-Delbr ck-Centrum for Molecular Medicine; Berlin-Buch, Germany;* [4]*University of Pennsylvania; Philadelphia, United States*

L.Savelyeva@dkfz-heidelberg.de

Germ line mutations of the BRCA2 gene account for a large proportion of familial breast cancer cases in females and the majority of familial breast cancer in males. Recent studies provide evidence for a role of the BRCA2 protein in the maintenance of genomic integrity by involvement in DNA repair and recombination. In pursuit of identifying in humans genetic damage resulting from mutated BRCA2 we have analyzed constitutional karyotypes of BRCA2 mutation carriers. FISH analysis from lymphocytes of patients of breast cancer families with germ line BRCA2 mutation revealed additional constitutional chromosomal alterations on 9p23-24. The rearrangements obseved include inversions, duplications and amplifications. Additionally, a high level of random somatic chromosomal abnormalities on 9p23-24 has been shown. The 9p rearrangements are complex in all families analysed showing that this chromosomal region has suffered a number of intrachromosomal recombinations. The topography of the 9p rearrangements can differ among family members, even within an individual that can have cell populations with different 9p rearrangements. Collectively these results raise point to an association of mutant BRCA2 with genomic instability and gene alteration in 9p23-24 in at least a subset of BRCA2 mutation carriers.

P0198. Copy Number Changes In Breast Tumors; Preliminary Findings

***S. Artan*[1]**, *U. Oner*[2], *M. Muslumanoglu*[1], *S. Isiksoy*[2], *H. Ozon*[1], *B. Yasar*[3], *H. Kiper*[3], *O. Cilingir*[1], *N. Basaran*[1]

[1]*Osmangazi University, Medical Faculty, Department of Medical Genetics; Eskisehir, Turkey;* [2]*Osmangazi University, Medical Faculty, Department of Pathology; Eskisehir, Turkey;* [3]*Osmangazi University, Medical Faculty, Department of Surgery; Eskisehir, Turkey*

sartan_61@yahoo.com

Breast cancer is one of the carcinomas that the extensive chromosome aberrations involve in tumor development. Analysis of chromosome abnormalities in solid tumors by the CGH has gained much importance since it is more sensitive than cytogenetics in respect to analysis of DNA gains and losses. Purpose ; Screening of breast tumors for copy number changes and comparing them with pathologic grades for tumor progression. Materials/Methods ; DNA samples from 11 ductal and 4 lobular carcinomas were analysed. The tumor and control DNAs were differentially labelled by the PCR labelling technique. Results ; Our preliminary results revealed a substantial degree of overlap with the previous published reports. However, 2 of 11 ductal carcinomas showed 5q13.3-q21 amplification and 2 of 4 lobular ones revealed 5p gains and 16q21-q23 deletion. Of 15 analysed samples, 8 (53%) showed various copy number changes. The correlation between the genetic changes and pathologic grades were discussed. Conclusion ; Gains and losses found by CGH are especially valuable because of determination of mechanisms and the effective genes involved in breast tumor development and progression.

P0199. A simple specific pattern of chromosomal aberrations at early stages of head and neck squamous cell carcinomas; PIK3CA but not p63 gene as a likely target of 3q26-qter gains

***R. R. Redon*[1]**, *T. Hussenet*[1], *D. Muller*[2], *J. Abecassis*[2], *S. P. du Manoir*[1]

[1]*IGBMC, CNRS/INSERM/ULP; Illkirch, France;* [2]*Centre Paul Strauss; Strasbourg, France*

redon@igbmc.u-strasbg.fr

By CGH analysis of 21 low-grade, node and metastasis negative HNSCC, we showed a simple and specific pattern of aberrations for pT1-2 but not pT3 tumors, with frequent gains on the long arm of chromosome 3 (found in 67% of cases), involving systematically the 3q26-qter area but with two alternative smallest region overlaps at 3q26 and 3q28-qter. First, to evaluate the relative contribution of two outstanding positional and functional candidate oncogenes, PIK3CA at 3q26 and p63 at 3q28, in the pathogenesis of HNSCC displaying a 3q gain, we measured their respective transcription levels in tumors with previously determined gene copy number. DNp63, the predominant p63 transcript, is overexpressed in tumors compared to normal tissues, but its expression level is independent to gene copy number. In contrast, a significant PIK3CA overexpression is associated with increased gene dosage. These results indicate that PIK3CA, contrary to DNp63, may participate to the progression of head and neck tumors consequently to a low-level 3q over-representation Second, we selected, by CGH and FISH on interphase nuclei, tumors exhibiting localized gains or amplifications in the 3q26-qter region. Analysis of these tumors by high resolution CGH on a BAC array dedicated to chromosome 3 will refine the mapping of 3q gains in head and neck cancer.

P0200. Analysis of Aneuploidy Frequency in Washing and Bioptic Samples of Blader Transitional Cell Carcinoma by FISH Technique

***M. Kaynak*[1]**, *M. Kilin* [2], *M. S. Yildirim*[1], *H. Acar*[1], *A. Cenker*[2]

[1]*Department of Medical Genetics, Selcuk University, Medical Faculty; Konya, Turkey;* [2]*Department of Urology, Selcuk University, Medical Faculty; Konya, Turkey*

murat.kaynak@turk.net

Blandder transitional cell carcinomas are heterogenous groups of tumors in biological and behavior terms. Most of them are diagnosed at the first presentation as low grade and superficial, but about two-thirds of patients present recurrences of which 10-25% will be of a higher stage. Karyotyping of tumors have been demonstrated to be an important prognostic index of cancers. However, accurate karyotype analysis of solid tumors is frequently insuficient by few number of recognizable metaphases. The detection of numerical chromosomal aberrations on interphase nuclei by fluoresence in situ hybridization (FISH) has been evidence to be important tool for bladder cancer. In this study, we performed FISH analysis with chromosome specific probes to detect numerical chromosome 8 and 11 aberations on washing and bioptic samples of bladder transitional cell carcinoma. The result showed that chromosomes 8 and 11 aneuploidies were increased. The incidence of aneuploidy increased in the paralel of the tumour stage.

P0201. The MN test in urothelial cells of cervix cancer patients

G. Ghandi, *P. Sharma*

Department of Human Genetics, Guru Nanak Dev University; Amritsar, India

baurs@glide.net.in

Cervix cancer is a very frequent cancer among women especially in developing countries while reduction in its incidence in the developed world has been reported through various mass screening approaches because of the possibility of treatment on its early detection.Urine samples of just- diagnosed cervix cancer patients (n=25; age-range 21-75 yr) and of those with other gynaecological problems (n=25; age-range 21-70 yr) , were processed for the MN test . The patients had married at an early age (11-15 yr) and had more number of abortions (n=21; 19 spontaneous and 2 induced) as compared to those in the controls (n=16; 5 spontaneous, 11 induced). The total number of pregnancies were 127 and 93 in the patients and control groups, respectively. The cancer patients had mostly (n=19) low socioeconomic status while there were more control individuals (n=14) belonging to middle socioeconomic status. An elevated frequency of cells with micronuclei was observed in 72% of the cancer patients which with the Student s t-test was significantly high (0.359–0.058) as compared to that in control ones(16.7%, 0.058–0.066). There were 8 individuals each in stages I and II and 9 in stage III with the highest frequency of MNd cells being observed in stage III cancer patients though statistical significant results were also observed when damage in stages I and II were compared with the control data. Highest frequency of cells with micronuclei was observed in patients married in the age range of 11-15 years, though the

damage in the other age-at-marriage groups was also significant. Maximum damage was also observed in 51-60 yrs age group though data in the different age groups when compared to their parallel and total age groups was significantly elevated too. The incidence of MNd cells was highest in the group with 4-6 number of pregnancies in the cancer patients but was also significant as compared to that in the parallel control groups. Significant results were also obtained on comparing the frequency of MNd cells in both, the low and middle SES groups with their parallel controls. The results of the present study for the MNT in urothelial cells have revealed an elevated frequency of cytogenetic damage in cervix cancer patients even in a tissue which is not the site where the cancer has developed.

P0202. Late Cytogenetic Changes after X-ray Treatment of Malignancy

J. Koblik
Department of Clinical Genetics, General Hospital, NsP; Spisska Nova Ves, Slovakia
nspspis@nextra.sk

A 30 year old man with azoospermia was routinely investigated in the Department of Clinical Genetics in our hospital. His karyotype was analysed after PHA stimulation of lymphocytes following short term cultivation of peripheral blood. Taking his history, it was ascertained that in childhood. Hodkgkin s disease had been confirmed histologically. He began treatm ent at 7 years, receiving several series of combined cytostatic treatnents supplemented with X ray therapy. The patient s history is summarised in table l./on the poster/. For the last l4 years, the patient has felt very well. He is not taking medication and he has no problems with the primary disease. He is examined by an oncologist twice a year/including scintigraphy/with negative findings. He has no complications,except for azoospermia. The karyotype was analysed using standard methods and chromosomes were stained by Giemsa, following G banding by the trypsin method. Chromosomal aberrations were detected in about 20% of the analysed mitoses and inclited neuploidy, monosomy and polysomy of the autosomes, balanced and unbalanced translocations, newly derived marker chromosomes, breaks, dicentric chromosomes, acentric fragments and other changes. Pictures of them are demonstrated on the poster.Three explanations can be proposed for these findings, which require further investigations. /a/the long term effect of X ray irradiation in childhood, interfering with the stem cells of the bone marrow, /b/ manifestation of the onset of secondary malignancy /leukemia?/ not yet presenting symptomatically,or /c/ Persistence of the primary disease. Patient s history of the disease is documented on the poster.

P0203. Characterization of secondary genomic changes in pancreatic tumors in transgenic TGFalpha/p53+/- and TGFalpha/p53-/- mice - a murine tumor progression model for pancreatic cancer in human

B. Schreiner[1], *U. Zechner*[2], *F. Greten*[2], *M. Wagner*[2], *S. Koschnik*[2], *C. Weber*[2], *W. Deppert*[3], *H. Kern*[4], *G. Adler*[2], *H. Hameister*[1], *R. Schmid*[2]
[1]*University of Ulm, Department of Human Genetics; Ulm, Germany;* [2]*University of Ulm, Department of Internal Medicine I; Ulm, Germany;* [3]*Universitiy of Hamburg, Heinrich Pette Institute for Experimental Virology and Immunology; Hamburg, Germany;* [4]*University of Marburg, Department of Cytobiology and Cytopathology; Marburg, Germany*
bettina.schreiner@medizin.uni-ulm.de

Transgenic mice overexpressing transforming growth-factor alpha (TGF-alpha) in exocrine pancreas show a transdifferentiation of acinar cells to duct-like cells. These cells form tubular structures, express ductal markers and develop pancreatic cancer with long latency and low incidence. As expected the Ras/Erk-pathway is activated in premalignant lesions in TGFalpha transgenic mice. To investigate the effect of p53 inactivation on tumor susceptibility, TGFalpha-transgenic mice were cross bred with heterocygous p53 knock out mice, to generate TGFalpha/p53+/- and TGFalpha/p53-/- mice. By p53 inactivation tumor formation is accelerated. TGFalpha/p53+/+ mice develop pancreatic tumors on average by day 360, TGFalpha/p53+/- by day 265 and TGFalpha/p53-/- by day 100 (TGFalpha/p53+/+ n=32, TGFalpha/p53+/- n=41, TGFalpha/p53-/- n=11). To identify recurrent, secondary chromosomal imbalances, pancreatic carcinomas were analyzed by comparative genomic hybridization (CGH) and by LOH using different markers. Tumors in TGFalpha/p53+/- mice segregate the wildtype p53 allel and acquire homozygous deletion of the p16INK4a / p19INK4a/ARF locus. CGH screening of these tumors revealed several gains and losses. The most common overrepresentations were observed on chromosome 11 proximal and on chromosome 15 distal and underrepresentations on chromosome 11 distal and on chromosome 14. In contrast to human pancreatic adenocarcinoma the total number of aberrations in the mouse tumors is low. But distinct genomic loci become affected. The increased copy number of some candidate genes on chromosome 11 and 15 was measured by quantitaive realtime PCR. We found a variable degree of amplification on chromosome 11 for Egfr, c-Rel and Stk10 and on chromosome 15 for c-myc. Further, cell lines were established from different tumors.

P0204. Cancer Incidence in High Background Radiation Areas; Facts Versus Fear

J. Mortazavi[1,2]
[1]*Iranian Nuclear Regulatory Authority; Tehran, Islamic Republic of Iran;* [2]*Rafsanjan University of Medical Sciences; Rafsanjan, Islamic Republic of Iran*
radioadaptive@angelfire.com

It has been reported that upon reaching a certain level of cell damage the production of repair enzymes is triggered which decrease the chromosome aberrations (Pohl-Ruling 1990, 1991). If so, prolonged exposure to high levels of natural radiation in areas with elevated levels of background radiation could decrease the frequency of chromosome aberrations. There are reports indicating that increased levels of chromosome aberrations in lymphocytes can be used to predict cancer risk in humans (Hagmar 2000,1998). One may conclude that a dose of ionizing radiation sufficient to produce a certain level of cell damage increases production of antioxidants and repair enzymes that decrease either the frequency of chromosome aberrations or the cancer risk. People in some areas of Ramsar, a city in northern Iran, receive an annual radiation dose from background radiation that is more than five times higher than the 20 mSv yr-1 that is permitted for radiation workers. Inhabitants of Ramsar have lived for many generations in these high background areas. If an annual radiation dose of a few hundred mSv is detrimental to health, causing genetic abnormalities or an increased risk of cancer, it should be evident in these people. Our cytogenetic studies show no significant differences between people in the high background compared to people in normal background areas. As there was no increased levels of chromosome aberrations, it may be predicted that the cancer incidence is not higher than the neighboring areas with normal background radiation level. Although there is not yet solid epidemiological information, most local physicians in Ramsar report anecdotally there is no increase in the incidence rates of cancer or leukemia in their area. There are no data to indicate a significant increase of cancer incidence in other high background radiation areas (HBRAs). Furthermore, several studies show a significant decrease of cancer death rates in areas with high backgrounds. To test for adaptive response, an in vitro challenge dose of 1.5 Gy of gamma rays was administered to the lymphocytes. Lymphocytes of Ramsar residents showed significantly reduced radiation sensitivity for chromosome aberrations compared to those of residents in normal background areas. Specifically, following this exposure inhabitants of HBRAs had about 56% the average number of induced chromosomal abnormalities of those in normal background radiation area (NBRA). These findings suggest that adaptive response can be induced by chronic exposure to radiation at levels lower than have been used in the laboratory. Given the apparent lack of ill effects in the populations of these high dose rate areas, these data further suggest that current dose limits are overly conservative. It can be concluded that prolonged exposure to high levels of natural radiation possibly triggers processes such as the production of antioxidants and repair enzymes, which decreases the frequency of chromosome aberrations and the cancer incidence rate.

P0205. Using cytogenetics methods in differential diagnostics of urothelial neoplasms

I. A. Bushkova, *G. E. Plugnikova*
Rostov Regional Institute of Pathology, Department of Experimental Pathology; Rostov-on-Don, Russian Federation
matsionis@aaanet.ru

Malignant urothelial neoplasms occupy the second place on frequency and on lethality among neoplasias of a bludder. The favourable location of the latter allows to utillize in treatment a local chemotherapy and immunotherapy, however, not always it is possible to reach desirable outcome. Thus, basing on histological research it is impossible to predict behaviour of a tumour - whether this tumor will be invasive, whether this invasive tumour will metastasize. A reason is hidden in heterogeneity of a tumour. The last researches in the field of molecular genetics have allowed closely to be approximated to solution of these problems. As is known, the neoplasia is accompanied by genom changes of malignant cells. Researches indicating link of a biological potential of tumours with particular violations DNA already have appeared. So, series of the authors bind development of a cancer of the bludder to deletions in particular districts of chromosomes 9p, 13q and 17p. Thus mapping here genes-supressors, such as p16

(encodes inhibitors of cyclin-dependent kinase), Rb (gene of a susceptibility to a retinoblastoma), p53 - so-called Lthe genom watchdogı- are exposed to mutations. These genes participate in a regulation of cell cycle, not admitting a cell with DNA damages to division. It was detected that the p16 deletion is a marker for a squamous metaplasia with greatest progressiveness. The mutations of p53 gene are correlated with a degree, stage and presence of a vascular invasion. The violations in Rb gene are authentically influence on a survival rate of the patients.
And though the proteins, products of pathological genes, are well detected by immunohistochemistry methods, it does not allow to reveal heterogeneity of a neoplasia. We have concentrated the researches on attempt to prove genetic heterogeneity of non-papillary cancer (primary carcinomas in situ) and secondary centers of carcinoma in situ at preexisting papillary tumour, as it is known, that the prognosis of current non-papillary cancer is much worse and it practically is not diagnosticated at cytoskopy. Knowledges about heterogeneity of these groups of urothelial tumours will give possibilities of development various protocols of treatment for them.

P0206. Interphase FISH for evaluation of minimal residual disease (MRD) in CML patients

R. Talwar[1], *K. Kucheria*[1], *V. Jobanputra*[1], *V. P. Choudhry*[2]
[1]*Department of Anatomy, All India Institute of Medical Sciences; New Delhi, India;* [2]*Department of Haematology, All India Institute of Medical Sciences; New Delhi, India*
vjobanputra@hotmail.com

Chronic Myeloid Leukemia (CML) is a clonal hematopoietic malignancy that progresses from a benign chronic phase to an ill-defined accelerated phase and subsequently blast crisis. Among the various drugs administered to CML patients, Interferon-alpha(IFN) is gaining importance due to its ability to induce cytogenetic response unlike the other myelo-suppressive drugs like Busulphan (BU), Myleran, Hydroxyurea (HU) etc. The present study was conducted in our laboratory to assess the response to IFN therapy in the Philadelphia (Ph«) positive CML patients by sequential cytogenetic analysis and to evaluate the importance of employing FISH as an adjunct procedure in evaluation of minimal residual disease (MRD). Cytogenetic analysis using standard protocols was carried out in 210 patients (including 12 in blast crisis). Out of these, 185 patients (167 Ph+ and 18 Ph-) were studied following presentations with clinical features of CML while 25 cases (MPDs, acute leukemias and leukemoid reaction) were analysed to exclude the possibility of having Ph translocation, t(9;22)(q34;q11). Further dual colour FISH using specific probes for bcr and abl genes was done in 28 patients and the results were compared with those of conventional cytogenetics. Standard Ph translocation was observed in 167 cases (89 were 100% Ph+ and the rest were mosaics ranging from 50-90%). One of the mosaic cases revealed the presence of double Ph translocation in 23% of metaphases. Thirty-six Ph+ patients on Interferon (IFN) therapy were followed up at intervals of 4 months till 3 years. Sequential cytogenetic analysis and FISH was done in these cases at every follow up. Twelve patients showed cytogenetic response after 12 months, 7 after 16 months, 3 after 20 months, 1 after 24 months and 1 after 32 months of IFN therapy. In all, varied degrees of cytogenetic response was observed- complete response (0% Ph+ cells) in 2 cases, partial response (1% to 35% Ph +cells) in 1 case, minor response (35% to 90% Ph+ cells) in 21 patients and no response (Ph+ cells >90%) in 14 cases. These results show that IFN therapy can suppress the Ph+ clone and reverse the bone marrow karyotype to normal in a significant number of patients. On comparing the results of cases analysed by FISH, in 25 cases the results were same as those obtained by cytogenetics. However 3 cases which were Ph-negative on karyotyping showed positive bcr/abl fusion signals on metaphase spreads and interphase nuclei. The results indicates that FISH is a highly sensitive and efficient tool for detecting even low levels of abnormal cells that appear Ph -negative by cytogenetics. The study shows the importance of using FISH for detecting response at the molecular level in CML patients on therapy and demonstrates the efficiency of FISH in detecting abnormal clone of small size.

P0207. Multicolor banding (MCB) of all human chromosomes based on region specific microdissection libraries

T. Liehr[1], *A. Heller*[1], *H. Starke*[1], *A. Weise*[1], *K. Mrasek*[1], *V. Trifonov*[2], *N. Rubtsov*[2], *U. Claussen*[1]
[1]*Institute of Human Genetics and Anthropology; Jena, Germany;* [2]*Institute of Cytology and Genetics; Novosibirsk, Russian Federation*
i8lith@mti-n.mti.uni-jena.de

The multicolor-banding (MCB) technique, allowing the differentiation of chromosome region specific areas at the band and sub-band level, 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. We present for the first time the complete set of appr. 160 regionspecific microdissection libraries covering the entire human genome and the resulting MCB patterns for all human chromosomes at the 550 band level. Clinical cases with congenital or acquired complex chromosomal rearrangements involving different chromosomes are presented. In some of these cases neither conventional GTG-banding nor 24-color-FISH could resolve the complex changes, e.g. interstitial deletions, insertions or inverted insertion. Using this new and straight forward MCB technique, complex rearrangements could be clarified with one single flourescence in situ hybridization (FISH) experiment. Acknowledgments; This work was supported by the Herbert Quandt Stiftung der VARTA AG, the Madeleine Schickedanz-Kinderkrebs-Stiftung, the DAAD, the DFG and the Wilhelm Sander-Stiftung.

P0208. Microdissection of spectral karyotyped Chromosomes; The new FISH-MD technique offers the most accurate karyotyping under SKY.

J. Weimer, *N. Arnold*
Universit ts-Frauenklinik Kiel; Kiel, Germany
joerg.weimer@t-online.de

A technique disclosing most information about chromosome modifications is the technique of choice for analysis of chromosome alterations. The high efficient way to detect chromosome modifications by spectral karyotyping (SKY) and their detailed specification with microdissection of chromosomes labeled by fluorescense paints (FISH-MD) incited us to combine both techniques to a new united technique called SKY-MD. This new technique gives an overview of derivative chromosomes by spectral karyotyping first and identifies such changed chromosomes through following microdissection in an united procedure. Reverse painting identifies the involved chromosome regions including the breakpoints of such microdissected chromosomes. SKY-MD was successfully applied on a prenatal case and unveils an invisible cryptic aberration.

P0209. Technical improvements for ultrastructure analyses on human chromosomes in the interphase and mitosis of the cell cycle with the laser scanning microscop LSM 510

V. Beensen[1], *T. Liehr*[1], *A. Heller*[1], *H. Starke*[1], *M. Wiederhold*[2], *P. M hlig*[3], *P. Ullmann*[4]
[1]*Institute of Human Genetics and Anthropology; Jena, Germany;* [2]*Clinics of Anesthesiology and Critical Care; Jena, Germany;* [3]*Institute of Molecular Biotechnology e.V.; Jena, Germany;* [4]*Carl Zeiss Jena GmbH; Jena, Germany*
vbee@mti-n.mti.uni-jena.de

The ultrastructure of human chromosomes and their structural and functional organization in the interphase nucleus are still largely unknown. Their exploration would be of great scientific interest. The simultaneous presentation of fluorochromed chromosomes in combination with fluorescence-immunohistochemical presentations of the cytoskeleton provides an innovative methodical approach to the study and solution of the open questions. For the simultaneous presentation of the cytoskeleton proteins with primary and secondary antibodies (antibodies labeled with FITC and Rhodamine) and metaphase chromosomes (amniocyte preparation; in situ technique; 4,6-diamidino-2-phenylindole - DAPI - staining), the common methanol-acetic acid fixation (3;1) was replaced with a fixing pretreatment of the cells with a mixture of triton X-100 (2%), formaldehyde (2%) and glutaraldehyde (0.5%). This kind of specimen-preserving pretreatment does not cause any visible spreading of chromosomes in the form of metaphase plates. Fluorochroming of individual chromosomes or chromosome regions (multicolor FISH) was performed after a method described by RUBTSOV et al. (Hum. Genet. 97; 705-709, 1996). An efficient analysis of specimens prepared by the new object-preserving method described is made possible especially by the capability for simultaneous excitation of all fluorochromic dyes used (including DAPI, FITC, Rhodamine) and the acquisition and analysis of data in three dimensions. For instance in the metaphase stage compared with an interphase nucleus a quantitative increase in the tubuline and tau-protein concentration can be correlated to the increase of chromosomal DNA concentration. In the case of simultaneous tested §-actin such a quantitative increase was not found. The obtained data allow conclusions to be drawn on the intracellular, intranuclear and intra-chromosomal localization of elements of the cytoskeleton (e.g. tubuline and tau-protein), and permits analyses of the location-specific interaction between DNA probe and target-DNA in the chromosome (e.g., by multicolor FISH). In addition to this 3D analyses we made image stacks (e.g. of FISH stained metaphase plates) to generate three-dimensional image information, such as the creation of stereo images and orthogonal or oblique image sections. These techniques allow us to further investigations on cell-biological questions and ultrastructure analyses. In the medical

field, this may deepen our understanding of the structure and function of the elements of the cytoskeleton and their interaction with other cell structures as the chromosome, the kinetochore and the centrosome.

P0210. Characterization of a small supernumerary marker chromosome by centromere specific multicolor-color FISH (cenM-FISH); case report

I. Hartmann[1], *H. Starke*[1], *A. Nietzel*[1], *F. von Eggeling*[1], *M. Rocchi*[2], *V. Beensen*[1], *B. Mitulla*[3], *U. Claussen*[1], *T. Liehr*[1]
[1]*Institute of Human Genetics and Anthropology; Jena, Germany;* [2]*Institute of Genetics; Bari, Italy;* [3]*Central Clinic, Genetic Counseling; Suhl, Germany*
i8lith@mti-n.mti.uni-jena.de

Cytogenetic diagnostic was done in a newborn boy who presented with typical features of Down syndrome like hypertelorism, simian crease and hypomotility. The suspicion of Down syndrome was confirmed in cytogenetics as a free trisomy 21 was detected. Additionally a small supernumerary marker (SMC) chromosome was detected in 28/35 analyzed metaphase spreads (karyotype; 48,XY,+21,+mar). Such stable small SMC often present without molecular cytogenetically detectable euchromatin and are uneasy to characterize in standard cytogenetic approaches, as neither GTG-banding nor 24-color FISH using whole chromosome libraries as probes are able to explain their origin. Microdissection and reverse painting could be used for their characterization, as well. However, this technique is used more reasonably for larger markers with larger portions of euchromatin. Recently, we developed a probe set, using all human centromeric probes labeled in different colors, allowing the simultaneous characterization and identification of all chromosomes by their centromeric region (Nietzel et al., Hum Genet., in press). The technique, called cenM-FISH has been applied in the presented case and revealed, that the small SMC was a derivative chromosome 4. As it was not stained by a whole chromosome painting probe for chromosome 4 it was described as a der(4)(p11q11). As the karyotypes of the parents were completely inconspicuous two de novo events must have taken place to lead to the detected numerical aberrations. Studies to exclude uniparental disomy 4 are in progress. In summary, the cenM-FISH technique is a very useful approach for the one step identification of all human chromosomes by their centromeres. Acknowledgments; This work was supported by the Herbert Quandt Stiftung der VARTA AG, the Madeleine Schickedanz-Kinderkrebs-Stiftung and the Wilhelm Sander-Stiftung.

P0211. The significance of microsatellite analysis in the routine diagnostics of MR/MCA syndromes

M. Czak [1], *. Morava*[2], *G. Kosztol nyi*[2]
[1]*University of P cs, MTA-PTE Clinical Genetic Research Group; P cs, Hungary;* [2]*University of P cs, Department of Medical Genetics and Child Development; P cs, Hungary*
mrita@apacs.pote.hu

In the recent years the basic role of the routine chromosomal examination in the clinical evaluation of mental retardation/multiple congenital anomalies (MR/MCA) syndromes has progressively been completed by FISH. However, even the time and labour consuming FISH techniques including YAC/BAC clones may be insufficient to give precise answer to the clinical geneticists. Cases are presented in which microsatellite marker analyses using dried blood spots on Guthrie cards resulted in significant additional information and completed the clinical diagnostics. In the first (patient with an unusual phenotype and a ring chromosome 15) the karyotype/phenotype correlation analysis was supplemented by precise data, in the second (a complex chromosomal rearrangement) the lost of significant parts of chromosomal material could be excluded with more certainty, in the third (an interstitial deletion of the X chromosome) the extent of the missing part could be defined more precisely. On the base of these observations, the authors suggest that it is worth enrolling microsatellite analysis to the routine protocoll of MR/MCA diagnostics, which may result in significant data essential not only for uniparental disomy but also for otherwise unsoluble clinical problems.

P0212. Chromosome 2 rearrangements precisely characterized by multicolor banding (MCB) and simultaneously with region specific probes

A. Weise[1], *H. Starke*[1], *M. Volleth*[2], *M. Stumm*[2], *A. Heller*[1], *N. Rubtsov*[3], *V. Trifonov*[3], *M. Rocchi*[4], *K. Mrasek*[1], *U. Claussen*[1], *T. Liehr*[1]
[1]*Institute of Human Genetics and Anthropology; Jena, Germany;* [2]*Institute of Human Genetics; Magdeburg, Germany;* [3]*Institute of Cytology and Genetics; Novosibirsk, Russian Federation;* [4]*Institute of Genetics; Bari, Italy*
i8lith@mti-n.mti.uni-jena.de

The multicolor-banding (MCB) technique, allows the differentiation of chromosome region specific areas at the band and sub-band level. It is based on microdissection derived region specific libraries producing changing fluorescence intensity ratios along the chromosomes, which are used to assign different pseudocolors to specific chromosomal regions. A MCB probe set for chromosome 2 was used in combination with region specific YAC probes to characterize aberrations with involvement of chromosome 2. Two clinical cases, both presenting mental retardation and multiple dysmorphic stigmata were analyzed up to now. Case 1 had a der(2)t(2;8) and the breakpoints according to GTG banding and CGH were located in 2q37 and 8q22. Using MCB these breakpoints were corrected and refined to 2q37.3 and 8q23-24.1. Case 2 had a deletion - again according to GTG-banding and CGH data - in 2q32-33. MCB showed, however, that the deletion was more proximally located and MCB combined with the region specific YAC 762E6 confirmed that breakpoints of the deletion were in 2q31 and 2q32. MCB analysis on two other patients with duplication in 2p are in progress. In summary, it has been demonstrated, that MCB can successfully be combined with region specific YAC probes and can be used for the refinement of uncertain chromosomal breakpoints. Acknowledgments; This work was supported by the Herbert Quandt Stiftung der VARTA AG, the Madeleine Schickedanz-Kinderkrebs-Stiftung, the DAAD, the DFG and the Wilhelm Sander-Stiftung.

P0213. Diagnostic applications of M-FISH analysis to chromosomal aberrations

M. Sone[1], *T. Iwai*[1], *S. Yamamoto*[1], *S. Ogita*[1], *S. Furukawa*[1], *Y. Hamada*[1], *I. Kondo*[2]
[1]*Kagawa Children s Hospital; Zentsuji, Japan;* [2]*Ehime University School of Medicine; Ehime, Japan*
sone@kagawasyo.hosp.gp.jp

Multiple fluorescence in situ hybridization (M-FISH) analysis allows the visualization of 22 human autosomes and two sex chromosomes, in 24 different colors. We have used M-FISH analysis in four patients with complicated chromosomal aberrations determined preliminary by GTG-binding methods; one case with 46, XY, t(8;16;11), one case with 45, X, -14, +t(1;14), one case with 46, XY, +12p, and one case with 46, XX., der(5), t(5;7)(p15.1;p15.3). Furthermore, region specific DNA probes were used to verify precise break points in abnormal chromosomes.Then, karyotypes wereidentified as follows; one was 46, XY, t(8;16;11)(q21.2;p11.2;p11.2), one was 45, X, -14, der(Y); t(Y;1)(q11.2; q25), t(Y;14)(p11.3;q13), ins(Y;3)(p11.3; q24 q25), one was 47, XY, +dup(12) (pter-q13.11) and one was 46, XX,der(5), t(5;7)(p15.1; p15.3)mat. In all cases, deleted and duplicated portions were discertained by GTG-binding methods. The M-FIHS analysis was able to identify easily chromosomal origins in marker chromosomes. The M-FISH analysis in addition to the GTG-binding method is provided with the most cytogenetic information in complicated chromosomal aberrations.

P0214. A new versatile technique to detect specific sequences within single DNA molecules; Multiphoton multicolor FISH (MM-FISH)

A. G hlert[1], *I. Riemann*[2], *A. Nietzel*[1], *N. Rubtsov*[3], *V. Trifonov*[4], *U. Claussen*[1], *T. Liehr*[1], *K. K nig*[2]
[1]*Institute of Human Genetics and Anthropology; Jena, Germany;* [2]*Laser Microscopy Division, Institute of Anatomy II; Jena, Germany;* [3]*Institute of Cytology and Genetics; Novosibirsk, Russian Federation;* [4]*Institute of Cytology and Genetics; Novosibirsk, Germany*
i8lith@mti-n.mti.uni-jena.de

Multiphoton multicolor fluorescence in situ hybridisation (MM-FISH) is based on non-resonant two or more photon excitation of multiple FISH fluorophores in a subfemtoliter excitation volume. We used a femtosecond laser source at 800 nm (near infrared = NIR) in combination with a laser scanning microscope and objectives of NA>1.2 to induce the fluorescence of a variety of FISH fluorophores and to perform pinhole-free fluorescence imaging with submicron spatial resolution. FITC-labeled 40 kb subtelomer probes and multicolor-labeled centromere probes that bind to repetitive sequences of 0.340 kb and 2.000 kb have been detected. Three-dimensional depiction of 10 m cryosections and of an amniocyte interphase nucleus have been done. Taking advantage of the simultaneous excitation of different FISH fluorophores we realized multicolor imaging with a single scan and a single excitation wavelength. Additionally, we have visualized Spectrum Orange and Spectrum Green labeled bcr and abl gene probes of 300 and 650 kb on single DNA-fibers with an estimated number of 75 - 225 m-1 fluorescent molecules. MM-FISH provides the possibility to detect specific DNA-sequences within interphase nuclei and tissue biopsies with high light penetration depth and without out-of focus fading. Additionally, the NIR laser microscope was used to create laser cuts on chro-

mosomal DNA. On one hand, the effects of these laser cuts was analyzed by paint generation from chromosomes after laser cuts of different size. On the other hand human chromosomes were dissected with laser in specific regions, the fragments were collected and amplified by DOP-PCR, to create chromosome region specific DNA-libraries. The labeled products were successfully tested with FISH on human chromosomes. In summary, MM-FISH and the possibility of using NIR laser microscopy for microdissection have potential applications in the field of molecular cytogenetics, prenatal diagnostic and molecular pathology - especially for 3-D-applications.

P0215. Assessment Of Ipm-fish, A New M-fish Technique Combining Combinatorial Labeling And R-banding; Study Of 18 Patients With Various Constitutional Chromosomal Rearrangements.

J. Aurich-Costa[1], *E. Gr goire*[1], *H. Mossafa*[2], *S. Tapia*[2], *D. Ch rif*[1]
[1]*Genset, S.A.; Evry, France;* [2]*Laboratoire Pasteur Cerba; Cergy Pontoise, France*
joan.aurich@genset.fr

The recently described M-FISH and SKY techniques are complementary to the standard karyotypic analysis identifying the origin of rearranged chromosomal fragments. However only the poor DAPI banding is usually used to define the breakpoints. Here, we report the analysis of 18 patients presenting different constitutional chromosomal abnormalities with a new M-FISH technique; IPM-FISH, which combines IRS-PCR painting probes and M-FISH, resulting in a simultaneous labeling and R-banding. Method; Patients presented constitutional abnormalities including numerical and structural chromosomal rearrangements. These patients have been previously karyotyped by RHG banding. Ten to thirty metaphases were analyzed by IPM-FISH. Painting probes were labeled with combinations of FITC, DEAC, Cy3, Texas Red, Cy5. Images were acquired and analyzed as already described (Aurich-Costa et al., Genes Chromosomes and Cancer, in press). Results; All chromosomal abnormalities were detected and completely described using IPM-FISH in a single experiment. There is a perfect correlation in the breakpoint definition between IPM-FISH and RHG banding. Only when the chromosomal abnormalities involved heterochromatic or repeat regions is IPM-FISH unable to determine the chromosomal origin. However, in those cases, whatever the technique used in the karyotypic analysis, other FISH techniques are required to determine the origin. Conclusion; Here we show that even for intrachromosomal rearrangements, IPM-FISH is a sensitive, precise and reliable technique.

P0216. High-resolution Mapping of the Human 4q21 and the Mouse 5E3 CXC Chemokine Cluster by Fiber-FISH.

M. Erdel[1], *M. Theurl*[1], *M. Meyer*[2], *H. C. Duba*[1], *G. Utermann*[1], *G. Werner-Felmayer*[2]
[1]*Institut f r Medizinische Biologie und Humangenetik der Universit t; Innsbruck, Austria;* [2]*Institut f r Medizinische Chemie und Biochemie der Universit t; Innsbruck, Austria*
martin.erdel@uibk.ac.at

The CXC or small inducible cytokine B subfamily (SCYB) includes the non ERL CXC T-cell chemoattractants MIG (SCYB9) and IP-10 (SCYB10). These gamma interferon-inducible chemokines were localized to human chromosome 4q21 and have been shown to be organized in a mini-cluster that is distant from another tight cluster of other CXC chemokine genes at 4q12. The physical separation together with data from phylogenetic analysis of the CXC proteins suggest that MIG and IP-10 form a distinct evolutionary lineage. We recently identified the human and mouse homologue of the closely related chemokine I-TAC (SCYB11) and mapped this gene to the same mini-cluster at 4q21 and the orthologous mouse locus 5E3, respectively, by fluorescence in situ hybridisation (FISH). Here, using isolated PAC and BAC probes and cloned sequences from SCYB9, SCYB10, and SCYB11 from man and mouse, we applied two-colour FISH on stretched DNA fiber preparations (Fiber-FISH). On both, human and mouse DNA fiber targets, the three chemokines were found in an identical arrangement as a cluster within a range of approximately 25 kilobases. We have determined the arrangement from centromer to telomere (SCYB11/SCYB10/SCYB9) and the distance separating the genes (5 kb and 7 kb, respectively). Sequence and FISH data strongly support the hypothesis that the three genes result from gene duplication which took place before emergence of muroid rodents.

P0217. Identification of high quality anchored BAC FISH probes by using REPuter program

D. Ehling[1], *J. V. Choudhuri*[2], *J. Weidner*[3], *R. Giegerich*[2], *J. Wirth*[1], *T. Schmitt-John*[1]
[1]*University of Bielefeld; Bielefeld, Germany;* [2]*Faculty of Technology; Bielefeld, Germany;* [3]*Praenadia GmbH; Muenster, Germany*
daniela.ehling@biologie.uni-bielefeld.de

To date, almost complete sequences of two human chromosomes are available and allow to search for chromosomal regions of interest if suitable computing programs are at hand. We have used the REPuter program to view the distribution of exact and degenerate repeats with a minimal length of 20 bp in the Down syndrome critical region and subtelomeric region of 21q22.3. The program allowed us to select for large regions without repeats and high gene content. Moreover, we have designed specific primers within these sequences and isolated several overlapping BAC clones by screening a BAC library. By sequencing the ends of these BAC clones the exact size and positions of the clones in the map of chromosome 21 were determined. These BAC clones are generich and are composed of fewer repeats compared to other sequences known on chromosome 21, thus they are suitable for Fluorescence in situ hybridisation (FISH) analyses. These clones yield high-intensity, region-specific FISH signals on both metaphase chromosomes and interphase nuclei and represent a major source for the identification of trisomy and subtelomeric deletions for chromosome 21. Here, we present a combined software and experimental approach and show the application to extract specific sequences that are useful for the detection of human chromosomal disorders.

P0218. Partial Trisomies and the Nature of Chromosomal Bands

A. I. Corominas, *E. A. Pastene, E. I. Aranda*
Centro Nacional de Gen tica M dica; Buenos Aires, Argentina
clamidia@radar.com.ar

The human genome is a mosaic of isochores, long segments of DNA with distinctive characteristics of gene richness and nucleotid composition (Bernardi et al.). The correlation between isochores and chromosomal bands has been extensively studied, but the relation with viability of these segments in triple doses was poorly explored. Brewer et al have described a map of partial trisomies and their correlation with different phenotypes. Over the last 25 years, partial autosomal trisomies have been important tools for gene mapping, with the aim of locate genes in contiguous gene syndromes, contributing towards the mapping of several loci. A systematical analysis of chromosomal segments not observed in partial trisomies can provide information about triple-dose lethal genes, and, on the other hand, those segments observed in tetradoses, those vertically transmited in triple doses, and those appeared in triple doses with normal phenotypes, can yield clues on the biological role of chromosomal organisation. We propose here a map of chromosomal duplications based on clinical information from reports appeared in Medline about autosomal partial trisomies, and analyse its correlations with different types of chromosomal bands.

P0219. The use of FISH for the assessment of results from transfection and transgenesis experiments.

A. Y. van der Veen, *H. H. Faber, T. Dijkhuizen, A. van den Berg, T. Timmer, A. ter Elst, P. M. J. McLaughlin, C. H. C. Buys*
Dept. of Medical Genetics, University of Groningen; Groningen, The Netherlands
A.Y.van.der.Veen@medgen.azg.nl

Results from transfection and transgenesis experiments are usually assessed by PCR with primers for the sequence of the cDNA or the genomic fragment under study. Sequences to be introduced either contain a selectable marker themselves or are being co- transfected with a plasmid carrying such a marker. We have used FISH analysis (i) to quickly assess the proportion of cells into which a cosmid had been co- transfected with a neomycin-resistant plasmid and to check the clones resulting after appropriate dilution to obtain monoclonal cultures for cosmid content, (ii) to determine integration sites for PACs with a selectable marker and to select those clones that had single copy or low copy integration; (iii) to determine integration sites of human gene constructs in human gene-expressing transgenic mice and rats. In all these applications, FISH analysis appeared to be an easy and excellent method of assessment of results. Where the latter occurs on the basis of many cells in culture or tissue, thus obliterating differences between individual cells, FISH analysis leads to a very precise picture on a cell by cell basis. Therefore, we consider FISH analysis a welcome if not necessary complement to assessment by PCR.

P0220. De novo der(5) identified as an interstitial insertion of chromosome 3 material by COBRA multi-colour FISH

H. Engels[1], *M. Krei§-Nachtsheim*[1], *A. Ehrbrecht*[1], *S. Zahn*[1], *R. Schubert*[1], *G. Schwanitz*[1], *C. Ergang*[1], *M. van der Burg*[2], *D. Hansmann*[3], *A. K. Raap*[2], *H. J. Tanke*[2], *J. Wiegant*[2]
[1]*Institute of Human Genetics; Bonn, Germany;* [2]*Department of Molecular Cell Biology, Leiden University Medical Center; Leiden, The Netherlands;*

[3]Zytogenetisches Labor Dr. Hansmann / Dr. Wei§; Meckenheim, Germany
hengels@meb.uni-bonn.de
A 5 year old boy presented with ASD II, bilateral cleft palate, strabism, bilateral optic nerve coloboma, sensorineural hearing loss, bilateral inguinal herniae, micropenis, seizures and a pronounced psychomotor developmental retardation. Height, weight and head circumference were well below the 3rd centile. The family history was inconspicuous. Cytogenetic analyses demonstrated a derivative chromosome 5 with additional material of unknown origin in the distal long arm. FISH with a whole chromosome paint / WCP 5 indicated a heterologous insertion of material within 5q3. Its banding pattern was inconclusive and did not allow identification of the inserted material. COBRA multi-colour FISH with 24 WCPs pointed out a chromosome 3 origin of the inserted material which was confirmed by FISH with a WCP 3. Even with this information, the breakpoints of the aberration could not be determined upon re-examination of GTG-, CBG- and QFQ-banded metaphases; Although a pericentromeric origin of the inserted material could be excluded after CBG banding, its banding pattern fit neither the short nor the long arm of chromosome 3 exactly. To clarify the origin of the insertion, studies by 48 colour COBRA-FISH are in progress. Using only 5 fluorophores, this novel technique achieves colour identification of 48 different targets, in our case the differential labeling of all human p and q arms by PQ-COBRA-FISH (Genome Res. 10;861-865). Results of the additional studies as well as a genotype-phenotype correlation will be presented. Supported by the DFG / Deutsche Forschungsgemeinschaft

P0221. Chromosomes in interphase are similar to metaphase chromosomes

U. Claussen[1], *J. Lemke*[1], *J. Claussen*[1], *I. Chudoba*[2], *I. Trifonov*[3], *N. Rubtsov*[3], *H. Starke*[1], *A. Heller*[1], *K. Sperling*[4], *T. Liehr*[1]
[1]*Institute of Human Genetics and Anthropology; Jena, Germany;* [2]*MetaSystems GmbH; Altlussheim, Germany;* [3]*Institute of Cytology and Genetics; Novosibirsk, Russian Federation;* [4]*Institute of Human Genetics; Berlin, Germany*
ucla@mti-n.uni-jena.de
To understand the regulation of gene expression and the function of genes at the 3D-level, the knowledge of the structure of chromosomes in interphase nuclei is of main interest. Here, using the high resolution DNA-based multicolour banding technique (MCB), we examined the shape and banding pattern of human chromosomes 5 on lymphocyte interphase nuclei and on nuclei of HeLa cells at the 2D-level arrested at different phases of the cell cycle (early, middle, late G1; early, middle, late S; early middle, late G2). Chromosomes 5 in 2 dimensionally flattened interphase nuclei are bent, folded, and show a MCB pattern similar to metaphase chromosomes. The length of their axis is comparable to that of metaphase chromosomes at the 700 band resolution. The MCB pattern is visible in all phases of the cell cycle and can even be used for the identification of small structural chromosome aberrations which may be of fundamental interest in cytogenetics.

P0222. FISH, MFISH and Subtelomeric Probes in Clinical Cytogenetics

C. B. Lozzio, *T. Ryan, E. Bamberger, E. Ralston, E. Holland, W. Carter, C. Worthington, K. Harmon*
University of Tennessee; Knoxville, TN United States
clozzio@mc.utmck.edu
From January 1, 1995 to December 15, 2000 we have performed 1375 molecular cytogenetic studies in 167 amniotic fluids, 5 CVS, 63 bone marrows, 115 tissues and 996 cultures of peripheral blood. Microdeletion syndromes, rearrangements and marker chromosomes have been detected or characterized by these techniques. We used wcp, alpha satellite and specific region probes for FISH. MFISH was performed with the Multicolor Spectra Vysion Assay from Vysis and the Applied Imaging CytoVision Software. The subtelomeres were investigated with either the Chromoprobe Multiprobe T from Cytocell or the ToTelVysion Vysis probes. Examples of chromosome markers identified by these techniques include two small markers derived from chromosome 5, a small marker 7 in a case that also had a pericentric inversion of the X chromosome and a marker 13q32-qter with neocentromere. Several cases with de novo translocations were recognized by these techniques. In two of these cases the translocated region was very small and included the subtelomeres. In these cases we used the multiple subtelomeric probes. We found that an add (18)(p11.2) was a der(18)t(18;14) with monosomy 18p and partial trisomy 14q and the other case had a der(8)t(8;13) with monosomy 8p and partial trisomy 13q. MFISH studies in bone marrows and solid tumors characterized the origin of multiple chromosome rearrangements and helped in the interpretation of the diagnoses and/or prognoses. We conclude that the availability of new probes and the use of specialized techniques such as MFISH and subtelomereric probes have improved significantly the services offered by clinical cytogenetic laboratories.

P0223. NOR activity of a bisatellited additional chromosome in karyotype of patient with the features of Turner phenotype

A. Pendina, *O. Chiryaeva, T. Kuznetzova*
Ott s Institute of Obstetrics and Gynecology; Saint-Petersburg, Russian Federation
pendina@mail.ru
A 13-years-old girl with typical clinical features of Turner phenotype (short stature, shield chest, short neck) subjected to cytogenetic analysis. Cultured lymphocytes studies identified the small supernumerary marker chromosome in 49% of cells. The both parents had normal karyotype. Multiple staining techniques were used for identification of the extra chromosome. With QFH-banding the marker appeared as monocentric with two major QFH-positive bands and with satellites of variable size on its both ends; with C-banding, two C-positive regions indicated that chromosome possessed two centromeres. Silver staining demonstrated the presence of NORs variety in size on both ends. NOR activity was estimated by the size of silver deposits using Arbitrary Scale Units of 0-3. The NOR activity of each acrocentric chromosome in patient karyotype was the same as in parental karyotypes, except both chromosomes 14 and maternal chromosome 22. Comparisons of the total cellular NOR activity of cells with marker and without it showed significant difference (P<0,05). This difference was conditioned by marker chromosome NOR activity. NOR activity of acrocentric chromosomes 13, 14, 15, 21, 22 was the same in both cell lines. The existence of compensatory mechanisms regulating Ag-NOR activity seemed to be rather improbable in that case.

P0224. Computer assisted d agnosis of chromosomal aberrations

A. Koehler[1], *B. Wolf*[2], *U. Tautenhahn*[2], *J. Pelz*[2]
[1]*Institut fuer Humangenetik; Giessen, Germany;* [2]*Institut fuer Humangenetik; Berlin, Germany*
angelika.koehler@humangenetik.med.ini-giessen.de
The diagnosis of human malformation syndromes, if not possible at a glance, requires much subsequent work. In the case of an unrecognised pattern of multiple congenital anomalies a chromosome karyotype is usually requested. Developmental delay, growth retardation and abnormalities of three different organ systems strongly suggest a chromosomal aberration. Some commercially available computerised data-bases like SYNDROC and the Human Cytogenetics Database provide information on the phenotypes of patients with cytogenetically ascertained chromosome aberrations. The diagnostic abilities of these programs were tested using the clinical findings of 101 patients with an established karyotype. Both programs are based on an algorithm which seeks and defines a diagnosis by a set of phenotypic components all having the same weight (descriptive algorithm). A second (Bayesian) algorithm is applied by SYNDROC to rank competing diagnoses in order of probability. We used three levels of precision to evaluate the proposed diagnoses; suggestion of the correct (1) chromosome, (2) chromosome arm, (3) aberration typ. The best results were yielded with the descriptive algorithm; the respective chromosome was diagnosed in nearly all cases, the correct chromosomal arm in about 60% of all tests and the aberration typ on the right chromosome arm was suggested in about 25%. With the Bayesian algorithm the respective numbers were 52%, 28% and 16%. The probabilities calculated with this algorithm were far from being convincing with values of less than 0.25 for the majority of cases. Since all patients had a cytogenetically confirmed diagnosis, it seems to be necessary to change some of the estimates that went into the Bayesian formula.

P0225. 21/21 Carrier As a Result Of Unexpected Segregation In Robertsonian Translocation 14/21

E. Kovacova, *F. Cisarik*
The Hospital of Zilina; Zilina, Slovakia
foxi@mail.viapvt.sk
We studied segregation of Robertsonian translocation (RT) 14/21 in large family. Besides carriers of RT 14/21, we found exchange in chromosomal composition from RT 14/21 to 21/21 in one sibling. He has one child with trisomy 21 and there was one spontaneous abortion. In the rest of family we found another 2 cases of trisomy 21 and 5 spontaneous abortions. We examined another 7 families with various RTs in our database. Alltogether, in 8 genealogies there were 40 carriers, 4 cases of trisomy of acrocentrics and 18 spontaneous abortions. We did not find the same case of exchange of acrocentrics in RTs in our database. The literature does also not refer to such identical unusual segregation in RTs. Chromosomes 13, 14 and 21

are preferentially engaged in RTs, therefore carriers of 13/14, 14/21 and 21/21 are most frequently found in the population of newborns as well as in groups of patients with various types of ascertainment. Preference of chromosomes 13, 14 and 21 in RTs explains Therman /1989/ in hypothesis about crossing-over in segment A-B, dealing with inversion of A-B in chromosome 14 relative to chromosomes 13 and 21. We assume that occurence of exchange of chromosomes in RT from 14/21 to 21/21 is extremely seldom, and may proceed via two mechanisms; a/ meiotic crossing-over with exchange in RT from 14/21 to 21/21, and subsequent loss of chromosome 21 after fertilisation in early trisomic zygota b/ mitotic crossing-over in early zygota, releasing the chromosome 14 from RT 14/21 and formation of RT 21/21. Risk of unbalanced offspring for male carrier RT 14/21 is about 2.5%, while risk for carrier RT 21/21 is 100%.

P0226. An interesting case of mosaic tetrasomy, trisomy, disomy and monosomy of chromosome 13

M. Zanganeh, *S. Voghouie, A. Karimin-Nejad, N. Naghashpour, R. Assaie, M. H. Karimi-Nejad*
Karimi-Nejad Pathology and Genetics Center; Tehran, Islamic Republic of Iran
mhkarimi@mavara.com

Mr. & Mrs. A.F., an unrelated couple were referred to our center for chromosomal study with history of three abortions. Mr. A.F. is 32 years old and Mrs. A.F., a 27 years old female with G3 P0 Ab3. Twenty metaphse spreads of Mr. A.F. revealed normal male karyotype. 50 metaphase spreads were studied from PHA stimulated lymphocytes of Mrs. A.F. showing four mosaic patterns with robertsonian translocation of chromosomes 13. 45, XX, der(13;13)9q10;q10)/ 45 cells (90%) showed 45 chromosomes with one copy of the derivative chromosome, 3 cells (6%) showed 46 chromosomes with 2 copies of the derivative chromosome without normal 13s (tetrasomy 13), 1 cell (2%) showed one derivative chromosome and one normal 13 (trisomy 13), 1 cell (2%) had one normal chromosome 13 (monosomy 13). Unfortunately further medical work up and examination was not possible because of lack of cooperation on the patient s part. We believe that the various mosaic cell lines are a result of pre and post zygotic nondisjunction????

P0227. Increased rate of meiotic and mitotic non-disjunction of chromosome 21 in an arab family

C. Ramel[1], *A. Krebsova*[1], *L. Al Gazali*[2], *H. Neitzel*[1], *K. Sperling*[1]
[1]*Institute of Human Genetics, Humboldt-University, Berlin; Berlin, Germany;* [2]*Paediatrics Department, United Arab Emirates University, Al Ain; Al Ain, United Arab Emirates*
christian.ramel@charite.de or rubberchris@web.de

Down Syndrome (DS) is usually the result of maternal meiotic non-disjunction. DS with free trisomy 21 in more than two siblings of chromosomally normal parents is due to gonadal mosaicism or premature centromere division (PCD). Here we report on a consanguineous arab family with 10 children of whom three had free trisomy 21. The parental karyotypes were normal and did not exhibit PCDs. Based on 22 evenly distributed microsatellites, it was shown that the extra chromosome was of maternal origin, resulting from an error in meiosis I (2 cases) and meiosis II. The recombination rate (1 and 2 exchanges in the siblings) was normal. In addition, we have analysed the rate of somatic malsegregation in interphase nuclei of cytochalasin B-blocked binucleated lymphocytes and lymphoblastoid cells (LCLs) in both parents, their trisomic offspring, the grandmother and greatgrandmother and three unrelated controls. Fluorescence in situ hybridization (FISH) analysis was performed using pericentric probes for chromosome 21 and 22 to score 1000 binucleated cells per individual. The frequency of non-disjunction of chromosome 21, but not of 22, was significantly increased in the lymphocytes and LCLs of the mother compared to the other family members and controls as well. To the best of our knowledge this is the first report that a high rate of maternal meiotic non-disjunction is paralleled by a specific increase in mitotic non-disjunction. Further studies are in progress to elucidate the genetic basis of this new phenomenon.

P0228. Assessment of aneuploidy rate in motile spermatozoa selected by three different methods

O. Samura, *N. Miharu, K. Ohama*
Department of Obstetrics and Gynecology, Hiroshima University School of Medicine; Hiroshima, Japan
samurao@clubaa.com

Using multi-colour fluorescence in situ hybridization, we compared the frequencies of disomy and diploidy of chromosomes 1, 16, 18, X and Y between spermatozoa of good motility with those of poor motility after separation by swim-up, glass-wool and two-layer discontinuous Percoll methods. Semen samples were collected from 7 normal males aged 26-31 years. A minimum of 6,000 sperm nuclei per sample for disomy and 12,000 sperm nuclei per sample for diploidy were evaluated for each chromosome (618,335 sperm nuclei total). Hybridization efficiency was 99.61%. We did not observe significant differences in disomy rates for any chromosome when comparing spermatozoa with good motility to those with poor motility. Diploidy rates in the discontinuous Percoll separated spermatozoa were not significantly different between spermatozoa with good motility and those with poor motility (p=0.31). However, after separation by glass-wool method the frequency of diploid sperm with good motility (mean=0.26%; range=0.16%-0.37%) was significantly higher than those with poor motility (mean=0.22%; range=0.12%-0.35%) (p=0.046). After separation by swim-up method the frequency of diploid spermatozoa with good motility (mean=0.18%; range,0.12%-0.30%) was significantly lower than those with poor motility (mean=0.25%; range,0.17%-0.43%) (p=0.010). Even though significant differences were observed between motile and poorly motile spermatozoa selected by swim-up and Glass-wool methods, the differences were so small that they could be ignored in clinical practice. Our results suggest that the separation of motile sperm by the methods described here does not have an effect on the selection of spermatozoa of disomy and diploidy.

P0229. A Rare Case; Mosaic Trisomy 22

M. Ozdemir[1], *N. Ay*[2], *B. Durak*[1], *H. Berkil*[1], *Y. H. Ozon*[1], *I. Kaya*[1], *N. Basaran*[1]
[1]*Osmangazi University, Medical Faculty, Department of Medical Genetics; Eskisehir, Turkey;* [2]*SSK Hospital Pediatrics Department; Eskisehir, Turkey*
mozdemir@ogu.edu.tr

The case, 9 years of age, the daughter of healthy parents (mother;43 years father;44 years) was referred to our center because of the mental retardation. The pedigree was normal, the two sibs of the proband were healthy. Her pysical examination revealed facial dysmorphism, microchephaly, hiperflexibility of the joints. The chromosome constitution of her was determined as 46,XX/47,XX,+22 (98/2) (2%). Because of the mosaic karyotype, skin biopsy was performed and then the FISH results were analysed. The mosaic trisomy was confirmed (46,XX/47,XX,+22) (73/27) (27%). The physical features of the case were in consistent with her mosaic karyotype. This case was reported since mosaic trisomy 22 is the rarely seen abnormality and it is a good example to show the importance of FISH in mosaic karyotypes.

P0230. Chromosome analysis in mental retardation.

R. Mikelsaar[1], *A. Salo*[2], *K. Varb*[1], *A. S vari*[3], *K. Muru*[1], *R. Teek*[1]
[1]*Institute of General and Molecular Pathology, University of Tartu; Tartu, Estonia;* [2]*United Laboratories Ltd; Helsinki, Finland;* [3]*Karula Homeschool; Karula, Estonia*
mikels@ut.ee

Mental retardation affects 2-3% of the population and is etiologically very heterogeneous. It has involved at least in 503 gene disorders and in most chromosomal anomalies. Routine cytogenetic analysis indicates that chromosomal anomalies are the cause at about 35% of cases of severe mental retardation. Multiprobe-FISH reveals submicroscopic subtelomeric chromosome defects in 7% of unexplained cases of moderate and severe mental retardation. We have performed cytogenetic analysis in 243 patients with severe mental retardation. Using standard cytogenetic techniques (Giemsa, C-, R-, Q banding) 54 (22.2%) cases of chromosomal anomalies were identified; 38 cases (15.6%) of aneuploidies and 16 cases (6.6%) of structural rearrangements. We have performed a pilot study of Multiprobe-FISH (Cytocell Chromoprobe Multiprobe-T System) in 8 patients with unexplained severe mental retardation and malformations /dysmorphisms and normal conventional 400-550 bands karyotype. From them in one case a subtelomeric rearrangement was identified; deletion Xq. The girl (21 year old) has etiologically unclear severe mental retardation, short stature (140cm, -2.3SD), slight microcephaly (48cm, -2.3SD), dysmorphic features as epicanthic folds, short fingers, V clinodactyly etc. These features coincide with previously published certain clinical diagnostic criteria that improve the detection rate of subtelomeric rearrangements. Results suggest that banding analysis combined with M-FISH increase the possibility to detect causes of mental retardation.

P0231. Korean Cytogenetic Database

D. Hwang, *S. Cho, J. Choe, Y. M. Choi, S. Y. Moon*
Hamchoon Women s Clinic; Seoul, Republic of Korea
doyhwang@hamchoon.co.kr

Korean Cytogenetic Database Study Group was organized in 1997 to establish Korean cytogenetic database. The Korean Cytogenetic Database Study Group consists of 23 contributing Cytogenetic Laboratories through-

out South Korea.
The Korean Cytognetic Database is critical 1) to study the epidemiololgy of cytogenetic abnormalities among Koreans, 2) to design clinical cytogenetic services, 3) to understand unique cytogenetic findings among Koreans, 4) to determine urgent cytogenetic research questions. Between 1997 and 1999, 7147 cases of abnormal karyotypes were registered. The classification based on tissue samples are follows; 4495 cases of peripheral blood, 235 of peripheral blood-oncology, 1060 of amniotic fluid, 1098 of chorionic villi, 141 of fetal blood, 394 of Product of conception, 607 of bone marrow and 17 of other specimens. Among these, the detailed analysis of 4401 cases of peripheral blood and 979 of amniotic fluid will be presented.
In numeric abnormalities, trisomy 21 was the most common abnormalites and followed by trisomy 18 and trisomy 13 among autosomes. Among sex chromosome, XXY was the most common and followed by 45,X. In structural abnormalites, reciprocal trnalocation and robertsonian translocation are two most common abnormalities. The t(13;14), t(14;21) and t(14;22) were the most common robertsonian translocation in descending orders among Koreans.
In conclusion, Korean cytogenetic database present the first comprehensive epidemiologic data on cytogenetic abnormalities among Koreans. Our data are compatible with other datasets reported in other countries.

P0232. Age Depending Changes In Contribution Of Aneuploidy Formation Mechanisms In Human Oocytes

***T. Kovalenko**, A. Stefanovich, I. Barilyak*
Ukrainian Scientific Center of Medical Genetics; Kiev, Ukraine
tamila@humgen.kiev.ua

Three main mechanisms of aneuploidy formation are known; meiotic bivalents nondisjunction, equal number of hypo- and hyperhaploid oocytes is result of it; anaphase lagging, its outcome is hypohaploid oocytes; and precocious division of centromeres of univalents at first meiotic division, aneuploidy by single hromatids is result of this mechanism. But up to our days reasons of aneuploidy formation by these mechanisms are still unknown. The aim of this work was to investigate the maternal age influence on the contribution of each mechanism of aneuploidy formation in oocytes retrieval during IVF programs. Oocytes from 67 women (average age 29.4–3.2 years) undergoing IVF were the object of this study. Women were divided into two groups; group1 — 35 patients under 30 years old, average age — 26.4–1.8; group2 — 32 patients from 30 years old, average age 33.5–2.5. Such air drying methods as described by Tarkowski, 1966; Mikamo and Kamiguchi, 1983; Wramsby, 1985; Almeida and Bolton, 1993 were used to prepare the oocytes for cytogenetic analysis. Our results showed that 50% of oocytes were aneuploid. Increase of hypohaploid oocytes number in the first group (41.2–4.9% versus 33.3–4.7%; in group 2) was showed. The main mechanism of aneuploidy formation in the group 1 was anaphase lagging (33.8%). In the group 2 two mechanisms of aneuploidy formation were predominate; anaphase lagging and precocious division of centromeres of univalents at first meiotic division (both 14.3%). In such way increasing of female age can change contribution of different mechanisms of aneuploidy formation.

P0233. Spectrum of congenital cytogenetic disorders encountered in north India

***R. Das**, S. Kaur, G. Garewal*
Postgraduate Institute of Medical Education & Research; Chandigarh, India
pallab@ch1.dot.net.in

Analysis of the spectrum of congenital disorders over the last ten years encountered in the Nehru Hospital, a referral hospital catering to four neighbouring states in north India, is presented. Aneuploidy of the autosomal chromosomes and the sex chromosomes are commonly encountered problems. The aneuploidies are a result of a non-disjunction error in meiosis I or anaphase lag. Karyotyping of GTG-banded metaphases from peripheral blood lymphocytes for genetic disorders on 1400 samples was performed. We found 339 cases of Downs syndrome with trisomy 21. Translocations to chromosomes 21, 13 and 14 were encountered in 6.99 percent of cases and mosaicism was found in 2.43 percent. Sex chromosome aberrations like Turner s syndrome (TS) was found in 76 cases with 29 percent patients having mosaicism. There were two cases with 45, XY/XO. In addition three cases of TS were mosaics for XXX or superfemale. There were 26 cases of Klinefelter s syndrome with four of them showing mosaicism. Nineteen individuals were diagnosed to be male pseudohermaphrodites with female phenotypes and 9 cases were female pseudohermaphrodites with male phenotypes. Of the 65 cases refered as ambiguous genitalia where no sex could be ascribed phenotypically, in 46 cases male genotype and in 19 cases female genotype could be assigned. Mullerian agenesis confirmed on ultrasonography and a normal karyotype was found in 40 cases. Two cases of Edward s syndrome (trisomy 18) and one case of Patau s syndrome (trisomy 13) was found. Sporadic cases of various translocations and other abnormalities like 46, XY t(9;18), 46, XY t(3;4), 46, XY(18p-), 46, XY isochromosome 17 and five cases of marker chromosome were also encountered. This data gives an idea of the spectrum of cytogenetic disorders seen in Indians from our region.

P0234. Genetics findings in 100 infertile men referred to Uromia s Cytogenetics and Molecular Medicine unit

M. Omrani
Mottahary Hospital; Uromia, Islamic Republic of Iran
Davood40@hotmail.com

Genetics findings in 100 infertile men referred to Uromia s Cytogenetics and Molecular Medicine Unit Dr.M.D.Omrani*, Dr. M.R.Fallah, Mrs. F. Azizi Cytogenetics and Mol. Med. Unit, Mottahary Hospital, Uromia, Iran Dept of Urology, Imam Hospital, Uromia, Iran Keywords; infertility, Azoospermia, Klienfilter s syndrome From 100 men who presented at infertility clinics and who had Azoospermia and severe oligozoospermia, blood and semen samples were taken and cytogenetics and PCR studies were carried out. The chromosomal studies revealed the following results; About 30% of cases were classified as classic Klienfilter s syndrome, 13.3% as Mosaic Klienfilter s syndrome (46XY/46XXY), 10% Familial Hypospadiasis, 6.6% Male with 46XX karyotype (SRY gene present, azoospermia factor gene absent), 3.3% Chromosomal deletion (Ypdel), 3.3% Hormonal problem (hyperprolactenemia), and 30% Idiopathic. In order to find out the causes of infertility in the idiopathic cases, PCR reactions were carried out using leukocyte DNA and sperm DNA. Y-chromosomes deletions in leukocytes DNA but not in sperm DNA were detected. No Y-chromosomes deletions were detected in the fathers of these men. AZF deletions in these men (65% of idiopathic cases) are de-novo mutations. The impact of these finding in this small city which has habitants with different cultural and belief backgrounds were very important. In some part of this area still there is an old belief that all infertilities of a couple back to the female and man has right for second marriage without paying any attention to his wife. Therefore these results are a relief for women from the sense of guilt. It also gives the opportunity to offer genetic testing and genetic counseling before starting assisted reproductive procedures.

P0235. Clinicogenetic profile of Variant Klinefelter Syndrome patients

***R. Dada**[1], K. Kucheria[2]*
[1]AIIMS All India Institute of Medical Sciences; New Delhi, India; [2]AIIMS; New Delhi, India
rdada@hotmail.com

Klinefelter Syndrome (KFS) is a numeric sex chromosomal aberration caused by meiotic nondisjunction. Approximately 15% KF patients are mosaics with variable phenotype. Available literature does not distinguish the variant cases from KFS but since these cases have a distinct phenotype we analysed these cases in detail. Cytogenetic analysis was done in 85 cases of male infertility. Of this 9 cases were diagnosed as KFS, 6 were mosaic KF and 3 were variant cases. Fluorescent In Situ Hybridization was done in variant cases to asses the percentage of different cell lines and this was compared with the cytogenetic results. In all variant cases detailed clinical examination was done. One Variant case with 47,XXY(60%)/48,XXXYY(26%)/49,XXXYY(14%) was 5.8 feet tall with poorly developed secondary sexual characters, scant body hair, gynaecomastia, bilateral soft testis, high gonadotrophin levels and normal testosterone levels. He also had congenital heart disease (CHD) with Mitral Valve Prolapse and coarse facial features. Testicular Fine needle aspiration cytology (FNAC) showed Sertoli Cell Only syndrome. The second variant with 46,XY(53%)/48,XXXY(40%)/49,XXXYY(7%) was 5.6 feet tall with poorly developed secondary sexual characters, euchnoid habitus and elevated gonadotrophins and low Testosterone level. He had low IQ, was mentally retarded and had difficulty in speech which led to temper tantrums. He also had delayed milestones. FNAC showed few germ cells in some semeniferous tubules but predominantly SCO type 11 syndrome The third KF variant had 46,XY(50%)/47,XXY(30%)/48,XXYY(20%) chromosome complement.MRI showed normal testis a nd gonadotrophin levels were in the normal range. He was 5.8 feet tall and had low IQ. The mean FSH , LH and Testosterone levels in these 3 KF variant was 36.6mIu/ml,16.6mIu/ml and 1.8ng/ml respectively. The percentage of mosaic cell lines identified cytogenetically was confirmed by FISH. These variant cases had additional clinical features than KF patients and thus variant cases should have a detailed clinical examination to look for CHD, behavioral and speech problems and skeletal abnormalities. Those variant cases with normal cell line may show spermatogenesis in some semeniferous tubules on FNAC and thus these couples can be advised to go in for

Assisted Reproductive Technology (ART) after genetic counseling . FISH in adjunt to conventional cytogenetics helps in identifying in low level mosaicism and enables us to calculate the accurate risk estimation in cases going in for ART.

P0236. Cytogenetic Evaluation of Infertile Males from Chennai (Madras), India

P. M. Gopinath[1], *C. S. Nagarajappa*[2], *N. Chandra*[3], *K. Baba*[4], *A. Rajashekaran*[5]
[1]*Genetics Department (IBMS) Madras University, Taramani; Madras, India;* [2]*Anatomy Department, JJM College of Medicine; Davanagere, Karnataka, India;* [3]*Department of Genetics; Chennai, India;* [4]*Pathology Department (IBMS); Chennai, India;* [5]*Madras Andrology Research Centre; Chennai, India*
gopinathpm@yahoo.com

A total of 204 infertile men comprising of 186 married men, referred for infertility lasting between 3 and 14 years and 18 unmarried individuals aged between 11 and 28 years, referred for congenital abnormalities of external genitalia or absence of secondary sexual characters were investigated. Data on family history, habits, occupational hazards, details of clinical examination and laboratory findings including hormone levels, ultrasound and X-ray report, semen analysis and infections and/or surgical operations were recorded using a questionnaire. Eighteen men were found to possess chromosomal abnormalities upon analysis of GTG — banded metaphases obtained from cultured lymphocytes from the married men (Azoospermia = 98; Oligozoospermia = 88). Seven showed 47, XXY karyotype, 9 were Klinefelter mosaics, one was a Klinefelter variant (48 XXYY) and one possessed an inherited translocation t (1; 22). Meiotic studies and histological evaluation were carried out in 38 cases employing testicular biopsy. Desynapsis, X-Y dissociation, a low number of chiasma, univalents, stickiness, spermatogenic arrest at various levels, germ cell degeneration and aplasia (Sertoli cell only syndrome and mixed SCO) were some of the abnormalities noted. Eleven unmarried men showed an abnormal karyotype — 4 had 47, XXY complement, 6 were Klinefelter mosaics and one had an inherited translocation t(Y; 15). These results suggest that chromosomal aberrations play an important role in the causation of male infertility. A correlation was also observed between the abnormal karyotype and the phenotypic characteristics.

P0237. FISH analysis of five 47, XXY patients support the hypothesis that only 46,XY gonia can complete meiosis.

J. Selva
CHI Poissy-St germain; Poissy, France
ampcyto_poissy@hotmail.com

The aim of our study was the evaluation of meiosis in 47,XXY or 46,XY gonia in azoospermic non mosaic Klinefelter patients by two ways ; -investigation of the correlation between the sex chromosomes complements of the biopsied testicular cells and the efficiency of spermatogenesis -evaluation of the aneuploidy rate in post meiotic cells, when found. Material and methods ; Both karyotype and FISH analysis on lymphocytes of five azoospermic men were homogeneously 47,XXY. Testis biopsy was performed for the 5 patients . In the IVF laboratory, if any testicular spermatozoa were recovered, all the biopsied tissue was frozen for a subsequent Intra Cytoplasmic Sperm Injection attempt, excepted one smear, which was prepared for FISH analysis, with three directly labelled probes for X,Y and 18 chromosomes (Chromoprobe¡Cytocell) Results ; A total of 280 labelled testicular cells was observed. The only patient for whom no spermatozoa was recovered had only diploid 47,XXY testis cells (n=56). The four other patients, displayed 46, XY and 47,XXYdiploid testis cells . All pachytene figures were 46,XY (n=21). The post meiotic aneuploidy rate, based on the observation of spermatids (n=66) and spermatozoa(n=26) was 6,5%.and involved mostly (4/6) Y meiosis II malsegregation and autosomal disomy. Our results support the hypothesis that only 46,XY gonia can achieve successfully meiosis in Klinefelter patients, with a low aneuploidy rate for sex chromosomes in resulting haploid cells.

P0238. Evolutionary breakpoints on the Y chromosomes of higher primates

R. Wimmer[1], *S. Kirsch*[2], *A. Weber*[1], *G. Rappold*[2], *W. Schempp*[1]
[1]*Institut f r Humangenetik und Anthropologie; Freiburg, Germany;* [2]*Institut f r Humangenetik und Anthropologie; Heidelberg, Germany*
wimmerra@ukl.uni-freiburg.de

Comparative FISH-mapping of human Y genes and gene families on chromosomes of higher primates have shown that Y chromosomal rearrangements are confined to the non-recombining parts of the Y chromosomes of higher primates, outside the pseudoautosomal and the sex-determining region. In the course of our search for evolutionary breakpoints we performed comparative FISH-mapping of individual PAC clones from a human Y-chromosomal PAC contig to chromosomes of humans, great apes and Old World monkeys. The PAC contig spans approximately 2.8 Mb in proximal Yq11.21 including the entire AZFa-region. Interestingly, our FISH-results revealed that some DNA segments within the PAC clones are not exclusively restricted to their place of origin in Yq11.21, but are found at additional Y chromosomal, X chromosomal and as well as at autosomal sites within the human karyotype. Similar results were obtained for great apes, although certain human PAC clones were observed to generate altered signal patterns on the Y chromosomes of great apes. These altered patterns indicate species-specific rearrangements during primate evolution and some of these are also of phylogenetic interest.

P0239. Comparative study of disomy and diploidy in spermatozoa of fertile and infertile men using FISH

S. Zahn[1], *G. Schwanitz*[1], *G. Haidl*[2], *A. Ehrbrecht*[1], *R. Schubert*[1]
[1]*Institute of Human Genetics; Bonn, Germany;* [2]*Institute of Dermatology; Bonn, Germany*
susanne.zahn@gmx.de

Infertility affects ~15 % of all couples. In about half of these cases the infertility can be attributed to the male partner. One possible cause of male infertility is an increased proportion of aneuploid and polyploid spermatozoa. In our study, sperm cells of eleven infertile patients, aged 28-47 years, and four healthy probands of proven fertility, aged 33-36 years, were investigated. Disomy rates of chromosomes 3, 7, 8, 9, 11, 18, X and Y as well as diploidy rates were analysed by single- and triple-colour fluorescence in situ-hybridization. Semenograms of the patients showed oligoasthenoterato- (OAT, n=4), oligoastheno- (OA, n=2), terato- (T, n=3), asthenoterato- (AT, n=1) and oligoteratozoospermia (OT, n=1). The mean disomy rate for all autosomes analysed was similar for infertility patients (0,11 %) and control subjects (0,09 %). In contrast, significant differences between fertile and infertile males were observed for gonosome aneuploidies and diploidy rates. The average frequency of sex chromosome aneuploidy in infertile patients was twice as high as in the controls. The frequency of XY spermatozoa, caused by meiosis I failures, was 0,05 % in fertile and 0,19% in infertile males. In contrast, XX and YY spermatozoa were demonstrated in 0,11 % of both groups. Diploid spermatozoa were detected with a mean frequency of 0,16 % in infertile males; the controls showed a mean of 0,05 %. A donor-adapted protocol for spermhead decondensation ensuring maximum probe penetration and hybridization efficiency will be presented. Furthermore, we discuss the correlation of sperm aneuploidy / diploidy and male infertility.

P0240. Sex reversal in a girl with an Xp;Yq translocation resulting in a duplication of the Dosage Sensitive Sex reversal locus

M. DesGroseilliers[1], *J. Michaud*[2], *E. Lemyre*[2], *N. Lemieux*[3]
[1]*D partement de Pathologie et biologie cellulaire, Universit de Montr al et Centre de recherche, H pital Ste-Justine; Montr al, PQ Canada;* [2]*Centre de recherche, H pital Ste-Justine; Montr al, PQ Canada;* [3]*D partement de Pathologie et biologie cellulaire, Universit de Montr al et Centre de recherche, H pital Ste-Justine; Montr al, PQ Canada*
desgrosm@magellan.umontreal.ca

Sex determination and development in humans is a very complex process. Many genetic or chromosomal disorders can alter the process and result in sex reversal. For example, XY females can result from an abnormal interchange between the X and the Y chromosomes, or from a mutation in the SRY (Sex-determining Region of the Y chromosome) or in the androgen receptor genes. Some other aberrations such as mutations of SF1, SOX9 or WT1, deletions of 2q, 9p or 10q, and duplication of Xp21 have been reported. We present the case of a girl with dysmorphism and delayed growth development associated with an abnormal male karyotype. FISH analysis with probes WCPY (Whole Chromosome Painting of the Y), WCPX (Whole Chromosome Painting of the X), DYZ3 (recognizing the Y centromeric region), DYZ1 (recognizing Yq12), SRY (Yp11.3), DXS142 (Xp21.1), and the co-hybridization of TelVysion subtelomeric Xp/Yp and Xq/Yq have shown a 46,X,der(Y)t(X;Y)(p11.3;q11.222~q11.23) karyotype. Probe SRY was positive. Thus, the sex-reversed patient has two copies of all the genes within the Xp11.3-Xpter fragment, including DSS (Dosage Sensitive Sex reversal). A review of the literature shows that the Xp-duplication is associated with sex reversal when DSS is duplicated. This case adds strength to the hypothesis that the double presence of active DSS in XY individuals, even in the presence of active SRY, might be sufficient to cause sex reversal. (This research was supported by grant from R seau de M decine G n tique Appliqu e-FRSQ).

P0241. The study of the frequency of heteroploidy in sperm from men with low-quality semen.

I. Fedorova, *J. Loginova, L. Petrova, T. Kuznetzova*
Ott s Institute of Obstetrics and Gynecology; Saint-Petersburg, Russian Federation
irendf@mail.ru

High frequency of heteroploidy in male spermatozoa may be attributed to male infertility. Heteroploidy frequency in spermatozoa from 11 healthy men and 39 men with low-quality semen was analyzed by means of single color FISH technique with centromere specific probes to chromosomes 18. Single-probe FISH revealed frequency sum of chromosome 18 disomy and diploidy in semen of control subject 0.71%. The frequency of chromosome 18 disomy and diploidy was higher in patients with oligoastenoteratospermia and oligoastenospermia. The moderate inverse correlation between frequency of chromosome 18 disomy and diploidy and sperm concentration of spermatozoa, percentage of spermatozoa with progressive motility and spermatozoa with normal morphology was found.

P0242. An increased frequency of aneuploid sperm in infertile men with teratozoospermia

R. H. Martin[1], *C. Templado*[2], *T. Hoang*[1], *C. Greene*[3], *A. Rademaker*[4], *J. Chernos*[1]
[1]*Dept Medical Genetics, University of Calgary; Calgary, AB Canada;* [2]*Departmento de Biologia Celular y Fisiologia e Immunologia, Universidad Autonoma de Barcelona; Barcelona, Spain;* [3]*Dept Obstetrics and Gynecology, University of Calgary; Calgary, AB Canada;* [4]*Cancer Center Biometry Section, Northwestern University Medical School; Chicago, IL United States*
rhmartin@ucalgary.ca

We have demonstrated that infertile men who are candidates for intracytoplasmic sperm injection (ICSI) have an increased frequency of chromosomal abnormalities in their sperm. Reports based on prenatal diagnosis of ICSI pregnancies have confirmed the increased frequency of chromosomal abnormalities in offspring. Most studies to date have lumped various types of infertility together. However, it is quite likely that some subsets of infertility have an increased risk of sperm chromosomal abnormalities whereas others do not. We have studied 9 men with severe teratozoospermia (0-13% morphologically normal forms) by multicolour fluorescence in situ hybridization (FISH) analysis to determine if they have an increased frequency of disomy for chromosomes 13, 21, XX, YY and XY, as well as diploidy. Eight of the men also had asthenozoospermia (<20% forward progression) but none of the men had oligozoospermia (<20 X 106 sperm/ml). The patients ranged in age from 20 to 49 years (mean 33.2 years) in comparison to 18 normal control donors who were 23-58 years (mean 35.6 years). The control donors had normal semen parameters and no history of infertility. A total of 180,566 sperm were scored in the teratozoospermic men with a minimum of 10,000 sperm analyzed/donor/chromosome probe. There was a significant increase in the frequency of disomy in teratozoospermic men compared to controls for chromosomes 13 (.23% vs .13%), XX (.13% vs .05%) and XY (.50% vs .30%) (p<.0001, 2-tailed Z statistic). This study indicates that men with teratozoospermia and asthenozoospermia but with normal concentrations of sperm have a significantly increased frequency of sperm chromosomal abnormalities.

P0243. Mosaicism In Turner Syndrome

M. Dobos[1], *R. Raff*[2], *J. Szabo*[1], *A. Ehrbrecht*[2]
[1]*2nd Department of Paediatrics, Semmelweis University; Budapest, Hungary;* [2]*Institute of Human Genetics,; Bonn, Germany*
Pgabi@gyer2.sote.hu

Most of the Turner patients (UTS) are mosaics. The variability in cytogenetic findings explains some of the phenotypic differences. In patients with mosaic UTS, clinical variability results from the unequal tissue distribution of the cell lines and from the type of chromosomal defect. We investigated 299 patients because of short stature with or without Turner stigmata or primary amenorrhoea. Chromosome analysis was performed from lymphocyte cultures. The karyotype analysis proved X - chromosome abnormalities in 135 cases including 45 mosaics. The non-mosaic karyotypes were 45,X [62 cases] , 46,X,i(X) [14], 46,X,del(Xp) [4], 46,X,del(Xq) [6], 46,X,r(X) [1], 46,X,+mar [3]. The number of mosaic karyotypes investigated by GTG was 30 . In addition the 45,X cell line we detected 46,XX [5 cases], 46,X,i(X) [4], 46,XX/47,XXX [2], 46,X,+mar [6], X-deletion [4], and Y-chromosome containing cell line in 9 patients (45,X/46,XY [7], 45,X/46,X,t(X;Y)[1], 45,X/46,X,r(Y) [1]. The karyotypes of 15 patients with Turner phenotype were normal. By reason of clinical symptoms we also studied the lymphocyte cultures of these patients by FISH using wcp X, DXZ1, wcp Y and DYZ3 probes. FISH demonstrated the presence of a second cell line with numerical gonosome aberration ; 45,X [7 cases out of 15], 47,XXX, [1 case], 46,X,+mar(X) [2 cases], and of Y-containing DNA in 3 patients. In lymphocyte cultures of two patients with UTS phenotype we detected 46,XX with both methods. We analysed interphase nuclei of buccal cells with Xcen in these patients. X-monosomy was found in both at lower (17 % and 9 %) frequency . The proportion of the mosaics in our material was thus 33,33%. The results of chromosome analysis in one cell system should be completed by investigation of a second one, preferentially originating from an other germ layer, if phenotype-karyotype discrepancies are diagnosed.

P0244. Two cases of extra marker chromosome in infertile men.

J. Lissitsina[1], *R. Mikelsaar*[1], *K. Varb*[1], *G. Timberg*[2], *M. Punab*[2]
[1]*Institute of General and Molecular Pathology, University of Tartu; Tartu, Estonia;* [2]*Clinic of Surgery, University of Tartu; Tartu, Estonia*
Jelena.Lissitsina@kliinikum.ee

The frequency of supernumerary marker chromosome is 0.3 - 1.0% in infertile men. Extra marker chromosome might be the cause of meiotic arrest and instability in spermatogenesis resulting in infertility of men. Cytogenetic analysis from peripheral blood culture of 9 infertile men (sperm density <10M/mL) revealed two patients with mosaic supernumerary marker chromosomes which were present in 93% (case 1) and 3% (case 2) of cells (n=200). Both marker chromosomes were smaller than G-group chromosomes and C banding positive. In case one, the AgNOR staining showed that the marker chromosome was bisatellited. There were also both t(14q;21p) and trisomy 21, confirmed by fluorescence in situ hybridisation (FISH) in 3% and 2.5% of cells (n=400), respectively. But they were absent in skin fibroblasts. The karyotype was mos 47,XY,+mar/46,XY,+t(14q;21p),+mar/48,XY,+mar,+21/46,XY. Case one showed that supernumerary marker chromosome might cause through the interaction with other chromosomes major chromosomal anomalies as trisomy 21 and t(14;21) which are the real cause of sterility. In case two, the marker chromosome was present only in 3% of metaphases and karyotype was interpreted as mos 47,XY,+mar/46,XY. This case indicates that in even with very low frequency the supernumerary marker chromosome might be associated with infertility of men.

P0245. Cytogenetic Findings In Azoospermic Men

B. Durak[1], *M. Turgut*[2], *Y. H. Ozon*[1], *C. Can*[2], *W. Schempp*[3], *Y. Ozyurek*[2], *N. Basaran*[1]
[1]*Osmangazi University, Medical Faculty, Department of Medical Genetics; Eskisehir, Turkey;* [2]*Osmangazi University, Medical Faculty, Department of Urology; Eskisehir, Turkey;* [3]*Albert Ludwigs University Institut for Humangenetics and Anthropology; Freiburg, Germany*
bdurak@ogu.edu.tr

The study was performed to determine the specific chromosomal abnormalities of the azoospermic infertile men from the department of Urology of Osmangazi University. The karyotypes of 30 cases were determined from GTG and C banded metaphases of peripheral blood samples. Of 30 analysed azoospermic infertile men, 25 had normal karyotypes but the remaining 5 cases showed some chromosome abnormalities. One case had mosaic 46,XY/47,XYY (80/20) karyotype, one case had 46,XY,isoYp, two cases showed 47,XXY, and the other showed 46,XY,t(15;19). The karyotypes of two cases with structural chromosomal abnormality were also confirmed by the FISH analysis. In the review of literature, it has been discussed that autosomal translocations, especially the ones of acrocentrics could possibly be the reason of infertility since the abnormalities in NOR regions of the translocated chromosomes cause false X/Y recombinations during the meiosis. The reported cases with autosomal translocations support this hypotesis. Our case is especially important because of being another example supporting this mechanism.

P0246. Molecular Cytogenetic Detection of Robertsonian Translocation in Sperm Nuclei of Carrier

M. S. Yildirim, *H. Acar, T. Cora, M. Kaynak*
Selcuk University, Medical Faculty; Konya, Turkey
mselman@dostmail.com

Robertsonian translocations are the most common structural chromosome anomalies in man. Carrier of the translocations may result in offspring with chromosomaly unbalanced which may cause spontaneous abortion or phenotypically abnormal individual,although the carriers are phenotypically normal. Therefore analysis of the chromosomal constitution of their sperm nuclei can be helpful in assesing this risk in individual situation. We have used dual color fluorescence in situ hybridization (FISH) on decondensed sperm heads from carrier of structural chromosome reorganizations t(21;21). In addition, in sperm nuclei, the behavior of the Robertsonian translocation pattern along with sex chromosome was determined. The

present study demonstrates that analysis of the segregation pattern of Robertsonian translocation in sperm nuclei gives further information about chromosomal abnormalities in sperm and offsprings.

P0247. Concurrent Use of Alpha Centromere and Heterochromatin Fluorescent in situ Hybridization Probes to Enumerate Disomy in Human Sperm

A. E. Frayna[1], *W. G. Kearns*[2], *S. F. Hoegerman*[1]
[1]*Department of Biology, The College of William & Mary; Williamsburg, VA United States;* [2]*Center for Medical Genetics, The Johns Hopkins University School of Medicine; Baltimore, MD United States*
aefray@wm.edu

Studies of aneuploidy frequencies in normal sperm by FISH have yielded variable results. Past inconsistency resulted, in part, from variable scoring of closely associated homologous signals. Spatially paired signals may be interpreted as either disomy or as fragmented target sequences (a split signal). Martin et al. (1995) proposed that two signals be scored as disomy only if separated by one signal s diameter or more. This reduced data variability but the criterion s appropriateness remains undetermined. To address this, we estimated disomy 9 frequency using two-chromosome, three-color FISH. Alpha satellite centromeric sequence and adjacent heterochromatic region probes were used concurrently. A probe for chromosome 18 was used to determine ploidy. We score essentially haploid cells as disomic for chromosome 9 only if we observe two signals for both its centromeric and heterochromatic targets regardless of the distance between them. A cell with three signals for 9, regardless of the distance between the two signals from the same target, is interpreted as monosomy 9 with a split signal. Based on 30,644 sperm from three normal donors, we estimate the disomy frequency to be 0.11%. Centromeric and heterochromatic sequence split signals occurred at frequencies of 0.9% and 1.2% respectively. Disomic cells with signals separated by less than one diameter were nearly three times as common as monosomic cells with greater split signal separation. With either chromosome 9 probe alone and the criterion of Martin et al., the disomy frequency would have been 0.07%, roughly a 50% underestimation. Chromosome 1 results will also be presented.

P0248. Chromosomal anomalies in referred couple with recurrent pregnancy loss

B. Kar[1], *D. Vasanthi*[2], *N. Jayshankar*[3], *G. Haripriya*[3], *G. Perumal*[3]
[1]*Apollo Hospital; Chennai, India;* [2]*Department of Medical Genetics and Obstetric Gynecology, Apollo Hospital; Chenni, India;* [3]*Department of Medical Genetics and Obstetric Gynecology, Apollo Hospital,; Chenni, India*
ahel@vsnl.com

Recurrent pregnancy loss is a well-known clinical manifestation of various etiological factors. Convincing evidence on the causes of recurrent pregnancy loss is meager. Several factors are associated with recurrent pregnancy loss few of them included genetic/chromosomal, environmental etiologies, infectious agents, maternal congenital, acquired anatomic abnormalities, immunologic, endocrinologic dysfunctions and some are partly influenced by hereditary factors. During the period of past 2 years a total of 96 couples were referred for cytogenetic analysis with a history of two or more abortions without recognizable gynecological causes for the confirmation of possible chromosomal anomalies. After the detailed family history and pedigree analysis, routine PHA stimulated peripheral blood cultures were set up and karyotyping was done after GTG banding and other banding techniques were performed as required. A high incidence of 12.5% (n=12) of chromosomal abnormalities was observed among 96 couples referred (i.e. both structural and numerical). Those included 4.2% with balanced reciprocal translocations (N=4), 3.1% with pericentric inversions (n=3) and 5.2% with gonosomal aneuploidies/mosaicisms (n=5). In addition to these chromosomal heteromorphisms and single cell abnormalities were also found in 11.4% (n=11) and 2.1% (n=2) respectively. These data suggests that couple with a history of two or more abortions should be investigated cytogenetically. DNA microsatellite and FISH analysis are in progress in few patients found with rare chromosomal translocations.

P0249. Cytogenetic study of menstruation disorders

K. Alavi, *K. Alavi, A. R. Mohammadinia, M. H. Karimi-Nejad*
Karimi-Nejad Pathology & Genetics Center; Tehran, Islamic Republic of Iran
mhkarimi@mavara.com

In this survey, 733 cases with abnormal menstruations have been investigated. All cases were referred to our Center, from 1979 till 1999, and cytogenetically have been studied by G banding method as routine and C and Q banding in some cases.554 of 733 cases (75.6%) had primary amenorrhea, 122 cases (16.6%) had secondary amenorrhea and other 57 cases (7.8%) had oligomenorrhea. The mean age (+/- SD) was 21.8 (+/- 5.7) years. The most common cause for referring was primary amenorrhea (66.0%), followed by secondary amenorrhea (15.1%) and oligomenorrhea (5.9%). Other main complaints were infertility and short stature. 561 cases (76.5%) were cytogenetically normal (46,XX) and 172 cases (23.5%) had abnormal karyotypes, mostly 46,XY (59 cases, 8.0%) and 45,X (30 cases, 4.1%), followed by 45,X/46,X,iso(Xq) and 46,X,iso(Xq) (respectively 2.0% and 1.9%). Other abnormal karyotypes were structural abnormalities of chromosome X, consist of deletion of Xp and Xq and ring (X), trisomy X (a 47,XXX cell line with or without 45,X and/or 46,XX cell lines), marker X, Robertsonian and X; autosome translocations. Also there were 14 cases of pericenteric inversion of chromosome 9. This study revealed that the most common cause of menstrual disorders among the patients reared, as woman is 46, XY, followed by Turner syndrome and its variants.

P0250. Translocations and Spontaneous Abortions

R. R. A. P. Pardo Vargas, S. A. C. Castillo Taucher, M. S. ***C. Calvo Prandi***, *P. P. S. Sanz, V. V. D. Daher, L. A. T. Tobella, S. D. S. Salazar*
Hospital Cl nico Universidad de Chile; Santiago de Chile, Chile
servgene@ns.hospital.uchile.cl

Chromosomal anomalies usually invoked as one important etiology that should be considered, when a couple has spontaneous abortions. In this work we present the results of 481 cytogenetic studies corresponding to 481 trophoblasts of spontaneous abortions in a 10 years period. We had 382 (79,4%) positive cultures, and 162 (42%) had chromosomal alteration. The most frecuent structural alterations were desbalanced translocations (6,2%). We studied five pairs(50%) of parents of this group, and we fond always a balanced translocation in one of them. We believe that the high ratio of translocations in the parents may be reflecting more frecuent chromosomal rearrangements in the descendants of couples with balanced translocations as has been recently suggested in preimplantational studies.

P0251. Is there an increased frequency of low level sex chromosome mosaicism in couples undergoing intracytoplasmic sperm injection?

F. Morel[1], *J. Amice*[1], *V. Amice*[2], *A. Herry*[1], *M. J. Le Bris*[2], *M. T. Le Martelot*[2], *S. Roche*[2], *V. Julou*[3], *A. Val ri*[4], *M. Collet*[1], *M. De Braekeleer*[1]
[1]*CHU Morvan & Universit de Bretagne Occidentale; Brest, France;* [2]*CHU Morvan; Brest, France;* [3]*CH; Lorient, France;* [4]*CHU Cavale Blanche; Brest, France*
morelfred@lycosmail.com

Intracytoplasmic sperm injection (ICSI) is now widely acknowledged as the most effective therapeutic approach to severe male infertility. However, one problem raised by ICSI is the potentially elevated genetic risk associated with this technique. Some of the genetic anomalies observed in children born after ICSI arise de novo without a predisposing parental chromosome aberration, others may be derived from a predisposing abnormality present in one of the parents. Moreover, several studies have shown an increased frequency of constitutional chromosome aberrations in male and female partners of couples examined prior to ICSI. Nevertheless, many questions have not been broached, such as the meaning of low level sex chromosome mosaicism. When an abnormality is seen in a low percentage of cells, sex chromosome mosaicism by banding is suspected to be an artefact but can we be too categorical? Cytogenetic investigations were performed in 379 patients (179 males and 200 females) prior to ICSI treatment. Control groups were obtained from 194 males and 227 females who underwent a standard cytogenetic examination because of family chromosomal abnormalities (excluding the gonosomes). At least 16 cells were karyotyped. The routine analysis was performed on R-banded chromosome preparations. Sex chromosome loss or gain was observed in at least one cell from 24.5% of ICSI females against 22.03% of control females (p=0.70). X chromosome loss was observed in at least one cell from 20.5% of ICSI females against 18.94% of control females (p=0.69). Nevertheless, we found a significant difference between these two groups (p=0.01) if we considered X chromosome loss in at least two cells; 8% for ICSI females against 2.64% controls. No significant difference in X chromosome gain (47,XXX or 48,XXXX) was found between ICSI females and controls. Sex chromosome loss or gain was observed in at least one cell from 10.61% of ICSI males against 9.79% of control males (p=0.79). No significant difference was observed between male groups concerning gonosome loss (45,X) or gain (47,XYY and 47,XXY). These results supported previously published studies indicating that the loss of one X chromosome in only one cell in females undergoing ICSI is probably an artefact. Nevertheless, these results suggest that when two 45,X cells are found, the patient could have true low level sex chromosome mosaicism. In this case, we recommend confirmation of mosaicism by fluorescence in

situ hybridization (FISH) before discussing the expected risk of chromosomal aberrations in the offspring of the infertile couple.

P0252. Cytogenetic Studies in patients with Bad Obstetric History

S. Nagaraj[1], *S. Rajangam*[2]
[1]*St.John s Medical College; Bangalore 560034, India;* [2]*St.John s Medical College; Bangalore, India*
n.srinivas@usa.net

The phrase Bad Obstetric History has been used because it avoids the stigmas engendered by abortion and habitual . The term bad obstetric history includes not only couples with recurrent abortions but also with stillbirths and offspring with multiple congenital anomalies. Abortion is the most common complication of pregnancy and is responsible for significant emotional distress to couples desiring children. Karyotyping of 104 couples with bad obstetric history was done, after ruling out other etiological factors (anatomical, infectious, hormonal, seminal). Using conventional as well as molecular cytogenetic techniques (FISH) detection of the chromosomal abnormality and confirmation has been done. In this study, cytogenetic analysis revealed chromosomal abnormalities in 20 out of 104 couples (19.23%). Major chromosomal abnormalities have been found in 5 out of 104 couples (4.80%) and minor chromosomal abnormalities (variants) in 15 out of 104 couples (14.42%). The major chromosomal abnormalities were X mosaicism (45,X/46,XX); 3 cases of Reciprocal translocation{46,XX,t(7;8)(q31;p22);46,XY,t(7;13)(q31;q32); 46,XY,t(4;6)(4;15)(p12q21;q15q26)} and Robertsonian translocation {45,XY,t(13;14)} During counseling the chromosomal cause of bad obstetric history has been explained, with a request for follow up including prenatal procedure.

P0253. Detection of sex chromosomes mosaics in lympfocytes of patients with Klinefelter«s and Turner«s syndrome

S. Kurkova[1], *Z. Zemanova*[2], *V. Hana*[2], *J. Musilova*[2], *K. Michalova*[2]
[1]*Institute of Hematology and Blood Transfusion; Prague, Czech Republic;* [2]*3rd Medical Department, General Faculty Hospital and 1st Medical Faculty,Charles University; Prague, Czech Republic*
kurkova@uhkt.cz

The aim of this study was to detect numerical changes of sex chromosomes in dividing and non-dividing nuclei of peripheral lymphocytes of males and females with previously diagnosed Klinefelter«s and Turner«s syndrome using interfase fluorescence in situ hybridization (I-FISH). We compared clinical findings with cytogenetic results, determined the differences in sensitivity of classical and molecular-cytogenetic methods and estimated control values for healthy males and females. 26 males with Klinefelter«s syndrome and 18 females with Turner«s syndrome were examined. In males with Klinefelter«s syndrome the mosaics with 47,XXY/46,XY karyotype was found by I-FISH in all patients, small cell lines with numerical sex chromosome changes were ascertained in 8 cases (33%). In 7 females with Turner«s syndrome I-FISH proved karyotype 45,X. Remaining 11 females had chromosome mosaics of two or more clones. In all females I-FISH confirmed the results of classical cytogenetic methods and in 6 females (30%) it revealed other clones previously not detected. As a control 10 healthy males and 10 healthy females were examined and mosaics of gonosomes were not detected. The cutt-off level for the VYSIS probes used was 2,5%. The examination of fibroblasts cultures by I-FISH would prove the existence of the sex chromosome mosaics in different tissue. This would be important finding specially in patients with Klinefelter«s syndrome. Study was supported by grants GA UK 30/96 and GACR 302/98/0071.

P0254. Replication of the X chromosome in gonadal tissues of a fetus with cystic hygroma and 46,X,del(Yp) karyotype.

H. A. Aviv
UMDNJ-New Jersey Medical School; Newark, NJ United States
avivha@umdnj.edu

The SRY gene on the short arm of the X chromosome is necessary for male development. Without SRY, patients with the 46,XY karyotype develop as females, fail to achieve normal puberty and have dysgenic gonads with high incidence of gonadoblastoma. We report a female fetus, aborted at 17 weeks of pregnancy, with a non-mosaic 46,X,del(Y)(p11.2).ish del(Y)(SRY-) karyotype diagnosed by classical cytogenetics and FISH. Ovarian tissue was full of oocytes and mitotic figures. FISH studies of paraffin embedded ovarian tissues with the X and Y centromere probes revealed extensive sex chromosome mosaicism manifested by loss of the Y chromosome and replication of the X chromosome. We propose that the X chromosome replication is a post-zygotic event that arises to facilitate gonadal differentiation in the absence of all the factors necessary for normal gonadal development.

P0255. Reciprocal translocation t(13;18) in a woman with reproductive failures; case report

A. R. M. Stana[1], ***A. G. A. L. Lungeanu***[2]
[1]*Filantropia Hospital; Bucharest, Romania;* [2]*National Institute; Bucharest, Romania*
genetica@vbabes.ro

A childless couple was reffered for cytogenetic examination because of their reproductive problems; after eight years period of sterility and two spontaneous abortions, the last pregnancy ws interrupted following ultrasound examination. The anomalies detected at 11 weeks of gestation by this early ultrasound evaluation suggested a fetal chromosomopathy. Later on we performed cytogenetic analyses of both partners, using conventional banding techniques. The husband had a normal karyotype, but the wife was found to be a carrier of a balanced reciprocal translocation t(13;18). The consequences of this gross structural chromosomal rearrangements are discussed. Our results are important for the genetic counseling and recommendation for prenatal diagnosis in future pregnancies.

P0256. Cytogenetic abnormalities in two cases of primary amenorrhea.

A. G. A. L. Lungeanu[1], *A. A. Rodewald*[2], *V. I. Stoian*[3]
[1]*Nationa Institute V.Babes Bucharest; Bucharest, United States;* [2]*Hamburg University, Institute for Human Biologie; Hamburg, Germany;* [3]*Faculty of Biology, Department of Biology, Bucharest University; Bucharest, Romania*
genetica@vbabes.ro

There are presented two phenotypically normal females of 21 and 23 years old, respectively, diagnosed as primary amenorrhea. First case, S.M., was reffered for cytogenetic investigation at 21 years age, because of her menstrual cycle began at 17 years and was intermittent. At the time she presented herself for karyotype exam she was under hormonal tretment. GTG banded karyotypes revealed cellular mosaicism 45,XX,-14,+18p/46,XX,+18pter,(20%/80%). The presence of supplementary fragment on the short arm of 18 chromosome was found both in 45 and 46 chromosome metaphases, which could explain the failure of prescribed hormonal therapy meant to regulate her cycle. Hypodiploidy was associated with chromosome 14 monosomy, and we cannot exclude that the supplementary fragment on 18p might come, possibly, from 14. We consider this feature, which is peculiar to this case, involved in the primary amenorrhea developed by our subject; meanwhile, we excluded X chromosome involvement. The second case, M.I., 23 years old with primary amenorrhea, exhibited a mosaicism 45,XX,t(20;21)/ 45,X0. By our knowledge, there are not other previous reports concerning involvement of this translocation in primary amenorrhea.

P0257. Rare Triple Transformations As The Cause Of Marital Infertility

T. Kranjc, *S. Brankovic-Niksic, S. Nikolic, G. Radivojevic-Pilic*
Institute of Mental Health; Belgrade, Yugoslavia
nbrankov@eunet.yu

The paper presents the case of two marital couples with a large number of miscarriages in the first trimester of gravidity. The analysis of karyotype of peripheral blood has indicated the presence of two very rare triple translocations. In one case, we had 46, xy, t (5; 8; 15) (p13; p21; q13) and in another; 46, xx, t (2; 8; 13) (p13; p23; q22). The paper also discusses the possibility for having healthy offspring.

P0258. Chromosome 21 Disomy In The Spermatozoa Of The Fathers Of Children With Trisomy 21

I. Kaya[1], *B. Durak*[1], *Y. H. Ozon*[1], *T. Donmez*[2], *N. Basaran*[1]
[1]*Osmangazi University, Medical Faculty, Department of Medical Genetics; Eskisehir, Turkey;* [2]*Osmangazi University, Medical Faculty, Department of Urology; Eskisehir, Turkey*
ankaya@yahoo.com

In this study FISH analysis has been carried out in the sperm samples of 10 normal males and 13 fathers of children with trisomy 21, totally 23 males. The purpose of our study is to detect the hybridization efficiency and segregation frequencies by using locus spesific probes of chromosome 21 and 13. In our study 23,420 nuclei have been examined and the hybridization efficiency is determined as 97.9 %. 13,420 nuclei of our case group have been examined. As the control group, a total of 10,000 nuclei were analysed (Table1). In conclusion, disomic spermatozooa can be effective in the formation of aneuploidic embryo. However, to increase the reliability of the method of aneuploidy screening on the fathers of children with Down syndrome , paternity test have to be performed and the number

of donor series have to be increased.
Table 1

	HAPLOIDY (%)	DISOMY 21(%)	DISOMY 13(%)	DIPLOIDY (%)
CONTROL	95.28	0.49	0.3	1.26
CASES	92.57	1.6	0.6	1.3

P0259. Direct duplication of 8p21.3p23.1; FISH delineation and clinical significance

Y. S. Fan[1], *J. H. Jung*[1], *V. M. Siu*[1], *S. A. Farrell*[2], *G. B. Cote*[3]
[1]*London Health Sciences Centre; London, ON Canada;* [2]*The Credit Valley Hospital; Mississauga, ON Canada;* [3]*Sudbury Regional Hospital; Sudbury, ON Canada*
fany@lhsc.on.ca

We report six cases in two families and a sporadic case with a dir dup(8)(p21.3p23.1). In one family, the duplication started in the mother and was transmitted to one son and one daughter. In the second family, the father was mosaic for the anomaly which was transmitted to his two daughters. In each case, the cytogenetic anomaly was initially described as a 8p+ with banding analysis. These were further studied with fluorescence in situ hybridization using whole 8 painting, 8p specific painting and 8p subtelomeric probes. Deletion was not detected in the subtelomeric region of the short arm of the abnormal chromosome 8 examined in one family and in the sporadic case. The phenotypic picture varies from normal to moderate mental reardation in the affected individuals. No obvious dysmorphism or congenital defects were consistently observed among these cases. After comparing the chromosome region involved in our cases with those in others having direct or inverted duplications of 8p, it is thought that the segment 8p21.1->21.3 might be the critical region for a 8p duplication syndrome. The parental origin of the duplication does not seem to impact on its clinical significance.

P0260. Clinical, cytogenetic and FISH findings in a 19y old girl with 45,X,del(18)(q21.3)de novo

O. Rittinger[1], *G. Kronberger*[2], *E. Ploechl*[2]
[1]*Clinical Genetics, Children s Hospitalh; Salzburg, Austria;* [2]*Clinical Genetics; Salzburg, Austria*
o.rittinger@lks.at

An increasing number of structural chromosomal aberrations is considered to be a clinically recognizable condition, mainly through a distinctive physiognomy and malformation pattern. This holds true also for terminal deletions of the long arm of chromosome 18,originally identified by Grouchy et al.1964. We report on a 19y old girl affected by a distal deletion of 18q and, in addition, pure monosomy X. We would like to discuss the impact of the respective aberrations on the clinical features. Interestingly, there is a considerable overlap of the features of both conditions; mild mental retardation, triangular face, carp like mouth, microcephaly, unusual concave deep set nails and bilateral club foot refer mainly to the autosomal deletion, some other facial features , eg the deep hair line, epicanthus, ptosis, the down-slanting palpebral fissures mirror the well-known Turner syndrome aspect.No hearing impairment or cardiac failure was diagnosed.Growth retardation, a common finding in both clinical pictures,resulted in a final height of 143cm.Cytogenetic investigation let assume the breakpoint in 18q22,but this remains to be proven by FISH to date. More complex rearrangements or only interstitial deletion were excluded by subtelomeric FISH analysis. Multiplex PCR investigation for possible Y chromosome sequences was uneventful.Hormonal results revealed the exspected hypergonadotrophic insufficiency of the ovary,but could not detect any abnormal response of GH to provocative agents. Self-esteem and social acceptance improved markedly by psychological and hormonal support. To our knowledge, this patient represents the first case of Turner syndrome involved in this particular autosomal anomaly.

P0261. Intrachromosomal triplication of 15q11-q13 ; a new observation.

F. Vialard[1,2], *C. Mignon-Ravix*[3], *D. Parrain*[4], *M. F. Portno* [1], *M. Le Lorch*[5], *S. Romana*[5], *M. G. Mattei*[3], *H. Moirot*[2]
[1]*Laboratoire de Cytog n tique, CHU Saint Antoine; Paris, France;* [2]*Laboratoire de Cytog n tique, CHU de Rouen; Rouen, France;* [3]*Unit Inserm U491, Faculte de M decine; Marseille, France;* [4]*Service de Neurophysiologie, CHU de Rouen; Rouen, France;* [5]*Laboratoire de Cytog n tique, CHU de Necker; Paris, France*
fvialard@hotmail.com

We report a new case of intrachromosomal triplication of the proximal 15q11-q13 region. At our knowledge, it is a rare chromosomal disorder. Our propositus is a 22 year old boy, with severe developmental delay and epilepsy. Cytogenetic R-bands analysis suggested the presence of additional material on the proximal region of one chromosome 15. FISH analyses with probes from the Prader-Willi/Angelman region showed this region is triplicated on the abnormal chromosome, the middle segment being inverted in orientation. Then, the patient is tetrasomic for the 15q11-q13 region. Molecular studies using microsatellite and RFLP analyses demonstrated that the triplication is maternal in origin, with two alleles being involved. It is interesting to note that tetrasomies 15q11-q13 with intrachromosomal triplication, as those associated with supernumerary inv dup (15) chromosome, most often result from rearrangements occuring at maternal meiosis. In both situations, the dosage of maternal/paternal imprinted regions is identical and, in the absence of mosaicism, phenotypes are expected to be similar. We compare the phenotype of our patient with that of patients from these two classes of tetrasomy 15q11-q13. Finally, we propose a mechanism to explain the formation of intrachromosomal triplication.

P0262. Unusual genotype / phenotype correlation in a patient with partial monosomy 15 and partial trisomy 14

M. Volleth[1], *J. B rger*[2], *P. Muschke*[1], *P. Wieacker*[1], *M. Stumm*[1]
[1]*Dept. of Human Genetics; Magdeburg, Germany;* [2]*Dept. of Human Genetics; Berlin, Germany*
Marianne.Volleth@medizin.uni-magdeburg.de

Deletion and other abnormalities of human chromosome 15q11-q13 are associated mainly with two developmental disorders, Prader-Willi syndrome and Angelman syndrome (AS). Despite the advanced analysis of this critical region and the identification of new genes, the exact genotype / phenotype correlation is still unclear, especially in translocation patients. We report on a girl with an unbalanced translocation whose breakpoint falls within the 15q11-q13 region. The patient presented some features of Angelman syndrome as mental retardation, speech impairment, developmental delay, microcephaly, hypopigmented skin, light hair and eye color. Other clinical features typical for AS such as ataxia are missing. GTG-banding and FISH analysis of the patient detected a derivative chromosome 14 due to an unbalanced maternal inherited translocation t(14;15)(q13;q11.2). The der(14) resulted in a partial monosomy 15p13-q11.2 and a partial trisomy 14p13-q13. SNRPN is not deleted and AS has been excluded by methylation analysis. YAC FISH was performed for the detailed breakpoint characterization. The breakpoint on chromosome 15 lies proximal from YAC 254b5 and very close to the centromere. Therefore, it is unlikely that the partial monosomy 15 mainly affects the patient«s phenotype. The break-point on chromosome 14 lies between YAC 855c06 and YAC 950b08, resulting in a trisomic segment of about 30 cM. However, the patient«s phenotype is not similar to that of other patients with proximal trisomy 14q. But pure trisomies of chromosome 14 are rarely described and a correct genotype / phenotype correlation is difficult. Our data suggest that a pure trisomy 14p13-q13 may generate an AS-like phenotype.

P0263. Complex chromosomal rearrangements of 6 postnatal cases; are they as rare and detrimental as we think?

S. M. Zneimer, *J. C. Kelly, S. D. Stewart*
Quest Diagnostics; Van Nuys, CA United States
susan.m.zneimer@questdiagnostics.com

We report 6 cases in which complex chromosome rearrangements (CCR s) were observed, each with varying clinical manifestations from normal to severe congenital anomalies, in a series of 5000 postnatal chromosome studies. In all cases, at least 3 breakpoints and 2 chromosomes were involved. In cases 1-3 (see karyotypes below), an inversion of one chromosome was present with a translocation involving a second chromosome. In cases 4-5, CCR s involved 3-way reciprocal translocations. Case 6 involved 2 chromosomes in which a portion of one chromosome was inserted into the X chromosome. The G-banded karyotypes are below;
Case 1; 46,XX,der(2)inv(2)(q14q23)t(2;3)(p15;q27)
Case 2; 46,XY,der(12)inv(12)(q21.3q24.1)t(1;12)(q42.3;q24.3)
Case 3; 46,XX,der(5)inv(5)(p13p15.1)t(5;8)(q23.2;q22.1)/46,XX
Case 4; 46,XY,der(7)t(7;13)(p11.2;q14.3)t(7;21)(q11.2;q22.3)
Case 5; 46,XY,t(1;7;14)(q32.3;p21.2;q21.2)
Case 6; 46,X,ins(X;8)(p22.1;p23.1p23.3)
All cases are apparently balanced rearrangements. Three of the CCR s (1,2 and 4) were phenotypically normal individuals with a history of reproductive problems. Three of these CCR s (3,5, and 6) were studied for an indication of developmental delay or phenotypic anomalies.
There are few reports of CCR s in the literature implying that these events are quite rare. However, it is possible that CCR s occur more frequently than expected from the literature. Our data suggests that balanced forms of these events may even be fairly common in the population. It appears that genomic material itself and not the number of breaks and chromosomes involved may influence phenotype. Only balanced forms of CCR s

were observed in this study. Although CCR s have the potential to giving rise to unbalanced forms, a high rate of gamete loss may explain their scarcity.

P0264. Dup 9p syndrome with multiplex hemangiomatosis and deafness in a familial t(10;22) carrier

J. K rteszi[1], . *Morava*[1], *M. Czak* [2], *T. Decsi*[3], *G. Kosztol nyi*[1]
[1]*University of P cs, Department of Medical Genetics and Child Development; P cs, Hungary;* [2]*MTA-PTE Clinical Genetics Research Group; P cs, Hungary;* [3]*University of P cs, Department of Pediatrics; P cs, Hungary*
judit.karteszi@freemail.hu

The characteristic features of duplication 9p syndrome include severe mental/growth retardation, microcephaly, deep set eyes, carp-mouth, VSD, umbilical hernia and 5th finger clinodactyly. Epilepsy may also be present in some patients. We report on a female infant with typical features of the 9p trisomy syndrome associated with multiplex hemangiomatosis and sensoneurinal deafness. High resolution analysis and fluorescent in situ hybridisation using the chromosome 9 specific painting probe detected a de novo partial duplication of 9p [dup 9(p21 p23)] as part of a complex chromosome rearrangement. A balanced translocation [t(10;22)(q26;q12)] was found in the patient and in her symptomless father without any alteration of the short arm of chromosome 9. Patients with balanced translocations involving chromosomes 10 and 22 are seldom described. The de novo duplication of a chromosome, other then the chromosomes involved in the rearrangement, in an offspring of a translocation carrier has not been reported yet. The new partial duplication of chromosome 9 in association with (10;22) translocation in our patient could be related to a possible instability of the balanced chromosomal rearrangement.

P0265. Maternal insertion of 18q11.2-q12.2 in 18p11.3 leading to recurrent unbalanced translocations in the offspring detected by microdissection and multicolor banding (MCB)

H. Starke[1], *G. Senger*[2], *M. Kossakiewicz*[3], *H. Tittelbach*[3], *N. Rubtsov*[4], *V. Trifonov*[4], *A. Heller*[1], *U. Claussen*[1], *T. Liehr*[1]
[1]*Institute of Human Genetics and Anthropology; Jena, Germany;* [2]*Practice of Medical Genetics and Gynecology; Regensburg, Germany;* [3]*Kossakiewicz; N rnberg, Germany;* [4]*Institute of Cytology and Genetics; Novosibirsk, Russian Federation*
b6sthe@mti-n.mti.uni-jena.de

A 24 year old women was referred to amniocentesis because of Down syndrome in the family. No chromosome 21 aberrations but a chromosome 18q- was detected by GTG-banding. After microdissection and reverse painting (the created microdissection library was called MIDI 58), the derivative chromosome 18 was defined as del(18)(q11.2q12.2) [see Figure]. Ultrasound examination of this child (in the following called child 1) was inconspicuous, however, pregnancy was terminated. The karyotype of the mother showed a morphologically altered chromosome 18, as well. It was classified as an inv(18)(p11.3q12.1) according to GTG banding. One year later an inconspicuous girl was born (child 2), showing the same aberrant chromosome 18 as in the mother. Now, in her third pregnancy (child 3), karyotypic analysis of the chorion cells revealed an 18q+. Multicolor-banding (MCB) analysis using a chromosome 18 specific probe set revealed, however, a normal banding pattern for 18q and changes in 18p. The identical changes as in the fetus were detected in the short arm of the so-called inverted chromosome 18 of the mother. Additional hybridization of the microdissection library MIDI 58 confirmed, that the maternal altered chromosome 18 was in fact an ins(18)(pter->p11.32:;q11.2->q12.2:;p11.31->q11.2:;q12.3->qter) [see Figure]. Application of the probe MIDI 58 on the aberrant chromosome 18 of child 3 showed a duplication dup(11.2q12.2) [see Figure]. As ultrasound diagnostics is inconspicuous, the parents decided not to terminate the pregnancy. In summary, the partial monosomy in child 1 and the partial trisomy in child 3 can easily be explained by unequal crossing over events during maternal meiosis. MCB in combination with microdissection and reverse painting were the prerequisite to determine the karyotype of the mother and her offspring correctly. Acknowledgements; This work was supported by the Herbert Quandt Stiftung der VARTA AG, the Madeleine Schickedanz-Kinderkrebs-Stiftung, the DAAD, the DFG and the Wilhelm Sander-Stiftung.

P0266. An unusual de novo inversion and duplication of chromosome 8; 46, XY,rec(8) dup(p11 p22), inv (8)(p23 q22) A relationship with San Luis Valley Syndrome ?

C. Hans[1], *D. Delneste*[1], *S. Graw*[2], *F. Christiaens*[3], *E. Vamos*[3], *L. Duprez*[3]
[1]*Hopital Erasme Universit Libre de Bruxelles; Bruxelles, Belgium;* [2]*Eleanor Roosevelt Institute; Denver, United States;* [3]*Hopital Universitaire Des Enfants Reine Fabiola; Bruxelles, Belgium*
chans@ulb.ac.be

The San Luis Valley Syndrome (SLVS) is due to a recombinant chromosome 8 resulting from a recurrent pericentric inversion in a population of Hispanic ancestry originating from the San Luis Valley in Colorado (AJHG,2000,66;1138). We report on a 4 years old boy born to unrelated Spanish parents. He presents with severe mental retardation, right sided spastic hemiplegia and brain malformations (agenesis of corpus callosum, left frontal cortical dysplasia, hydrocephaly). He shows facial dysmorphism with large low set ears, hypertelorism, pterygium colli, pectus excavatum, but no cardiac defect. Prometaphase chromosome analysis reveals a recombinant chromosome 8 with a pericentric inversion (p23 q22) and duplication 8(p11 p22). This duplication is confirmed by CGH. The breakpoints of the pericentric inversion 8(p23 q22) are similar to those reported in the SLVS chromosome 8 variant. However, whereas the rec(8) observed in the SLVS consists in dup(8)(q22-qter), and del(8)(p23-pter), in our case the rec(8) includes dup(8)(p11-p22) and no detectable deletion by CGH. The clinical features in our patient are indeed compatible with the dup(8) syndrome. Molecular tools are now used in order to study the breakpoints regions and explore a possible link with the SLVS.

P0267. Duplication (4)(q31.1qter) in a newborn with suspicious clinical diagnosis of Nijmegen breakage syndrome

I. Cernakova[1], *M. Kvasnicova*[2], *Z. Lovasova*[2], *N. Badova*[3], *E. Seemanova*[4], *K. Spackova*[5]
[1]*Lab. experimental medicine; Olomouc, Czech Republic;* [2]*Dept. Clin. Genetics; Banska Bystrica, Slovakia;* [3]*Dept. Paediatrics; Banska Bystrica, Slovakia;* [4]*Inst. Genet.; Prag, Czech Republic;* [5]*Lab. exptl. med., Dept. Paed., Palacky Univ.; Olomouc, Czech Republic*
iveta.cernakova@hotmail.com

Nijmegen breakage syndrome (NBS) is a rare autosomal recessive condition characterised by progressive microcephaly, early retardation of growth, chromosomal instability, hypersensitivity to ionising radiation and immunodeficiency predisposing to recurrent infections and malignancies in childhood. We report a case of a newborn with some clinical features of NBS and usefulness of combination of clinical examination, classical cytogenetics, FISH and molecular methods in establishing of correct diagnosis. The newborn is the 2nd child of young, healthy and genetic unrelated parents. He is hypotrophic with unspecific somatic stigmatization; microcephaly,facial dysmorphism and heart abnormality (ASD). The karyotype of the child was 46,XY, add(4)(q35) de novo with chromosomal breaks in 4% of metaphases. We revealed the origin of additive material on 4q as dup(4)(q31.1qter) using the whole chromosome painting probe 4 and telomere probe 4q combined with G-banding technique. The translocation t(14q;21q) was also in 4 metaphases by FISH detected. The suspicious clinical diagnosis of NBS was excluded because the deletion 657del5 in NBS1 gene on 8q21 was not detected. We compare the similarity of clinical picture of our patient with cases with duplication of distal part 4q described in the literature.

P0268. Report of 6 cases of trisomy 10p resulting from parental pericentric inversion (10)

R. Karimin-Nejad, *H. Karimi, H. Miri, N. Nabavi-Nia, M. Rahimi, K. Shirneshan, M. H. Karimi-Nejad*
Karimi-Nejad Pathology and Genetics Center; Tehran, Islamic Republic of Iran
mhkarimi@mavara.com

During the past 20 years and among more than 25000 peripheral blood karyotypes, we have detected 6 cases of add(10)(q25). All of these cases were referred between 1-12 months of age with multiple congenital anomalies including; severe growth retardation, congenital dislocation of the hip, bilateral club feet, high bossing forehead, narrow face with sagging cheeks, broad nasal bridge, harelip or cleft palate, small round chin, large posteriorly rotated low set ears, hyperflexed limbs, hypoplastic genitalia and severe hypotonia. In 5 of the cases, their parents karyotypes were studied, and 3 of the fathers and 2 of the mothers had a pericentric inversion in chromosome 10. The inversion involves the short and the long arm with breakpoints at p11 and q25, resulting in a long acrocentric chromosome 10. This identified the karyotype of the children as rec(10)dup(10p)inv(10)(p11q25). One of the families did not participate in the study. Prenatal diagnosis has been performed for two of the families, revealing a normal fetus in one and a trisomy 10p in the other.

P0269. DiGeorge Syndrome in Czech Republic

M. Nemeckova[1], *E. Kocarek*[2], *A. Puchmajerova*[2], *T. Klein*[3]
[1]*Gennet CZ, s.r.o.; Prague, Czech Republic;* [2]*Institute of Biology and Medical Genetics, University Hospital Motol; Prague, Czech Republic;* [3]*Kardiocentrum, University Hospital Motol; Prague, Czech Republic*
michaela.nemeckova@gennet.cz

90 percent cases of DiGeorge syndrome (DGS) with variable clinical manifestation -conotruncal congenital heart diseases, craniofacial dysmorphia, hypocalcemia, immunodeficiency, psychiatric disorders- are due to 22q11.2 microdeletion. Estimated incidence of 1/4000 live births is ranging DGS among the most frequent microdeletion syndromes. Peripheral blood of 86 patients suspect from DGS was investigated by FISH using LSI probe (Vysis) between 1997-2000. At 24 patients the microdeletion was found. 23 from them had congenital heart defect. Tetralogy of Fallot (ToF) —39 %- and interrupted aortic arch (IAA) —35 %- were the most frequent ones. At one case the microdeletion was inherited from mother with forme fruste of DGS. Epidemiological and clinical consequences of DGS are discussed.

P0270. A molecular cytogenetic and clinical study of patients with the Prader-Willi and Angelman syndromes.

P. Kugl k[1], *R. Gaillyov* [2], *H. Radilov* [1]
[1]*Department of Genetics and Molecular Biology, Faculty of Science, Masaryk University; Brno, Czech Republic;* [2]*Department of Medical Genetics, Children«s Hospital, Faculty Hospital; Brno, Czech Republic*
kugl@sci.muni.cz

The Prader-Willi (PWS) and Angelman (AS) syndromes are two distinct mental retardation syndromes that are caused by either microdeletions or uniparental disomy of the same chromosomal region. The deletions responsible for PWS and AS have been localized to band 15q11-q13 of chromosome 15. PWS is characterized by infantile hypotonia with feeding problems, short stature, small hands and feet, almond-shaped eyes, hypogonadism, psychomotor retardation, hypopigmentation, and development of obesity. AS is characterized by severe mental retardation, seizures, inappropriate laughter, ataxic gait, puppet-like upper limb movements, lack of speech, large mandible, hypopigmentation, an open mouth with protruding tonque, and microcephaly. We report the results of molecular cytogenetic and clinical studies on 29 patients suspected for PWS or AS. During the last 2 years we diagnosed 4 patients with deletion 15q11-q13 using fluorescence in situ hybridization (FISH) with D15 S10 and SNRPN DNA probes. In patients without a cytogenetic deletion, molecular study of uniparental disomy for chromosome 15 was performed. Based on our results we analyzed the clinical criteria and typical phenotype features for patients having deletion 15q11-q13 or uniparental disomy.

P0271. A Case of Marfanoid habitus with mental retardation associated with a subtelomeric translocation

M. C. Vincent[1], *S. Julia*[1], *P. De Mas*[1], *D. Lacombe*[2], *G. Bourrouillou*[1], *P. Calvas*[1]
[1]*Hospital Purpan; Toulouse, France;* [2]*Hospital Pellegrin; Bordeaux, France*
vincent.mc@chu-toulouse.fr

The fluorescence in situ hybridization (FISH) technology has made new approaches available for cytogenetic screening such as subtelomeric rearrangement examination which would be missed by conventional chromosome banding methods and painting probe in molecular cytogenetics. We report here a case of unbalanced cryptic translocation in a family with both recurrent miscarriages and three children with mental retardation. The index case (IC), a 14 years old girl, is the second among two girls born from non consanguineous parents. Since infancy, she presented a developmental delay, a marfanoid habitus and dysmorphic facies features including epicantal fold, suborbital fullness of rubber tissues, prominent nose with squared and wide nasal root and dysplastic ears. Echocardiogram, renal ultrasound examination, skeleton radiographies and metabolic analyses were normal. Conventional cytogenetic analyses failed to detect numerical or structural chromosome aberration. FISH analyses using Multiprobe panT CYTOCELL kit were performed in order to detect putative subtelomeric rearrangement. The analysis demonstrated in the index case a p-arm telomere monosomy and a p-arm telomere trisomy for the chromosome 8 and 12 respectively. The molecular cytogenetic examination of the father involved the identification of the balanced subtelomeric translocation t(8,12)(pter,pter). This cryptic chromosome anomaly resulted from an unbalanced subtelomeric translocation by malsegregation adjacent-1 type. Accurate genetic counselling and the opportunity for early prenatal diagnosis can now be offered to this family. This type of molecular cytogenetic telomerics analysis allowed the description of putative new syndromic association which do not share any features with described trisomy 12p and monosomy 8p.

P0272. Translocation (4;13)(p16;q11) giving rise to different unbalances; duplication 4p and deletion 4p (Wolf-Hirschhorn Syndrome)

M. I. Melaragno, *S. S. Takeno, M. Corbani, D. Brunoni*
UNIFESP; Sao Paolo, Brazil
melaragno.morf@epm.br

Wolf-Hirschhorn syndrome (WHS) is characterized by severe growth and mental retardation, typical facial appearance, and seizures. It results from the partial deletion of the short arm of chromosome 4 (4p16.3). In most reported cases, the deletion can be detected by conventional chromosome analysis, while in some cases, a microdeletion can only be detected by molecular probes. This study presents a male patient with WHS who died with 37 years. He carried a karyotype 45,XY,der(4)t(4;13)(p16;q11)mat,-13. A balanced translocation could be observed in his mother and grandmother. His brother presents mild mental retardation, minor phenotypic alterations and a karyotype 47,XY,+der(13) t(4;13)(p16;q11). The same extra derivative chromosome 13 was also present in one of his three children, a girl who has the same phenotype as her father. Fluorescent In Situ Hybridization (FISH) was performed using LIS WHS Microdeletion Probe (Vysis) revealing two signals in the balanced translocation; one in the normal chromosome 4 and one in the derivative chromosome 13. Thus, both girl and her father have partial trisomies for 4p16-pter and 13pter-q11, while the WHS patient had monosomy for the same segments, although the unbalance for the chromosome 4 is the relevant one for the phenotype. It is an unusual balanced translocation as it results in two different unbalanced gametes from 3;1 disjunction, both giving viable individuals. One of them could be fertile and transmit the extra derivative chromosome. The unbalanced gametes arising from 2;2 disjunction in this translocation would probably be less compatible to life. (CNPq).

P0273. Clinical variability in ring chromosome 15 syndrome; implication of deletions of subtelomeric regions ?

B. Doray, *F. Girard-Lemaire, O. Caron, E. Flori*
Laboratory of Cytogenetics; Strasbourg, France
doray_s@club-internet.fr

Ring chromosome 15 is an uncommon chromosomal abnormality (about thirty cases reported since 1975). The common clinical phenotype includes severe short stature, microcephaly, triangular face and variable mental retardation. We report here two patients with ring chromosome 15. The first patient is a girl followed-up from birth to the age of 18 months. She presented a severe phenotype including pre and postnatal growth retardation, psychomotor delay, microcephaly with triangular face, congenital heart disease and renal malformation. A ring chromosome 15 was identified in 95 % of the analysed blood lymphocytes. FISH using subtelomeric probes showed deletion of the subtelomeric regions. The second patient was a 33 year-old man. He was referred in genetic department for infertility. He had a small stature, but no significant dysmorphism and he was normally intelligent. Chromosome analysis revealed a ring chromosome 15 in all analysed blood lymphocytes. FISH using subtelomeric probes was performed and proved the presence of subtelomeric regions in this ring chromosome. This report emphasises the large clinical variability of ring chromosome 15 and may suggest that deletions of subtelomeric regions would be associated with a more severe phenotype. However, other similar reports would be necessary to support this hypothesis.

P0274. Recurrent pure maternal 12p trisomy

D. Souza[1], *R. A. Bicudo*[2], *M. S. G. Silva*[2], *C. C. Benetti-Filho*[2], *D. Moretti-Ferreira*[1]
[1]*Dept. of Genetics, IBB-UNESP; Botucatu, Brazil;* [2]*Genetic Counselling Service, IBB-UNESP; Botucatu, Brazil*
sag@fmb.unesp.br

12p trisomy phenotype is a subject that has been debated in the literature . Even though patients with pure 12p trisomy are rare, there are several reports on patients carrying it by translocation with other autosomes. Two brothers have been recently referred to our service. Their phenotypes were similar and consisted of turricephaly, round facies, prominent front, epicantic fold, scarce eyebrows, wide nasal bridge, short nose, anteverted nostrils, long nasal filter, thin upper lip, malformed ears, short neck, severe hypotonia and Neuro-Pshycho-Motor-Development delay. Both children were males and aged 8 and 2 years when genetically and clinically assessed. Cytogenetic analyses of both patients revealed karyotypes 47, XY, der (21)t(12; 21)(12 pter -> 12p11.21;;21q 11.1-> 21p 11.1) analyzed by banding techniques GTG, CBG, high resolution and FISH. Cytogenetic analysis of the mother revealed karyotype 46, XX t (12; 21)(12 qter-> 12p 11.2 ;; 21q 11.1 ->21 qter). The father showed normal karyotype. The patients two sisters presented balanced translocation 12/21, an extra chromosome of the sexual pair and karyotype 47, XXY, t (12; 21) (Klinefelter Syndrome associated with balanced translocation). The patients have the 12p trisomy classical phenotype. The brother of the patient with Klinefelter Syndrome is likely to be another example of interchromosal effect on maternal meiosis, resulting in the chromosome X non-disjunction.
Supported by FUNDA O LUCENTIS

P0275. Trisomy 14q syndrome; confirmation by two rec dup(14q) in two generations with familial pericentric inversion (14)(p12q24)

A. Dufke[1], *P. Ruppersberger*[2], *R. H. Sch ffel*[3], *P. Kaiser*[1]
[1]*Department of Medical Genetics; Tuebingen, Germany;* [2]*Gemeinschaftspraxis Dr. Limbach; Heidelberg, Germany;* [3]*Pediatrician in Private Practice; Lichtenau-Fuerstenhagen, Germany*
andreas.dufke@uni-tuebingen.de

In a girl presenting with conspicuous craniofacial features and psychomotoric retardation cytogenetic analysis revealed a rearranged chromosome 14 with pure trisomy of the distal bands 14q24 to 14qter (rec dup(14q)) due to a paternal pericentric inversion (14)(p12q24). Familial examination gave the same pericentric inversion in the two oldest sibs of the proposita (Kaiser et al., 1984; Clin Genet 26; 73-76). Examination of the younger sister had been refused by the parents. This younger sister had three consecutive miscarriages. Chromosomal analyses had not been carried out on the fetuses. Only during the fourth pregnancy she was diagnosed to carry the familial pericentric inversion. Chorionic villus sampling was performed which led to the loss of a chromosomal healthy fetus. In a fifth pregnancy no prenatal cytogenetic analysis was carried out and a boy was delivered by caesarean section because of fetal distress at 35 weeks of gestation. Stenosis of the aortic isthmus was noted. At the age of 5 months he presented with developmental delay. He had minor facial anomalies, including eye abnormalities and a characteristic shape of the ears. The phenotype was very similar to the proposita. Cytogenetic investigation showed the same imbalanced karyotype (rec dup(14q)). We therefore propose a distinctive multiple congenital anomaly / mental retardation (MCA/MR) syndrome due to partial trisomy of distal chromosome 14q. We discuss the recombination risk of the given inversion and compare the clinical and cytogenetic data with previously reported cases on trisomy 14q. A long term follow up of the proposita is given.

P0276. A new case of partial trisomy 6p — monosomy 6q from a familial inversion of chromosome 6.

M. Gregoire[1], *Y. Leroux*[1], *V. Bourdon*[1], *M. Krier*[2], *J. Andre*[2], *P. Jonveaux*[1], *B. LeHeup*[1]
[1]*Department of Medical Genetic CHU de Nancy-Brabois; Vandoeuvre, France;* [2]*Pediatric Nephrology CHU de Nancy-Brabois; VANDOEUVRE, France*
leheup@facmed.u-nancy.fr

This report describes a new patient with unusual clinical signs which are related to a partial trisomy 6p and monosomy 6q. The patient, born in 1983, is the first of a normal father born in 1956 and a normal mother born in 1959. Pregnancy has been uneventful. She was born at the term of 38 weeks with normal birth lenght and weight. Head circumference was slightly decreased at —2,5 SDS. During the first days of life biological evidences of renal insufficiency lead to the diagnosis of a left renal dysplasia with bilateral ureteral reflux. Cytogenetic examination was reported as normal. Developmental milestones were delayed with the walk present after 18 months of life. Language did not appear before 4 years of life with evidence of nasal speech. At 13 years height and weight were in the 50th centile. Pubertal development was normal. The mean IQ was evaluated at 53 with mental progression around 7 years of age. The facial dysmorphism was mild with microcornea, strabism, asymetrical right ptosis, prominent nasal bridge, low set ears, and prognathism. Halluces and thumbs were broad and symetrical shortening of the 4th metacarpal and metatarsal bones were also reported. The progression of renal insufficiency lead to kidney transplantation in 1998. Her mother reported two early miscarriages at the 6th week of gestation in 1998 and 1999. The cytogenetic reevaluation of the patient and her parents gave the following results ; mother ; 46, XX, inv(6)(p23q27); patient ; 46, XX, rec(6)dup(6p)inv(6)(p23q27)mat. The patient has a trisomy of the 6pter - 6p23 and monosomy of the 6q27-6qter regions. Compared to the data of the literature the cytogenetic anomalies are identical to the case reported in 1993 by Wauters. To the difference of that clinical report our patient did have neither short stature, blepharophimosis, nor cardiac malformations . The shortening of metacarpal and metatarsal bones have not been reported so far.

P0277. A case of pure partial trisomy of 5q34-qter associated with asthma, allergies and hyper IgE.

S. Demczuk[1], *B. Dion*[1], *P. Lepage*[2], *B. R. Hadad*[3], *T. Hudson*[2], *V. M. Der Kaloustian*[4]
[1]*Montreal Children s Hospital Research Institute; Montreal, PQ Canada;* [2]*Genome Center, Montreal General Hospital; Montreal, PQ Canada;* [3]*Institute for Molecular and Human Genetics, Georgetown University; Washington, DC United States;* [4]*Medical Genetics, Montreal Children s Hospital; Montreal, PQ Canada*
suzanne.demczuk@muhc.mcgill.ca

Unbalanced chromosomal anomalies can lend information on the position of genes and on the understanding of pathologic mechanisms of common diseases. Thus, one locus for asthma and allergic disease, a multifactorial trait, has been mapped to the long arm of chromosome 5. We report on a patient with a partial trisomy of chromosome 5 and significant asthma and allergies.

The patient presented, at 6 years of age, congenital heart disease (ASD, VSD, PDA), developmental delay, severe eczema, asthma, numerous food allergies and elevated serum IgE. The blood karyotype showed extra material on the short arm of chromosome 22. Spectral karyotyping (SKY) revealed that the extra material on chromosome 22 originated from chromosome 5. FISH studies with BAC probes were undertaken to determine the extent of chromosome 5 trisomy. BACs mapping to 5q34 and 5q35 all hybridized to the der(22). The child s karyotype is thus; 46,XX, der(22)t(?;22)(?;p11.1). ish der(22)t(5;22)(q34;q11.1) (wcp5+, D22Z4+, D5S400+, D5S2006+). Parents karyotypes were normal.

The patient illustrates a case of pure partial trisomy of 5q34-5qter, as loss of 22p material is non-significant. The distal 5q trisomy genotype has been associated with clinical signs that include growth and mental retardation, eczema, craniofacial anomalies, and malformations of heart, lungs, and genitalia; many of which our patient presents. The trisomic region does not include the chromosome 5 interval (5q31) linked to asthma and allergic disease. However, numerous genes potentially involved in heart development (hCsx, MSX2), IgE levels (H2R) and asthma and allergic response (LCT4 synthase, LCP2, H12-3) have been mapped to this chromosomal region.

P0278. Psu dic(21;21)(21pter->21q22;;21q22->21pter) in two newborns with Down syndrome

A. Polityko[1], *N. Shorokh*[2], *J. Jaroshevich*[1]
[1]*Institute for Hereditary Diseases; Minsk, Belarus;* [2]*Regional Genetic Centre, Brest; Brest, Belarus*
lazjuk@bcsmi.minsk.by

Dicentric structure having only one active centromere is called pseudodicentric. Such chromosome is able to overcome the cell divisions successfully and is a stable structure in constitutional karyotype. We report on two unrelated infants with the typical for Down syndrome phenotype features in both. Their karyotypes had pseudodicentric chromosome constructed due to two chromosomes 21q22 junction. No prenatal chromosome studies has been done in both cases. Chromosome analyses were performed after standard preparation of PHA stimulated lymphocyte cultures with subsequent GTG and C banding, respectively. Cytogenetic studies of GTG-banded metaphases showed that proposita I, a newborn female, have karyotype 46,XX,psu dic (21;21)(21pter->21q22;;21q22->21pter),+21. The analysis of C banded chromosomes showed the presence of the second centromere region in pseudodicentric chromosome. The parental karyotypes were found normal. Proposita II was newborn male with typical Down syndrome features, his karyotype was 46,XY,psu dic (21;21)(21pter->21q22;;21q22->21pter),+21. The parental karyotypes were normal too. Cytogenetic aspects of de novo pseudodicentric chromosomes are discussed.

P0279. De novo rea(13q13q); Most are Isochromosomes Rather Than Robertsonian Translocation

B. Wang[1], *J. Williams*[2], *L. G. Shaffer*[3]
[1]*Changhua Christian Hospital; Changhua, Taiwan Republic of China;* [2]*The Prenatal Diagnostic Center of Southern California; Los Angeles, CA United States;* [3]*Baylor College of Medicine; Houston, TX United States*
baotyan@cch.org.tw

Robertsonian translocations that most commonly give rise to secondary trisomy 13 are rob(13q13q). Over 90% of rob(13q13q) are ascertained as de novo rearrangements in trisomy 13. Recent studies (Shaffer et al. 1994) demonstrated that 3 of 4 cases with homologous rearrangements of chromosome 13 resulting in trisomy 13 were isochromosomes. We present results of molecular analyses in a case of de novo homologous chromosome 13 rearrangement [rea(13q13q)]detected in an abortus specimen. An abortus specimen was collected from a 37-year-old, G3, P0, SAB2 woman following diagnosis of a missed abortion at 8 weeks gestational age. Cytogenetic studies revealed the karyotype of the abortus to be 46,XX, -13,+rea(13q13q)de novo. Using 10 micro-satellite markers from chromosome 13, it was determined that this rea(13q13q) was a maternally derived isochromosome and that there was no recombination involved in the rearrangement. This would suggest that the isochromosome formed postzygotically. However, a meiotic origin can not be excluded. These posible mechanisms of isochromosome formation will be discussed.

P0280. Cytogenetic and molecular analysis of a familial translocation with breakpoint on 12p11 cosegregating with brachydactyly E

J. Wirth[1], *A. Vester*[2], *H. C. Hennies*[3], *K. Hoffmann*[3], *A. Kobelt*[4], *H. G. Nothwang*[2], *H. H. Ropers*[2], *T. F. Wienker*[5], *S. Baehring*[3]
[1]*Developmental Biology, University Bielefeld; Bielefeld, Germany;* [2]*Max Planck Institute of Molecular Genetics; Berlin, Germany;* [3]*Max-Delbrueck-Centrum Berlin-Buch; Berlin, Germany;* [4]*Institute of Medical Genetics; Chemnitz, Germany;* [5]*Institut f r Medizinische Statistik, Dokumentation und; Bonn, Germany*
jutta.wirth@biologie.uni-bielefeld.de

Autosomal dominant brachydactyly type E associated with severe hypertension has been described in a large Turkish kindred. Linkage analysis of this pedigree localized the critical region to an interval of 6 cM on chromosome 12p11. Interestingly, chromosome rearrangements associated with similar phenotypes involving the same chromosomal segment were reported (Nagai et al. 1995, Baehring et al. 1997). We have previously described a translocation with breakpoint on 12p11 cosegregating with typical features of brachydactyly E in four generations. Cytogenetic and molecular studies revealed that the breakpoint is located 3 Mb proximal to the critical interval for brachydactyly E and hypertension. Breakpoint spanning YAC and PAC clones were identified. Partial sequencing of clones provided a complete sequence of 150 kb spanning the breakpoint. By Southern blot analysis we could demonstrate that the open reading frame of the parathyroid hormone-like hormone gene (PTHLH), which was supposed to be a good positional candidate for brachydactyly, is not disrupted. Other candidate genes are now under investigation. The identification of the breakpoint will enable us to discover genetic factors contributing to brachydactyly E and might give insight into the etiology of brachydactyly E associated with hypertension.

P0281. Charge Association and Interstitial Chromosome 22q11 Deletion; Molecular, FISH and Clinical Study

S. Guarducci, *M. Lapini, U. Ricci, E. Lapi, L. Ombroni, E. Andreucci, I. Sani, L. Giunti, M. Giovannucci Uzielli*
Genetics and Molecular Medicine Unit, Department of Paediatrics, University of Florence, A.Meyer Children Hospital; Florence, Italy
uzielli_ml@unifi.it

CHARGE Association is a non random occurrence of congenital malformations that has been described in clinical series; inclusion criteria for the CHARGE Association are at least four of the major features (monolateral or bilateral coloboma of the eye, heart defect, atresia of the choanae, retarded growth and development, genital hypoplasia, criptorchidism, ear anomalies and hearing defects). Cleft lip and palate or high-arched palate, velopharingeal insufficiency, micrognathia, facial palsy, esophageal atresia, malakoplakia of the colon, cerebral anomalies, have been also described in the clinical spectrum. All of the features of CHARGE Association occur, more or less expressed, in the series of patients who have deletions within chromosome band 22q11 (Ryan et al., 1997). Viceversa, not all cases of CHARGE Association show the 22q11 deletion. We report the observations of a long follow-up study of four children, two females and two males, with CHARGE Association; in two of them, FISH and molecular analysis revealed a deletion in the critical region 22q11. In the other two children, who were negative for the 22q11 deletion, we are now analysing the PAX2 gene for deletion and nucleotidic variations of the coding sequence, because the pattern of expression of PAX2 suggests that genes encoding downstream targets effectors could be candidate gene for the CHARGE Association (Tellier et al, 2000).

P0280. Unbalanced translocation 8/Y (45,X,-Y,t(Y;8)(q12p23)); case report and review of literature

K. Bosse[1], *H. Engels*[1], *K. Van der Ven*[2], *R. Raff*[1]
[1]*Institute of Human Genetics; University of Bonn, Germany;* [2]*Department of Reproductive Medicine; University of Bonn, Germany*
bosse@meb.uni-bonn.de

A 25 year old healthy G1P0 female underwent amniocentesis at 16 weeks of gestation because of abnormal triple test results. Ultrasound examination demonstrated isolated bilateral choroid plexus cysts. Karyotype analysis from amniocytes revealed an unbalanced de novo Y/autosome translocation [45,X,t(Y;8)]. A molecular genetic investigation with 30 specific sequence tagged sites (STS) located between Yp11.32 and Yq11.23 excluded partial deletions in Y euchromatin. At that time no subtelomeric probes for chromosome 8p were available and the parents were informed about the remaining risk of a terminal deletion of 8p. At birth the boy presented with average body measurements and an unremarkable physical examination. FISH with a probe of the subtelomeric region 8p (Locus D8S2333) confirmed a monosomy 8pter to 8p23. The boy showed no developmental peculiarities until a speech delay was diagnosed at the age of two years. On examination at 42 months he presented with very mild dysmorphic features including a dolichocephalus, supraorbital fullness of subcutaneous tissue and a bulbous nasal tip. The Denver Developmental Screening Test revealed a psychomotor delay of almost 18 months. Terminal deletions of the short arm of chromosome 8 reported previously demonstrated a common breakpoint 8p23 in the majority of patients. In general the children show mild to moderate mental retardation and only minor facial dysmorphisms. Physical abnormalities mainly include heart defects, seizures and behaviour difficulties. Although the factors responsible for a normal spermiogenesis (TDF, AZF) are present in the boy infertility in adulthood due to disturbed XY pairing in meiosis I must be expected

P0281. A new case of interstitial 6q deletion with Prader-Willi-like phenotype

L. Faivre, *M. De Blois, S. Jacquemont, O. Raoul, C. Turleau, M. Prieur, A. Munnich, M. Vekemans, V. Cormier-Daire*
D partement de G n tique; Paris, France
lfaivre@hotmail.com

More than 30 cases of interstitial deletion of chromosome 6q have been reported in the literature, with variable breakpoints and clinical features. Four of them displayed clinical features suggestive of Prader-Willi syndrome. Here we report a fifth case of interstitial 6q deletion and Prader-Willi like phenotype. The probant was described as floppy and with feeding problems in early infancy. He sat at age 2, walk at age 3 ?, and the speech was very poor at age 5. His behaviour was hyperactive, with short attention span and intolerance to frustration. Excessive weight began at age 3, with a big appetite and food-seeking behaviour. At physical examination at age 5, weight was +5.5 SD, height was -1 SD, and OFC was + 2.5 SD. He had slight dysmorphic features including a square face, large forehead, small palpebral fissures, mild strabismus, thin nose, and thin lips. Hands and feet were small, external genitalia were normal. Investigations showed absence of malformations, a normal methylation assay in the SNRPN region, and normal FISH study with the SNRPN probe. However, cytogenetic examination showed an interstitial deletion of chromosome 6q, del(6)(q16.1q21). Karyotypes of the parents were normal. Parental origin of the deletion is in progress. This observation confirmed that individuals with a Prader-Willi phenotype and normal cytogenetic and molecular studies of the 15q11-q12 region should be examined for a deletion 6q. It is worth noting that the 6q16.2 band is common in 4 out of the 5 cases.

P0282. Extreme growth retardation in a child with a de novo interstitial 20q13.2 deletion.

D. Genevi ve[1], *L. Faivre*[1], *O. Raoul*[1], *D. Sanlaville*[1], *F. Monceaux*[2], *M. Bonnet*[1], *P. Gosset*[1], *C. Ozilou*[1], *A. Munnich*[1], *M. Vekemans*[1], *M. Prieur*[1], *V. Cormier-Daire*[1]
[1]*D partement de g n tique h pital Necker Enfants Malades; Paris, France;* [2]*Service d Endocrinologie P diatrique h pital Necker Enfants Malades; Paris, France*
gendav@club-internet.fr

Deletions of chromosome 20q are extremely rare and have not been associated with a particular phenotype. Here we report on a girl with a chromosome 20q deletion. Mensurations at birth were 1320 g for weight, 38 cm for height, and 27.5 cm for OFC and severe feeding difficulties were noted in the first months of life. Nocturnal enteral feeding was started with no significant effects on height. At age 11 months, weight was 4180 g (-5 SD), height was 55 cm (-7 SD), and OFC was 39.5 cm (-5 SD). Clinical examination showed rare hair, high forehead, irregular eyebrows, broad nasal bridge, bulbous nose, iris dysplasia, large ears, short philtrum, thin upper lip, micrognathia, and numerous skin dimples. She could not sit without aid and was hypertonic. Cardiac and renal ultrasound, and cerebral MRI were normal. High resolution blood chromosomes of the child and her parents showed a de novo interstitial 20q13.2 deletion in the proband. In situ hybridisation studies confirmed the presence of the telomeres. Analysis of the parental origin of the deletion is in progress. The other reports of chromosome 20q deletion were reviewed but none of them had similar breakpoints and similar striking phenotype.

P0283. A hereditary case of distal short arm deletion of the X chromosome

S. Vjatkina[1], *T. Kuznetzova*[2], *T. Kuznetzova*[2], *J. Loginova*[2], *O. Chiryaeva*[2], *L. Petrova*[2], *L. Petrova*[2], *L. Petrova*[2]
[1]*Ott s Institute of Obstretrics and Gynecology; Saint-Petersburg, Russian Federation;* [2]*Ott s institute of Obstetrics and Gynecology; Saint-Petersburg, Russian Federation*
pendina@mail.ru

Partial deletions of the short arm of the X chromosome are known to be

compatible with fertility in some cases but they cause a significant ovarial insufficiency with Turners signs or gonadal dysgenesis in other cases. We report a family in which a mother (54 years old) and two daughters (27 years old) with short stature but without other Turners stigmata had the same chromosomal anomaly. The standard cytogenetic technique (QFH) and FISH with locus-specific DNA probe (Xp21.1) revealed non-mosaic karyotype 46,X,del(X)(qter-p21.1;). As revealed by RBA technique the abnormal X was always late replicating in all the examined cells. One of the sisters has an eight month old daughter with normal karyotype 46,XX. The other sister has now 37 weeks pregnancy. Prenatal diagnosis demonstrated normal female karyotype in this fetus. Our investigation suggest that plural genetic factors on both arms of human X chromosome are involved in ovarian development.

P0284. Unusual mosaicism due to non-homologous postzygotic recombination involving chromosomes 6 and 10 in the mother, and 46,X,der(X)t(X;6) in her daughter.

I. Petkovic[1], *I. Barisic*[2]
[1]*University Childrn s Hospital Zagreb; Zagreb, Croatia;* [2]*University Children s Hospital Zagreb; Zagreb, Croatia*
iskrapetkovic@vip.hr

It is well known that unbalanced translocations are mostly familial, and cytogenetic analysis of parents usually reveal the reciprocal exchange of chromosome segments identified in a given unbalanced rearrangement. In this report we present an unexpected cytogenetic finding of chromosome rearrangement transmission. Our patient is 23-month old girl with short stature, failure to thrive, moderate mental retardation, deficiency if IgG, mild facial dysmorphism, no malformation or abnormalities of visceral organs. Cytogenetic analysis of peripheral blood and fibroblast revealed a karyotype of 46, X, der(X)t(X;6)(q22; p11). Cytogenetic investigation of the father revealed normal karyotype (46,XY), while the mother presented 46,XX/46,XX, der(10)t(6;10)(p11; p11) in the peripheral blood, and 46,XX was observed in fibroblasts culture. We suggest that unusual mosaicism in the mother is due to unstable 6p11 region leading to the jumping non-homologous postzygotic recombination involving chromosome 10 (observed) and X (not observed) at S/G2 phase of the cell cycle. The mother transmitted to her daughter der(X)t(X;6) and normal chromosome 6.

P0285. Trisomy 9p22p24 Due To A Maternal Insertion Translocation

P. F. Ippel[1], *J. M. Engelen*[2], *J. M. de Pater*[1]
[1]*University Medical Center; Utrecht, The Netherlands;* [2]*Clinical Genetics Center; Maastricht, The Netherlands*
pfippel@knmg.nl

We report on a boy with partial trisomy 9p, due to a maternal insertion translocation ins t(12;9)(q24.1;p22p24)mat. His phenotype is characteristic for partial trisomy 9p; his clinical manifestations are mental retardation, growth retardation, microcephaly, epicanthus, low-set ears micrognathia, clinodactyly of the fifth fingers and extremely small genitals. The mother and 2 carrier brothers did not show any dysmorphic features but had major learning difficulties not present in related non-carriers. The inserted segment is very small and the GTG banding findings were confirmed by using microdissection and FISH.

P0286. Screening for Cryptic Chromosome Rearrangements using subtelomeric probes; The experience of a UK diagnostic cytogenetics laboratory.

N. Friend, *D. Corney, R. W. Palmer*
Great Ormond Street Hospital; London, United Kingdom
deborah.corney@gosh-tr.nthames.nhs.uk

The availability of commercial kits containing subtelomeric FISH probes has allowed the application of these probes for the screening of diagnostic cases. Since August 1998 the North East London Regional Cytogenetics Labortory has successfully screened 95 cases for the presence of cryptic rearrangements using these probes. These cases were all seen by a clinical geneticist and referred specifically for subtelomeric screening. A total of 6 cryptic rearrangements were identified which had apparently normal G-banded karyotypes. In addition, 5 cases were found to have chromosome abnormalities which were detectable on high resolution G-banding and two patients had microdeletions detected by commercial microdeletion probes. The finding of 6 cryptic rearrangements out of 95 cases gives a frequency of cryptic rearrangements of approximately 6% which is consistent with other published reports. In total, 13 out of the 95 cases (13.6%) were cytogenetically abnormal. These findings demonstrate the usefulness of this technique in a diagnostic setting since using conventional cytogenetics, approximately half of the chromosomally abnormal patients in this group would not have been identified.

P0287. A case of the balanced translocation between the 12 and X chromosomes, accompanied by the mental deficiency.

O. A. Kotkova, *E. Staroverova, O. Udalova*
Medical Diagnostic Centre; Nizhny Novgorod, Russian Federation
medcentr@sandy.ru

A proband - patient, age 17, was born from the third pregnancy. The family history is not aggravated. The age of mother is 36. The toxicosis were observed in the first half of pregnancy, pneumonia in 16 weeks.. The girl was born in time, precipitated labor, the mass at birth is 3850 g., height-56 cm. At examination; the weight is 51 kg., the height is 162 cm. Middle constitution, the subcutaneous fat is poorly developed. The skin is dry, the turgor is decreased. A diffusive muscular hypotony. The forehead is convex, the occipitalbone is impressed. There re the supplementary cartilages at the external surface of the floor of the auricle. The features are right. A macroglossia, the gothic palate. The first degree scoliosis. The longitudinal, transverse platypodia, the sandals-like foot. The physical development is according to the age. The tonicoclonic fits are observed since 3,5 months. The fits, expressed in spontaneous vomiting, headache without a loss of consciousness, have appeared since 3 years 3- 4 times a year. She is reading by syllables, the handwriting is unstable with mistakes. The calculation is within 10 by means of fingers. At self- service and communication she is rapidly exhausted and becomes indifferent, fretful, hypomimic. The infatility in judgements, the scanty vocabulary are noted, infatility in judgements, the scanty vocabulary are noted, she is egocentric. The extension of the brain large cistern, petrification of the anterior falx. The areas of pathologic density in the brain tissue are not revealed. The cortical sulci are visualized clearly. The rheoencephalogram is a small asymmetry of the blood flow. The EEG - the significant alterations of the brain bioelectrical activity in the form of disfunction of the diencephalic - truncal structures. The brain spastic readiness threshold is decreased. The elements of the epileptiform activity are registered. There re no focal alterations. The karyotype of mother is 46,XX, the karyotype of father is 46,XY. The karyotype of proband is 46,X,t(X;12)(X q ter -> X p 21.1 ;;12 q 13.1 -> 12 q ter; 12 p ter -> 12 q 13.1 ;; X p 2.11 -> X p ter). Taking into consideration the presence of the phenotype microabnormalities in combination with a mental deficiency, spastic syndrome it is possible to presure a presence of the monosomy (microdeletion) 12 chromosome.

P0290. De novo structural rearrangement with duplication of 11p15 in a boy with prenatal onset of overgrowth, dysplastic kidney, hydronephrosis and dysmorphisms.

K. B. J. Gerssen- Schoorl[1,1], *J. B. G. Verheij*[1], *B. Sikkema-Raddatz*[1], *A. H. van der Vlugt*[2], *A. Y. van der Veen*[1], *L. K. Leegte*[1], *A. J. van Essen*[1]
[1]*University of Groningen, Department of Medical Genetics; Groningen, The Netherlands;* [2]*University Hospital; Groningen, The Netherlands*
K.B.J.Gerssen-Schoorl@medgen.azg.nl

We present a boy with some features suggestive of Beckwith-Wiedemann syndrome and additional congenital anomalies; prenatal onset of overgrowth; birth weight 3,710 g, birth length 50 cm and head circumference 35.4 cm at 33+4 (or possibly 35+5) weeks gestation (all above P97), dysmorphic ears (but no clear earlobe crease), no evident macroglossia, hypertelorism, a right epicanthic fold, deafness, transient hypotonia with hypoventilation, transient cardiac hypertrophia of ventricles, internipple distance >P97, abdominal hypotonia with diastasis recti, right dysplastic kidney and left congenital hydronephrosis due to pelviureteric obstruction. The G-banding pattern of lymphocyte metaphases was interpreted as 46,X,?t(Y;11) (q11.2;q13.3). FISH using whole chromosome paints of chromosomes Y and 11 confirmed the translocation. Despite a negative DAPI staining result, presence of Y heterochromatin could be demonstrated with probe pY3.4. The der(11) chromosome showed a hybridisation signal on 11q13.4 with BAC 120P20. A subtelomeric probe specific for 11p hybridised to both arms of the der(11) chromosome. A subtelomeric probe specific for 11q hybridised to the q-arm of the der(Y) chromosome, but not to the der(11) chromosome. Therefore the karyotype was eventually defined as 46,X,?t(Y;11)(q11.2;q13.3). ish der(Y)t(Y;11)(q12;q13.4), der(11)t(11;11)(q13.4;p15.5).This resulted in a duplication of 11p15.5. The possible mechanism of origin will be presented. Trisomy 11p15.5 of paternal origin results in an overexpression of insulin-like growth factor 2 (IgF2). This is probably responsible for the partial expression of features of the Beckwith-Wiedemann syndrome in this case, in particular the overgrowth.

P0291. Partial monosomy 13q associated with anophthalmia due to a familial translocation (9;13)(p24.3;q31.3)

S. Sodia, *E. Petek, W. Emberger, H. Zierler, K. Wagner, P. Kroisel*
Institute of Medical Biology and Human Genetics, University of Graz; Graz, Austria
sigrun.sodia@kfunigraz.ac.at

We report on a newborn boy with a partial monosomy 13(q31.3-qter). After an uneventful pregnancy he was born at term, birth weight was 3170 g, length 50 cm, head circumference 33 cm. Because of hypospadia in combination with bifid scrotum, blepharophimosis and anophthalmia, cytogenetic analysis was performed. An unbalanced translocation 46,XY,der(13)t(9;13)(p24.3;31.3) was found. His mother shows a balanced translocation 46,XX,t(9;13)(p24.3;q31.3) Therefore the patient has a partial monosomy 13q31.3-qter combined with a partial trisomy of the telomeric segment of 9p. MRI of the brain showed hypoplasia of corpus callosum, absence of optic nerves and chiasma opticum with only tiny hypointense structures in both orbits. A critical region for major organ development has been assigned to 13q32. Genes involved in various types of eye anomalies were mapped to 13q, but non for anophthalmia so far. By subsequent cytogenetic analysis of the family, 4 carriers of the balanced familial translocation in three generations were identified. One 27 years old uncle of the patient, with mild mental retardation, congenital heart defects, microcephaly and clinodactyly showed the corresponding unbalanced translocation with partial trisomy 13q and partial monosomy for the very telomeric segment of 9p. Detailed mapping of both chromosomal breakpoints will improve our understanding on the molecular basis of malformations observed in cytogenetically unbalanced members of this family.

P0292. Genetic heterogeneity in 3 Tunisian patients with microdeletion 22q11.2

L. Ben Jemaa Khemakhem, *H. Kamoun, L. Hila, N. Bayou, R. Mrad, F. Maazoul, M. Chaabouni, H. Chaabouni*
Service des Maladies Cong nitales et H r ditaires EPS Charles Nicolle, Laboratoire de G n tique Facult de M decine; Tunis, Tunisia
lamia.khemakhem@rns.tn

The syndrome of microdeletion 22 can includes cardiomyopathy, aplastic or hypoplastic thymus and parathyroid glands, cleft palate, and a peculiar dysmorphic facies. Heart malformations usually include interrupted aortic arch arteries, truncus arteriosus, and tetralogy of Fallot; numerous associated abnormalities are observed. The disorder frequently progresses to psychomotor retardation, with a 50% chance of mental retardation. The affection is due to a microdeletion on chromosome 22 in the q11.2 area. We describe here three cases of microdeletion 22.
The first case is a Cayler syndrome. The patient is one year old boy. He has consanguineous healthy parents, two brothers and one sister normal. The patient has asymmetrical crying facies; Asymmetry of the mouth when crying with drooping of the left corner, Retrognathism, high arched palate, abnormal ears, umbilical hernia, bilateral cryptorchidism and congenital heart disease ; ventricular septal defect associated to septal anevrism. He has a delayed psychomoteur developement.
Cardiofacial syndrome is described by Cayler (Omim 125520) in 1967. This syndrome associates asymmetrical crying facies (ACF) due to hypoplasia or absence of the depresor muscle of the angle of the mouth and congenital heart disease. Incidence of ACF is estimated of 0.63% to 0.82% per live birth; 2.5% to 8% of these children have congenital cardiac defects. The second case was a girl with psychomoteur retardation, she has speech defect ,retroverted ears, bulbous nose ,micrognatia,heart disease, microcephaly,arachnodactyly and ear anomaly.
The third case was a girl with a malformative syndrome with tetralogy of fallot, ptosis of the reight eye, epicanthus, hypertelorism and deficit of cellular immunity.
In the three cases cytogenetic study by double FISH; D22S75 (DGCR) localised in 22q11.2 and D22S39 (Control Probe) in 22q13.3 showed a microdeletion of 22q11.2 region (D22S75).
This deletion has been described in Cayler syndrome, included in CATCH 22 group.
The other cases are member of CATCH 22 group with different clinical signs who push us to ask the question ; when we must search the microdeletion 22 ? In most cases microdeletion is de novo but we have to detect it in parents for genetic counselling.

P0293. Mosaicism for a duplication of 17p11.2 region in Charcot-Marie-Tooth 1A disease detected by ECFs-FISH analysis.

M. Zawada[1], *M. Jarmuz*[1], *J. Wigowska-Sowinska*[2], *J. Wronka*[2]
[1]*Institute of Human Genetics, Polish Academy of Sciences; Poznan, Poland;* [2]*University of Medical Sciences; Poznan, Poland*
mzawada@man.poznan.pl

Charcot-Marie-Tooth 1A disease (CMT1A) is a peripheral neuropathy associated with a submicroscopic tandem duplication on the short arm of chromosome 17. The 17p11.2 region contains the peripheral myelin protein 22 gene (PMP-22), the dosage of which plays a crucial role in the aetiology of the disease. The CMT1A duplication is not visible by standard cytogenetic analysis, but is detectable by FISH analysis of interphase nuclei and extended chromatin fibers (ECFs-FISH). We have analyzed 24 members of 7 families; 9 patients with clinical symptoms of CMT1A disease and 15 normal individuals. The use of fibers-FISH with the pVAW412R3, pVAW409R3 and 17 SpectrumOrange probes has resulted in the detection of a duplication in 8 patients. However, 100% of duplication was only in 4 of them. In the next 4 patients the mosaicism for 17p11.2 duplication was detected in 30-80% of analysed chromatin fibers. 6 normal individuals were shown to have the mosaicism in 10-80% of analysed chromatin fibers. We conclude that mosaicism for the CMT1A duplication in patients and members of their families could be successfully diagnosed by well-established ECFs-FISH method. This work was supported by State Committee of Science - Grant No 4.PO5A.081.13. The FISH results were worked out owing to computer system (MetaSystems GmbH) purchased by Foundation for Polish Science.

P0294. Prenatal detection of mirror (reverse tandem) duplication of chromosome 21. Cytogenetic evidence for postzygotic origin.

J. J. van der Smagt, *Y. M. Hendriks, E. Bakker, D. Verbove, C. Schouten, M. M. Bartelings, G. C. Beverstock*
Leiden University Medical Center (LUMC); Leiden, The Netherlands
j.j.vandersmagt@kgc.azl.nl

Mirror duplications of chromosome 21 are a rare cause of Down syndrome, different from the usual i(21q) or homologous Robertsonian translocations. Theoretically they could arise as a result of telomeric fusion or subtelomeric translocation between two chromosome 21 homologues, or as a result of illegitimate exchange between sister chromatids. Pangalos et al. (1992) demonstrated by molecular methods in their three cases that they were in fact reverse tandem duplications with a deletion of distal 21q22.3. As no mosaicism was observed they favoured prezygotic origin of the rearranged chromosome. Our case concerns a prenatal diagnosis for Duchenne muscular dystrophy. At CVS the short term culture demonstrated a 46,XX karyotype in 14 cells, while in all 14 cells of the long term culture a 46,XX,-21,+der(21)t(21;21)(q22.3;q22.3) karyotype was observed. The same abnormality was found in all 31 amniocytes analysed, and after termination of the pregnancy in fetal skin, kidney and liver. No evidence of mosaicism was found. Both parents have normal karyotypes. Most likely the rearranged chromosome has arisen after fertilisation. Other mechanisms are conceivable but would require an unlikely second event. Ultrasound at 19 weeks showed no abnormalities suggestive of a Down phenotype. After termination only minimal dysmorphic signs consistent with Down syndrome and no heart defect were found. The results of DNA-analysis, characterising this unusual chromosome rearrangement are presented.

P0295. Prader-Willi and Angelman Syndromes in Chilean Patients. Clinical and Molecular Diagnosis.

B. Curotto, *L. Santa Mar a, A. Alliende, F. Cort s*
INTA University of Chile; SANTIAGO, Chile
malliend@uec.inta.uchile.cl

Prader Willi (PWS) and Angelman AS) syndromes are multigenic disorders characterized by developmental and neurobehavioral abnormalities. Different underlying genetic defects cause loss of expression of genes located either in paternal or maternal 15q11-q13. Approximately 70% of PWS and AS patients have a deletion in 15q11-q13, whereas uniparental disomy (UPD) accounts for 25% of PWS cases and 2-3% of AS. A small number of patients have a mutation at the imprinting center. UBE3A gene is mutated in 20% of AS. To confirm the presumptive clinical diagnosis of PWS or AS in 62 Chilean patients we used a methylation specific PCR assay, FISH analysis and classical cytogenetics; 23 out of 37 PWS patients had positive scores according to the Holm«s consensus diagnostic criteria (PWS-A group), the remaining 14 patients were not completely evaluated under this criteria (PWS-B group). AS patients were evaluated with the consensus criteria. In 17 out of 62 cases (27.4%) the molecular analysis confirmed the clinical diagnosis of PWS or AS; 34.8% in the PWS-A group, 21.4% in the PWS-B group and 24% in AS patients. Those cases with a positive methylation test were analyzed by FISH. Classical cytogenetics showed structural rearrangements in 2 PWS cases; 46,XY,del(15)(q11-q13);45,XX,del(15)(q11-q13),der(13;14)mat; and one with normal molecular analysis; 46,XY,t(5;7)(q12;q31). These results confirm that Holm«s criteria is of great assistance in PWS and suggest that definitive clinical diagnosis of PWS and AS must be confirmed by molecular and cytogenetics analysis.

P0296. Familial partial 9p trisomy, six cases and four carriers in three generations

I. Dimofte[1], *A. Popa*[1], *D. Balaban*[1], *T. Poalelungi*[1], *L. Enache*[2], *V. Broasca*[1], *S. Nedelcovici*[1]
[1]*Faculty of Medicine; Constanta, Romania;* [2]*Faculty of Medicine; Iasi, Romania*
revarsarea@lycos.com

Six cases of translocation trisomy for distal half of the short arm of a number 9 chromosome and four asymptomatic balanced translocation carriers are presented in a three -generation pedigree. The clinical features are remarkably similar to those recently recognized and increasingly reported in full short arm (9p) trisomy and should be considered a modification of the same syndrome. In addition to non-specific mental retardation and short stature, there is, in common, a characteristic facies, including downturned corners of the mouth, a slightly bulbous nose, moderately large ears, suggestively wide-set eyes with an anti-mongoloid slant, dysplasia and hypoplasia of the nails, clinodactyly of the 5th fingers, and abnormal dermatoglyphs. It appears that the trisomy 9p syndrome in its variant forms, including trisomies for more or less than just the short (p) arm, is one of the most common clinical autosome anomalies in humans, exceeded only by trisomy 21 (Down s syndrome) and possibly trisomies of chromosomes 13 and 18.

P0297. Molecular cytogenetic analysis of a de novo balanced X-autosomal translocation with predominant inactivation of the derivative X chromosome in a girl with multiple malformations

K. Shirneshan[1], *B. Gl ser*[2], *K. Bink*[3], *J. Wirth*[4], *S. K. Bohlander*[1]
[1]*Institute of Human Genetics; G ttingen, Germany;* [2]*Institute of Human Genetics; Ulm, Germany;* [3]*Institute of Pathology, Technische Universit t; M nchen, Germany;* [4]*Developmental Biology, University of Bielefeld; Bielefeld, Germany*
kshirne@gwdg.de

We report on the characterization of a de novo reciprocal t(X;15)(p11.3;q26) in a girl with multiple congenital malformations. Delivery occurred at term (39 weeks), with 2220 g birth weight .Head circumference (34 cm) and length (54cm) were normal. Several dysmorphic features were observed including facial stigmata, hands with abnormal morphology and ulnar deviation, hypoplastic fifth toe and shortened fifth metatarsal. Radiographically, a hypoplastic corpus callosum and variant Dandy-Walker cyst were observed. She had a poorly differentiated liver, a neonatal muscular hypotonia and suffered from a not treatable epilepsy. At the age of 16 months she died. Cytogenetic analysis demonstrated an apparently balanced reciprocal translocation with breakpoints in Xp11.3 and 15q26. Replication banding analysis from EBV-transformed lymphocytes showed inactivation of the derivative X-chromosome in 98%. FISH analysis resulted in the identification of a spanning YAC (ICRFy900C1228) for the Xp11.3 breakpoint and BAC bA89K11 for the 15q26 breakpoint, respectively. No smaller clone was identified for Xp breakpoint. Interestingly, the serine threonine-protein kinase, PCTK1, was mapped within a BAC that flanks the region at the telomeric site and extends with its 5 end into the breakpoint region.Thus, we suggest that disruption of PCTK1 is not well tolerated and forces activation of the non-rearranged X-chromosome. This could lead to functional disomy for most of Xp and functional monosomy of 15q26. The hypothesis that these functional aneuploidies are responsible for the phenotype is supported by data from the literature where similar phenotypic abnormalities are described for patients with monosomy 15q26 and functional disomy of Xp.

P0298. A newborn with a de novo partial deletion of the short arm of chromosome 7(p21?pter) confirmed by fluorescence in situ hybridization (FISH) analysis

A. Divane[1], *V. Peristeri*[2], *A. Andreou*[2], *N. Morrison*[3], *L. Florentin*[1]
[1]*Molecular Biology and Cytogenetics Center, Alfalab Medical Institute of Research and Diagnosis; Athens, Greece;* [2]*Neonatal Intensive Care Unit, Ippokratio Hospital; Thessaloniki, Greece;* [3]*Institute of Medical Genetics; Glasgow, United Kingdom*
tir_div@yahoo.com

The patient a newborn boy born at term from healthy parents had a variety of abnormalities. Initial examination immediately after birth, showed an apparently normal boy. Eleven hours later the boy was transferred to the intensive care unit due to feeding difficulties. More detailed examination revealed a number of facial, hand and nail, heart and genital malformations. These included microopisthognathia , low set ears, bilateral malformed elicosis of ears, broaden nasal tip, lycostoma, ulnar deviation of the 5th finger, clinodactily of the 5th finger, toe nail hypoplasia, cardial anomalies, abnormal external genitalia, hypospadias, muscle hypotonia, hypertelorism, craniosynostosis, polysyndactily etc. The patient was referred to our center for cytogenetic analysis. Chromosome preparations were obtained from PHA-stimulated lymphocytes. Chromosomes were stained using G-banding techniques. The karyotype revealed one structurally abnormal chromosome 7, specifically a deletion of the short arm of chromosome 7. The breakpoints were on the short arm of chromosome 7 bands p21 to pter. The karyotype is; 46,XY,del(7)(p21?pter). Fluorescence in situ hybridation using a Cytocell Chromoprobe-T 7ptel/qtel showed 7ptel and qtel signal on one chromosome 7 but only 7qtel signal on its homologue. No consistent 7ptel signal was detected elsewhere. FISH results confirmed a deletion of a 7ptel sequence on one chromosome 7 that has not been translocated to another chromosome. In our case both parents had normal karyotypes indicating a de novo partial deletion of the short arm of chromosome 7.

P0299. Xp21.2 deletion in a mother and three of her four daughters

P. Trioche[1], *G. Tachdjian*[1], *F. Perreaux*[1], *M. Portnoi*[2], *A. Aboura*[1], *P. Chevalier*[1], *P. Labrune*[1]
[1]*H pital Antoine B cl re; Clamart, France;* [2]*H pital Saint Antoine; Paris, France*
philippe.labrune@abc.ap-hop-paris.fr

Short stature in females is often caused by monosomy X or deletion Xp. We report on a mother and three of her 4 daughters with short stature as sole phenotypic anomaly. Patients and methods ; The first and the third daughter of healthy unrelated parents were referred for retarded growth at ages 5.5 and 3 years, respectively. The father1s height was 170 cm, and the mother1s 156 cm. In both sisters, growth had been delayed since birth, below the 3 percentile. Except short stature, physical examination proved entirely normal. Abdominal and cardiac ultrasound examinations were normal. Blood laboratory tests gave normal results for renal functions, RBC and WBC counts, thyroid hormones, cortisol, LH and FSH. Bone maturation was delayed in both girls. The second daughter did not present any growth retardation, whereas the fourth daughter was examined at the age of 24 months while her growth was slowing down to the 5th percentile. Chromosomal analysis were performed in both parents and the four sisters. The three affected girls were put on growth hormone therapy with good initial results. A fifth pregnancy began a few weeks ago. Both conventional and molecular cytogenetic analyses showed deletion of band Xp21.2 in affected patients. Conclusion ; Isolated Xp21.2 deletion resulted in this family in isolated retarded growth whereas all other physical abnormalities encountered in Turner syndrome were absent. Moreover, the hemizygoty of the chromosomal region Xp21.2 is not associated with ovarian function anomalies. These results are useful for genetic counseling of individuals with partial monosomy X.

P0300. Williams And Catch22 Syndromes - Clinical And Genetical Diagnosis

I. Malcic, H. Kniwald, I. Baric, D. Begovic, M. Jelusic, ***R. Lasan***
Institute for Medical Research and Occupational Health; Zagreb, Croatia
iva.trcic@zg.tel.hr

Williams and CATCH22 syndormes represent two clinical syndromes with multiple involvmen,with well recognized genetical varities. We present 14 patients (7 with Williams and 7 with CATCH22 syndrome), in whom the diagnosis was confirmed by demonstration of deletions with fluorescent in sytu hybridization (FISH). Williams syndrome is generally characterized by mental deficiency, gregarious personality, unusual elfin faces, supravalvural aortic stenosis and idiopathic infantile hypercalcaemia. Patiens with Williams syndrome show a hemizygous submicroscopic deletion of 7q11.23 detectable by FISH. The deleted portion of the choromosome corresponds to the elastic gene. We report 7 patient (3 girls and 4 boys) with characteristics of Williams syndrome and FISH varified deletions of 7q11.23. These patients showed the characteristics faces and gregaroius personalities. Cardiac evaluation revealed in all 7 children presence of supravalvular aortic stenosis (2 underwent surgical procedure by Doty), and in all 4 patients peripheral pulmonary stenosis. Hypercalcaemia was not documented in these patients. Learning difficulties are present in all patients. Chormosome analysis done on peripheral blood were found to be normal in all patients. CATCH22 syndrome, the acronym (Cardiac defect, Abnormal face, Thymic hypoplasia, Cleft palate, and Hypocalcaemia), as a concept that compromises DiGeorge syndrome, velo - cardio - facial syndrome and conotruncal anomaly face syndrome, is connected with submicroscopic 22q11.2 deletion. We present 7 patients with CATCH22 syndrome with 22q11.2 microdeletion. Major cause of morbidity in those children was congential heart disease (CHD) (4 with tetralogy of Fallot, 2 with persistent truncus arteriosus and 1 child with pulmonary atresia). Other phenotypic characteristics included abnormal facies (hyper-

telorism, low set small ear lobes and micrognatia). Clinical findings of immunodeficiency were present in 4 and hypocalcaemia in 3 children, respectively. One child has cleft of soft palate. All of the CHD s are succesfully corrected surgically.

P0301. Cytogenetic and molecular-cytogenetic investigation of Rett syndrome

S. G. Vorsanova[1], Y. B. Yurov[2], V. Y. Ulas[1], I. A. Demidova[1], V. O. Sharonin[1], A. D. Kolotii[1], A. K. Beresheva[1], I. V. Soloviev[2]
[1]Institute of Pediatrics and Children Surgery, Ministry of Health; Moscow, Russian Federation; [2]National Centre of Mental Health, RAMS; Moscow, Russian Federation
yurov@pediatr.mtu-net.ru

Rett syndrome (RTT) is a severe neurodevelopmental disorder with the incidence of 2.5% in mentally retarded girls in Russia. We have performed cytogenetic studies of 70 patients (67 girls and 3 boys) with clinical picture of RTT, selected according to the criteria for diagnosis of RTT developed by B. Hagberg (Hagberg et al., 1996). Collection of DNA samples and fixed cell suspensions of RTT patients (37 girls and 2 boys) and theirs parents (27 patients) was established for molecular studies, for example, analysis of MECP2 mutations in Russian cohort of RTT patients. Among 70 patients; 67 girls with clinical picture of RTT were with normal female karyotype (46,XX); one boy was with normal male karyotype in cells of blood (46,XY) and two boys were with mosaic form of Kleinfelter s syndrome (47,XXY/46,XY) in blood and muscle cells. 24 mothers and parents of RTT girls were with normal karyotype, two mothers — with mosaic forms of Turner syndrome (45,X/46,XX) and one - mosaic karyotype (47,XX,+mar/48,XXX,+mar). We analysed chromosome X in lymphocytes of 67 affected girls with clinical picture of RTT using BrdU + Gimsa staining technique (Vorsanova et al., 1996). Specific type of inactive chromosome X (so-called type C) with unusual staining of chromatin in long arm of the chromosome X was found in 66 (from 67) girls with RTT. This technique was positively used for presymptomatic diagnosis of RTT in five girls in affected families. We believe that the phenomenon of altered chromatin conformation in inactive chromosome X could be used as laboratory test for preclinical diagnosis of the RTT. This work was supported in parts by INTAS, IRSA and COPERNICUS-2000 grants.

P0302. FISH detection of telomeric microdeletions in children with mental retardation

I. V. Soloviev[1], S. G. Vorsanova[2], V. O. Sharonin[2], Y. B. Yurov[3]
[1]National Centre of Mental Health, Russian Academy of Medical Sciences; Moscow, Russian Federation; [2]Institute of Pediatrics and Children Surgery MH; Moscow, Russian Federation; [3]National Research Center of Mental Health RAMS; Moscow, Russian Federation
yurov@rcmh.msk.ru

Molecular-cytogenetic studies of chromosomal micro-anomalies at subtelomeric chromosomal regions have been conducted by two-step fluorescence in situ hybridization (FISH) in 407 children with mental retardation. A large set of cosmid, BAC and PAC clones, containing simple telomeric sequences (TTAGGG)n was identified by FISH mapping. These clones were located in the most telomeric and subtelomeric regions as well as non-telomeric regions of p- and q- arms of different chromosomes. We used original simple two-step FISH protocol. Availability of large-insertion PAC probes, which contain DNA sequences, common for telomeric and, partially, subtelomeric regions of all human chromosomes, including p- and q-arms, allowed us to marl all telomeric regions in one FISH study. Therefore, first FISH study for each patient was performed with large-insertion PAC probe, specifically marking telomeric and subtelomeric regions of all human chromosomes. If some chromosome arm had absence of telomeric hybridization signals, or the intensity of hybridization signal was reduced in comparison to homologues chromosome arm, we used second DNA probe, which is strongly specific to definite telomeric region of individual chromosome. Deletions of chromosomal material, involving telomeric regions were detected in 14 (3,4%) cases from 407 patients with severe and mild forms of mental retardation and apparently normal karyotypes revealed by classical banding techniques. Microdeletions were detected in following chromosomal loci; 4q35.2; 5p15.33; 9q34.3; 10q26.3 (two cases); 11q25; 13q34; 16q24.3; 18p11.32 (three cases); 18q23; 21q22.2 and 22q13.33. These data demonstrate that undifferentiated forms of mental retardation could be associated with nonspecific telomeric micro-aberrations of different chromosomes. FISH studies using specially developed panel of telomeric DNA probes could be useful for identification of generally undetermined forms of mental retardation. This work was supported in parts by COPERNICUS-2000 grants.

P0303. Detection of subtle chromosome abnormalities by subtelomere analysis.

A. N. Lamb[1], C. Lytle[1], A. S. Aylsworth[2], C. M. Powell[2], K. W. Rao[2], M. Hendrickson[3], J. C. Carey[3], J. M. Opitz[3], D. H. Viskochil[3], A. R. Brothman[3], J. Proffitt[4], M. Irons[5]
[1]Genzyme Genetics; Santa Fe, NM United States; [2]University of North Carolina; Chapel Hill, NC United States; [3]University of Utah; Salt Lake City, UT United States; [4]Vysis, Inc.; Downers Grove, IL United States; [5]Children s Hospital; Boston, MA United States
allen.lamb@genzyme.com

Subtelomere FISH analysis was performed in two separate studies. The first was performed on individuals with dysmorphic features and developmental delay/mental retardation selected by clinical geneticists, primarily from two centers. The second group consists of individuals with DD/MR referred to our clinical service laboratory. In both groups, all individuals had previous G-banded karyotype analysis, with the first group at the 500-band level or higher; the banding level is unknown for most of the second group. Probes developed by Vysis, Inc. were used in both studies. Seventy-one patients have been analyzed in the first group, with only one abnormality detected (1.4%). An additional 35 patients from this group will be reported at the meeting. The one rearrangement is a der(18)t(2;18)(pter;qter). A reciprocal t(2;18) was detected in one of the parents. In the clinical referral population, 5 abnormalities were detected in 124 patients (4.0%). One of these abnormalities, i(Yp), does not explain the child s phenotype, leaving four abnormalities that are potentially clinically significant (4/124 = 3.2%). The abnormalities detected are (1) der(18)t(10;18)(pter;qter), (2) del(9)(qter)de novo, (3) del(18)(q23), and (4) der(18)t(16;18) (pter;pter). In case (4), the 16p subtelomere probe is distal to the intact 18p subtelomere probe. Parental studies have not been completed on this case. When karyotypes were re-reviewed, cases (1), (2), and (4) were cryptic rearrangements, and (3) had a cytogenetically visible deletion. A more detailed phenotypic analysis will be presented on a subset of these patients. Both groups show a lower hit-rate than the largest published study (7.4% + 3%; Knight et al., Lancet 354;1676-81, 1999). In addition to the severity of MR, studies may also show differences in the detection rate depending on the quality of previous karyotyping, and on the number of polymorphisms encountered. No confirmed polymorphisms were seen with this probe set in either group reported here, such as the common 2q variant as seen with other probes. Some cross-hybridizations were noted with this probe set, but these have not been bright enough to present problems.

P0304. Delineation of the critical deletion region for mental retardation, behavioural phenotype and microcephaly of the 8p23.1 deletion

A. Moncla[1], C. Missirian[1], J. Mancini[2], B. Chabrol[3], N. Philip[4]
[1]Departement de genetique medicale; Marseille, France; [2]Service de Neurop diatrie-H ptal Enfants-Timone; Marseille, France; [3]Service de Neuropediatrie-H pital Timone-Enfants; Marseille, France; [4]Departement de genetique m dicale-H pital Timone-Enfants; marseille, France
amoncla@ap-hm.fr

We report 3 unrelated patients with deletion in the distal region of chromosome 8p. Two of them presented similar clinical manifestations. They were referred respectively at age 4 and 8 years for unexplained mental retardation. They had a mild developmental delay with microcephaly. Both patients had a facial dysmorphism associating a round face with broad cheeks, slightly upslanting palpebral fissures, long eyelashes, small nose, and thin upper lip. Both had puffy, tapering fingers. They presented epilepsy and a remarkable behavioural phenotype including hyperactivity, short attention span, aggressive outbursts and sleep disturbance. A congenital heart defect (CHD) was present in one case. These clinical findings were suggestive of the del8p phenotype. We confirmed this chromosomal anomaly by conventional chromosomal analysis in both cases. Parent s chromosomal analysis was normal. The third patient, referred at age 16 years, was a mildly retarded boy with a tall stature. He never presented seizures or behavioural problems. Conventional chromosomal analysis showed an abnormal 8p;parent s chromosomal analysis was normal. We performed a molecular analysis using FISH with 8p subtelomeric probe and YAC clones within the 8p23.2 and 8p23.1 regions and analysis of polymorphic DNA markers. An interstitial deletion in the sub-band 8p23.1 was detected for the first two patients. A commonly deleted region of ~ 6Mb associated with del8p had previously been described by Devriendt et al. Interestingly, our patients, exhibiting the full clinical phenotype except for CHD, carried a different deletion of only part of the commonly deleted region with a small overlap of ~ 600kb. Our results led us to define the minimal critical deletion region for microcephaly and behavioural phenotype. In addition, the third patient carried a subtelomeric 8p deletion without overlap with del8p23.1, and, additional chromosomal material from subtelomeric 4p region. His

clinical phenotype is probably explained by this dup4pter. Our study confirmed that the apparently terminal deletions in 8p were in fact interstitial deletions. We suggested that the subtelomeric deletion of 8p would result in a milder or normal phenotype.

P0305. A patient with the first microdeletion of the Wolf-Hirschhorn-Syndrome Critical Region refines the genotype-phenotype correlation

A. Rauch[1], *S. Schellmoser*[2], *C. Kraus*[1], *H. G. D rr*[2], *U. Trautmann*[1], *M. R. Altherr*[3], *R. A. Pfeiffer*[1], *A. Reis*[1]
[1]*Institute of Human Genetics, Friedrich-Alexander University of Erlangen-Nuremberg; Erlangen, Germany;* [2]*Department of Pediatrics; Erlangen, Germany;* [3]*Los Alamos National Laboratory; Los Alamos, United States*
arauch@humgenet.uni-erlangen.de

Deletions within human chromosomal band 4p16.3 cause Wolf-Hirschhorn/Pitt-Rogers-Danks syndrome (WHS/PRDS), which is characterized by profound mental and developmental defects. Although a Wolf-Hirschhorn syndrome critical region (WHSCR) of approximately 165 kb has been defined based on two atypical interstitial deletions the genotype-phenotype correlation remains controversial, due to the large deletion sizes of usually several megabases. We report the first known patient with a small de novo interstitial deletion restricted to the WHSCR who presented only with a partial WHS phenotype consisting of low body weight for height and mild facial anomalies. Further hallmarks of WHS such as shortness of stature, microcephaly, seizures and major mental retardation were missing. The deletion was initially demonstrated by FISH analysis and breakpoints were narrowed down with a mini-FISH technique with 3-5kb amplicons. A breakpoint spanning PCR assay allowed to define the distal breakpoint as disrupting the WHSC1 gene within intron 5, exactly after an AluJb repeat. The proximal breakpoint was not found to be associated with a repeated sequence or a known gene. The deletion size encompasses 191.5 kb and includes WHSC2, but not LETM1. Symptoms attributable to this deletion are therefore low body weight for height, mild facial anomalies and some learning and fine motor deficiencies, while seizures may be associated with deletions of LETM1.

P0306. Replication Status As A Marker For Genomic Instability In Amniocytes Originating From Pregnancies With Balanced Rearrangments

A. Amiel[1], *E. Levi*[1], *O. Reish*[2], *M. D. Fejgin*[1]
[1]*Meir Hospital; Kfar-Saba, Israel;* [2]*Assaf Harofeh Hospital; Zrifin, Israel*
alizaamiel@hotmail.com

Earlier studies demonstrated a monoallelic mode of expression in some pre-cancerous and invasive squamous carcinoma of the cervix as well as in multiple myeloma . A high rate of asynchronic pattern has been described in; 1. lymphocytes of individuals with solid tumors as well as in other malignancies. 2. amniocytes of genotypes with an extra chromosome 13,18 and 21. 3. lymphocytes of young mothers of a Down syndrome pregnancy. The asynchronic pattern was not locus specific and was found in all loci analyzed. These findings suggested that the mechanism controlling the temporal order of replication could be altered in cells with a genetic predisposition to cancer or aneuplody. In this study we found a higher rate of asynchronic pattern in genotypes carrying inversions 2 and 9 and in balanced heritable translocations ($p<0.01$) and even higher rate in in cases with a de novo balanced translocations ($p<0.05$). The process of tumor genesis may begin with change in cell cycle regulation which includes the duplication replication and segregation of genetic information. However, it remains unknown whether individuals with balanced chromosome rearrangement are at increased risk of developing cancer later in life.

P0307. Premature chromosome condensation in a new genetically determined syndrome

H. Neitzel[1], *L. M. Neumann*[1], *D. Schindler*[2], *A. Wirges*[1], *K. Sperling*[1]
[1]*Institute of Human Genetics; Berlin, Germany;* [2]*Institute of Human Genetics; Wuerzburg, Germany*
heidemarie.neitzel@charite.de

The entry of mammalian cells into mitosis is regulated by the activation of an evolutionary highly conserved protein complex (cyclinB/CDK1) leading to nuclear envelope breakdown, chromosome condensation and spindle assembly. Premature chromosome condensation can be induced experimentally if the activated complex is introduced into an interphase cell. Here, we report on two sibs of consanguineous parents, born after uneventful pregnancies in 1993 and 1995. At birth they presented with microcephaly, cranial and body growth remain well below the 3rd centile. Neuromotor development was delayed and combined with profound mental retardation. They had a normal karyotype with poor chromosome morphology, normal rate of sister chromatid exchanges and a slightly elevated rate of spontaneous chromosomal aberrations. Most conspicious was the high frequency of prophase-like cells with intact nuclear membrane (10%) in lymphocyte, lymphoblastoid, and fibroblast cultures. Already 1h after 3-H thymidine application a significant percentage of these cells was labelled, indicating that the length of the G-2 phase is drastically reduced. Flow cytometric analysis revealed no obvious disturbances in the cell cycle progression. Thus, all data are consistent with premature entry of the cells into mitosis. To the best of our knowledge this combination of cytogenetic and clinical findings has not been reported before. It is logical to assume that a gene involved in cell cycle regulation is mutated in these patients.

P0308. Interphase FISH studies of replication timing at Rett syndrome using chromosome X specific DNA probes

Y. B. Yurov[1], *S. G. Vorsanova*[2], *A. D. Kolotii*[2], *I. V. Soloviev*[3]
[1]*National Centre of Mental Health , Russian Academy of Medical Sciences; Moscow, Russian Federation;* [2]*Institute of Pediatrics and Children Surgery, Russian Ministry of Health; Moscow, Russian Federation;* [3]*National Centre of Mental Health, Russian Academy of Medical Sciences,; Moscow, Russian Federation*
yurov@rcmh.msk.ru

Rett syndrome (RTT) is, probably, caused by mutations in the methyl-CpG binding protein-2 (MeCP2) gene on chromosome Xq28. In RTT patients the defective MeCP2 might fail to keep other genes silent . Mutations in MeCP2, as well in other genes, encoding proteins that functionally interacting with MeCP2, could be involved in pathogenesis of RTT. The dysregulated genes, which are under control of MecP2, are still unknown. Therefore, the identification of target genes whose expression is under control of MeCP2 will help to fully explain the cause of RTT and develop adequate treatment of RTT. FISH using chromosome X specific genomic DNA probes, was applied to determine the loci with altered replication and transcription at RTT. Randomly selected PAC clones for Xp, Xce and Xq were used. It was found that two clones from Xq28 (anonymous clone PAC 24.23.0 and PAC 671D9, containing MeCP2 locus) are early replicating in both X chromosomes and, probably, escape inactivation in late-replicating chromosome X in RTT patients. Therefore, region Xq28 could contain the genes escaping X- inactivation and expressing from the human active and inactive chromosomes X. These results support the hypothesis (Vorsanova et al., 1999) proposing the disturbances in dosage compensation effect due to aberrant activation of genes in inactive chromosome X at RTT genes (di-allelic expression instead of mono-allelic). Our results indicate that MecP2 itself is under unusual epigenetic control and could escape X-inactivation. Activation of normal allele of MeCP2 from inactive chromosome X could reduce pathogenic effect of mutated allele at RTT. Supported in parts by INTAS and IRSA grants.

P0309. Chromosome aberrations in lymphocytes of residents living in buildings constructed with radioactively contaminated rebar

S. Wang-Wuu, *K. D. Wuu*
Institute of Genetics, National Yang-Ming University; Taipei, Taiwan Republic of China
sww@ym.edu.tw

Using the G-banding technique, we examined lymphocytes from ninety individuals (43 males and 47 females, median age 31) living in buildings constructed with radioactively contaminated rebar. Forty-five non-exposed control subjects (22 males and 23 females, median age 30), matched to the radiation-exposed individuals by sex and age, were selected for comparison. At least 500 metaphases were checked for each individual. All recognizable structural aberrations of chromosomes or chromatids were recorded. After adjusting for age and smoking status, both the precentage of cells with aberrated chromosomes (PCAC) and the number of aberrated chromosomes per 100 cells (NAC) were found to be significantly higher in the radiation-exposed females than in the control females ($p<0.05$ for PCAC and NAC). This difference, however, was not observed in the comparison of radiation-exposed vs. control males. It suggests a possible interaction between sex and radiation exposure in their effects on chromosome aberrations. Neither the duration of exposure nor the cumulative exposure showed a significant correlation with PCAC or NAC.

P0310. Cytogenetic analysis of patients dosed with 131-Iodine for scintigraphy of the thyroid

I. P. Aranha[1], *C. L. Rodrigues*[2], *R. Corbo*[2], *B. Gutfilen*[3]
[1]*Universidade do Estado do Rio de Janeiro; Rio de Janeiro, Brazil;* [2]*Universidade Federal do Rio de Janeiro; Rio de Janeiro, Brazil;* [3]*Univ. Estado do Rio de Janeiro/Univ. Federal do RJ; Rio de Janeiro, Brazil*
aranha@uerj.br

The radioisotope 131-Iodine has a half-life of 8.02 days and is used in

Nuclear Medicine for scintigraphy of the thyroid with an average activity of 3.7 MBq. It is also used in other dosages for metastasis detection and in case of ablation. There are no conclusive results, yet, as to the effect of the dosage of 131-Iodine used in scintigraphy of the thyroid on patients. The objective of this study was to evaluate the effect of that dosage on chromosomes from human lymphocytes. Cells from 21 patients from Hospital Universit rio Clementino Fraga Filho were placed in enriched 1640 RPMI medium and incubated for 48 and 72h at 37oC. Blood was collected 24 hours after administration of the radioisotope. Blood cells collected from the same patients prior to the administration of 131-Iodine were used as control. Chromosomes were stained with Giemsa Gurr (2%, pH=6.8) and analyzed under optical microscope. Of 6300 metaphases analyzed from the 48h cultures,1146 gaps and 682 breaks were found in the test group. Of 6300 metaphases analyzed from the control group, 291 gaps and 119 breaks were found. These results are significant in a paired t-test (p<0.05). In the 72h cultures, of 6300 metaphases analyzed, 216 gaps and 52 breaks were found in the test group. Of 6300 metaphases analyzed from the control group, 10 gaps and no breaks were found. These results are also significant in a paired t-test (p<0.05). Our results show that 131-Iodine is responsible for the chromosomal alterations observed.

P0311. An approach for characterization of individual features of the karyotype instability.

S. R. Rushkovsky[1], *V. F. Bezrukov*[2], *I. R. Barilyak*[1]
[1]*Ukrainian Scientific Center of Medical Genetics; Kyiv, Ukraine;* [2]*Kyiv Taras Shevchenko University; Kyiv, Ukraine*
rsr@bvf.carrier.kiev.ua

We suggest an approach that allows to individualize the karyotype instability evaluation. This approach is based on simultaneous analysis of several parameters of karyotype instability. Any person may reveal high (H) or low (L) level of instability by each of parameters. A series of levels of instability (H or L) of all parameters is an individual characteristics of person s genome instability. A number of individual variants is equal 2^n, where n is a number of investigated parameters of genome instability and number 2 is a number of groups (H or L) of instability.

We have studied three parameters of karyotype instability (levels of chromosomal aberrations, polyploid mitoses and diplochromosomes) under two-way cultivation of human lymphocytes. First way (52-hours cultivation) reveals immediate karyotype instability in first mitosis; second way (144-hours cultivation) allows to detect delayed karyotype instability in fifth mitosis. The additional information obtained under two-way cultivation allowed us to separate donors into four groups (instead of the two) of instability (***LL***, ***LH***, ***HH***, ***HL***) according to their individual characters of karyotype instability by each parameters. The number of individual variants that may be distinguished with this approach is 4^3=64. Some individual variants were statistically significantly more frequent and some less than it could be expected from distribution of the donors by every studied parameters. We may explain it by two reasons; by common mechanisms that lead to different manifestations of genome instability and by selective elimination of persons with certain individual characteristics of karyotype instability.

P0312. The in vitro effects of selective and non-selective non-steroidal antiinflammatory drugs on the frequency of sister chromatid exchanges

S. Ozturk[1], *H. Kocak*[2], *S. Palanduz*[1], *K. Cefle*[1], *H. Erkal*[1], *B. Aydil*[2], *B. Gurkan*[2]
[1]*Istanbul University, Istanbul Medical Faculty, Department of Internal Medicine, Division of Medical Genetics; Istanbul, Turkey;* [2]*Istanbul University Faculty of Dentistry, Department of Oral Surgery; Istanbul, Turkey*
sozturk@istanbul.edu.tr

Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) have wide use in medical as well as surgical clinics for their analgesic, antiinflammatory and antipyretic effects. In this study we investigated the in vitro effects of non-selective (naproxen) and selective (etodolac and meloxicam) NSAIDs on the frequency of sister chromatid exchange (SCE) in their maximum therapeutic concentrations. SCE is a reliable test for the evaluation of DNA damage caused by various physical and chemical agents. We found that the mean SCE frequencies in the naproxen (8 microgram/ml), etodolac (15 microgram/ml) and meloxicam (2 microgram/ml) groups were 7.81 – 2.18, 7.63 – 2.18 and 7.75 – 2.00 per metaphase, respectively. The mean SCE frequency in the control group was 6.89 – 1.82. There was no statistically significant difference between the NSAIDs group and the control group in regard to SCE frequency (p >0.05). These findings imply that the anti-inflammatory drugs used in this study do not have a genotoxic effect in vitro.

P0313. Results of cytogenetic inspection of the persons working on nuclear - chemical plant and inhabitants living in this region

N. Popova, *S. Nazarenko, Y. Yakovleva, E. Vasilyeva*
Research Institute of Medical Genetics; Tomsk, Russian Federation
natasha@img.tsu.ru

The frequency and spectrum of chromosome aberrations in peripheral blood lymphocytes was evaluated in workers of the Siberian Chemical Plant (SCP) with various integral doses of external gamma-irradiation (I group - 18-37 cGy; II group - 93-157 cGy) and in inhabitants, living in the vicinity of SCP Seversk city (10-km zone) and in radiation safe Kargala settlement (control group). The frequency of aberrant cells in Seversk city population and two groups of SCP staff was significantly higher in comparison with the control (p< 0.05). High interindividual differences in the frequency of cells with chromosome damages in I and II groups of SCP workers were detected. 20% of the inspected workers belongs to a category of high genetic risk having frequency of aberrant cells 4-fold more of spontaneous level. 8,5% of the inspected workers belongs to a category of the persons with superhigh genetic risk. For them the frequency of cells with chromosome aberrations exceeds a spontaneous level in 10 times and more. A level of chromosome exchange aberrations (biomarkers of radiation effect) for the population of Seversk city and control group does not differ from a spontaneous level (about 0,1%). For the II group of SCP workers it exceed at 3 times in comparison with a control level, but significant differences were not detected. The significantly increase of the frequency of chromatide aberrations in Seversk town inhabitants and both groups of SCP workers was revealed (p< 0.05). It testifies for the effect of other then radiation factors to somatic cells in investigated individuals.

P0314. Genetic Approach In The Study Of Some Aspects Of Bronchial Asthma Pathogenesis

V. V. Semenov, *A. V. Semenov, E. S. Koshpaeva, A. P. Pigalov*
Kazan State Medical University; Kazan, Russian Federation
medbiogen@mail.ru

The problem of bronchial asthma is considered to be the burning question among children s diseases. Available data suggest that destruction of genetic stability of the cells is a key component of bronchial asthma pathogenesis. Measurement of clastogenic and anticlastogenic activity of asthmatic and nonasthmatic children blood plasma were investigated using Crepis capillaris seeds (chromosome aberrations assay). In our studies, the plasma of 9 nonasthmatic and 13 asthmatic children aged 7-14 with different degree of the severity of disease were examined. The results obtained, show that clastogenic effect of plasma of nonasthmatic patients (1.65 – 0.39) and asthmatic group (1.37 – 0.35) doesn t differ from the level of spontaneous mutation 1.14 – 0.31 (p > 0.05). It is pointed out that the plasma of children with asthma has the low ability to decrease the level of ethylmethane sulphonate-induced chromosome aberration, compared to the plasma of nonasthmatic children — 1.96 – 0.42 (p> 0.05). Thus, in our investigation, the new approach for studying bronchial asthma is proposed. This method (chromosome aberrations assay) may be used for determination of severity of bronchial asthma manifestations. One may conclude that destruction of normal function of antimutagenic system of asthmatic patients blood. Therefore, for rise of genoprotection activity of blood is necessary to use the drugs with antimutagenic activity.

P0315. Chromatide Aberrations In Lymphocytes Of Asthmatic Children Peripheral Blood And Possibility Of Heir Correction By Dimephosphone

A. V. Semenov
Kazan State Medical University; Kazan, Russian Federation
arsen_s@mail.ru

According to literature data, the rise of chromosomal aberration level was recorded in inflammatory processes. Since the asthma is related to chronic inflammatory diseases, therefore in our investigation chromosome aberration level was studied using lymphocytes of peripheral blood of patients with bronchial asthma. The first group of asthmatic patients received the traditional treatment. In addition to that treatment the other group of patients received dimepfosphone (30-50 mg/kg, per os exposed 7 days). In experimental study the genoprotection activity of dimephosphone was demonstrated. The mechanism of genoprotection activity of that drug was manifested by activation processes of excision repair and inhibition of active oxygen radical. In our studies, asthmatic children with different severity of manifestation of disease aged 7-14 were examined. Chromosomal aberration level from asthma patients and nonasthmatic controls was compared. According to the obtaining results, level of chromosomal changes from asthmatic tended to be higher than that from the nonasthmatic group (p < 0,001). Reduction of level of chromosomal aberration in

lymphocytes of peripheral blood of patient with light form of asthma by dimephosphone-treatment was observed (p< 0.001). At the same time, decrease of the number of lymphocytes with chromosome aberration from children with severe of manifestations of bronchial asthma was not observed (p> 0.05).

P0316. In vitro genotoxic effects of cadmium (II) in human lymphocytes evaluated by electron microscopy in situ end-labelling (EM-ISEL) and sister chromatid exchange (SCE).

M. Cojocaru[1], *F. Depault*[1], *S. Chakrabarti*[2], *F. Fortin*[1], *N. Lemieux*[1]
[1]*D partement de Pathologie et Biologie cellulaire, Universit de Montr al; Montr al, PQ Canada;* [2]*D partement du Travail et d Hygi ne du milieu, Universit de Montr al; Montr al, PQ Canada*
cojocarm@magellan.umontreal.ca

Cadmium is a known carcinogenic metal but evidence concerning its genotoxic effects is conflicting. Human daily exposure to cadmium compounds occurs primarily through diet, drinking water and cigarette smoke. The objective of this work was to study DNA damage in human peripheral blood lymphocytes after a 2-hour in vitro cadmium chloride (CdCl2) exposure. The induction of genotoxic effects using both the electron microscopy in situ end-labelling (EM-ISEL) assay and the sister chromatid exchange (SCE) test was investigated. The inhibition of cell-cycle kinetics due to cadmium(II) was also evaluated. Cytotoxicity was assessed by the Trypan Blue exclusion test in order to select the correct doses of CdCl2 for the genotoxic assays. The values of labelling intensities obtained by EM-ISEL in cells treated with slightly cytotoxic (250 uM) or non-cytotoxic (125uM, 25uM) doses were superior to the values obtained in control cells. A significant increase in single-strand break frequencies was observed at both chromosomal and nuclear chromatin level. In contrast, there was no significant difference between the SCE frequency in cells treated with cadmium and the control cells. However, both the proliferative and the mitotic indexes were significantly decreased. These results suggest that cadmium(II), even at non-cytotoxic doses, remains an active clastogenic and mitotoxic agent, but that the DNA lesions do not induce an increase in SCE frequency neither at non-cytotoxic nor at slightly cytotoxic doses. This project was supported by a grant of Redicy Inc.

P0317. The results of 15-year cytogenetic monitoring for the groups of high priority suffered from the action of Chernobyl accident factors

M. A. Pilinskaya*, S. S. Dibskij, Y. V. Shemetun, Y. B. Dybskaya, L. R. Pedan*
Research Center of Radiation Medicine; Kiev, Ukraine
lena@leed1.kiev.ua

During about15 years the selective cytogenetic monitoring among the critical groups of Chernobyl accident victims in Ukraine have been fulfilled. Conventional, G-banding and molecular (FISH-WCP) cytogenetic methods have been used for the examination of the high priority groups. In the delayed terms following the accident in all exposed groups (patients recovered from acute radiation sickness - ARS, clean-up workers, Chernobyl power plant personnel, persons evacuated from Pripyat in 1986, self-settlers from 30-km alienation zone, children and adults from regions of obligatory and voluntary evacuation) the frequency of chromosome aberrations (as integral as specific for radiation exposure) significantly elevated the control levels.The increased number of radioinduced cytogenetic markers (mainly stable) have been discovered in some exposed groups (patients recovered from ARS, liquidators with stable lymphopenia, personnel of Sarkophagus, children evacuated from Pripyat, persons living and/or working in contaminated by radionuclides territories) even with the help of conventional cytogenetics. Introduction in1996 of modern FISH technique essentially improved the possibilities of cytogenetic monitoring in terms of the discovery of stable chromosome aberrations (including clones), although under as traditional as molecular cytogenetics a wide interindividual variability of cytogenetic effects for the identical radiation exposure had been revealed. Comparison of the results of the conventional and fluorescence chromosome analysis showed that the discovery of translocations under the conventional staining with group kariotyping consisted no more than 29 % from the level of FISH translocations per whole genome. These data have been confirmed by the results of G-banding analysis. The calculation of the biological doses on the frequency of reciprocal translocations in comparison with the official dose records and results of EPR (tooth enamel) dosimetry showed that FISH technique can be used for retrospective group dosimetry under the doses above 25 cGy and for the individual dosimetry in dose range ~ 50 - 300 cGy (in view of aging factor).

P0318. The Effects of Gonadotropin-Releasing Hormone Analogue (GnRHa = Buserelin asetate) on the Frequency of Micronucleus, Some Biochemical Parameters, Ovarian and Uterus Histology in Rats

A. Basaran[1], *M. Dikmen*[1], *I. Degirmenci*[1], *H. Veysi G nes*[1], *H. Kurt*[1], *D. Cosan*[1], *M. Cengiz st ner*[1], *V. Sahint rk*[2]
[1]*Department of Human Biology; Eskisehir, Turkey;* [2]*Histology- Embryology, Osmangazi University Medical Faculty; Eskisehir, Turkey*
abasaran@ogu.edu.tr

The aim of this study was made to investigate effects of GnRHa, which is a inhibitor agent of ovarian activity, on the frequency of micronucleus, some urine and blood parameters, ovarian and uterus histology. In this experimental study, 20 Sprague-Dawley female cycling rats, aged 2,5-3 mounts were used. They were divided into 2 groups. Serum physiological saline to the first group (Control) and 15 mg/day/rat GnRHa to the second group were injected subcutaneously during the experimental period. On the 26 th day urine samples were collected for 24-hours from each of the animals. The end of the experiment (27th day) rats were anaesthetised with either and blood samples were collected, tissue samples were removed and fixed immediately. As a result of the experiment, in the 2nd group micronucleus frequency, SGOT and SGPT activities, creatinine clearence, urine creatinine, urea nitrogen and uric acid increased. Histologically, GnRHa increased the number of corpus luteum and primer follicles in the ovaries. In addition, gland development in uterus tissue of the 2nd group was decreased.

P0319. Cytogenetic Studies of Patients with Behcet s Disease

G. Gullulu[1], *S. Oztas*[2], *N. Astam*[1], *I. Akyol*[1], *A. G. Tatar*[2], *A. Karakuzu*[3], *A. Odabas*[4]
[1]*Ataturk University Medical Faculty Department of Ophthalmology; Erzurum, Turkey;* [2]*Ataturk University Medical Faculty Department of Medical Genetics; Erzurum, Turkey;* [3]*Ataturk University Medical Faculty Department of Dermatology; Erzurum, Turkey;* [4]*Ataturk University Medical Faculty Department of Immunology; Afghanistan*
sitkioztas@asteoclub.com

Behcet s disease (MIM#109650) is an immunogenetically based multisystem disorder of unknown pathogenesis and mode of inheritance is not clear. Behcet s disease is characterized by uveitis, arthritis, orogenital ulcerations and pustular skin lesions. It is most frequently seen in Turkey and Japan. HLA-B5 and its subtypes HLA-Bw51 has been found to predominate in cases. Recently, a triplet repeat (GCT/AGC) microsatellite polymorphism in the MICA gene was shown that its frequencies were significantly higher in the Behcet s patients than they were in healthy controls. In this study, we investigated the chromosomal abnormalities and analysed sister chromatid exchange in patients with Behcet s diseases. Thirty-five patients with Behcet s diseases (15 patients with uveitis and 20 patients without uveitis) and 20 healthy subjects as control were analyzed. The chromosomal abnormalities and sister chromatid exchange analysis has been performed on lymphocytes. All patients were analysed prior to treatment. We observed some chromosomal configurations that may indicate centromer association in patients, along with the increased rate of sister chromatid exchange. No numerical chromosomal abnormality was observed in patients with Behcets disease.

P0320. Radiation-induced chromosomal abnormalities in the germ cells of testicular cancer patients

A. C. Velazquez-Wong[1,2], *E. Sloter*[1,3], *F. Salamanca-Gomez*[2], *M. L. Meistrich*[4], *A. J. Wyrobek*[1]
[1]*Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory; Livermore, CA United States;* [2]*Unit of Investigation in Human Genetics, Mexican Institute of Social Security; Mexico City, Mexico;* [3]*Genetics and Developmental Biology Program, West Virginia University; Morgantown, WV United States;* [4]*Department of Radiation Oncology, M. D. Anderson Cancer Center; Houston, TX United States*
velazquez1@llnl.gov

Ionizing radiation is an effective treatment for testicular cancer. Incidental exposure to the healthy testis during radiotherapy (RT) has been shown to induce deleterious effects on spermatogenesis and semen quality. In a study of semen quality in 10 seminoma patients, sperm concentrations were significantly reduced at 60 days following RT with partial recovery occurring in some patients after 500 days. To investigate the long-term genetic effects on germ cells, we applied the multicolor sperm ACM FISH method (Sloter et al. 2000) to semen samples from seminoma patients collected before and after RT. The ACM assay detects (a) partial chromosomal duplications and deletions, which are the sperm products of premeiotic or meiotic breakage events or rearrangements, (b) chromosomal breaks within 1cen-1q12, and (c) extra or missing copies of chromosome 1. In a

pilot study of six samples from two patients (estimated testicular dose; 38 and 55 cGy), a significant 2 to 3-fold increase in the frequency of partial chromosomal duplications and deletions was detected 159-174 days following RT (P<0.01) which returned to pretreatment range 531-848 days after RT. This coincided with a transient 2 to 5-fold increase in numerical abnormalities (P<0.01). RT did not affect the frequency of sperm with chromosomal breaks (i.e., postmeiotic damage) at the time points surveyed. This work is important for understanding the long-term genetic effects of RT on spermatogonial stem cells. [Work was conducted under the auspices of the U.S. DOE by LLNL under contract W-7405-ENG-48 and support from the Mexican Institute of Social Security.]

P0321. Spectral karyotyping of Werner«s syndrome fibroblast cultures and observation of a near tetraploid clone with exceptional growth potential

R. Melcher[1], *R. von Golitschek*[2], *C. Steinlein*[2], *M. Schmid*[2], *H. Hoehn*[2]
[1]*Department of Medicine; W rzburg, Germany;* [2]*Institute of Human Genetics; Wuerzburg, Germany*
r.melcher@medizin.uni-wuerzburg.de

Werner«s syndrome (WS; MIM #277700) is an uncommon autosomal recessive condition characterized by many features usually associated with aging. Patients with WS also have an elevated risk of cancer. Skin fibroblast cell cultures from WS patients are usually composed of one or several clones, each marked by a distinctive, apparently balanced translocations, designated as «variegated translocation mosaicism«. WS fibroblast cultures have a limited capacity to proliferate in vitro and a deletion mutator phenotype. In the present study spectral karyotyping (SKY) was applied to two WS fibroblast cell lines (WL and AA) to clarify if there are subtle chromosomal alterations despite the gross chromosomal alterations published so far and to analyse if any chromosomal alterations are responsible for a growth advantage. Using the high resolution SKY techniques, we discovered a new type of cyclic rearrangements and could confirm that a subset of metaphases in WS fibroblast cultures appear to maintain a normal karyotype. Furthermore, we succeeded in establishing a subclone of a WS fibroblast culture (WL) that achieved an unprecedented 43 population doublings in vitro. This long-lived subclone has a pseudotetraploid karyotype, with tetrasomy for all autosomes except chromosomes 4 and 6 which are trisomic. Although it is too early to consider this unique subclone immortal or transformed, the mechanism of polyploidization could have been a first step in that direction and explain the exceptional gain in growth potential.

P0322. Autosomal fragile sites among Mentally retarded subjects; Genetic implications and review of literature

K. R. Manjunatha[1], *G. K. Chetan*[1], *R. Arathi*[1], *G. V. Bhaskar Rao*[1], *P. Latha*[1], *S. Padma*[1], *S. R. Girimaji*[2]
[1]*Dept. Of Human Genetics, National Institute of Mental Health and Neurosciences; Bangalore, India;* [2]*Dept. Of Psychiatry, National Institute of Mental Health and Neurosciences; Bangalore, India*
krmanjunatha@yahoo.com

Genetic screening program for mentally retarded subjects routinely carried out at our centre revealed the association of a variety of autosomal fragile sites such as 1p36,2q21,2q23,2q31,2q33,3p14, 4 q 3 1 , 5 q 2 1 , 5 q 3 1 , 6 p 2 3 , 6 q 2 1 , 6 q 2 6 , 7 q 3 1 , 8 q 2 4 , 9q32,10q21,10q25,10q26,12q24,13q21, 14q32 and 22q13 with a frequency of 2-20% of expression, even in repeated blood cultures and some of them are new sites, showing association with mental retardation as compared with the available literature. The possible role of phenotype-genotype correlations leading to establishing genetic implications is attempted keeping inview of the recent literature.

P0323. Cytogenetic Studies In Traffic Police.

H. P. Mundluru, *S. Varre, S. D. Gundimeda, P. P. Reddy*
Institute of Genetics and Hospital for Genetic Diseases; Hyderabad, India
sandymund@hotmail.com

Traffic police are highly exposed to vehicular pollutants like carbon monoxide ,nitrogen oxides, lead oxide particles, suspended particulate matter and diesel exhaust particles as they are on duty for long periods.Peripheral blood lymphocytes were collected from fifty traffic police and analysed for chromosomal aberrations.Their age ranged from 25 to 58 years.and duration of service ranged from 2-32years.For control data blood samples from 30 men who are not exposed to any pollutants and belonged to the same age and socio _-economic status were collected. A significant increase in the frequency of chromosomal aberrations like deletions,breaks ,gaps, dicentrics and fragments was observed in the exposed group when compared to the control group.

P0324. Effects of Iodine 131 Treatment on Micronucleus Formation in Patients with Tyroid Disease

M. Aksoy[1], *B. Aslan*[1], *H. Arikoglu*[1], *A. B. Turhan*[1], *M. Kaynak*[1], *G. Tastekin*[2]
[1]*Department of Medical Biology, Selcuk University, Medical Faculty; Konya, Turkey;* [2]*Department of Nuclear Medicine Radiology, Selcuk University, Medical Faculty; Konya, Turkey*
bigearslan@hotmail.com

Radioactive iodine (131I) has been considered as a safe and effective treatment for thyroid diseases such as hyperthyroidism and thyroid cancer. However, the side effects of this treatment were reported by different authors. In addition, various forms of cytogenetic damages including high rate of micronucleus (MN) and chromosomal aberrations related to radiation exposure were reported. The micronucleus assay is an effective and alternative method for evaluating genetic damages induced by chemicals and physical agents. MN assay is a sensitive, simple and fast technique to detect effects of clastogenic and genotoxic agents. In our study, MN frequency in peripheral blood lymphocytes of 131I treated patients was analysed to detect the possible chromosomal damages related to 1) 131I treatment, 2) in vitro culture statute and 3) the use of colchicine. It was also determined the effects of 131I in the different period, after the treatment. The results obtained before treatment were compared with those obtained at different periods after treatment, and other parameters.

P0325. Analysis of the interaction of papillomavirus episomes with mitotic chromosomes

S. Kivi, *I. Ilves, A. Abroi, R. Kurg, M. Ustav*
Institute of Molecular and Cell Biology, Tartu University; Tartu, Estonia
skivi@ebc.ee

DNA viruses that possess episomal genomes, such as papillomaviruses, must maintain their genomes as stable extrachromosomal elements in host cells. Long-term episomal maintainance of papillomaviruses is shown to be dependent on a chromatin attachment ability of viral genomes. To address the mechanisms responsible for that, we examined nuclear compartmentalization of plasmids containing the bovine papillomavirus type 1 full-length URR and URR deletion constructs in the Chinese hamster ovary (CHO) cell line based model system. From this study we concluded that BPV1 URR-dependent chromatin attachment is determined by viral E2 protein *in trans* and its binding sites on plasmid DNA *in cis*. Now, using E2 antibodies and immunofluorescence as well as FISH analysis, we have studied the determinants of the BPV1 E2 protein that are necessary for URR-dependent chromatin attachment. The analysis of different E2 deletion constructs confirmed the previous reports that both N-terminal transactivation domain as well as DNA-binding dimerization domains are needed for E2-mediated linking of the BPV1 episomes to the chromatin. Our data suggest that the binding of the E2 protein to host chromosomes is achieved through the interaction of the specific determinants within N-terminal domain of E2 with chromatin. These interactions guarantee the proper partitioning and nuclear retention of the viral genomes, and are likely to be separable, at least partly, from interactions involved in transactivation and viral replication supporting activities.

P0326. Laser Pressure Catapulting; Preparation of single cells, cell areas or chromosomes in a non-contact way

R. Burgemeister[1], *A. Clement-Sengewald*[2], *G. Lahr*[3], *K. Sch tze*[1]
[1]*P.A.L.M. Microlaser Technologies; Bernried, Germany;* [2]*Women s Hospital of the Ludwig-Maximilians University; Munich, Germany;* [3]*Laser Laboratory and Molecular Biology, Academic Hospital Harlaching; Munich, Germany*
Renate.Burgemeister@PALM-Mikrolaser.com

Pure sample preparation is one of the most thrilling tasks in modern molecular biology and medicine. The technique of Laser Pressure Catapulting (LPC) uses only the force of focused laser light to eject a selected specimen from the object slide or from a living cell layer and to directly catapult it into the cap of a routine microfuge tube. Subsequent molecular genetical examinations can be carried out immediately after centrifugation of the catapulted material into the bottom of the PCR tube. This procurement of single cells, small homogeneous cell areas or chromosomes from different sources occurs in an entirely non-contact and locally confined manner. This non-contact Laser Pressure Catapulting technology avoids the danger of contamination with unwanted specimen. The procurement of a specimen is mandatory for the subsequent analysis of cell or chromosome specific genetic alterations such as in cancer research, prenatal diagnosis, preimplantation diagnosis, cell biology or developmental biology. Any kind of tissue from different sources (also archival samples) and even subcellular structures can be captured using this laser method. There is no heat involved in this technique, and the applied laser wavelength does not affect the biological information. Wherever the procurement of homogenous

samples is mandatory for the subsequent analysis of a cell, a cell area or a chromosome, LPC is a key technology. LPC is the state of the art tool for quick and pure sample preparation for a variety of applications - a prerequisite for reliable genetic and proteomic analysis.

P0327. A Case of Translocation between Chromosomes 5 and 6; (5;6)

I. V. Soprunova, *A. B. Bashilina, S. V. Boulytcheva*
Astrakhan Medico-Genetic Consulting Centre; Astrakhan, Russian Federation
coolworld@astranet.ru

The proband, an eight-month old girl was born from the first pregnancy, occurring against the background of hestosis and anemia. The proband s parents are healthy; her mother is 17, Russian and her father is 22, Azerbaijanian. The girl s weight at birth was 2750 gr., her length was 50 cm., the head was 33 cm in circumference. During the first days after her birth the syndrome of depression was dynamically substituted by the syndrome of the central nervous system excitement. During the neurosonographical examination there was found the lobar form of goloprozencephalia and the disgenesis of the callous body. At the age of 8 months the girl s weight was 4000gr., her length was 60 cm., the head was 36 cm in circumference, the greater fontanelle was almost closed (0,3 * 0,3 cm). The examination discovered microcephalia, the cranium asymmetry, brachicephalia, flat occipit, low forehead, hypertelorism, apicant, Mongoloid form of the eyes, wide flat nose bridge, long filter, low-placed dysplastic auricles, short neck, a brown spot (0,5 * 0,5 cm) on the skin of the pubis. There are some symptoms of heavy disorders of the girl s neuropsychological and physical development. The child can not carry her head properly, she can not sit or stand, does not respond to the surroundings. There were signs of convulsion syndrome and hypothrophy. The citogenic examination by the method of G-colouring discovered the proband having translocation between 5 and 6 chromosomes, karyotype 46, xx,t (5;6). The mother s karyotype is normal, 46, xx. The proband s father was not cytogenetically examined.

P0328. The structure and shape of chromosomes in three-dimensionally intact interphase lymphocyte nuclei

J. Lemke[1], *P. Ullmann*[2], *I. Chudoba*[3], *S. Michel*[1], *U. Claussen*[1]
[1]*Institute of Human Genetics and Anthropology; Jena, Germany;* [2]*Carl Zeiss Jena GmbH; Jena, Germany;* [3]*MetaSystems Inc.; Altlussheim, Germany*
johanneslemke@gmx.net

The DNA-based high resolution multicolour banding (MCB) technique covers a whole chromosome with differently labelled microdissection probes and thereby allows to investigate the chromosome structure in detail in all stages of the cell cycle. It has been shown that normally prepared chromosomes in lymphocyte interphase nuclei show a similar MCB pattern as metaphase chromosomes. To analyse the structure and the shape of human chromosomes 5 in interphase, lymphocytes were routinely cultivated and harvested for chromosome analysis. Three-dimensionally intact interphase nuclei were obtained by drying of the suspension on the slide at relative humidity of less than 7%. The nuclei were then hybridised with the MCB probe set for chromosome 5. With the help of a confocal laser scanning microscope (LSM 510, Zeiss) it was possible to deline the axes of both chromosomes, to analyse their shapes and to localise the coloured chromosome sub-regions. Human chromosomes 5 in interphase nuclei are similar to metaphase chromosomes. In many cases they are cylindrically shaped almost as in three-dimensionally intact metaphases. In contrast to metaphase chromosomes interphase chromosomes are always bent. The combination of both MCB and 3D analysis using confocal laser scanning microscopy seems to be a powerfully technical combination for functional analysis of the ultrastructure of interphase nuclei.

P0329. Cytogenetics And Molecular Cytogenetic Analysis Of The Patients With Psoriasis

H. Berkil[1], *B. Durak*[1], *I. Sabuncu*[2], *C. Ataman*[1], *N. Basaran*[1]
[1]*Osmangazi University, Medical Faculty, Department of Medical Genetics; Eskisehir, Turkey;* [2]*Osmangazi University, Medical Faculty, Department of Dermatology; Eskisehir, Turkey*
hberkil@ogu.edu.tr

Psoriasis is a chronic disease of the skin characterized with silvery scales demercated and clear-cut borders, localized on ping-red papuls or macules. Psoriasis affects about 2 percent of the population. The disease appears with the hyperproliferation of epidermal cells and inflammation resulting from infiltration of activated T helper cells and mononuclear cells and release of pro-inflamatory cytocines. In studies, the psoriasis susceptibility locus is restricted on a genomic sequence of 60 kb (6p21.3) on the HLA region. Recently, genome wide scans have established the presence of various gene localizations different from HLA and these are suggested to be responsible of psoriasis. But up to date only 1q, 6p21 and 17q are confirmed in various studies. In our study by using cytogenetic analysis and FISH with Cyclin D1 probe, the 11q13 region, that is one of the responsible candidate regions is inspected to determine the possible chromosomal disorders in peripheral blood samples of 31 patients. Additionally, we evaluated the cases for the hereditary model of the disease. As the results of cytogenetic and FISH analyses, the chromosomal structure and the 11q13 region were determined to be normal in all of our patients. The pedigree analysis of some cases suggested the mode of inheritance of the psoriasis as dominant, as well.

P0330. Heterochromatic der(15) in a male infertility patient; Case report and review of the literature

G. Bujdos [1], *G. Schwanitz*[2], *E. Koltai*[1], *A. Ehrbrecht*[2], *R. Raff*[2]
[1]*Institute of Forensic Medicine of Semmelweis University, Research Unit; Budapest, Hungary;* [2]*Institute of Human Genetics; Bonn, Germany*
dunay@igaz.sote.hu

Chromosome analyses have been performed in a sample of infertility patients. In the case reported, a supernumerary marker chromosome was found by Q- and G-banding after lymphocyte culture. The male patient showed no clinical abnormalities besides abnormal spermatogenesis /azoospermia/. The relatives in the ascending and collateral line of the index patient were included in the cytogenetic investigation. Three of them, all females, were also carriers of the der(15). They showed neither an increased abortion rate nor decreased fertility. Molecular analysis of the AZF gene in the index patient gave normal results. The marker chromosome was identified by FISH as a heterochromatic deviate of chromosome 15. The role of heterochromatic marker chromosomes in male sterility will be discussed.

Supported by the PPP-program of DAAD/M B and the Hungarian Academy of Science.

P0331. Digital Photography in Cytogenetics; An Innovative and Inexpensive Approach

K. Singh, *C. B. Jha, N. Kumar*
B P Koirala Institute of Health Sciences; Dharan, Nepal
kulpra@hotmail.com

Introduction; Karyotyping involves a time consuming laborious process of cell culture, chromosomes preparation, banding and staining. Documentation requires conventional photography based on 35 mm optical camera and results are known only after development and printing. Commercial imaging karyotyping equipments are available which have simplified the analysis but are beyond the reach of small laboratories. Here we would like to share our experience of digital imaging using video camera. Aims; To demonstrate utility of digital imaging using simple video camera as a means to document karyotype. Methods;GTG banded metaphase chromosomes prepared from peripheral blood of ten subjects were analyzed both by conventional film photography and by images captured via CCD video camera. Multi-frame analog videos of metaphases under simple microscope were recorded and digitized with the help of video capture card using PII computer. Individual frames were saved as BMP format and were enhanced using MGI Photosuite software. Images were printed on photoglossy paper using Canon inkjet printer. Karyotypes were prepared from the photographs of same metaphases. Results; A total of 50 complete metaphases were examined. Average time consumption to printing and print loss due to intermediate processes were 12 hours and 6% respectively using conventional method as against 45 minutes and 2% respectively using video-digital method. The contrast between bands among individual chromosomes was better with conventional method. However, identification of chromosomes poses no problem with either modalities. The cost involve in conventional printing was higher. Conclusion; This innovative video-digital microphotography is less time consuming, inexpensive and reasonably cost-effective and with some improvisation can produce good quality photographs. Further, the technique makes storing and archiving the images easier and can have wider applications in small labs in developing countries.

P0332. Regular type of Down s Syndrome as a major indication for early amniocentesis

I. Tonkovic Djurisevic, *D. Muzinic, D. Begovic*
Division of Genetics and Metabolism, Department of Pediatrics, University Hospital Centre; Zagreb, Croatia
tonkicaa@yahoo.com

Objective; To establish the existence of increasing risk for a chromosome aberration in mothers with previous child with Down syndrome. From 16604 prenatal cytogenetic investigations, performed since 1978 until

1999 in our centre, 831 pregnant women have had a previous fetus with Down syndrome. Routine chromosomal investigations in cultured amniotic fluid cells were performed according to well known standard procedures. Results; 712 women were under the age of 35 and among them 5 (0.70%) had fetus with a chromosome aberration. Four cases (3.36%) of pathological karyotyp were found in a group of 119 patients over the age of 35. Conclusion; The parents with a previous child with Down syndrome have an increased risk for having a second affected child. Advanced maternal age increase the risk.

P0333. Pericentric inversion of chromosome 9 in recurrent reproductive loss

L. R. Martelli[1], *S. R. Pereira-Martins*[2], *S. A. Santos*[1], *L. A. F. Laureano*[3], *A. S. Martinez*[4], *J. G. Franco Jr*[5]
[1]*School of Medicine of Ribeirao Preto, University of Sao Paulo; Ribeirao Preto, Brazil;* [2]*Federal University of Maranhao; Sao Luis, Brazil;* [3]*University Hospital, HCFMRP - USP; Ribeirao Preto, Brazil;* [4]*Faculty of Philosophy, Sciences and Letters,University of Sao Paulo; Ribeirao Preto, Brazil;* [5]*Human Reproduction Center, Sinha Junqueira Maternity; Ribeirao Preto, Brazil*
lrmartel@rge.fmrp.usp.br

Chromosome heteromorphisms involving the pericentromeric region of chromosome 9 have been associated to early recurrent abortions. However, results and opinions about the clinical significance of pericentric inversions remain contradictory. We have performed cytogenetic analysis of 120 unrelated couples who had presented two or more spontaneous abortions due to indeterminate causes. The control group was made up of 384 individuals. Chromosomal studies have included GTG, CBG, NOR and high resolution bandings. Ten couples (8.3%) presented a major chromosome aberration and 33 couples (27.5%) showed a polymorphic chromosome 9. Pericentric inversion involving the 9q region was detected in 9.1% of the couples and in 1.3% of the samples from the control group. This finding was characterized by F.I.S.H. technique using CEP 9 alpha satellite (VYSIS), beta (D9Z5) and satellite III (D9Z1) DNA probes. In all cases, the breakpoints were located in euchromatic regions, confirming the cytogenetic diagnosis. The origin was maternal in 80% of the cases. Frequencies of individuals and of couples carrying inv(9) were compared to the frequencies shown by the general population and to the frequencies reported by 11 authors, revealing a significant difference between observed and expected ratios. Our results suggest a correlation between pericentric inversions of chromosome 9 and recurrent reproductive loss.

P0334. Clinical, Genetics and Environmental Studies in Birth Defects

N. A. Meguid[1], *M. A. Awadalla*[2], *M. E. Zaki*[3], *G. S. Ali*[4], *S. A. Temtamy*[3], *A. M. Dardir*[3]
[1]*National Research center, Head of the Department of Human Genetics; Giza, Egypt;* [2]*Faculty of Medicine, Ain Shams University; Cairo, Egypt;* [3]*National Research Center, Department of Human Genetics; Giza, Egypt;* [4]*Institute of Postgraduate Childhood Studies, Ain Shams University; Cairo, Egypt*
nmeguid@email.com

The present study included 2600 patients referred to our Department of Human Genetics, National Research Center, Giza; through 8 years study period. The main aim of the present study was to classify cases of birth defects whether they are isolated or multiple, to identify the genetic etiology of cases and to evaluate the genetic versus environmental components of birth defects. Patients were subjected to complete clinical and geneic evaluation. Construction of special computer program for data registry and search was performed. Syndrome diagnosis and identification was carried out by the use of special database programs. Our results showed that; genetic syndromes were the most frequent group. Genetic etiologic classification of the studied cases showed that the monogenic group of disorders was the most predominant group (53.1% of the studied cases) and autosomal recessive pattern of inheritance is the most frequent pattern of inheritance (34.2%). The effect of environment was detected in 3.7% of cases emphasizing a mild impact of environmental effect in our present study. Rate of consanguinity among the referred patients was 53.6%, 71% were first cousins. Mental reardation represented 44.9% of all referred cases. Chromosomal breaks were the most predominant structural aberrations (26%) and were increased in the genetic syndromes, indicating the use of FISH to detect any cryptic aberrations. Our study supported the hypothesis that consanguinty is an important risk factor in the causation of birth defects, environmental factors have a definite effect in the present study and computer is an important tool in the diagnosis of genetic diseases. We concluded that, the high incidence and recurrence risk of single gene defects in the present study indicates that there is a need to develop a comprehensive preventive program. This will form the basis of a community based genetic service, which is an integral part of preventive health care.

P0335. Queensland Clinical Genetics Service Telemedicine Project

I. Meinecke[1], *J. C. MacMillan*[1], *M. Gattas*[1], *M. Loane*[2], *R. Wootton*[2]
[1]*Queensland Clinical Genetics Service; Brisbane, Australia;* [2]*Centre for Online Health; Brisbane, Australia*
ingrid_meinecke@health.qld.gov.au

Queensland covers over 1.7 million square kilometres of the continent of Australia. The statewide genetics service, Queensland Clinical Genetics Service (QCGS), is based in Brisbane where 44% of the state s population live. Outreach clinics are held in 9 other cities, however for many rural patients attending these clinics often involves travelling large distances. Queensland Health has over 160 telemedicine sites to facilitate access and support to rural patients and health practitioners. Mental health, paediatrics, and intensive care are the main users of this technology, however the potential exists for expansion into other areas of medicine, including genetics (telegenetics). In order to evaluate the effectiveness, efficiency and acceptability of telegenetics, we have conducted a study, comparing clinical telegenetics consultations with face-to-face consultations. Patients suitable for inclusion in the study were invited to participate and sent information, a survey on pre-clinic expectations and a consent form. Those patients consenting to participate were randomly allocated to either a traditional face-to-face clinic or to a telegenetics clinic. Post-consultation the geneticist and genetic counsellor involved completed a survey on their perception on the clinical session. Four weeks after the consultation participants had telephone follow-up by the project officer to complete a survey examining whether the patients needs were met by the consultation. At the time of abstract submission 12 patients had entered the study. Early results suggest telegenetics has been a suitable method for service delivery from a patient and health professional perspective.

P0336. Two siblings of familial idiopathic brain calcification presented with cerebellar ataxia

M. Watanabe
University of Tsukuba; Tsukuba, Ibaraki, Japan
masa-wat@igaku.md.tsukuba.ac.jp

We report a family with brain calcification, predominantly in the carebellar nuclei, and no evident cause such as abnormal calcium or phosphorus metabolism. The proband, a 53-year-old man, showed slowly progressive dysarthria and gait disturbance. Upper limb diadochokinesis was impaired bilaterally. The finger-to-nose test is ataxic bilaterally and worse on the left side. His gait was awkward, wide-based, and short stepped, but was not festinating. Sensation and sphincteric function were normal.Fundic examination failed to reveal pigmentary macular degeneration. There were no bone deformities or subcutaneous calcification suggestive of Albright s osteodystrophy. Results of blood chemical tests were not remarkable; creatinine, 0.7 mg/dl; urea-nitrogen, 14.2 mg/dl; calcium, 9.9 mg/dl; inorganic phosphorus, 3.2 mg/dl; magnesium, 2.7 mg/dl; free triiodothyronine, 2.4 pg/ml; free thyroxine, 1.4 ng/dl; thyroid-stimulating hormone, 1.10 mU/ml; intact parathyroid hormone, 11 pg/ml. He had bilateral and symmetric calcification of the cerebellar nuclei, basal ganglia, thalamus, and subcortical white matter on CT. Calcified areas showed central low signal intensity areas surrounded by high-signal intensities on MRI T1- and T2-weighted images. The elder brother was asymptomatic but showed calcification of cerebellar nuclei and basal gangria. Familial idiopathic brain calcification is a rare disorder with less than 20 previously reported families. Mental deterioration, parkinsonis, and cerebellar ataxia appear in adult life and progress gradually.

P0337. Incidence of chromosomal abnormalities in patients with mental and developmental delays in a referred Iranian population .

F. Behjati[1], *M. T. Akbari*[2], *Z. O. Ashtiani*[2], *M. Khaleghian*[2]
[1]*University of Welfare and Rehabilitation; Tehran, Islamic Republic of Iran;* [2]*Division of Cytogenetics, Medical diagnostic laboratory of Pars; Tehran, Islamic Republic of Iran*
maryamneishabury@yahoo.co.uk

We report the cytogenetic findings in 73 patients with mental and development delays out of a total of 383 cases referred for suspected chromosomal abnormalities . Routine and High resolution technique for peripheral blood culturing was carried out. All cases were evaluated by GTG banding (ISCN-1995) . Other banding techniques including C-NOR and Q - were also performed . A total of ten metaphase spreads for each patient was invesigated . In mosaic cases, 30-50 cells were examined . the overall

abnormality rate for all 383 cases was 16.5% . The mentally and developmentally delayed referred cases comprised three major groups ; Down syndrome, failure to thrive and non -Down Syndrome mental retardation. The chromosomal abnormality rate among them was 87%, 7% and 70% respectively. The total rate of abnormality amongst the mentally and developmentally delayed cases was 8%, which is comparable to Similar studies.

P0338. X-linked immunodeficiency, inflammatory bowel disease and hypohydrotic ectodermal dysplasia in a large family

G. Knudsen[1], *M. Kristiansen*[2], *K. Storhaug*[3], *T. Abrahamsen*[4], *J. Steen-Johnsen*[5], *A. Smahi*[6], *G. Courtois*[6], *K. flrstavik*[1]
[1]*Department of Medical Genetics, National Hospital; Oslo, Norway;* [2]*Institute of Medical Genetics, University of Oslo; Oslo, Norway;* [3]*Resource Centre for Oral Health in Rare Medical Conditions, University of Oslo; Oslo, Norway;* [4]*Department of Pediatrics, National Hospital; Oslo, Norway;* [5]*Department of Pediatrics, Telemark County Hospital; Porsgrunn, Norway;* [6]*Service de G n tique, H pital Necker-Enfants-Malades; Paris, France*
gun.peggy.stromstad.knudsen@rikshospitalet.no

A possible new immunodeficiency syndrome was reported in 1978. The male patient had a transient block in the normal maturation of B cells and intractable diarrhoea, and died at age 8 months. Three diseased brothers probably had the same disorder. Their two sisters have since had two and one affected sons respectively. All seven affected boys were dystrophic at birth. They were stillborn or died in early infancy, except one boy who died at age 5 years. This boy developed signs of hypohidrotic ectodermal dysplasia, with thin hair, small cone-shaped teeth and oligodontia, but with no periorbital wrinkling. At age 8 months he started having serious pneumococcal infections which were difficult to treat. He also developed an inflammatory bowel disease. All three obligate female carriers had small teeth and reported lack of sweating. One carrier had a highly skewed X-inactivation, whereas two carriers had a random X inactivation. Haplotype analysis showed that both affected and unaffected family members shared markers flanking the hypohidrotic ectodermal dysplasia (ED1) gene. Affected members shared markers in the regions Xp21.1-p11.4 and Xq27-qtel. A novel X-linked disorder of immunodeficiency and hypohidrotic ectodermal dysplasia has been reported. This disorder is allelic to incontinentia pigmenti, and the patients had mutations in exon 10 of the IKK-gamma (NEMO) gene. The findings in our family bear some similarity to this novel immunodeficiency. However, no mutations in exon 10 were found. Our family may represent a mutation in other parts of the NEMO gene or yet another X-linked immunodeficiency syndrome.

P0339. The genetic basis of inter-individual differences in cytokine release amongst patients undergoing cardiac surgery with cardiopulmonary bypass -preliminary results -

M. Seyfarth[1], *A. Roth-Isigkeit*[2], *L. Hasselbach*[3], *P. Schmucker*[2]
[1]*Department of Immunology and Transfusion Medicine; Luebeck, Germany;* [2]*Department of Anaesthesia; Luebeck, Germany;* [3]*Department of Clinical Chemistry, Medical University of Luebeck; Ratzeburger Allee 160, D-23538 Luebeck, Germany*
liu.hasselbach@medinf.mu-luebeck.de

Cardiac surgery with cardiopulmonary bypass (CPB) leads to a systemic inflammatory response with cytokine secretion. Cytokine release measurements during cardiac surgery with CPB showed a high inter-individual variability in cytokine secretion. We studied patients who underwent a cardiac surgery with CPB. Previous results from our laboratory have showed that the inter-individual differences in IL-6 release were accompanied by differences in the release of other cytokines such as TNF-A, IL-1§ and sIL-2R. This phenomenon might in part be due to variations in individual genetic true nature. Evidence for a relationship between genetic variations at the HLA and/or cytokine gene loci and the expression of cytokines have been reported elsewhere. The regulation of the expression of some cytokines is in part genetically determined. To examine whether genetic background might play a role in affecting individual cytokine serum levels and influencing the degree of the inflammatory response during surgical trauma (e.g. cardiac surgery with CPB), we investigated the distribution of polymorphic elements within the promoter regions of the TNF-A and IL-6 genes in our samples and determined their genotype concerning the BAT2 gene involved in the HLA region. Our preliminary data suggest that the A allele at -308 of the TNF-A gene promoter region might be responsible for higher release of serum TNF-A in patients undergoing cardiac surgery with CPB. Regulatory polymorphisms in or near the TNF locus, i.e. allele set 140/150 of the BAT2 microsatellite marker on the HLA region combined with the G allele at -308 of the TNF-A gene, might represent one construction providing a genetic background for a less sensitive response to various stimuli. Further studies involving a larger sample size should be done in order to confirm our preliminary results.

P0340. Difficulties in the early diagnosis of sporadic Carney Complex.

L. G. Flores-Pe a, *V. Del Castillo, A. Gonz lez del Angel, R. Rodr guez, L. De la Torre, M. Esmer*
Instituto Nacional de Pediatr a; Mexico, D.F., Mexico
lauraflorespena@hotmail.com

Carney complex is an autosomal dominant syndrome, charecterized by lentigines, myxomatous skin tumors; and heart, breast or endocrine tumors (primary nodular adrenocortical disease, pituitary adenoma, Sertoli cell tumors). Myxomas are the main finding suggesting Carney complex, however they can be mistaken as schwannomas and other bening or malignant tumors. Also the presence of lentigines and neurofibromas can be confused with lesions of neurofibromatosis or LEOPARD. We report on a girl with a lentiginous syndrome, first evaluated at 6years/old due to the presence of pigmented spots on buttocks, chin and legs. At age 9 years, she developed a mediastinal tumor which was surgically removed and diagnosed as lymphangio-hemangio-lipoma. On the clinical examination at the genetic consultation she showed low nasal bridge, hipertelorism, hyperpigmented lip and sclera spots, high arched palate,low set ears. Her cardiologic examination disclosed cardiomiopathy. At this moment she was diagnosed as having LEOPARD syndrome, but later the cardiomiopathy was discarded. Four years later she had a neurilemoma on left hand and developed hypothyroidism, obesity, an ovarian teratoma, a pituitary microadenoma and tall stature. Theses features were all consistent with the diagnosis of Carney complex. This case ilustrates the difficulties on the early diagnosis of the disease, mainly in casas without other affected family members. In the present case the definitive diagnosis was reached only after the apparition of important features such as the mediastinal lymphangio-hemangio-lipoma and the shwannoma of the hand. The histologic confirmation of the nature of these lesions proved to be an essential diagnostic tool. The genetic loci of carney complex were recently identified on chromosomes 2p16 and 17q2, therefore the molecular diagnosis can be useful in cases like ours.

P0341. Preliminary Findings In The Diagnostic Evaluation Of A Sample Of 103 Individuals With Pervasive Development Disorders

C. E. Steiner, *M. M. Guerreiro, A. P. Marques-de-Faria*
Unicamp; Campinas, Brazil
dgmfcm@unicamp.br

Pervasive development disorders (PDD) are a heterogeneous group of neurobehavioral disorders of infancy. In order to identify etiologic factors in a sample of individuals with PDD, a total of 103 subjects (84 males and 19 females) referred for autistic behavior were submitted to a protocol including clinical evaluation, screening for inborn errors of metabolism, karyotype in folic acid deficient medium, and molecular test for the FRAXA mutation. Until now, the following conditions were detected; Down syndrome (n = 3), untreated phenylketonuria (n = 2), Rett syndrome (n = 2), tuberous sclerosis (n = 1), fragile X syndrome (n = 1), 5 other dysmorphic conditions, 2 non-genetic etiologies, and 2 individuals with chromosomal polymorphisms. Besides those, 4 individuals presented no PDD and 3 individuals had infantile psychosis. These data suggest that, in at least 15% of this sample, a main etiologic factor could be identified. Although the diagnosis of a specific genetic condition do not modify the treatment of autistic individuals in most cases, it is of significant importance to the genetic counseling of their relatives. For this reason, we reinforce the importance of careful dysmorphologic examination and complementary tests in any person presenting with PDD, besides neurologic and psychiatric evaluation.

P0342. Congenital Anomalies on 279 necropsies of children; A prospective study at Ribeir o Preto, Brazil.

J. M. Pina-Neto[1], *A. Piram*[2], *L. C. Peres*[3]
[1]*S o Paulo University; Ribeir o Preto, S o Paulo, Brazil;* [2]*S o Paulo Universisty, Faculty of Medicine; Ribeir o Preto, S o Paulo, Brazil;* [3]*S o Paulo University, Faculty of Medicine; Ribeir o Preto, S o Paulo, Brazil*
jmdpneto@fmrp.usp.br

We analyzed prospectively 1127 autopsy of children aged 0 to 14 years performed at Universitary Hospital, Faculty of Medicine of Ribeir o Preto, from January 1995 to December 1998.Congenital anomalies were present in 279 cases (24.8%); most cases were children aged 0 to 28 days(40.9%)and 1 to 12 months(36.2%). Sample was separate in two groups; evaluated for clinical geneticist in life or autopsy (157 cases) and not evaluated (122 cases).In the group of evaluated cases the diagnosis can be established in 126 (80.3%) where 21(13.4%) were of single anomalies, and 105 (66.8%) of multiple anomalies. In the group of not evaluated cases 62 (50.8%) had the diagnosis defined, where 28 (23.0%) were single anomalies, and 34 (27.9%) were multiple ones.Considering the

cases with established clinical diagnosis (188 cases), 40 (21.3%) were monogenic, 33 (17.6%) were chromosomal, 38 (20.2%) multifactorial, 6 (3.2%) environmental, 54 (28.7%) were of unknown etiology, and in 17 (9%) the etiology cannot be determined. The recurrence risk was considered low in 121 cases (64.4%), moderate in 21 (11.2%), high in 28 (14.9%), dependent of handling in 1 (0.5%), unknown in 7 (3.7%) and not defined in 10 (5.3%). The cases without diagnosis (91 cases) had the etiology and the risk of recurrence considered undetemined. These data will be discussed and compared with similar studies. Financial Support; FAPESP.

P0343. Valproic Acid Embryopathy; Report of Two Siblings with Further Expansion of the Phenotypic Abnormalities and a Review of the Literature.

C. Kozma
The Child Development Center. Georgetown University Medical Center.; Washington, D.C., United States
kozmac@georgetown.edu

Fetal Valproate syndrome (FVS) results from prenatal exposure to Valproic acid (VPA). It is characterized by a distinctive facial appearance, a cluster of minor and major anomalies, and central nervous system dysfunction. In this report, two siblings who were exposed to monotherapy with VPA are described with documentation of long-term follow up. Both children had craniofacial findings, multiple systemic and orthopedic abnormalities, an overgrowth pattern, and developmental deficits. In an effort to further delineate the phenotypic features of FVS, the literature from 1978 to 2000 is reviewed. A total of 70 cases that were solely exposed to VPA with adequate phenotypic description were identified. Cases that did not have adequate phenotypic description were excluded as well as cases that were exposed to VPA and other anticonvulsant treatment The clinical manifestations of FVS encompass a wide spectrum of abnormalities including consistent facial phenotype, multiple systemic and orthopedic involvement, central nervous system dysfunction, and altered physical growth. The facial appearance is characterized by a small broad nose, small ears, flat philtrum, a long upper lip with shallow philtrum, and micro/retrognathia. In this review, 62% of the patients had musculoskeletal abnormalities, 30% had minor skin defects, 26% had cardiovascular abnormalities, 22% had genital abnormalities, and 16% had pulmonary abnormalities. Less frequently encountered abnormalities included brain, eye, kidney, and hearing defects. Neural tube defects were seen in 3% of the sample. Twelve percent of affected children died in infancy and 30% of surviving patients had developmental deficits/mental retardation. While 15% of patients had growth retardation, an overgrowth pattern was seen in 9%. The data from this comprehensive review especially the developmental outcome should be added to the teratogenic risk, which arises in association with the use of VPA during pregnancy.

P0344. The impact of prenatal diagnosis and folic acid (FA) supplementation on prevention of Neural Tube Defects in British Columbia

M. I. Van Allen[1], *E. Boyle*[2], *D. McFadden*[3], *B. Irwin*[4], *P. MacLeod*[5], *G. Chambers*[6]
[1]*Dept. Medical Genetics, University of British Columbia; Vancouver, BC Canada;* [2]*U.B.C. Medical School; Vancouver, Canada;* [3]*Dept. Pathology, B.C. Children s Hospital; Vancouver, Canada;* [4]*Spina bifida clinic, B.C. Children s Hospital; Vancouver, Canada;* [5]*Victoria General Hospital; Victoria, Canada;* [6]*Family Practice Dept., U.B.C.; Vancouver, Canada*
mvallen@cw.bc.ca

The apparent declining rate of NTD affected births is being attributed to prenatal diagnosis and to folic acid (FA) use. The simultaneous initiation of these preventative programs makes it difficult to assess the impact of either intervention. We present results of a descriptive, retrospective study on the apparent incidence (stillbirths (SB) and livebirths (LB))of registerable births (i.e. >20 weeks gestation) compared to the actual incidence (medical abortions (TAB), SB, and LB) of NTD affected pregnancies. We identified 144 NTD affected fetuses/SB/LB born between 1997 and 1999. During this time period the apparent incidence of NTDs was 52/133,959 or 0.39/1000. The actual incidence was 144/134,041 or 1.07/1000. FA supplementation was used peri-conceptionally in 38.7% (36/93) pregnancies. Affected pregnancies were prenatally diagnosed in 67% (96/144), with 89% (85/96) of those diagnosed at <24 weeks, and 84% (81/96) resulting in TAB. Severe associated anomalies were present in 31% (45/144), with 9.5% (13/144) having chromosomal abnormalities and 15% (21/144) having recognisable syndromes and MCA disorders. Family history of NTDs was reported in 12% (17/144). In conclusion; TABs following routine prenatal diagnosis are a significant contributor to the declining incidence of NTD affected births. FA use is rising and is contributing to the decline in the actual NTD incidence from 1/795 in 1958-84 to 1/930 in 1997-99, representing a 15% reduction. There may be a rising proportion of NTDs due to underlying chromosomal, syndromic and genetic disorders unresponsive to FA supplementation.

P0345. Cytogenetic and molecular analysis in 300 infertile males of Austrian centres of andrology

O. Rittinger
Clinical Genetics, Children s Hospitalh; Salzburg, Austria
o.rittinger@lks.at

Background; Male infertility is caused by many different exogenous and endogenous factors. Today screening for chromosomal aberrations and deletions of Y chromosomal sequences belongs to the standard procedures of andrology centers. We report on the largest cohort of infertile males in Austria comparing clinical data with cytogenetic and molecular findings. Methods; Clinical examination, seminal fluid and hormonal analyses were carried out on 300 infertile males. Cytogenetic investigation was performed according standard methods (GTG-banding). For fluorescence in situ hybridization (FISH) we used Y alpha satellite-(DYZ3), Y-classical satellite-(DYZ1) and Quint Essential Y probes. Molecular analysis; we are routinely screening for the presence of a total of 12Y-chromosomal regions (centromer and AZF a-c) by amplification using 3 multiplex PCR mixes and additional Yq and/or Yp single PCR if necessary. Results; Klinefelter Syndrome was the most frequent gonosomal anomaly (19 patients) followed by the mosaic karyotype 45,X/46,XY (15 patients). Structural aberrations included 6 translocations (one involving the Y chromosome), 13 small deletions and 9 larger deletions (AZFb and AZFc.). The most frequent small deletion concerned sY153 (8 patients), 4 larger deletions started with sY153. Discussion; Klinefelter Syndrome, 45,X/46,XY dysgenesis and XX maleness are well known causes for infertility. Larger Y chromosome deletions concerning AZFa, AZFb and AZFc cause infertility with decreasing probability. In small deletions the analysis of the paternal Y chromosome may help to establish causal relationsship. Frequent polymorphisms may be a reason for Y-chromosome fragility as observed in our sY153-deleted patients.

P0346. Characterization of the phenotypic and developmental aspects of an infant with 5p duplication

N. Scribanu[1], *S. A. Schonberg*[2], *J. M. Meck*[1]
[1]*Georgetown University; Washington, DC United States;* [2]*American Medical Laboratories; Chantilly, VA United States*
scribann@georgetown.edu

We report the cytogenetic and developmental findings in a 6 month old African American female infant who was evaluated for a stridorous cry and dysmorphic features at birth. The proposita was the product of a third pregnancy of an 18 year old woman and was delivered at 39 weeks gestation with a birth weight of 2.29 kg. She presented with a stridorous hoarse cry, poor suck, tracheomalacia, malrotation of the gut, generalized hypotonia, congenital heart disease and enlarged clitoris. She was at the 5th percentile for height and weight. Her head, at the 50th percentile, was disproportionately large. Her anterior fontanelle was large, full, and extended to the metopic suture which was gaping. The proposita had a prominent forehead and upward and outward slanting of the palpebral fissures. The nasal bridge was flattened and had a prominent crease. Her ears were slightly low-set, large and prominent. Her fingers and toes were very elongated (arachnodactylic). Severe feeding difficulties required gavage feeding. G-band chromosome studies showed an inverted duplication of most of the short arm of chromosome 5 (46,XX,inv dup (5)(p15.32p12). Maternal chromosomes were normal; the father was not available for study. Fluorescence in situ hybridization (FISH) studies were performed to confirm that the extra material was derived from chromosome 5 and also to determine if the cri du chat critical region was deleted as a result of this structural abnormality of 5p. Whole chromosome paint for chromosome 5 showed hybridization to the entire abnormal #5 confirming that the extra material was derived from 5. The EGR1 probe to the cri du chat critical region at 5p15.2 showed one signal on the normal #5 as well as a double hybridization signal in the middle of the short arm of the abnormal #5 chromosome, indicating trisomy for this locus. The FISH result was consistent with the G-band findings of an inverted duplication of 5p, resulting in trisomy for most of the short arm of chromosome 5. The cri du chat region was included in the duplicated portion and, therefore, was trisomic, not monosomic as in individuals with cri du chat syndrome. Duplication of 5p is associated with a specific phenotype which is dependent upon the extent of the trisomic region. Based on published reports, we concluded that the proposita was on the severe end of the phenotypic spectrum for trisomy 5p syndrome which is consistent with the cytogenetic finding that virtually all of her 5p was trisomic. At followup, it was evident that this infant had global developmental delays. Additional information on the natural history of this syn-

drome will be presented.

P0347. Deletion syndromes and ectrodactyly; phenotype shows an hierarchy of the genes

I. Lurie
University of Maryland at Baltimore; Baltimore, MD United States
lugo15@excite.com

Ectrodactyly and its equivalents are relatively common in 4 deletion syndromes; del(2)(q31), del(4)(q33qter), del(6)(q21) and del (7)(q21.3). Manifestations of the defect may be different even in sibs with exactly the same deletion. Nevertheless, analysis of phenotypic picture may shed some light on the hierarchy of the genes, involved in the origin of ectrodactyly. In these patients defect of each extremity may be scored from 5 (maximal defect with absence of the whole limb) to 1 (equivalent forms of ectrodactyly). Each person will be characterized by the sum of scores for defects of both upper and (after colon) both lower limbs. In such a case it is possible to compare 1) severity of defects of the upper and lower limbs for each type of deletion, 2) general severity of limb defects in different deletions. The results of the analysis of the reported cases (after summation of all individual s scores) are shown in the Table.

Deletion	Number of cases	Absolute score	Mean score
2q31	11	39;35	6.7
4q33qter	7	14; 8	3.1
6q21	9	28;6	3.9
7q21.3	13	12;54	5.1

It can be seen that in persons with deletions of 2q31 upper and lower limbs are affected with the same severity. Deletion 6q21 affects mostly upper limbs, deletion of 7q21.3 affects mostly lower limbs. It can be presumed, that a gene on 7q21.3 acts on later stages of embryogenesis than a gene on 6q21. Generally, the most severe defects are found in del(2)(q31), the smallest defects in del(6)(q21) and del (4)(q33qter). Therefore, this formal analysis can provide some information concerning hierarchy of the genes, involved in development of ectrodactyly.

P0348. Where to look for the genes of esophageal atresia?

O. LURYE[1], *I. Lurie*[2]
[1]*Belorussian Institute of Herediary Disorders; Minsk, Belarus;* [2]*University of Maryland at Baltimore; Baltimore, MD United States*
olury001@umaryland.edu

Phenotypic manifestations of structural autosomal imbalance (SAI) give a clue to the location of genes causing congenital malformations. Data on ectrodactyly, holoprosencephaly (HPE), and other defects confirmed the fruitfulness of this approach. A similar analysis was performed for esophageal atresia (EA), genes for which have not yet been mapped (except one gene on 2p23p24 [Celli et al., 2000]). EA is a rare manifestation of chromosomal defects. 27 cases of SAI with EA were found in the literatire. Deletions of 3 segments; del(17)(q22q23), del(14)(q32.3qter) and del(9)(p22pter) were associated with EA more than once. Most likely, segments 17q22q23 and 14q32.3qter harbor genes responsible for EA. The role of 9p22pter is questionable; in both cases with EA monosomy 9p was associated with partial trisomies for another autosome but EA was not found in any case of pure del(9p). Duplications of 3 other segments [dup(3)(p25p26), dup(5)(q34qter) and dup(18)(q12qter)] were associated with EA more than once. Minimal duplication 18q with EA was 18q12-qter, that actually does not help to localize the gene, responsible for this defect. Two of three persons with EA and dup(3)(p25p26) also had HPE. EA was not found in 16 other persons with dup(3p) and HPE and was found in one of 76 persons with dup 3p without HPE. The most likely explanation; in trisomy 3p HPE and EA may be different manifestations of the same triplicated gene. 18 out 27 persons with EA and SAI also had congenital heart defects, 6 out 27 had anal atresia, but not a single had diaphragmatic hernia.

P0349. Fanconi anemia in Iran; a profile

S. H. Sayar[1], *S. Tootian*[1], *F. Farzanfar*[1], *A. K. Hajianpour*[2]
[1]*Iranian Blood Transfusion Organization; Tehran, Islamic Republic of Iran;* [2]*Alfigen, the genetics institute; Pasadena, CA United States*
sayar111@yahoo.com

During a period of 3 years, from November 1997 to November 2000, a total of 116 patients under the age of 25 with the diagnosis of aplastic anemia have been referred to our center for chromosome breakage study to rule out Fanconi anemia. Using Mitomycin-C to induce chromosome breakage, we found 57 positive cases; this is indicative of a high frequency of this detrimental disease in Iran and we would directly attribute it to the high rate of consanguineous marriages in our country that is estimated to be as high as 30%. All patients but one were products of consanguineous marriages; 5 had distant relative parents and all other parents were first cousins. On physical examination, 2 patients (3.5%) were entirely normal, 15 (26.3%) had only short stature and/or skin findings and other 40 patients (70.2%) showed at least one major abnormality. The breakage study in 3 patients (5.3%) revealed 2 cell populations, one with normal and the other with increased breakage rate.

P0350. Case report; a newborn child with ectrodactyly and partial trisomy 16q.

P. Ilisson[1], *K. ˝unap*[1], *T. Ilus*[1], *O. Bartsch*[2]
[1]*Medical Genetics Center, Tartu University Clinics; Tartu, Estonia;* [2]*Department of Clinical Genetics, Medical Faculty, Technical University; Dresden, Germany*
Piret.Ilisson@kliinikum.ee

Cases of ectrodactyly are usually sporadic, but cases of autosomal inheritance or demonstrating chromosomal rearrangements have also been described. So far 4 different genes or regions for ectrodactyly have been mapped on 7q21, Xq26, 10q24, and 3q27. In addition deletions of 2q24-q31 and 6q16-q23 have been reported. We report a newborn girl demonstrating ectrodactyly of hands and feet (aplasia or hypoplasia of II-III fingers and toes), heart defect - hypoplasia of left ventricle, interrupted aorta; genital anomalies - hypoplasia of labias; and facial dysmorphic features - down-slanting palpebral fissures, blepharophimosis, upturned nose, long philtrum, broad nasal tip, retrognathia. Standard chromosome studies of blood lymphocytes showed a pathological karyotype - 46,XX,add(21)(p11). Parental karyotypes were normal. Fluorescence in situ hybridization (FISH) revealed partial trisomy for 16q - karyotype after FISH 46,XX,add(21).ish der(21)t(16;21)(q12;p11). There are some 20 cases about the trisomy 16q reported so far, but only one case with similar duplicated region (trisomy 16q12.1-qter). To our knowledge, the association of ectrodactyly and partial trisomy 16q has not been reported previously, but some cases demonstrated clinodactyly and flexion contractures. Therefore, our case suggests that possibly another gene(s) causing ectrodactyly in a subset of patients maps on chromosome 16q12-qter. In contrast to the other genes and regions for ectrodactyly identified so far, this possible gene(s) or region on 16q may be predicted to cause ectrodactyly by overexpression (trisomy).

P0351. Cytogenetic and molecular analysis in 300 infertile males of Austrian centres of andrology

E. Ploechl[1], *G. Kronberger*[1], *G. Sander*[1], *M. Dunzinger*[2], *A. Jungwirth*[3], *E. Plas*[4], *I. Vlasak*[1], *O. Rittinger*[1]
[1]*Clinical Genetics; Salzburg, Austria;* [2]*Urologische Abteilung, KH Barmherzige Schwestern; Linz, Austria;* [3]*Urologie; Salzburg, Austria;* [4]*Urologie, KH Wien-Lainz; Wien, Austria*
e.ploechl@lks.at

Background; Male infertility is caused by many different exogenous and endogenous factors. Today screening for chromosomal aberrations and deletions of Y chromosomal sequences belongs to the standard procedures of andrology centers. We report on the largest cohort of infertile males in Austria comparing clinical data with cytogenetic and molecular findings. Methods; Clinical examination, seminal fluid and hormonal analyses were carried out on 300 infertile males. Cytogenetic investigation was performed according standard methods (GTG-banding). For fluorescence in situ hybridization (FISH) we used Y alpha satellite-(DYZ3), Y-classical satellite-(DYZ1) and Quint Essential Y probes. Molecular analysis; we are routinely screening for the presence of a total of 12Y-chromosomal regions (centromer and AZF a-c) by amplification using 3 multiplex PCR mixes and additional Yq and/or Yp single PCR if necessary. Results; Klinefelter Syndrome was the most frequent gonosomal anomaly (19 patients) followed by the mosaic karyotype 45,X/46,XY (15 patients). Structural aberrations included 6 translocations (one involving the Y chromosome), 13 small deletions and 9 larger deletions (AZFb and AZFc.). The most frequent small deletion concerned sY153 (8 patients), 4 larger deletions started with sY153. Discussion; Klinefelter Syndrome, 45,X/46,XY dysgenesis and XX maleness are well known causes for infertility. Larger Y chromosome deletions concerning AZFa, AZFb and AZFc cause infertility with decreasing probability. In small deletions the analysis of the paternal Y chromosome may help to establish causal relationsship. Frequent polymorphisms may be a reason for Y-chromosome fragility as observed in our sY153-deleted patients.

P0352. Bartsocas Papas syndrome in four children of consanguineous parents

K. Boduroglu, *E. Tuncbilek*
Hacettepe University; Ankara, Turkey
kbodur@gen.hun.edu.tr

Bartsocas-Papas syndrome (BPS) is the autosomal recessive form of popliteal pterygium syndrome which is first described by Bartsocas and Papas in 1972, in four children of third-cousin parents. So far, 18 patients from 9 families have been reported, vast majority originating from the Mediterranean region. Here, we report on a Turkish family with four affected children. The proposita, an 8-month-old female, was the eighth child of second-cousin parents. Physical examination showed, oral cleft, filiform and cutaneous bands between the jaws, ankyloblefaron, corneal ulceration, syndactyly of fingers and toes, popliteal pterygium and genital anomalies. Radiological studies revealed synostosis of phalanges of toes and fingers, and mild pulmonary stenosis. Family history showed that three previous siblings were born with similar clinical features. Although the preceeding sibs had died in early days of life, the index case survived until one year of age.

P0353. Clinical And Molecular Studies Of A Family With Severe Brachydactyly Type B (BDB)

A. Fortuna[1], *M. Pinto*[1], *M. Oldridge*[2], *A. O. M. Wilkie*[2]
[1]*Instituto Genetica Medica; Porto, Portugal;* [2]*Institute of Molecular Medicine, John Radcliffe Hospital; Oxford, United Kingdom*
ana.fortuna@igm.min-saude.pt

Brachydactyly type B, an autosomal dominant disorder, is the most severe of the brachydactylies and is characterized by terminal reductions of the fingers and toes. We report the results of a clinical and radiological study of a father and son affected with BDB. These two individuals demonstrated a severe phenotype — bifid thumbs with split or rudimentary nails, several phalanges are entirely missing (absence of medial and distal phalanges in digits 2 and 5), the 3rd L metacarpal is short with hypoplasia and synostosis of medial and distal phalanges with camptodactyly and partial cutaneous syndactyly of digits 2-4 in the hands. The feet showed a broad 1st metatarsus, absent distal phalanges of digits 2-4, an additional transverse phalanx forming an incomplete osseus bridge between digits 2-3 and complete cutaneous syndactyly of digits 2-4 in the feet. We designed primers suitable for cDNA amplification of the receptor tyrosine kinase gene ROR2 and analysed the coding region by SSCP of restriction-digested RT-PCR products. Fragments showing SSCP changes were directly sequenced in the affected individuals, which enabled us to find a heterozygous 1 bp delection (2249delG), which leads to a frameshift at Gly750 with an arginine/ proline-rich sequence of 23 novel amino acids before the first stop codon. Allele-specific oligonucleotide (ASO) hybridization was used to confirm correct identification of the mutation, and showed that it arised de novo in the father as it was not present in either of his unaffected parents. This report is the most severe hand phenotype associated with ROR2 mutation to date.

P0354. Alport syndrome and mental retardation; clinical and genetic dissection of the contiguous gene deletion syndrome in Xq22.3 (ATS-MR).

A. Renieri[1], *I. Meloni*[1], *F. Vitelli*[1], *B. R. Lowry*[2], *O. Zuffardi*[3], *M. Rocchi*[4], *B. Pober*[5]
[1]*Medical Genetics; Siena, Italy;* [2]*Dept Medical Genetics Albert Children s Hospital; Calgary, Canada;* [3]*Biol genetics, Genetics Med; Pavia, Italy;* [4]*Istituto di Genetica; Bari, Italy;* [5]*Dept Genetics , Yale Univ Sch Medicine; New Haven, CT United States*
renieri@unisi.it

X linked Alport syndrome (ATS) is a semi-dominant nephropathy due to point mutation or, less frequently, to intragenic deletion of the COL4A5 gene located in Xq22.3. We describe two families with ATS-MR contiguous gene deletion syndrome in Xq22.3 characterized by Alport syndrome and mental retardation. The first family has a complex phenotype previously reported as AMME (Alport syndrome, midface hypoplasia, mental retardation and elliptocytosis) and a deletion extending about 2 Mb. The second family was identified in the literature using nephropathy and mental retardation as a query. This second family has a smaller deletion of about 1 Mb. A detailed characterization of both deletions and a comparison with a third large deletion causing only ATS identified the critical region for mental retardation. Moreover detailed analysis of gene content has suggested putative candidate genes for mental retardation as well as for additional phenotypic features. The three carrier females belonging to the two families showed ATS features only, i.e. microhematuria, suggesting that additional phenotypic features in affected males are X-linked recessive. However, X-inactivation in the carrier females is completely skewed, possibly due to a selective mechanism. This peculiar finding opens the possibility that the mental retardation, cardiopathy or elliptocytosis, are caused by dominant mutations in one or more genes found in the ATS-MR critical region. In conclusion, we have demonstrated the existence of a new contiguous gene syndrome in Xq22.3, which we propose to call ATS-MR which adds to the previously known ATS-DL (Alport syndrome and diffuse leiomyomatosis). Both contiguous gene deletion syndromes involve COL4A5, but while ATS-DL extends centromerically, ATS-MR extends telomerically with respect to the collagen gene. Due to relative simplicity of identification of the second family, just by screening the literature, we suggest that this syndrome may be less rare than thought. Since ATS in children is confined to microhematuria, this clinical sign may be missed or hidden by the more severe phenotype such as mental retardation. We suggest that a relative simple analysis such as urinalysis be used to screen males with MR of unknown etiology.

P0355. Dominant inheritance of cleft palate, microstomia and micrognathia

F. McKenzie[1], *A. Turner*[1], *S. Withers*[2], *P. Dalzell*[3], *S. Bhattacharjee*[3], *M. McGlynn*[1], *E. P. E. Kirk*[1]
[1]*Sydney Children s Hospital; Sydney, Australia;* [2]*Logan Hospital; Brisbane, Australia;* [3]*Prince of Wales Hospital; Sydney, Australia*
campbrook@msn.com.au

Cleft palate without cleft lip is a causally heterogeneous condition. There are several reports in the literature of autosomal dominant inheritance of cleft palate either in isolation or associated with other features. The combination of cleft palate, microstomia and micrognathia has been reported previously by Bettex et al (1998) in association with oligodontia and normal intelligence. All the affected members of Bettex family who had karyotypes done were demonstrated to have a fragile site at chromosome 16q22. We report a family in which four members in two generations have microstomia, micrognathia, a partial or complete cleft of the hard palate and normal intelligence. All have been shown to have the fragile site at chromosome 16q22, suggesting the possible presence of a closely linked gene in this region that is important in the development of the palate and lower face.

P0356. Breakpoint analysis in a SOTOS Patient with a Constitutional Translocation t(3;6).

K. Kok[1], *T. Draaijers*[1], *H. H. Faber*[1], *A. Y. van der Veen*[1], *J. Bullerdiek*[2], *C. T. R. Schrander-Stumpel*[3], *C. H. C. Buys*[1]
[1]*University of Groningen; Groningen, The Netherlands;* [2]*University of Bremen; Bremen, Germany;* [3]*Maastricht University; Maastricht, The Netherlands*
k.kok@medgen.azg.nl

In 1964 Sotos et al described five children with large body size and early accelerated growth, acromegaloid features, advanced bone age, and a non-progressive neurological disorder with mental retardation. Since then many cases have been reported of what is now known as the SOTOS syndrome or cerebral gigantism. Two case reports have suggested a possible involvement of the region 3p21; one describing a 6-year-old boy with clinical features consistent with SOTOS syndrome and showing a balanced translocation t(3;6)(p21;p21), and one describing a nonsmoker female with Sotos syndrome who -at the age of 22- died of small cell lung cancer, a tumour characterized by deletions in 3p21. A precise determination of the breakpoints of an EBV-transformed lymphoblastoid cell line from the patient carrying the balanced translocation was obtained by fluorescent in situ hybridizations of YACs from the regions 3p21-3p25 and 6p21-p22, respectively. The chromosome 3 breakpoint appeared to be in band p22, and does not coincide with any of the regions of chromosome 3 suggested to be involved in tumour development. We established a PAC contig for each of the breakpoint regions. Both contigs appear to contain expressed sequences. The precise position of these genes relative to the breakpoints, in combination with expression studies in six SOTOS patient-derived cell lines, may provide a first indication of a possible causative relationship with the clinical features of the SOTOS syndrome in general. (K.kok@medgen.azg.nl).

P0357. PCR-detection of the Glutatione S-transferase M1 null genotype in patients with sepsis, septical shock and multiple organ failure.

V. Viktorov, *D. Mekhdiev, V. Timerbulatov*
Bashkir Medical University; Ufa, Russian Federation
tvict@imail.ru

Polymorphism at the glutatione S-transferase M1 (GSTM1) gene locus have attracted much interest because the homozygous GSTM1 deletion (GSTM1 0/0) seems to modify the risk for severed types of diseases. The frequency of GSTM1 null genotype in 89 healthy individuals was 0,51. For search of association between variants of clinical current of a surgical infection and GSTM1 polymorphism the study of frequencies of genotypes at the patients with sepsis (N = 31), septical shock (N = 46) and multiple organ failure (N = 20) was carried out. The frequencies of GSTM1 null genotype in different groups varied from 37,76 % in septical patients to

65,29 % in septical shock patients and 54,23 % in patients with multiple organ failure. The data of this study demonstrate that observed differences in distribution of GSTM1 allele frequencies between patients with multiple organ failure and healthy individuals was not significantly (p>0,05). But the frequency of the null genotype for GSTM1 was higher in septical shock patients as compare to septical patients and healthy individuals. Our results demonstrate that the possibility of development of septical shock is increased for individuals with GSTM1 null genotype.

P0358. Microcephaly,encephalopathy and intracranial calcification; is there an overlap between congenital intrauterine infection-like syndrome and Aicardi-Goutieres syndrome

G. M. H. Abdel-Salam[1], *A. Halasz*[2], *A. E. Czeizel*[3]
[1]*Department of Human Genetics, National Research Center; Giza, Egypt;* [2]*EEG Department, Heim Pal Children's Hospital; Budapest, Hungary;* [3]*Foundation for Community Control of Hereditary Diseases; Budapest, Hungary*
ghasala@hotmail.com

Microcephaly and intracranial calcification with repeated normal TORCH constitute the recognized diagnostic criteria of congenital intrauterine infection-like syndrome (Mckusick 251290) and Aicardi-Gouti res syndrome (Mckusick 225750). It was reported that periventricular calcification and abnormal EEG and / or seizures characterize the former while the later is characterized by calcification of the basal ganglia, dystonic movements and chronic low grade CSF pleocytosis. The delineation between the two syndromes is not clear because of the expanding phenotype spectrum and the intrafamilial variability of Aicardi-Gouti res syndrome and the lack of diagnostic test that could characterize the congenital intrauterine infection-like syndrome. Here, two Hungarian siblings with microcephaly, encephalopathy, hypotonia, spastic tetraparesis generalized cerebral demyelination and intracranial calcifications are described. The elder boy had basal ganglia calcification while his younger brother had scattered supratentorial calcification and presented with abnormal eye movements and dystonic movements, in addition. The repeated TORCH and CSF examinations showed normal results. The presented cases are more with the diagnosis of Aicardi-Gouti res syndrome, however, congenital intrauterine infection like syndrome could not be excluded because of the absence of the CSF pleocytosis and the clinical variability of the two cases. This report provides evidences that there is an overlap between the two syndromes. Difficulties in making clear demarcation between the two syndromes as well as wider differential diagnosis will be discussed.

P0359. Clinical And Genetic Heterogeneity In Frontometaphyseal Dysplasia; Severe Progressive Scoliosis In Two Families

. Morava[1], *T. Ill s*[2], *J. Weisenbach*[3], *J. K rteszi*[1], *G. Kosztol nyi*[1]
[1]*University of P cs, Department of Medical Genetics and Child Development; P cs, Hungary;* [2]*University of P cs, Department of Orthopedics; P cs, Hungary;* [3]*University of P cs, Department of Pediatrics; P cs, Hungary*
emorava@yahoo.com

Frontometaphyseal dysplasia is a rare genetic syndrome affecting the skeletal system and connective tissue. It is believed to be inherited as an X-linked recessive trait however, descriptions of moderately affected female carriers suggest genetic heterogeneity. Prominent supraorbital ridges, radiologic evidence of cranial hyperostosis and flared metaphyses are characteristic. Scoliosis, a rare associated finding, is usually mild and familial progressive scoliosis has not been reported yet. The skeletal dysplasia and the associated clinical findings show significant intra- and interfamilial variability and the syndrome has been suggested to be an allelic variant of the Melnick Needles osteodysplasty, an X-linked (or autosomal) dominant entity. We present two families with frontometaphyseal dysplasia, in which also females exhibited the facial and skeletal features of the disease in association with progressive scoliosis. Some of the affected members also had hearing loss and urogenital anomalies supporting the existence of the recently suggested entity oto-frontometaphyseal-osteodysplasty syndome .

P0360. Is 3C (Cranio-Cerebello-Cardiac) syndrome caused by monosomy 6pter ? Report of three affected foetuses with a familial unbalanced translocation t(6;16) (p25.2;q23).

A. Toutain[1], *A. Guichet*[1], *A. D l zoide*[2], *F. D Hermies*[3], *C. Paillet*[4], *C. Moraine*[1]
[1]*Genetics Department, University Hospital; Tours, France;* [2]*Foetopathology Unit, Necker-Enfants Malades Hospital; Paris, France;* [3]*Ophthalmology Department, Hotel-Dieu; Paris, France;* [4]*Ultrasonography Department, University Hospital; Tours, France*
toutain@med.univ-tours.fr

3C syndrome is a rare MCA/MR syndrome considered as an autosomal recessive condition. It associates Dandy-Walker malformation, heart defects and characteristic craniofacial features but ocular problems such as coloboma and glaucoma, are frequent. We report three foetuses from the same sibship, two 27 weeks monozygotic twins and a 14 weeks foetus, who had malformations consistent with 3C syndrome. They all had a Dandy-Walker malformation. The twins had characteristic dysmorphic features and unilateral microphthalmia with posterior coloboma. The first twin had a very unusual atrial septal malformation and the third f˜tus had a pulmonary valve anomaly. Chromosome analysis revealed a subtle balanced t(6;16) (p25.2;q23) translocation in the mother and showed that all three foetuses had inherited the derivative 6 chromosome and were monosomic for the 6p25.2-6pter region and trisomic for the 16q23-16qter region. No chromosome abnormalities have been reported so far in 3C syndrome. However patients with terminal 6p deletion share many features with 3C syndrome. They may have brain defects such as hydrocephaly, Dandy-Walker malformation or cerebellar hypoplasia, cardiac malformations, mostly septal defects, and ocular abnormalities, including glaucoma and coloboma, consistent with the effects of FKHL7 haploinsufficiency. Moreover, the BMP6 gene is located in the 6p25 region and two 3C syndrome patients had anomalies of the sternum ossification, a feature which is also present in the BMP6 knock-out mice. These observations suggest that 3C syndrome could be caused in some cases by subtle chromosomal anomalies resulting in monosomy for the 6p terminal region.

P0361. Phenotypic effects of a 47,XXX cell line in Ullrich-Turner syndrome

V. P. Sybert
University of Washington; Seattle, WA United States
flk01@u.washington.edu

Phenotype-karyotype correlations in UTS are problematic; prognostic counseling may be difficult. Analysis of individuals with different X chromosome complements is confounded by overt or covert mosaicism. A proportion (3-4%) of females with Turner syndrome are mosaic for a 47,XXX cell line, either 45,X/47,XXX or 45,X/46,XX/47,XXX. 47,XXX alone is associated with a decrement in intellectual function, normal stature and normal ovarian function. The findings in 17/505 individuals in our UTS study clinic who are mosaic for a 47,XXX cell line, as well as 80 published case reports are compared with those of 227 females in our clinic with 45,X alone. Short stature is less common (48% compared with 73% in the 45,X group). Spontaneous menses (71% vs.10%) and fertility (40% vs. .05%) were much more likely in the mosaic women. A 46,XX cell line did not increase the likelihood of menarche/fertility, but did contribute to a greater likelihood of normal stature compared with those with 45,X/47,XXX. Pregnancy outcome was poor; more than ? resulted in spontaneous loss. The risk for cardiac and renal malformations does not differ between the mosaic and 45,X groups. Congenital edema, found in 38% of 45,X individuals, occurred in none with a 47,XXX cell line, although nuchal webbing is equally prevalent in both. None of our 17 patients has intellectual dysfunction, compared with 9% with 45,X. Although 21% of the women reported in the literature are mentally retarded, selection bias was present; several were detected in screening of institutions or for a family history of MR.

P0362. Pachygyria, hypertrichosis, micromelia, dysmorphic features and hypoparathyroidism; a new syndrome?

L. Basel-Vanagaite[1], *N. Marcus*[1], *G. Klinger*[1], *M. Shohat*[2], *O. Levit*[1], *C. Legum*[1], *L. Sirota*[1]
[1]*Schneider Children s Medical Center of Israel; Petah Tikva, Israel;* [2]*Rabin Medical Center; Petah Tikva, Israel*
baseldov@netvision.net.il

The term pachygyria covers a heterogenous group of brain malformations. We report two siblings with a previously undescribed pattern of malformations, including pachygyria, seizures, hypertrichosis, dysmorphism, micromelia, hypoparathyroidism, and early death. The proband is a one month old girl born to healthy unrelated Jewish parents. Before birth, repetitive fetal mouth movements were noted, indicating intrauterine fetal seizures, and there was marked polyhydramnios. Head circumference at birth was normal. Soon after birth, she developed generalised convulsions and apnea. Physical examination revealed multiple dysmorphic features including double eyebrows, hypertrichosis, short nose, anteverted ears, short neck, micromelia, left simian line, bilateral clasp thumbs, bilateral increased distance between the first and the second toes, edematous labia majora, and 11 pairs of ribs. Neurological examination revealed absence of the primitive reflexes. Metabolic studies were normal. The chromosomes were normal as well as fluorescent in situ hybridization using a commercially available probe for the Miller-Dieker critical region at 17p13.3. MRI studies revealed pachygyria and mild hypoplasia of the cerebellar vermis.

The family s first child had the same clinical picture and was weaned off the respirator at the age of six weeks. At the age of three months, she developed hypoparathyroidism. She died at the age of five months. CT revealed pachygyria with hypoplastic corpus callosum. The inheritance in this family could be consistent with an autosomal recessive pattern. We suggest that this combination of anomalies constitutes a unique syndrome.

P0363. Early diagnosis of Prader-Willi syndrome in infants with methylation-specific PCR

W. H. Tsai[1], *P. L. Kuo*[2], *S. J. Lin*[2]
[1]*Chi Mei Foundation Hospital; Tainan, Taiwan Republic of China;* [2]*National Cheng Kung University Hospital; Tainan, Taiwan Republic of China*
briant@seed.net.tw

Prader-Willi syndrome (PWS) is characterized by infantile hypotonia, feeding problems, obesity, small hands and feet, hypogonadism, and mental retardation. Early diagnosis of PWS is important not only for the avoidance of needless diagnostic procedures but also for the consideration of preventive and therapeutic options. However, the presenting features of PWS evolve with time and are usually subtle in infancy, making diagnosis difficult. In order to facilitate the diagnosis of PWS early in infancy, we specifically analyzed the clinical presentation of patients who were diagnosed at less than 1 year of age. From May 1, 1999 to April 30, 2000, 71 cases of suspected PWS were collected. Methylation-specific PCR was performed to amplify the CpG island of the SNRPN gene. Two sets of PCR primers were used specifically for the methylated and unmethylated versions of the SNRPN gene. Of the 10 infants suspected of PWS, 7 (70%) showed the 174 bp maternal-specific band only and proved to be PWS. The occurrence of the major consensus diagnostic criteria for PWS in the seven infants were as follows; neonatal and infantile hypotonia, 7 patients (100%); feeding problems in infancy, 6 patients (86%); excessive weight gain, 0 patients (0%); characteristic facial features, no less than 3 items present in 2 patients (29%); hypogonadism, 4 patients (57%); global developmental delay, 3 patients (43%); hyperphagia, 0 patients (0%). The average score according to major diagnostic criteria was 3. We conclude that in infants with obscure characteristic features of PWS, methylation-specific PCR is a useful tool for the rapid screening of PWS.

P0364. Clinical and genetic study of neurofibromatosis type 1 in Croatia

H. Kapitanovic[1], *S. Kapitanovic*[2], *T. Cacev*[2], *K. Pavelic*[2], *Z. Sabol*[1]
[1]*Private Pediatric Clinic; Zagreb, Croatia;* [2]*Rudjer Boskovic Institute, Division of Molecular Medicine; Zagreb, Croatia*
kapitan@rudjer.irb.hr

Neurofibromatosis type 1 (NF1) is the most common autosomal dominant disorder of humans appearing in childhood. Features defining the disease are multiple cafe-au-lait spots, multiple neurofibromas and Lisch nodules, but other features such as short stature, intellectual handicap, central-nervous tumors and other malignant diseases are also found. The gene NF1 was mapped to 17q11.2 and has been found to contain the mutations in NF1 patients. The mutation rate in the NF1 gene is one of the highest known for human genes with approximately 50% of all NF1 patients presenting as sporadic cases. Molecular analysis and genetic counseling is limited to the identification of the specific mutation in each patient or family or to the use of DNA polymorphisms and linkage analysis. We analyzed 46 families with neurofibromatosis type 1. NF1 was diagnosed clinically according to the NIH criteria. A positive family history was found in 47.8% (22 of 46) and 52.2% (24 of 46) of affected patients were considered to be the result of a new mutation. DNA was obtained from peripheral blood of patients and related individuals. We used PCR-RFLP and VNTR analysis for linkage and LOH analysis in the affected families and individuals. Twenty-four families in the Croatia population with the de novo mutation were studied using four intragenic markers. For VNTR analysis PCR products were separated on polyacrylamide gels or were analyzed by submerged gel electrophoresis. Loss of heterozygosity (LOH) in the affected individual revealed a gross NF1 gene deletion in 3 (13.6%) families; in 2 (67%) of them, the deletion was maternally and in one (33%) paternally derived.

P0365. Spondylothoracic dysplasia (Jarcho-Levin syndrome) and Spondylocostal dysostosis, the confusing vertebral malsegmentation syndromes. Report of five cases

N. El ioglu[1], *H. Bilgen*[2], *S. Yal in*[3], *T. Biren*[4]
[1]*Department of Pediatric Genetics, Marmara University Hospital; Istanbul, Turkey;* [2]*Dep. of Neonatology, Marmara University Hospital; Istanbul, Turkey;* [3]*Dep. of Orthopedics, Marmara University Hospital; Istanbul, Turkey;* [4]*Dep. of Radiology, Marmara University Hospital; Istanbul, Turkey*
nelcioglu@hotmail.com

Jarcho- Levin Syndrome (JLS, Spondylothoracic dysplasia) is the severest form of vertebral malsegmentation syndromes with reduced stature resulting from axial skeleton. The main features are short, immobile neck, short thorax with the patognomonic crab-like rib cage associated with multiple vertebral defects and ribs that flare in a fan-like pattern. The small size of the thorax in newborns frequently leads to respiratory problems and death in infancy. Inheritance is autosomal recessive. Carefully prenatal ultrasound examination during the second trimester should done for subsequent pregnancies. A clinically similar disorders is Spondylocostal dysostosis (SCD). The main features are abnormalities of vertebral segmentation and of the ribs, including multiple hemivertebrae, vertebral clefting and fused, hypoplastic vertebrae, rib fusions and deletions with a non-progressive kyphoscoliosis. Survival is much better and neural tube defects only rarely occur. Both recessive and dominant autosomal inheritance has been reported. Recently, mutations in the recessive form were demonstrated in the DLL3 gene, mapped at 19q13. Segmental spinal dysgenesis is a rare anomaly where there is dysgenesis or agenesis of one or more vertebrae usually at or near the thoraco-lumbar junction. We describe here four cases of multiple vertebral segmentation defects, two with the classical features of JLS and two other with SCD, and a case with segmental spinal dysgenesis associated with tethered cord. Both young infants with JLS had respiratory problems with fan-like deformities of the rib and one of them presented also a thoracic meningomyelocele and club foot deformity. The other two with the SCD, both suffer from short neck and short trunk associated with kyphoscoliosis without any significant respiratory restriction. All patients were sporadic and parental consanguinity were present only in one of them. We believe that appropriate classification of these similar phenotypes will improve molecular research and genetic counselling concerning recurrence risk, management, prognosis and prenatal diagnosis.

P0366. Lethal multiple pterygium syndrome with parental consanguinity

J. M. Coll e, *J. M. Cobben, F. Poppelaars, J. M. G. van Vugt, L. Wijnaendts*
Vrije Universiteit Medical Centre; Amsterdam, The Netherlands
jm.collee@azvu.nl

We report on a male fetus with the lethal multiple pterygium syndrome (LMPS). The parents of the fetus were first cousins. Clinical features were hydrops, a cystic hygroma colli, facial dysmorphism, cleft palate, pterygia, muscle hypoplasia, joint contractures, lung hypoplasia, cardiac hypoplasia and malrotation of the gut. LMPS is rare and although its pathogenesis is still unknown, the absence or important reduction of fetal movements and jugular lymphatic obstruction appear to play a major role in the development of contractures, webbing and facial features. LMPS was originally classified as an autosomal recessive trait. An excess of males in reported cases with LMPS and families suggestive of X-linked inheritance have led to the conclusion, that X-linked recessive inheritance cannot be excluded in (isolated) male cases. Consanguinity between parents was reported only once in the literature. Our case is to our knowledge the second report describing a fetus with LMPS and consanguineous parents and the first report of consanguineous caucasian parents. This finding supports autosomal recessive inheritance of LMPS.

P0367. Natural History Of Wolf-hirschhorn Syndrome; A Study Of 50 Patients

A. Battaglia[1], *J. C. Carey*[2], *A. Meloni-Ehrig*[3]
[1]*Stella Maris Research Institute; Calambrone (Pisa), Italy;* [2]*Division of Medical Genetics- University of Utah; Salt Lake City, UT United States;* [3]*Cytogenetics Laboratory- University of Utah; Salt Lake City, UT United States*
abattaglia@inpe.unipi.it

Accurate information on the natural history of a clinical condition is of paramount importance to professionals, the families and the patient. It is particularly so for WHS, for which still little data are available in the literature. To help delineate more accurately the natural history of WHS and to obtain better information to answer parents questions in a clinical setting, we wrote out an exaustive questionnaire dealing with all the possible clinical manifestations of the syndrome and with the psychosocial development. The questionnaires were sent to the families of children with WHS, through their national support groups in the USA and Italy, nationwide. Of 200 questionnaires sent out to USA families, 28 came back filled out; while of the 35 questionnaires sent out to Italian families, 22 came back filled out. Overall there were data on 50 patients (34 females, 16 males). 15 of the patients had been followed up by us from 4 months to 17 years. 35 cases were detected by standard cytogenetics, other 14 required FISH, while 1 did not show a detectable molecular deletion within the WHSCR. Our experience with natural history greatly expands literature reports; 48/50 (96%) had a seizure disorder; 21/50 (42%) underwent gastrostomy; 26/50

(52%) had heart lesions; 18/50 (36%) had oral facial clefts; 21/50 (42%) had hearing defects, that were sensorineural in 5 (10%); 30/50 (60%) had skeletal anomalies; 27/50 (54%) had abnormal teething; 13/50 (26%) had genitourinary tract defects. Of note, only 32/50 (64%) had severe/profound developmental retardation; while retardation was moderate in 12/50 (24%), and mild in 6/50 (12%). This is different from the literature, where it is conventionally stated that severe mental deficiency is an hallmark of WHS. Moreover, 9/50 (18%) patients were able to walk with support (between age 2 and 12 years), whereas 15/50 (30%) patients were able to walk unassisted (between age 15 months and 7 years). 6/50 (12%) patients also achieved sphincter control (by day). 11/50 (22%) patients were helping with food, dressing and undressing themselves, and doing household tasks. 7/50 (14%) patients became self-feeders between age 4 and 12 years. The 34/50 (68%) patients receiving serial EEG studies showed fairly distinctive abnormalities, usually outlasting seizures. Although difficult to control in early years, seizures tend to disappear with age. No apparent correlation was found between size of deletion and phenotypic severity. All the patients have a characteristic facial appearance which is the most important clinic diagnostic sign. A slow, but constant progress in development was observed in all cases during the follow-up period. In conclusion, our combined cases represent considerable experience, providing new information on several aspects of this important deletion syndrome.

P0368. Ablepharon macrostomia syndrome; possible localization to 4q?

M. Zenker[1], *U. Trautmann*[2], *V. Kalscheuer*[3], *C. Kraus*[2], *A. Rauch*[2], *A. Reis*[2]
[1]*Univ.-Klinik f r Kinder und Jugendliche; Erlangen, Germany;* [2]*Institut f r Humangenetik; Erlangen, Germany;* [3]*Max-Planck-Institut f r Molekulare Genetik; Berlin, Germany*
martin.zenker@kinder.imed.uni-erlangen.de

Ablepharon macrostomia syndrome (AMS; OMIM 200110) is a very rare condition characterized by a distinctive pattern of facial anomalies, malformed genitalia, webbed fingers, and cutaneous abnormalities. Only 8 case reports exist in the literature including one sib pair. AMS is listed as an autosomal recessive trait based on one report of apparent overlap between AMS and cryptophthalmos syndrome. A very recent report, however, suggested autosomal dominant inheritance with variable expression. The gene locus is uncertain. We present a male newborn with typical features of AMS; severe deficiency of the anterior lamella of both eyelids and defect of lashes, fishlike mouth with shortened philtrum, malformed nose with hypoplastic alae and nares, hypoplastic, abnormal ears, dry, patchy skin, webbed fingers, and hypospadia. Additional findings not previously mentioned in AMS were; renal anomalies (unilateral hypoplasia and contralateral pyelectasia) and cerebellar hypoplasia. By the age of one year developmental delay and microcephaly became evident. Karyotyping revealed a de novo paracentric inversion in the long arm of chromosome 4. Preliminary results of FISH analysis confirmed the inversion. Further investigations are under way. Our observation strongly suggests a localization of the AMS gene within one of the breakpoint regions on 4q which are currently under molecular cytogenetic investigation. The spectrum of clinical features in AMS is extended to renal and cerebellar anomalies. However, it cannot be excluded that more than a single gene is involved in our patient. The presumable causal relationship between AMS and the 4q inversion in our patient argues in favor of a dominant new mutation rather than a recessively inherited defect.

P0369. Polymorphism of the serotonin transporter gene (hSERT) in patients with opiate drug addiction.

A. Gareeva, *A. Galeyeva, E. Khusnutdinova*
Institute of Biochemistry of Ufa Scientific Center, Russian Academy of Sciences; Ufa, Russian Federation
ganna@anrb.ru

Analysis of VNTR polymorphism in intron 2 of the hSERT was analysed for allelic association in 160 patients in the age between 13-40 years with the 2nd stage opiate drug addiction (ODA). The decreasing tendency (P=0,06) of the geterozygotes was determined in patients compare to the control group first of all on the account of increasing of the fraction of the long (12-repeat) allele gomozygotes. The most frequent was genotype 12/12 (0,44), the second was geterozygotic genotype - 10/12 (0,34) among all ODA patients. The opposite results were found in the control group; the geterozygotes 10/12 were in the prevalence (0,45), when 12/12 genotype frequency was much lower (0,35). 10/10, 9/10, 9/12 genotypes frequencies were practically equal in the both groups (for patients; 0,21, 0,00, 0,01; for control; 0,17,0,01, 0,02 accordingly). In the hSERT gene allelic allocation frequencies, any veracious differences were not found. The 12-repeated allele in the patients and control samples (0,62 and 0,59 accordingly) was the most common. The shortest 9-repeated allele frequency was very low in both patients (0,03) and control group (0,02). The 10-repeated allele in the patients and control samples (0,38 and 0,40 accordingly) was almost equal. Thus, the results of analysis of VNTR in intron 2 of the hSERT show that there is a 10/12 geterozygotes descent tendency in ODA patients compare to the control group first of all on the account of the 12-repeat allele gomozygotes frequency rising.

P0370. Trisomy 16q24-qter only causes typical features of partial trisomy 16q syndrome

B. R. B. Schulze[1], *N. Breitbach-Faller*[2], *G. Tariverdian*[1], *D. Hager*[1], *A. Jauch*[1]
[1]*Institute for Human Genetics; Heidelberg, Germany;* [2]*Childrens Hospital, University of Heidelberg; Heidelberg, Germany*
Birgit_schulze@med.uni-heidelberg.de

Although trisomy 16q was supposed to be mainly responsible for prenatal lethality, some liveborn patients with partial trisomy 16q of varying extension have been reported. The phenotype is characterized by facial dysmorphism, congenital heart defects, urogenital abnormalities, failure to thrive and early death. Houlston et al. (1994) suggested that duplication of 16q22-qter is a critical region for the facial features seen in partial trisomy 16q syndrome comprising a high forehead, prominent metopic suture, hypertelorism, down slanting palpebral fissures, small mandible, and ear anomalies. There is little information about phenotypic severity in patients with small distal trisomic segments. To our best knowledge only three patients have been reported with trisomy of 16q24-qter, the most distal segment in 16q. We present two brothers with partial trisomy 16q24-qter and partial monosomy 1q44-qter. The clinical phenotype varies but both patients exhibit facial features described in partial trisomy 16q syndrome. Because of clinical findings resembling Rubinstein-Taybi syndrome (facial dysmorphism, small stature, hirsutism) in one of the two boys FISH investigation was performed. We used a probe corresponding to the 16p13.3 region and a paint of 16q (reference). Therefor we happened to find the cryptic translocation of 16q on 1qter. The boys phenotypes show similarities with partial trisomy 16q syndrome and less distinct symptoms of monosomy 1q44-qter and will be discussed in detail. We suggest, that trisomy 16q24-qter only may cause typical features of partial trisomy 16q syndrome.

P0371. Laboratory Findings in a Male Patient with Suspected X-Fragile Syndrome; Discovery of Mosaicism of Normal and Methylated FMR-1 Genes

P. Battaglia[1], *E. De Fanti*[1], *R. Grimau-Merino*[2], *L. Giardina*[2], *R. Schiavon*[1]
[1]*Laboratory of Clinical Pathology and Molecular Biology, Legnago Hospital; Legnago (VR), Italy;* [2]*Department of Pediatry, Legnago Hospital; Legnago (VR), Italy*
de_fanti_emanuela@hotmail.com

A 15 year-old man with severe mental retardation , autism, hyperactivity and language deficit, was suspected to have X-fragile syndrome. This patient and his family members were screened for FMR-1 gene mutations with PCR technique and Southern blot hybridization using a FMR-1 probe(pFxa1NHE,ONCOR) . The PCR analysis failed to show any abnormality of FMR-1 gene in all the subjects examined. The Southern Blot analysis revealed the unusual presence of two alleles in a male patient; a normal allele (2.8 Kb) and a methylated allele corresponding to the normal allele present in females. This methylated allele is 5.2 Kb in size due to the presence of a methylated site resistant to EagI restriction. We therefore hypothesize that a mosaicism of FMR-1 normal/FMR-1 methylated gene alleles may lead to the development of X-Fragile syndrome in this male patient. Examination of family members demonstrated the presence of two normal alleles in the proband s mother and a mutated allele in his grandmother .These findings may represent an atypical predisposition to FMR-1 methylation. Since the FMR-1 full mutated allele found in the grandmother was not transmitted to her daughter, the proband s mother could inherited the normal X chromosome, followed by a de-novo methylation mutation of FMR-1 in the proband. The Southern-Blot hybridization analysis revealed a FMR-1 mosaicism in the presence of a negative PCR study. It is therefore mandatory to employ the hybridation technique in the setting of strong clinical suspicion of FRAXA alteration even when initial PCR screening is negative.

P0372. Detection of subtelomeric rearrangements by FISH in patients with idiopathic mental retardation or autism.

E. Ostergaard, *J. M. Hahnemann, H. Hjalgrim, K. Nielsen, H. M. Nielsen, K. Brondum-Nielsen*
John F. Kennedy Institute; Glostrup, Denmark
els@kennedy.dk

The subtelomeric regions are often involved in chromosomal rearrange-

ments. They are gene rich and therefore rearrangements in these regions are more likely to have phenotypic consequences than rearrangements in other regions.
The 41 subtelomeric regions were screened for rearrangements with fluorescent in situ hybridization (FISH) using the Chromoprobe T-Kit (Cytocell, UK).
Two groups of patients, all with normal standard chromosome analysis, were investigated; One group consisted of 14 patients with autism, and with at least one other relative affected with autism. DNA analysis for fragile X was normal in all and no subtelomeric rearrangements were found.
The other group consisted of 41 patients with idiopathic MR and dysmorphic features or a family history of MR. In this group five subtelomeric rearrangements were found;
A 1pter deletion, resulting in a well characterized del(1)(p36.3) syndrome, was found in a girl born 1999. She had MR, growth retardation, and dysmorphism.
In a girl born 1986 a deletion of 4p was found. Using the FISH probe for Wolf syndrome confirmed del(4)(p16.3p16.3). The girl had MR, epilepsy, microcephaly, and dysmorphism, consistent with Wolf syndrome.
A previously not described derivative chromosome 9, der(9)t(9;22)(q34.2q34.3;q13.2q13.3) was found in a girl born 1999. She had MR, microcephaly, an atrial septum defect, and dysmorphism. Her father had a cryptic balanced reciprocal t(9;22) translocation.
A recently described polymorphism of 2q was found in two patients.
In conclusion three out of 41 (7.3%) patients with idiopathic MR were found to have subtelomeric rearrangements, in concordance with the prevalence (5-10%) in previous studies.

P0373. Toriello-Carey syndrome; further delineation and report of three new cases

I. Barisic[1], *B. Peter*[2], *E. Juretic*[2], *L. Mikecin*[3]
[1]*Childrens Hospital Zagreb; Zagreb, Croatia;* [2]*Clinical Hospital Centre, Dpt. of Obstetrics and Gynaecology; Zagreb, Croatia;* [3]*Childrens Hospital Zagreb, Dpt. of Paediatrics; Zagreb, Croatia*
ibarisic@white.kdb.hr

Toriello-Carey is a rare mutliple malformation/ mental retardation syndrome characterised by dysmorphic features including telecanthus/hypertelorism, short palpebral fissures, small nose, malformed ears, and Pierre Robin sequence. Affected patients also show several midline field defects; agenesis of the corpus callosum, laringeal anomalies, and congenital heart defect. Brachydactyly, hypotonia and developmental delay were present in most reported cases . The autosomal recessive inheritance was proposed, but recently X-linked or sex influenced gene disorder was suspected. We report on three patients, two of whom are sibs. Presented patients show clinical findings typical for this condition, but also some additional traits expanding further the phenotypic spectrum. Affected brother and sister had severe clinical presentation with death in early infancy. The third patient with milder phenotype also showed marked somatic and developmental delay. Specific malformation pattern observed in present patients and in the previously reported cases suggests an early midline developmental filed disruption, presumably caused by a developmental regulatory gene mutation.

P0374. Duplication of proximal 22q (q11.2-q13.2) in a 13-year-old boy

M. Addor[1], *V. Froidevaux*[2], *A. Gaide*[1], *D. Schorderet*[1]
[1]*Department of Medical Genetics; 1011 Lausanne CHUV, Switzerland;* [2]*Paediatrician; 2300 La Chaux-de-Fonds, Switzerland*
Marie-Claude.Addor@chuv.hospvd.ch

Isolated duplication of the proximal portion of chromosome 22q is extremely rare. A distinct phenotype has emerged for distal partial 22q trisomy or distal 22q syndrome. However, as very few cases of proximal duplication 22q are reported, there is no real consensus on the phenotype correlated to this cytogenetic anomaly. We report a 13-year-old boy, referred with the clinical suspicion of Seckel syndrome. Pregnancy and delivery at 38 weeks of gestation were normal ; birth weight was 2 550 g (10th centile), length was 50 cm (50th centile) and OFC was 33 cm (<10th centile). At birth, our patient presented hypotonia, respiratory distress, cardiac murmur (small VSD), microretrognathia and epicanthal folds. Initial routine chromosome analysis was reported as normal, as did cerebral and abdominal US. On radiograph of the chest, the number of rib was normal. He sat at 14 months, crawled at 19 months and began walking at 2 ? years. Audiological testing at 6 years was normal. At 13 years, he presented with a history of severe mental retardation, absence of speech and lack of daily living skills, hyperactivity, postnatal microcephaly (OFC 49.5 cm <3rd centile) and short stature (142 cm, 3rd centile) and dysmorphism. The physical findings included short and narrow forehead, low posterior hairline, downslanting palpebral fissures, epicanthic folds, strabismus, beaked nose, microretrognathia, high vaulted palate, dental malpositions, 5th finger clinodactyly and sandle gap between toes I and II. The patient didn t meet the clinical criteria for Seckel syndrome and a new cytogenetic study showed additional material on 22q. FISH analysis revealed a de novo 22q11.23-q13.2 duplication. Phenocopy of Seckel syndrome could be due to 22q duplication and high quality cytogenetic work up is recommended.

P0375. Evidence For 621+1g>t Mutation In Croatian Population

J. Knezevic[1], *A. Milic*[2], *I. Bari?ic*[3], *J. Pavelic*[2]
[1]*Rudjer Boskovic Institute, Division for molecular medicine; Zagreb, Croatia;* [2]*Rudjer Boskovic Institute, Division for moleculare medicine; Zagreb, Croatia;* [3]*Childrens Hospital Zagreb, Departement of Paediatrics; Zagreb, Croatia*
etna_doo@yahoo.com

ABSTRACT Since today more than 960 mutations and 200 polymorphysms in cystic fibrosis transmembrane conductance regulator gene (CFTR) have been reported. In the population of cystic fibrosis patients from Croatia, delF508 is the most common mutation (70%) followed by G542X (5.4%), N1303K (3.6%), R117H (1.8%), 1717-1G>A (1.8%) and R1162X (1.8%). In our regular diagnostic procedure we have recently found, for the first time, the case of the 621+1G>T splice mutation, placed in the intron 4. Its incidence is 0.5% for South European countries. Our usual laboratory procedure for detection of 15, in Europe most common, CF mutations includes PCR reaction followed by either heteroduplex or restriction fragment length polymorphism (RFLP) analysis. In a case of 621+1G>T mutation (RFLP, MseI restriction enzyme) mutated allele appeared as three bands sizes of 234, 142 and 54 bp. Considering that of 15 mutations, for which we usually test the samples, only 6 have been detected so far, the appearance of, in Croatian population novel, 621+1G>T, mutation confirms our strategy and the necessity for testing the large number of mutations.

P0376. Scoliosis, blindness and arachnodactyly syndrome of a large consanguineous Turkish family

M. Dundar[1], *K. Erkilic*[2], *Z. Candemir*[1]
[1]*Erciyes University, Medical Faculty, Department of Medical Genetics; Kayseri, Turkey;* [2]*Erciyes University Medical Faculty, Department of Ophthalmology; Kayseri, Turkey*
mdundar@hotmail.com

In this report we have described an affected sib in a large consanguineous Turkish family who appears to have a new distinct dominantly-inherited blindness, scoliosis and arachnodactyly syndrome. The proband is a 16 years old boy. The parents also had another 2 affected sibs with similar clinical features. The father also has blindness, thoracic scoliosis, and arachnodactyly. On admission, the proband was 153 cm height with 76 cm upper segment and 156 cm length of two arms. There was arachnodactyly at both fingers and toes. Forth and fifth toes bilaterally were situated in back position. He has been suffered from progressive vision lost and strabismus since his 8 years age. Clinical examination of the eyes showed hypermetropia and microcornea, bilateral lens luxation to the degenerated vitreous. There were atrophy of retinal elements and hypertrophy of retinal pigment epithelium. In the family, all affected individuals have blindness and scoliosis with variable degree as well as arachnodactyly. Biochemical analysis of all family members were normal. All skeletal survey was consistent with the clinical findings. Urine analysis of the family for aminoaciduria and other routines were normal. Our recent review of the literature, the London Dysmorphology Database (Winter and Baraitser, 1993) did not show up any other known syndrome equally or approximately matching the blindness, scoliosis and arachnodactyly syndrome proposed by this report.

P0377. Monozygotic Twins with the DiGeorge Syndrome

V. Krut Ikov [1], *M. Havlovicov* [1], *E. Koc rek*[1], *T. Klein*[2], *P. Goetz*[1]
[1]*Institute of Biology and Medical Genetics of 2nd School of Medicine, Charles University; Prague, Czech Republic;* [2]*Centre of Pediatric Cardiology and Cardiac Surgery of the 2nd School of Medicine, Charles University; Prague, Czech Republic*
vera.krutilkova@lfmotol.cuni.cz

The incidence of DiGeorge syndrome is estimated as 1;4000. Most cases are sporadic, only about 4-10% of all cases are familiar. We present a familiar case of 22q11.2 microdeletion with fenotypic differences. We have investigated monozygotic twins from the second pregnancy of 32 years old mother. From the first pregnancy a girl was born and died at the age of 16 days due to congenital heart (conotruncal) defect. A fenotypical stigmatisation and also anal atresia were present. Twin A- despite of her sister,

stigmatisation for DiGeorge syndrome not so typical, heart anomaly (subarterial ventricular septal defect, FOA, a.lusoria dx.) and thymic hypoplasia. 22q11.2 mocrodeletion was also confirmed. Twin B- typical DiGeorge syndrome fenotype, conotruncal heart anomaly and 22q11.2 mikrodeletion was confirmed. Mother of the babies had typical DiGeorge syndrome stigmatisation and mental defect, 22q11.2 microdeletion was confirmed, too. There is a suspect that the first child had the same finding.

P0378. Brachydactyly-Symphalangism-Deafness Syndrome - a Clinical-Genetic Study of 11 Cases

M. Bembea[1], *S. Bembea*[2], *O. Vancsik*[1], *C. Jurca*[1], *M. Barbu*[3], *G. Samuila*[2]
[1]*Clinical Children Hospital; ORADEA, Romania;* [2]*Clinical Children Hospital, ORL Department; Oradea, Romania;* [3]*Clinical Children Hospital, Department of Radiology; Oradea, Romania*
bembea@yahoo.com

Introduction; Symphalangism-Brachydactyly S. (Multiple Synostoses with Brachydactyly; WL S.; Hermann s Deafness-Symphalangism; Facio-Audio-Symphalangism S.; 186500, MIM) is a rare entity, and diverse names reflect the uncertainty in the definition of the syndrome. Objective; Correlation of 11 personally examined cases with data obtained from review. Material and method; Our clinical-genetic study comprises 11 cases examined in a family where the disease has been present for the last 5 generations. Results; The cases offer a complete and well defined clinical picture of a rare syndrome that has as definitory elements; brachydactyly + symphalangism + progressive deafness + autosomal dominant mode of inheritance. They are surprisingly similar in what the extension of finger morpholesions is concerned, but different in what the onset age and the deafness degree are concerned. Conclusions; As the largest and most complete series communicated, authors propose a definition of the syndrome as Brachydactyly-Symphalangism-Deafness syndrome (BSD Syndrome), in order to avoid confusion.

P0379. Abdominal lipomatosis in a patient with Proteus syndrome.

C. A. Kim, *I. R. Assumpcao, Y. K. L. Koda, V. M. P. Simone, M. Valente, W. C. Andrade, L. M. Albano, D. R. Bertola*
Instituto da Crianca; Sao Paulo, Brazil
chong@icr.hcnet.usp.br

Proteus syndrome is a complex disorder with malformations and overgrowth of multiple tissues in a mosaic manner. Clinical findings are very variable and the presence of subcutaneous lipomas, lymphangiomas or hemangiomas is common. However, abdominal lipomatosis is very rare. We report one black, 2 year-old girl with abdominal lipomatosis and isolated macrodactily. She was born from unrelated parents, with normal birthweight and length, in good conditions. Since four months of age, she presented failure to thrive with progressive abdominal protusion and severe mal nutrition. Macrodactily of the right middle finger and left preauricular tag were noted. Plantar surfaces of the feet were normal. Mental development was normal. Abdominal ultrasound and CT showed large lipomatosis with displacement of the bowel to midline. Laparotomy at age 2y2mo detected giant lipomatous mass involving greater omentum and mesocolon.The large lipomatosis resected weighted 2950g and was constituted by normal mature adipocytes. Abdominal, pelvic, and other visceral lipomatosis were reported in Proteus and Bannayan-Zonana syndrome, raising some diagnostic difficulties between them. As recurrent abdominal lipomatosis is already reported after a surgical resection, a follow up is required.

P0380. A Case Of Bardet-Biedl Syndrome Associated With Pancytopenia

K. Cefle[1], *S. Palanduz*[1], *S. Ozturk*[1], *M. A. Karan*[2], *N. B. Erten*[2], *B. A. Esen*[2], *A. B. Sozen*[3], *N. Serakinci*[4], *C. Tascioglu*[2]
[1]*Istanbul Medical Faculty, Department of Internal Medicine, Division of Medical Genetics; Istanbul, Turkey;* [2]*Istanbul Medical Faculty, Department of Internal Medicine, Division of General Internal Medicine; Istanbul, Turkey;* [3]*Istanbul Medical Faculty, Department of Internal Medicine; Istanbul, Turkey;* [4]*Danish Cancer Society, Dept. Cytogenetics; Aarhus, Denmark*
ceflek@istanbul.edu.tr

Bardet-Biedl Syndrome is characterized with polydactyly, pigmentary retinopathy, obesity, mental retardation, hypogenitalism and renal abnormalities. Herein we describe a patient with the classical findings of Bardet-Biedl syndrome associated with pancytopenia.The patient, a 43-year old woman, had been referred to our hospital due to pancytopenia. Her history included obesity beginning in early childhood, blindness, and polydactyly which had been corrected surgically. Physical examination revealed truncal obesity and small, short hands and feet. Her height was 151 cm. and weight was 80kg . There were scars in the hands and feet due to the surgical operation performed in the past for the correction of polydactyly. An ophtalmological examination revealed pigmentary retinopathy and subcapsular cataracts. Abdominal ultrasonography showed fetal lobulation in the kidneys. A complete blood count showed pancytopenia; The leukocyte count was 3300/mm3, the haemotocrite was %33.5, and the platelet count was 126000/mm3. Bone marrow biopsy revealed dysplastyc erythtroid cells. Further laboratory investigation in regard to the other causes of pancytopenia (Anti-ds DNA, blood B12 and folate levels, hemosiderinuria) gave negative results. In haemotological consultation it was recommended that the patient should be followed for a possible development of myelodysplastic syndrome. The clinical and laboratory features of this patient are compatible with a diagnosis of Bardet-Biedl. However, to our knowledge, coexistance of pancytopenia associated with Bardet Biedl syndrome has not been reported before.

P0381. Chromosomal aberation in a boy with Noonan syndrome

M. Havlovicova[1], *V. Krut lkov* [2], *A. Puchmaierov* [2], *D. Novotn* [2], *E. Koc rek*[2], *S. Kolou?kov* [3], *D. Zemkov* [4]
[1]*Institute of Biology and Medical Genetics of 2nd School of Medicine, Charles University Prague; Prague, Czech Republic;* [2]*Institute of Biology and Medical Genetics; Prague, Czech Republic;* [3]*Second Paediatric Department of the 2nd Medical Faculty Charles University; Prague, Czech Republic;* [4]*Second Pediatric Department of the 2nd Medical Faculty Charles University; Prague, Czech Republic*
marketa.havlovicova@lfmotol.cuni.cz

We present a case of a boy with small growth with the finding of chromosomal aberation. We have investigated 8,5 years old boy, which was reffered to our department by the endocrinologist with the suspection of Noonan syndrome. Fenotype; height before 3rd percentile, disproporcional stature, stigmatisation typical for Noonan syndrome, delayed bone age. The parents and the sister of the proband have also their height below 3rd percentile, but their stature is proporcional, they all have normal fenotype. Cytogenetic investigation of the proband 46,XY, t(2;12)(q37;q24). The same translocation was confirmed in the proband«s father. A gene for Noonan syndrome is located in the 12q22-qter region. The translocation is in the critical region of the gene for Noonan syndrome. We assume, that the translocation may be the cause of a mutation in the gene for the Noonan syndrome. DNA analysis of the critical region is further proceeding in collaboration with the Institute of Medical Genetics University Hospital Dresden (Dr. med. Bartsch).

P0382. Particular Aspects Of Osteochondrodysplasias During Puberty

C. Jurca, *M. Bembea, R. Spineanu, M. Bembea, M. Barbu, I. Vancsik, M. Nevelits*
Clinical Children Hospital; Oradea, Romania
benchea@yahoo.com

Puberty is characterised by rapid growth of long bones, opened epiphyses, increased traction on muscles insertion sites. All these contribute to the fact that, during this developmental stage, an increase in the incidence of orthopaedic problems and of medical conduct is noticed. Osteochondrodysplasias are part of this context. The objective of this work is to identify particular aspects of osteochondral dysplasias during puberty. In order to achieve this purpose, we have been studying 40 cases of osteochondrodysplasias, representing 1.45% of the 2749 cases of genetic diseases and congenital malformations in the evidence of the Genetics Department of the Clinical Hospital for Children, Oradea. The incidence is 2.84 in 10000 new-borns. Osteochondrodysplasias represent 47% of the total number of children with growth retardation at the age of puberty. The study comprises cases of Achondroplasia, Cleido-cranial Dysplasia, Marfan Syndrome, McCune-Albright Fibrous Dysplasia, Mucopolysaccharidoses, Osteogenesis Imperfecta, Multiple Exostoses Syndrome, Multiple Symphalangism, Ellis-van Creveld Syndrome. Diagnostic, evolutive, therapeutic, psychological and socio-professional aspects were taken into account. Conclusions refer to the necessity of a particular attention that osteochondrodysplasias cases that reached the age of puberty need, in an interdisciplinary manner, including the collaboration of paediatricians, orthopaedists, geneticians, medical recovery services, sociologists, psychologists and teachers.

P0383. Three dimensional facial analysis using stereophotogrammetry.

J. S. G. Bamforth, *D. Shaner, O. Beattie, A. Peterson*
University of Alberta; Edmonton, AB Canada
jbamfort@ualberta.ca

Diagnosis of a dysmorphic syndrome depends both upon the ability to recognise an abnormal appearing face and to match that face to a known pattern of abnormalities. This requires recognition skills and a good visual memory, often augmented by visual catalogues. Less than half of the dysmorphic patients presenting to our clinic will receive a diagnosis We are investigating the possibility of using three dimensional information to assist with diagnosis of dysmorphic features. Six cameras erected on a steel frame are calibrated using a surveyed calibration frame. Six overlapping images of each face are obtained so that 30 previously marked facial landmarks are visualised by at least two cameras. The x, y and z coordinates of each point can then be deduced by trigonometry. Images are translated to a common origin and rotated to a common plane (to ensure similar orientation). Measurements are expressed in units of standard deviations form an age appropriate norm. Distances between the landmarks can be computed and compared. We have imaged 160 faces, including three two generation families and patients with the dysmorphic syndromes. We found that variation between landmark positions is less between siblings in the same family irrespective of sex when compared with children of the same age who were unrelated. Syndrome affected patients show less left-right facial asymmetry compared to normal children. Patients with the same syndrome (unrelated) showed no statistically significant difference in landmark position compared to normal subjects, however, they show a variation in distances similar to siblings. This suggests that the resemblance between faces of patients with the same dysmorphic syndrome is as close as the resemblance between siblings. We are exploring more sophisticated shape analysis using three dimensional analysis models and obtaining more images of children affected with the same syndrome.

P0384. Role of the Web-Internet and Birth Defects (BD) in Ukraine

L. Yevtushok[1], *I. Baryliak*[2], *S. Lapchenko*[3], *T. Vihovska*[3], *W. Wertelecki*[4]
[1]*Ukrainian-American Birth Defects Center; Rivne, Ukraine;* [2]*Birth Defects Ukrainian Alliance; Kyiv, Ukraine;* [3]*Ukrainian-US Birth Defects Center; Lutsk, Ukraine;* [4]*University of South Alabama; Mobile, AL United States*
bdp@rivne.com

Aim; Introduce international standards for monitoring, diagnosis, treatment, prevention, and coding of BD. Method; USAID funds provided for the creation of BD Centers in two regions of Ukraine (Rivne and Volyn, both impacted by the Chornobyl disaster). Both BD Centers were provided with Web-Internet information resources. In January 2000, population-based BD Surveillance applying international standards began. Digital cameras were provided to neonatologists to enhance BD ascertainment and to medical geneticists to enhance clinical diagnosis, care, prevention, and coding. Illustrated clinical histories are shared on an on-going basis with Ukrainian and international experts. Results; Tele-consultations and use of electronic information have attracted contributions by national and international experts toward the care and prevention of BD in relatively remote areas of Ukraine. BD Centers have attracted students, interns, neonatologists, obstetricians, other clinicians, as well as parents and support groups seeking Web-Internet access to information resources. Conclusions; Implementation of Web-Internet strategies in rural areas of Ukraine are key elements that upgrade BD diagnosis care and prevention.

P0385. Mutational Analysis Of The HOXD13 Gene In The Triphalangeal Thumb-brachyectrodactyly Syndrome

J. Zenteno, *A. Perez-Cabrera, A. Jimenez, S. Kofman-Alfaro*
Genetics Department, Hospital General de Mexico-Facultad de Medicina, UNAM; Mexico City, Mexico
cayguash@yahoo.com

The triphalangeal thumb-brachyectrodactyly syndrome is a very uncommon autosomal dominant limb malformation in which polydactyly and triphalangeal thumb(duplication anomalies) coexist with ectrodactyly and syndactyly (absence malformations). To our knowledge, four familial and two sporadic cases have been described and interestingly three of these families were of Mexican origin, reflecting a geographic distribution of the disease. To date no molecular defects have been identified as the cause of the disease. Induced disruption of the homeotic gene HOXD-13 gene in mice produce a malformative pattern characterized by reduction of the legth of some bony elements, loss of phalanges, bone fusions and the presence of an extra element (duplication and absence anomalies). As this phenotype is strikingly similar to that seen in the triphalangeal thumb-brachyectrodactyly syndrome, we decided to carry out molecular analysis of the HOXD13 gene (located in 2q31) in three affected members of a Mexican family with the triphalangeal thumb-brachyectrodactyly malformation. We perform PCR amplification of the complete coding region of HOXD13 and automated sequence analysis of PCR products. After sequencing the two exons and the intron/exon boundaries of HOXD13 we were not able to detect any deletereous mutation in these subjects. A previously unidentified silent polymorphism was observed at nucleotide position 291 in exon 1(GCA to GCG) without changing the encoded alanine. Our data excludes that mutations in the coding region of HOXD13 are the cause of the triphalangeal thumb-brachyectrodactyly syndrome.

P0386. Involvement of CFTR gene in Male Infertility

J. Kusic[1], *D. Radojkovic*[1], *A. Nikolic*[1], *S. Niksic*[2], *V. Maletic*[3], *A. Savic*[1]
[1]*Institute of Molecular Genetics and Genetic Engineering; Belgrade, Yugoslavia;* [2]*Institute of Mental Health; Belgrade, Yugoslavia;* [3]*Medical School, Department of Urology; Belgrade, Yugoslavia*
qwert@eunet.yu

The majority of men with cystic fibrosis (CF) are infertile due to a bilateral congenital absence of the vas deferens (CBAVD). However, clinically affected CF patients present a spectrum of genital phenotypes ranging from normal fertility to severely impaired speramogenesis and CBAVD. In men with isolated CBAVD, with none of the clinical symptoms of CF, an increased frequency of CFTR mutations was found. The present study was undertaken to test whether CFTR gene may be involved in male infertility caused by reduced sperm quality. We analyzed 21 infertile men, without symptoms of CF, for the presence of mutations and polymorphisms within 17 exons (and flanking sequences) of CFTR gene. One of 11 men with infertility due to oligospermia or non-obstructive azoospermia and 5 of 10 men with obstructive azoospermia had at least one mutation in CFTR gene - deltaF508 (one male with obstructive azoospermia was a compound heterozygote - deltaF508 / 711+3 A-G). Three men with obstructive azoospermia and 2 with oligospermia or non-obstructive azoospermia had 5T allele in Tn polymorphic site on one chromosome. Haplotype analysis of (TG)m/Tn polymorphic loci reveald (TG)11/7T haplotype as the most frequent one in both groups of patients. Because the frequency of mutations in our sample of infertile males (16.7%) was significantly higher (P=0.00138) than the expected mutation frequency in general population (2%) it seems likely that CFTR gene is associated with infertility due to reduced sperm quality in some cases. In the group of men with obstructive azoospermia the frequency of mutations was higher compaired to the group of men with oligospermia or non-obstructive azoospermia suggesting that involvement of CFTR gene is much stronger in aetiology of obstructive azoospermia than its participation in the process of spermtogenesis or sperm maturation. The results of this study are also relevant with regard to genetic counseling of couples treated by artificial reproductive technology - the high incidence of CFTR mutations in men with obstructive azoospermia implies that they bear an increased risk for CF offspring and molecular genetic analysis of the CFTR gene are indicated in these couples.

P0387. The Role of Neonatologists in Birth Defects (BD) Monitoring in Ukraine

T. Vihovska[1], *O. Shevchuk*[2], *O. Sulima*[3], *H. Vashchylin*[4], *W. Wertelecki*[5], *L. Yevtushok*[6]
[1]*Ukrainian-US Birth Defects Center; Lutsk, Ukraine;* [2]*Neonatology National Center; Rivne, Ukraine;* [3]*Neonatology National Center; Kyiv, Ukraine;* [4]*Lutsk Children s Regional Polyclinic; Lutsk, Ukraine;* [5]*University of South Alabama; Mobile, AL United States;* [6]*Ukrainian-American Defects Center; Rivne, Ukraine*
bdp_lutsk@fk.lutsk.ua

USAID funding prompted the creation of two BD Centers with BD Monitoring in two regions in Ukraine impacted by the Chornobyl disaster. Scarcity of Medical Geneticists prompted charging neonatologists with the task to ascertain BD s while the geneticists categorize, manage, and formulate prevention strategies. In January 2000, eighty neonatologists began BD population-based surveillance in Rivne and Volyn regions. Clinical data is illustrated by digital images. The BD focused activities by neonatologists soon prompted provincial health authorities to expand the BD registry to include all neonates. In addition, the health authorities are sponsoring a growing number of inter-disciplinary activities to further integrate obstetrics, perinatology, neonatology, pediatrics, pathology, and other disciplines that impact pregnancy outcomes and the prevention of birth defects. Conclusion; One key element of Ukrainian-US BD Centers and surveillance is the role played by neonatologists who have introduced medical genetics, teratology, and BD prevention strategies to relatively remote areas of Ukraine.

P0388. Sibs with Dyggve-Melchior-Clausen syndrome- Clinical presentation and review of the literature

B. Lemcke, *M. Herrmann, J. Horst, I. Kennerknecht*
Institute of Human Genetics; M nster, Germany
lemcke@uni-muenster.de

Dyggve-Melchior-Clausen syndrome (DMC) is a rare genetic condition with short trunk dwarfism, skeletal dysplasia and in part of cases associated with mental retardation. During pregnancy and at birth there are no gross abnormalities. In infancy and by early adulthood the dysmorphic signs become evident. Diagnostic criteria are often hypoplasia of the odontoid process, platyspondyly, lace-like appearance of the iliac crests, dysplastic heads of the humeri and femora, subluxation of shoulder and hip joints, irregularities of carpal bones. Involvement of the limb bones is predominantly proximal. Clinically a waddling gait, disproportionate dwarfism, rhizomelic shortening of limbs, prominent jaw, scoliosis, sternal prominence, genua valga and clawing of fingers can be observed. Cytogenetic anomalies were excluded.

Here, we report on two families of German and Lebanese origin, respectively, with one son and one daughter both affected with DMC. All probands developed a disproportionate dwarfism, severe mental retardation and speech delay. The faces became a coarse expression. Severe scoliosis and genua valga occured.

After exclusion of keratan sulfaturia the differential diagnosis of mucopolysaccharidosis type IV (Morquio) could be rejected. In addition restricted joint mobility and mental retardation of the probands is not compatible with the Morquio condition. A spondyloepiphyseal dysplasia is clinically similar but could be dismissed by radiological examination. X-ray with predominantly proximal dysplasia of epiphyses and metaphyses is typical and especially lace-like appearance of the iliac crests is pathognomonic. Those patients with DMC and regular mental development are proposed by several authors to be classified as Smith-McCort dysplasia suggesting a heterogeneity of DMC. Pictures of the affected individuals and a literature review are presented.

P0389. Rare origin of MODY 2 in 7/19 de novo translocation

N. Misovicova, *J. Volna*
Martin Faculty Hospital; Martin, Slovakia
misovic@enelux.sk

MODY represents a specific form of non-insulin-dependent diabetes mellitus. The disease is characterised by an early onset before the age of 25 years and by autosomal dominant inheritance. Its classification into 5 subtypes is based on gene mutation. Our patient is a young woman treated for diabetes mellitus since her 15th year. She has no family history for diabetes mellitus. The chromosomal analysis revealed an apparently balanced translocation between 7p and 19q. The break on 7p15 is considered to be the locus for glucokinase gene. The both parents have a normal karyotype. Mutations in the glucokinase gene cause diabetes in kindreds with MODY 2. There are some hypotheses to explain why patient with apparently balanced translocation have abnormal phenotype; gene disruption, position effect, submicroscopic deletion. Each of them can cause the glucokinase deficiency. The molecular genetic analysis is carried out to confirm the origin of MODY 2 in our patient.

P0390. Molecular Diagnosis Of Myotonic Dystrophy In Costa Rica.

F. Morales[1], *P. Cuenca*[2], *R. Brian*[3], *M. Sittenfeld*[4], *G. del Valle*[4], *D. Monckton*[5], *K. Johnson*[5]
[1]*University of Costa Rica; San Jose, Costa Rica;* [2]*Universidad de Costa Rica; San Jose, Costa Rica;* [3]*Hospital Nacional de Ni os; San Jose, Costa Rica;* [4]*Hospital san juan de Dios; San Jose, Costa Rica;* [5]*University of Glasgow; Glasgow, United Kingdom*
famorale@cariari.ucr.ac.cr

Myotonic dystrophy (MD) is the most common type of muscular dystrophy in adults. It s inheritance is complex since the genetic defect causing it is a unstable mutation due to the expansion of the triplet CTG at the DMPK gene on chromosome 19. The objective of this work was to achieve molecular diagnosis of the disease in order to improve the clinical management and the genetic counseling offered to patients and their families. Two technical diagnostic procedures were used, Southern blot (using the p5B1.4 probe after DNA digestion with EcoRI and BgII enzymes) and PCR. Fifty families have been localized dispersed in the whole country, 84 of their members have been studied at the molecular level, obtaining the following results; 21 asyntomatic individuals without the mutation, four asyntomatic members carriers of the mutation, among the syntomatic ones, 30 have the mutation and the others 29 belong a patients and relatives with DM-like symptons and do not have the mutation. The size of the mutation is positively correlated with severity of the symptoms and is negatively correlated with the onset age. Considering the inter-generational behavior of the mutation, Costa Rica shows no difference compared to the rest of the world. MD is a disease of importance at the national level.

P0391. A Case Of Autosomal Dominant Form Of Emery-Dreifuss Muscular Dystrophy In Bashkortostan (Russia)

R. V. Magzhanov[1], *D. Hilton-Jones*[2], *L. Akhmadeyeva*[1]
[1]*Bashkirian State Medical University; Ufa, Russian Federation;* [2]*Oxford Neuromuscular Centre; Oxford, United Kingdom*
medic@agidel.ru

We report a family with two girls, identical twins, living in Ufa (Bashkortostan, Russia). They were born from the first pregnancy in a young non-consanguineous married couple (both 22 years old). The girls developed normally until 18 months when the parents noticed difficulties in walking and both twins started to complain of fatigue. From the age of 2 years they were unable to run. The disorder is progressing slowly. Contractures of the elbows were first noticed at the age of 4. The limbs deformities and a pathognomonic posture with flexion of the elbows, scapular winging, mild equinovarus deformities at the ankles, limitation of the neck flexion due to the contracture of the posterior cervical muscles and slight lumbar hyperlordosis are seen in both twins. The girls have muscle weakness mainly of humeral and peroneal muscles, with pelvic girdle involvement and tendon areflexia; slight elevation of muscle enzymes (2.5 times for CK); and EMG evidence of myopathy. They present with waddling gait, difficulty climbing stairs and in walking on toes and they can t walk on their heels. No facial weakness, calf hypertrophy, mental and cardiac disturbances were noticed. We also observed slight difficulty in walking on her heels, limitation of dorsiflexion of feet, and mild weakness of the limb girdle muscles in the mother of these girls. This case is a presentation of a rare condition of autosomal dominant form of Emery-Dreifuss muscular dystrophy. The DNA studies for a mutation of the lamin A/C gene (1q11-q23) are in progress.

P0392. Small Mutations and Polymorphisms in the Dystrophin Coding Region in Japanese Patients with Duchenne Muscular Dystrophy.

J. Kawamura[1], *T. Ishihara*[1], *R. Kato*[1], *S. Kato*[2]
[1]*National Higasisaitama Hospital; Hasuda, Japan;* [2]*Microbiology, School of Medicine, Keio University; Tokyo, Japan*
kawamura@esaitama.hosp.go.jp

Duchenne muscular dystrophy (DMD) is a severe X-linked lethal myopathy with an incidence of approximately 1 in 3500 male births. The primary biochemical defect in DMD is dystrophin deficiency. The dystrophin gene, which spans approximately 2.4Mb of Xp, has a high mutation rate. About 60% of DMD patients has a deletion mutation and 10% has duplication mutation. Remaining one third of patients with DMD have no gross rearrangements in the dystrophin gene identifiable by Southern blot analysis or multiplex PCR. These Southern-negative cases presumably resulted from small mutations that cannot be detected by current diagnostic screening strategies. We sequenced dystrophin coding region for small mutations in 50 Southern-negative DMD patients by using reverse transcription PCR and direct sequencing, and identified 40 nonsense mutations or small deletions/insertions and 3 one exon skippings, all of which were unique to single patients. There was no clustering of small mutations similar to the deletion/ duplication distribution, and no characteristic phenotypes for small mutations were observed. As a consequence of detecting small mutations in the coding region of dystrophin in 86% of DMD patients without deletions/duplications, we believe that reverse transcription-nested PCR and direct sequencing is effective to screen for small mutations in dystrophin gene.

P0393. Singular And Syndromatic Anorectal Malformations

C. Skrypnyk[1], *O. Vancsik*[1], *M. Bembea*[1], *D. Baloiu*[2], *I. Vancsik*[3], *M. M. Bembea*[1]
[1]*Clinical Children Hospital; Oradea, Romania;* [2]*Clinical Children Hospital, Surgery Department; Oradea, Romania;* [3]*Clinical County Hospital; Oradea, Romania*
cristinaskrypnyk@usa.net

Introduction; Anorectal malformations include stenosis, atresia and ectasia. They occur with a frequency of approximate 1;5000 newborns. They occur isolated or associated in different syndromes. At least 35 dysmorphic syndromes that associate ano-rectal malformations have already been described. Etiology may be genetic (mendelian or chromosomal) or non-genetic. Study group and methods; The 11 cases of anorectal malformations that were admitted to the Clinical Children Hospital of Oradea during the last 20 years (1980-1999) have been studied clinically, in a retrospective manner. Results; The frequency of anorectal malformations for Bihor county is estimated at 1;10000 new-borns. 60% of them were isolated

anomalies, whereas 40% of them were associated in syndromes or by chance. A rare case of Townes-Brocks syndrome that associates anal imperforation, polydactyly and polyotia is discussed in detail. Particular genetical and clinical characteristics of these anomalies are also discussed. Conclusions; The relatively rare anorectal malformations give rise to particular problems of genetical and clinical diagnosis and of genetic counselling.

P0394. Case report; inverted triplication of distal 3q with features of Brachmann de Lange syndrome and other anomalies.

K. ˝unap[1], *T. Ilus*[1], *O. Bartsch*[2]
[1]*Medical Genetics Center, Tartu University Clinics; Tartu, Estonia;* [2]*Department of Clinical Genetics, Medical Faculty, Technical University; Dresden, Germany*
Katrin.Ounap@mail.ee

Intrachromosomal triplications producing partial tetrasomies are rare. At least 13 patients have been reported so far. Most cases involved chromosome 15q, but triplications of 2q, 5p, 7p and 13q have also been described. We report here the first case of an inverted triplication of 3q. The proposita was born at term, birth weight 3175 g, length 45 cm. Clinical examination on her 1st day of life showed disproportionate short stature with short limbs, generalized hirsutism, peculiar face, synophrys, small upturned nose, micrognathia, low set malformed ears, short and webbed neck, postaxial polydactyly of left hand, and omphalocele. Additional findings included hydrocephalus, Dandy-Walker malformation, spina bifida, cardiac anomaly (ventricular septal defect), and multiple renal cysts. Standard chromosome analysis from peripheral blood lymphocytes showed cytogenetic mosaicism with an abnormal chromosome 3 (45%) and normal cells (55%). The abnormal chromosome 3q was initially interpreted as a duplication of 3q21-q29, but fluorescence in situ hybridization (FISH) revealed an inverted triplication of distal 3q / karyotype 46,XX,trip(3)(q25.3q29)[27] /46,XX [32]. Parental karyotypes were normal. Partial duplications of chromosome 3q have been reported previously. Interestingly, a form of the Brachmann de Lange syndrome has been described with duplications including the 3q26.3-q27 region in several cases. Our patient demonstrates similar facial features and malformations and a triplication of 3q25.3-q29 (mosaicism). Reddy and Logan (2000) recently concluded that triplications can be mistaken for duplications and therefore, in assessing duplications, recommended confirmation by FISH. Our case supports their suggestion.

P0395. Duchenne Muscular Dystrophy In Clinical Children Hospital Oradea, Romania, In 20 Years

M. Praja, *M. Bembea, R. Spineanu, L. Ritli, A. Benedek, G. Miculschi, C. Cladovan, S. Cheregi*
Clinical Children Hospital; Oradea, Romania
pongo@texnet.ro

In any patient who presents with muscle wasting and weakness for no apparent cause and in whom there is no involvement of the central or peripheral nervous system, the possibility of muscular dystrophy should be entertained. The Clinical Children Hospital Oradea serves the population of Bihor county. Our study covers a period of 20 years (1977-1997). In this period we recorded nine clinical observations of Duchenne muscular dystrophy, which represent an incidence of 1;9700 live born male. Annual distribution of the incidence is unequal and there are no geographical particularities. However, the study revealed two affected brothers descendent from parents living in a special social and cultural environment resembling a genetic isolate. Among the clinical manifestations, cardiomyopathy was a constant feature in four cases which evolved with progressive and severe intractable congestive heart failure to death. The two brothers died at 10 and 13 years respectively, the interval between the two deaths being two months. It was obvious that the fatal outcome of the brother had a profound emotional influence on the other child who had previous psychological depression which accompanies the disease.

P0396. Misclassification Risk of Patients with Bilateral Cleft Lip and Palate and Features of Median Facial Dysplasia — A New Variant of Del(22q11.2) Syndrome?

A. Stellzig[1], *G. Komposch*[1], *G. Tariverdian*[2], *B. R. B. Schulze*[2]
[1]*University of Heidelberg; Heidelberg, Germany;* [2]*Institute for Human Genetics; Heidelberg, Germany*
Angelika_Stellzig@med.uni-heidelberg.de

The generic term median facial dysplasia (MFD) describes a subgroup of patients with cleft lip and palate exhibiting characteristic craniofacial defects; (1) short prolabium, (2) absence of frenulum labii, (3) hypoplasia of premaxilla, (4) single upper central and lateral incisors of the cleft side, and (5) deficient septal cartilage and nasal spine. Gross brain malformations are usually absent in MFD. The typical facial features of MFD are also present in patients with holoprosencephaly sequence (HPE-S). This clinical overlap can be explained by similar embryological origin of MFD and HPE-S. We report on two male patients with bilateral cleft lip and palate showing the facial findings of MFD or HPE-S. Additional congenital malformations were anal atresia in one and severe cardiac defect in the other patient. Both patients showed single maxillary incisors but the diagnosis of holoprosencephaly was excluded by brain MRI. Although uncommon brain anomalies were detected consisting of multiple white matter lesions in the one and unusual enlargement and tortuosity of intracerebral blood vessels in both patients. Further vascular anomalies were multiple teleangiectasias seen in the integument in both patients. In addition to facial anomalies, the patients also had psychiatric problems typically seen in velo-cardio-facial syndrome (VCFS). Fluorescence in-situ hybridization analysis confirmed a 22q11.2 microdeletion in both.

P0397. Ring chromosomes 17 and 18 cases report.

S. Amelina[1], *E. Timoljanova*[2], *G. Gurevich*[1], *G. Baibikova*[3], *M. Lukashevich*[4], *S. Kriger*[5]
[1]*Research Institute of Obstetrics and Pediatrics; Rostov-on-Don, Russian Federation;* [2]*Research Institute of Obstetrics and Pediarics; Rostov-on-Don, Russian Federation;* [3]*Research Institute of Obstetric and Pediatrics; Rostov-on-Don, Russian Federation;* [4]*State Medical University; Rostov-on-Don, Russian Federation;* [5]*Research Institute of Obstetics and Pediatrics; Rostov-on-Don, Russian Federation*
S.Amelina@rniiap.ru

Structural aberrations of separate chromosomes led to a wide range of clinical effects in a man. The changes of phenotype of this pathology can be characterized with multiple malformations in one cases and insignificant deflection in physical, somatic and sexual development in the others. Approximately 2,6% of all the cases of aberrations of somatic chromosomes in live birth children compose ring chromosomes. The discription of 2 children with ring chromosomes from unrelated families are presented here. Proband 1. The boy of 12 years old with border-line intellectual insufficiency has suffered with the loss of consciousness from 6 years old. He had multiple pigmentari spots of uncertain diameter placed diffusively on the skin surface. Besides, the child had short stature, stretching face, mongoloid palpebral fissures and spinal hypertrichosis. Karyotype is 46,XY,r(17)(p12q22) Parents karyotypes are normal. Proband 2. The boy of 14 months old was observed in connection with two-sided cryptorchidism. Psychophysical development of this child corresponds to his age. During examination acrocephaly, synophrys, depressed nasal bridge, epicantic folds, prominent mandible were discovered. Karyotype is 46,XY, r(18)(p11q22). Parents karyotypes are normal. Unlike previously described cases cryptorchidism proved to be the only manifistation of these chromosome aberrations.

P0398. Nephronophthisis and ulcerative colitis in siblings - a new association

S. Ala-Mello[1], *H. K ri inen*[2], *O. Koskimies*[3]
[1]*University of Helsinki; Helsinki, Finland;* [2]*The Family Federation of Finland; Helsinki, Finland;* [3]*Hospital for Children and Adolescents; Helsinki, Finland*
sirpa.ala-mello@helsinki.fi

Nephronophthisis (NPH) is a chronic tubulointerstitial nephritis leading to terminal renal insufficiency. The main symptoms are polydipsia and polyuria, caused by decreased urinary concentrating ability, and anemia. The disease is heterogeneous, but usually the inheritance pattern is autosomal recessive. In 80% of cases, the disease is caused by a homozygous deletion in NPHP1 gene in chromosome 2q13. Tapetoretinal regeneration, congenital hepatic fibrosis, neurologic findings and skeletal changes may occur as extrarenal manifestations with NPH. Ulcerative colitis is an inflammatory bowel disease with chronic diarrhea, rectal bleeding and characteristic histological findings. Its etiology is suggested to be multifactorial, consisting of genetic susceptibility and unknown exogenous factors. We present two siblings with NPH and ulcerative colitis and describe their clinical features and laboratory findings.

P0399. A ROR2 mutation in a family with Brachydactyly Type B in the hands and normal feet

C. Bacchelli, *F. R. Goodman, L. C. Wilson, P. J. Scambler*
Institute of Child Health; London, United Kingdom
C.Bacchelli@ich.ucl.ac.uk

Brachydactyly Type B is a dominantly-inherited limb malformation characterised by short 2nd to 5th fingers and toes with hypoplastic or absent nails, small or absent distal phalanges, small middle phalanges and variable symphalangism. Broad thumbs with bifid distal phalanges and central

syndactyly may also occur. In 10 affected families, mutations have recently been identified in *ROR2*, a gene encoding an orphan receptor tyrosine kinase essential for normal chondrocyte differentiation. These probably exert a specific gain-of-function effect (*ROR2* loss-of-function mutations cause Robinow syndrome when homozygous, but no phenotype when heterozygous). We have reviewed a 6-generation Welsh family with limb abnormalities first described by Schott in 1978 as a novel form of hereditary brachydactyly with nail dysplasia . Affected individuals have typical moderately severe Brachydactyly Type B in their hands, with variable involvement of the 2nd to 5th fingers, but no thumb abnormalities or syndactyly. Their feet, however, are completely normal both clinically and radiologically. Sequencing of the entire coding region of *ROR2* in the proband revealed a 2247G to A transition, predicted to convert Trp749 to a stop codon, thereby truncating the protein after its intracellular tyrosine kinase domain. Four similar mutations, including a nonsense mutation in the same codon, have previously been reported to cause a severe amputation-like phenotype in the hands and typical abnormalities in the feet. Our findings extend the range of phenotypes resulting from *ROR2* mutations to include involvement of the hands alone, and suggest that the effects of individual mutations may be significantly modified by genetic background.

P0400. The first case of FRAXE mental retardation in Croatia

S. Hecimovic[1], *R. Bago*[1], *D. Muzinic*[2], *D. Begovic*[2], *K. Pavelic*[1]
[1]*Rudjer Boskovic Institute; Zagreb, Croatia;* [2]*Clinical Hospital Rebro; Zagreb, Croatia*
shecimov@rudjer.irb.hr

FRAXE mental retardation (Xq28) is rare condition that is caused by expansion of the CGG repeat region in the FMR2 gene. Its much more frequent form is known as FRAXA fragile X syndrome (Xq27.3). FRAXE mental retardation is characterized with very mild mental handicap and more atypical clinical picture compared to FRAXA. We report the first FRAXE family diagnosed in Croatia. A pregnant woman (age 26, 16 weeks of gestation) was genetically counseled since one of her two brothers attended a special school for children with learning difficulties. DNA analysis of FRAXA and FRAXE form of the fragile X syndrome showed that she is a full mutation carrier of FRAXE mental retardation. Close clinical examination detected no phenotype characteristics associated with FRAXE full mutation. The couple decided to perform prenatal diagnosis after 18 weeks of gestation. The sex of the fetus was determined to be male. DNA analysis of primary culture of amniotic fluid detected mosaicism of full mutated and normal alleles for FRAXE locus. Linkage analysis of DXS548 and DXS1691 markers also showed that the fetus has inherited the same allele as its FRAXE affected uncle. After genetic counseling the couple decided to retain the pregnancy. Our molecular and clinical data show that FRAXE mental retardation is phenotypically very nonspecific and mild, and that every woman with any mentally retarded family member should be analysed for FRAXA and FRAXE loci of the fragile X syndrome.

P0401. Facio-oculo-acustico-renal syndrome (FOAR) and diaphragmatic hernia in a consanguineous family

V. Klamroth, *M. Herrmann, I. Kennerknecht, J. Horst*
Institut f r Humangenetik,Westf lische Wilhelms-Universit t; M nster, Germany
klamroth@uni-muenster.de

The first patients with facio-oculo-acustico-renal syndrome (FOAR-syndrome, MIM 227290) were described in 1971 and showed facial anomalies with broad forehead, hypertelorism with telecanthus, short nose, severe myopia, hearing loss and proteinuria. In 1993 a diaphragmatic hernia, exomphalos, absent corpus callosum, hypertelorism, myopia and sensorineural deafness syndrome (MIM 222448) was published which showed striking facial similarities with FOAR syndrome. Devriendt et al. (J Med Genet 1998,35;70f) described a male infant combining features of both syndromes further suggesting that these are variations of the same autosomal recessive disorder. We report on a 2-years-old girl with the typical facial appearance, severe myopia and sensorineural hearing loss of FOAR syndrome. She is the second child of a second degree cousin marriage. In the first pregnancy a diaphragmatic hernia was diagnosed by ultrasound scan. The girl was stillborn and showed prominent front, hypertelorism, downslanting palpebral fissures, flat nasal bridge and short nose. Based on our observations and on a review of the literature we postulate that the syndrome of diaphragmatic hernia, exomphalos, absent corpus callosum, hypertelorism, myopia and sensorineural deafness and the facio-oculo-acustico-renal syndrome are variable expressions of the same autosomal recessive syndrome.

P0402. Investigation of the relation between gene expression and genetic variants of FGF2 in patients with coronary artery disease (CAD)

P. Greiser[1], *U. Schagdarsurengin*[1], *S. Schulz*[1], *U. M ller-Werdan*[2], *C. Schn rer*[2], *T. S §*[1], *D. Rehfeld*[1], *A. Nordwig*[2], *L. Weidhase*[2], *K. Werdan*[2], *C. Gl ser*[1]
[1]*Inst. of Human Genetics & Med. Biology, University Halle; Halle, Germany;* [2]*Dep. of Internal Medicine, University Halle; Halle, Germany*
christiane.gl ser@medizin.uni-halle.de

FGF2 is regarded as a specific promoter for proliferation and differentiation of endothelial and smooth muscle cells and as an important risk factor of the atherosclerosis. We determined the FGF2 mRNA-expression in human native monocytes, a cell system srongly involved in the fundamental atherosclerotic processes at the vessel wall, and screened for mutations. 416 patients with angiographically proven severe CAD were included (83% male, mean 50.34y). All patients had associated risk factors like hypertension (47%), diabetes mellitus (22.7%), smokers (77%), cholesterol (5.8 mmol/l). The gene expression was determined by use of a competitive RT-PCR. The mutation screening was carried out by SSCP and sequencing analysis. We identified four genomic variants, all situated in exon 1, the C+242T polymorphism and the mutations C250T, G+19A, G+97C. To reveal the possible effect of the polymorphic genetic variant C242T we calculated the mRNA folding structure (mfold version 2.3, Zucker and Turner) and found a new loop in the region of the functional important first start codon, suggesting a direct influence on functional properties. In accordance with the importance of FGF2 for the atherosclerosis development we found significant differences dependent on the coronary risk; we identified significantly less mutation carries in the high risk patient subgroup who suffered from CAD before 45y (0.091 vs. 0.186, $p<0.05$) suggesting a protective influence of this genetic constellation. Our functional studies of the FGF2 gene product supported this thesis; mutation carriers had a protective significantly decreased gene expression compared with the wild type carriers (0.45ag/U vs. 1.054ag/U, $p<0.04$).

P0403. Cardiac evaluation of 25 Brazilian patients with Friedreich s ataxia.

L. M. J. Albano[1], *R. L. Moyses*[2], *J. Wagenfuhr*[2], *S. A. D. Nishioka*[3], *D. R. Bertola*[1], *S. M. M. Sugayama*[1], *C. A. Kim*[1]
[1]*Genetics, Instituto da Crianca; Sao Paulo, Brazil;* [2]*Cardiology, Instituto da Crianca; Sao Paulo, Brazil;* [3]*INCOR, Instituto de Cardiologia; Sao Paulo, Brazil*
chong@icr.hcnet.usp.br

Friedreich s ataxia (FA), a neurodegenerative disorder, is the most frequent form of hereditary ataxia with a prevalence of 1;50,000. We reported cardiac aspects of 25 Brazilian patients with clinical diagnosis of FA related to the frequency and size of expanded GAA repeats. The cardiac study included physical examination with electrocardiogram and echocardiogram of all patients. Molecular analysis to detect (GAA)n repeat length was performed by PCR, according to Filla et al. (1996). Homozygous GAA expansion repeats were detected in 17 cases (68%) - all typical cases. In 8 patients (32%) (6 atypical and 2 typical), none expansion was observed, therefore they were not FA. All patients with GAA expansion (100%) presented electrocardiogram abnormalities, but only 25% without GAA expansion showed it. The main electrocardiogram abnormality was related to ventricular repolarization (50%). Only 6% of patients referred cardiac signals and/or symptoms. The molecular analysis is essential to confirm the diagnosis of FA, however, a complete clinical and cardiac evaluation, specially an electrocardiogram, may help us to better select the cases that should perform these tests.

P0404. Genetically Determined Reproductive System Disorders (RSD)

J. Kristesashvili, *N. Kopaliani, N. Shervashidze, N. Aladashvili*
Zhordania Institute of Human Reproduction; Tbilisi, Georgia
aladn@access.sanet.ge

157 patients (ages ranging from 5 to 42 years) with different forms of RSD were analyzed (9-intersexes; 89-phenotypically men with hypogonadism, spermatogenesis disorders and infertility; 59-phenotypicallly female with hypogonadism, amenorrhea, delay of physical and sexual development. Clinical, genealogical, genetic, hormonal, ultrasound and other investigations were carried out. Standard methods were used for peripheral blood lymphocyte cultures. G, C, and Q banding were applied for analysis. Results; Out of 9 intersexual patient abnormal karyotypes were found in 2 cases; 47,XXY and 46,XY/47,XXY; In one patient, a heterochromatic polymorphism was detected; 46,XY,15p+ From 69 infertile men with azoospermia, abnormal karyotypes were detected in 22 patients; 47,XXY (5 cases), 46 ,XY/47XXY (16 cases), 46,X del (Yq)(q2.21-qter)- (1 case). From 12

men with severe oligozoospermia and/or teratozoospermia, abnormal chromosomal complements were detected in 4 patients; 47,XYY (1 case), 46, XY,inv(9)(q12q22)-(1 case); 45,XY,t(13;14) (q11;q11) - (1 case), 46,XX (1 case). From 8 male patients with hypogonadism and /or hypospadias, gynecomastia, chromosome aberrations were detected in 5 cases; 47,XXY -(1 case); 46,XY/47, XXY (1 case); 46,XX (2 cases) 46,XY/47,XYY (1 case). From 59 phenotypically female patients, 5 patients revealed androgen insensivity - 46,XY one familial case; 23 cases of Ullrich-Turner syndrome; 45,X (6 cases), 45,X/46,XX (10 cases), 46,X i(Xq) (4 cases), 45,X/46,Xi(Xq), (2 cases), 45,X/46,X r(X) (1 case). 31 from 59 female patients with amenorrhea and hypogonadism revealed the karyotypes; 45,X/46,XX (18 cases), 46,XY (2 cases) pure gonadal dysgenesis,46,XX - (11 cases.) Molecular-cytogenetic investigations were performed in 3 cases to improve the reliability of the cytogenetic analysis(Institut fuer Medizinische Genetik der Universitaet Zurich,Zuerich, Switzerland by U.Wiedemann and A.Baumer) The investigations revealed the high incidence of genetic causes in patients with RSD.

P0405. Osteopathia Striata with Cranial Sclerosis (OS-CS); Multiple Malformations in a Male of an Affected Mother

H. Rott[1], *P. Krieg*[2], *H. R schke*[2], *A. Reis*[1]
[1]*Institute of Human Genetics; Erlangen, Germany;* [2]*St. Annastiftskrankenhaus, Pediatric Dept.; Ludwigshafen, Germany*
hdrott@humgenet.uni-erlangen.de

We report on a family in which mother and two daughters were affected with Osteopathia Striata with Cranial Sclerosis (OS-CS). All patients showed striated bones and cranial sclerosis. One of the daughters gave birth to a male with a dysmorphic syndrome with multiple malformations including severe cranial sclerosis with frontal bossing, cleft palate, thoracic dysplasia, skeletal malformations including bilateral syndactyly of 3. and 4. fingers, bilateral polydactyly of the distal phalanx of both 2. fingers, bilateral fibular aplasia, and anal atresia. There were, however, no bony striations. Karyotype was normal. Linkage analysis with X-chromosomal polymorphic markers is in progress. This is the 13. observation of a severely affected male from women with OS-CS. These sons suffered from a characteristic syndrome with cranial sclerosis, cleft palate, conductive or mixed hearing impairment, thoracic dysplasia, respiratory distress, and various vertebral and intestinal malformations. The majority of cases deceased within the first year of live. Our patient is the first reported case with hypertrophic pylorus stenosis and anal atresia. OMIM quotes OS-CS as an heterogeneous disorder with an autosomal dominant (*166500)and a rare X-linked variant (311280). However, in genetic diseases striated tissue affections are characteristic for genetic mosaicism, either by X-inactivation in females or by early somatic mutation in males. Moreover, among all reported family observations there is no family with convincing autosomal dominant segregation. Therefore, we hypothesise that all cases of OS-CS are X-linked and there is no indication to assume genetic heterogeneity with an autosomal dominat variant.

P0406. A Case with Smith-Lemli Opitz Syndrome Presenting 46, XY, t(7;9)(p14;q21

Z. Candemir[1], *M. Dundar*[1], *Y. Ozkul*[1], *C. Saatci*[1], *S. Kurtoglu*[2]
[1]*Erciyes University, Medical Faculty, Department of Medical Genetics; Kayseri, Turkey;* [2]*Erciyes University, Medical Faculty, Department of Pediatrics; Kayseri, Turkey*
zcandemir@hotmail.com

Smith-Lemli Opitz syndrome is an autosomal recessive disorder characterized by growth retardation, mental deficiency, ptosis of eyelids, anteverted nostrils,limb anomalies, genital abnormalities including hypospadias, micro penis, cryptorchidism. In this report, we present a proband with Smith-Lemli Opitz Syndrome showing a reciprocal translocation between 7p14 and 9q21. The proband is 8 months old Turkish male who referred to Clinical Genetics Department for ambiguous genitalia. He had been admitted to the Pediatrics department for respiratory distress and genitally abnormality. The parents are not consanguineous. On admission, he was 5700 gr weights, 65 cm length and head circumference of 41 cm. All of these measurements were below the 3 percentile. Clinical examination showed wide forehead, inner epicanthal folds, blue iris, strabismus, short nose, anteverted nares, low set and back turned-malformed ears, micrognathia, short fingers with flexion contractures, short phallus, hypoplastic scrotum, cryptorchidism and hypospadias. There was also motor developmental delay and peripheral facial paralysis on the right side. Routine blood biochemical investigations were normal. Abdominal ultrasonography, echocardiography, cranial CT and MR were found to be normal. Chromosome analysis with G and C banding revealed a male karyotype with reciprocal translocation between 7p14 and 9q21. The parent s karyotypes were normal. The explanation of the clinical feature of the patient should be loss of genetic material while recombination of the chromosomes during meiosis.

P0407. State of the art of etiological evaluation of mental retardation in the Netherlands; the Maastricht experience.

U. Moog[1], *E. E. J. Smeets*[1], *L. M. G. Curfs*[1], *C. E. M. de Die-Smulders*[1], *C. T. R. Schrander-Stumpel*[1], *J. P. Fryns*[2]
[1]*Maastricht University; Maastricht, The Netherlands;* [2]*University Hospital Gasthuisberg; Leuven, Belgium*
ute.moog@gen.unimaas.nl

In the Netherlands, the total number of people with mental retardation (MR) is estimated to be 120.000. About 45.000 of them live in institutions or in small group homes (partially) integrated into normal neighbourhoods, and 75.000 live with their families or on their own. Knowledge of the etiology of MR is known to have advantages with regard to prognostic, therapeutic and reproductive counseling. The etiological work-up of MR forms a central part of the work of the 8 Dutch clinical genetics centers and is realized on request of the family and/or general practitioner, or as part of standardized evaluation offered to the institutions. We present our approach to the evaluation of MR which follows an extensive protocol including pre- and postnatal history, physical examination focussing on dysmorphism and the behavioural phenotype, laboratory testing and discussion in a multidisciplinary expert team. The procedure and results are exemplified by presenting data of one of the institutions in South Netherland.

P0408. An amino acid substitution in the HOXD13 homeodomain causes a novel brachydactyly-polydactyly syndrome

F. R. Goodman[1], *C. Bacchelli*[1], *C. M. E. McKeown*[2], *P. J. Scambler*[1]
[1]*Institute of Child Health; London, United Kingdom;* [2]*Birmingham Women s Hospital; Birmingham, United Kingdom*
fgoodman@hgmp.mrc.ac.uk

Mutations in *HOXD13* are known to cause synpolydactyly but have not previously been identified in any other congenital limb malformation. Here we report a 6-generation English family with a novel dominantly-inherited combination of brachydactyly and polydactyly resulting from an amino acid substitution in the homeodomain of HOXD13. All 12 living affected individuals have brachydactyly of the 5th fingers and toes, with rudimentary or absent distal phalanges, small middle phalanges and hypoplastic or absent nails. Most also have similar involvement of the 3rd and 4th fingers and toes, often with shortening of the 3rd to 5th metacarpals. In addition, four individuals have partial duplication of the 4th fingers. This pattern of brachydactyly closely resembles Brachydactyly Type B, recently shown to be caused by mutations in *ROR2*. The central polydactyly, however, is similar to that seen in synpolydactyly, and prompted us to search for a mutation in *HOXD13*. In affected family members, we identified a 940A to C transversion which is not present in unaffected family members or in 50 unaffected unrelated controls. This base change converts a highly conserved isoleucine residue, one of the 4 key amino acids in the recognition helix responsible for directly contacting target DNA, to a leucine. Such a conservative substitution appears likely to alter rather than abolish the DNA binding capacity of HOXD13, thereby perturbing the regulation of target genes and exerting a novel gain of function effect. Studies are now under way to isolate the targets and binding sites affected.

P0409. Molecular analysis in 74 patients with craniosynostosis

I. Weber[1], *M. Ninkovic*[2], *A. R. Janecke*[1], *B. Utermann*[1], *M. Witsch-Baumgartner*[1], *H. Anderl*[2], *G. Utermann*[1]
[1]*Medizinische Biologie und Humangenetik; Innsbruck, Austria;* [2]*Universit tsklinik f.Plastische- und Wiederherstellungschirurgie; Innsbruck, Austria*
ingrid.weber@uibk.ac.at

Craniosynostosis is a common congenital malformation occuring in approximately 1in 2500 live births. Most of the cases are isolated forms. Only few cases represent well known craniosynostosis syndromes. Mutations in fibroblast growth factor receptors (FGFRs)1-3 have been identified in several syndromic forms as well as in non-syndromic craniosynostosis. We enrolled 74 consecutive, unrelated Austrian (58) and South Tyrolean (16) patients with craniosynostosis in a clinical genetic and molecular investigation of exons of FGFR1-3 genes. So far we identified FGFR2 (S252W, P253R, C278F, C342S) and FGFR3 (P250R) mutations in 14 patients (17%). The clinical diagnosis of Apert syndrome was confirmed in all 6 patients. In 3 out of 8 cases of Crouzon syndrome we identified a mutation in FGFR2. In 5 patients with a clinical diagnosis of brachycephaly (3 cases), plagiocephaly (1), and Seathre-Chotzen syndrome (1) we identified the P250R mutation in FGFR3 causing Muenke syndrome. In each of

3 families with the Muenke mutation affected family members had the same type of craniosynostosis. Thus FGFR mutations are a common cause of syndromic and non-syndromic craniosynostosis in our large Austrian/South Tyrolean patient sample, too. Screening for the P250R mutation in FGFR3 is recommended especially in cases of unclassified craniosynostosis. In a next step 6 families without identified mutations will be analyzed for linkage.

P0410. Clinical presentation of adult Rett syndrome with detectable MeCP2 deletion

E. E. J. Smeets[1], *L. M. G. Curfs*[1], *M. J. Descheemaeker*[2], *U. Moog*[1], *J. P. Fryns*[2]
[1]*Maastricht University; Maastricht, The Netherlands;* [2]*Center of Human Genetics; Leuven, Belgium*
eric.smeets@gen.unimaas.nl

Rett syndrome (RS) is a childhood neurodevelopmental disorder that affects females(almost exclusively) with an incidence of 1 in 10.000 to 15.000. After an apparently normal period of development, acquired milestones are lost at an age of 6—18 months and a specific neurological disorder evolves with stereotypic hand wringing or clapping and preserved visual eyepointing as the most typical symptoms. Loss of speech and dystonic movement disorder together with severe cognitive deficit and epilepsy are predominant in this complex and puzzeling disorder leading over decades into severe multi-impairment. In 1999, RS was shown to be caused by deletions in the MECP2 gene by Zoghbi et al.. MECP2 deletions account for up to 75% of cases with classical RS. We present 8 cases of adult females with a deletion in the MECP2 gene. Diagnosis of RS was made conform to the clinical criteria although it was not always easy to confirm this diagnosis on the basis of retrospective clinical data alone. The clinical, neurological, behavourial and habilitation data will be presented and an attempt is made to phenotype-genotype correlation.

P0411. Kallmann Syndrome in a Patient with t(3;14)(q29;21)

E. F. Litvinovich[1], *A. Y. Savtchenko*[1], *I. Lapoutin*[2], *N. Gerasimenko*[2], *G. Stephanenko*[2], *A. Touritsa*[3], *O. Levtchenko*[3], *V. Volchenko*[3]
[1]*Omsk State Medical Academy; Omsk, Russian Federation;* [2]*Regional Medico-Genetic Center; Omsk, Russian Federation;* [3]*State Medical Academy; Omsk, Russian Federation*
levtchenko77@mail.ru

Most cases of Kallmann syndrome are caused by mutations or deletions of the KALX gene on the Xp22.3. However, Kallmann syndrome seems to be heterogeneous with at least one autosomal form. Syndrome has been reported in several patients with unbalanced (Schinzel et al., 1995) or balanced (Best et al., 1990; Cassamassima et al., 1993; Kroisel et al., 2000) autosomal rearrangements. We have observed the young man with Kallmann syndrome and apparently balanced translocation. The patient, age 22, has anosmia, eunuchoid habitus, hypogonadism and hypogenitalism, absence of body hair, underdevelopment of axillary and pubic hair, shortening of the 4th metatarsal bones, dry and flaccid skin. The results of endocrinological investigations were compatible with hypogonadotropic hypogonadism; very low basal LH (0.5 IU/l) and FSH (0.8 IU/l) and prepubertal testosterone (2.7 nmol/l). Cytogenetic study showed a balanced translocation t(3;14)(q29;q21). Therefore, the segments 3q29 and 14q21 should be added to the list of possible locations of autosomal gene of Kallmann syndrome.

P0412. Molecular Diagnosis Of Fraxa In Costa Rica.

P. Cuenca[1], *F. Morales*[2], *I. Castro*[3]
[1]*Instituto de Investigaciones en Salud y Escuela de Biolog a; San Jos , Costa Rica;* [2]*Instituto de Investigaciones en Salud y Escuela de Biolog a Universidad de Costa Rica; San Jos , Costa Rica;* [3]*Instituto de Investigaciones en Salud y Escuela de Medicina, Universidad de Costa Rica; San Jos , Costa Rica*
pcuenca@cariari.ucr.ac.cr

Fragile X chromosome syndrome is the most frequent form of hereditary mental retardation. Costa Rica s health system does not offer molecular assessment as part of the diagnostic tools to the patients and families. The objectives of this project are; 1. To investigate the presence of unstable mutations in the FMR1 gene, to determine it s methylation status and the number of repetitions of the CGG triplet in affected individuals and their families. 2. To offer genetic counseling to the families. 3. To enhance the knowledge of this disorder among health personnel and special education teachers. We have worked with children who had a previous cytogenetic diagnosis of fra(X) syndrome (group one, N=13), their near relatives (group two, N=30) and with children referred to us by teachers, pediatricians and psychologists (group three, N=15). Southern blot and PCR have been used to perform 58 molecular studies. Among the group one children, nine had a full mutation of the FMR1 gene and four were negative since both methods showed normal results. In group two there were two females with the full mutation and 12 individuals with the pre-mutation; a normal transmitting male and eleven female carriers. The rest of the relatives showed normal results. In group three only a girl was detected with the full mutation, the rest were normal, both at the molecular as well as the cytogenetic level.

P0413. Smith-Magenis syndrome; where genetics and behavioural sciences meet

L. M. G. Curfs[1], *D. Willekens*[2], *U. Moog*[1], *E. E. J. Smeets*[1], *J. P. Fryns*[2]
[1]*Maastricht University; Maastricht, The Netherlands;* [2]*Center of Human Genetics; Leuven, Belgium*
curfs@msm.nl

In this presentation, we emphasize the contribution of the behavioural approach in diagnosing Smith-Magenis syndrome (SMS). SMS is a clinically recognisable multiple congenital anomaly/mental retardation syndrome caused by an interstitial deletion of chromosomeregion 17p11.2. In our clinical practice, 11 subjects were diagnosed to have SMS. Data on psychopathology and examination of cognitive and behavioural profiles in these patients demonstrate that the behavioural phenotype serves as a useful diagnostic marker for SMS. After reviewing the published data on the distinctive behavioural features, recommendations are made for the indication of a diagnostic test by FISH with a probe specific for 17p11.2.

P0414. Neuropsychological Profile In Kabuki Syndrome

C. T. R. M. Schrander-Stumpel[1], *H. van der Vlugt*[2], *K. J. Berndsen-Peeters*[2], *L. M. G. Curfs*[1]
[1]*Maastricht University; Maastricht, The Netherlands;* [2]*University of Tilburg; Tilburg, The Netherlands*
connie.schrander@gen.unimaas.nl

The Kabuki (Niikawa-Kuroki) syndrome was reported in 1981 by Niikawa et al. and Kuroki et al. in a total of 10 unrelated Japanese children with a characteristic array of multiple congenital anoma-lies and mental retardation. The syn-drome is characterized by a distinct face, mild to mode-rate mental retardati-on, postna-tal growth retarda-tion, derma-toglyp-hic and skeletal abnor-malities. In Japan, the syndrome appears to have an incidence of about 1;32,-000 newborns. Outside of Japan, a growing number of patients has been recognized. In 2/3 of the non-Japanese pa-tients serious neurologic problems were pre-sent, most notably hypot-onia and feeding problems. Behavioural characteristics and neuropsychological profile are not wellknown with only a limited numbers of case reports in the literature. In close collaboration with the national Kabuki network a neuropsychological research aimed at the cognitive and behavioural phenotype was stated. The present paper reports the results of the neuropsychological evaluation in a series of 10 children with Kabuki syndrome ranging in age from 5 — 11 years.

P0415. Encephalopathy in a Person with t(2;15)(q37;q21)

A. Savtchenko[1], *E. Litvinovich*[1], *I. Laputin*[1], *T. Kulakova*[1], *N. Gerasimenko*[1], *L. Kopylova*[2], *A. Touritsa*[1], *A. Scherbakov*[1], *V. Voltchenko*[1], *O. Levtchenko*[1]
[1]*Omsk State Medical Academy; Omsk, Russian Federation;* [2]*Regional Medico-Genetic Center; Omsk, Russian Federation*
levtchenko77@mail.ru

A tall (185 cm) young man, age 16, presented with muscular hypotonia, postural instability, fine tremor of fingers, nystagmus, arachnodactyly, thick and long eyelashes. Papilledema was found upon examination of ocular fundi. Abdominal diagnostic ultrasound; characters of chronic cholecystitis. MRT showed asymmetric internal hydrocephalus. Wilson's disease was excluded by normal content of copper and ceruloplasmin in blood and urine. Content in blood serum; Alat — 0,18 mol/l (N 0,1 — 0,68), Asat — 0,5 mol/l (N 0,1 — 0,45), thymol test — 2.1 units (N until 5), total bilirubin — 12,8 mol/l (N 8,5 — 20,5), ?-lipoproteins - 30 FE (N 35-55). Cytogenetic examination showed an apparently balanced translocation t(2;15)(q37;q21). According to the amount of chromosome imbalance, the risk of posterity with the developmental defects (25-28 %) and spontaneous abortions (22-25 %) exists.

P0416. Clinical trial in 18 patients with features of Kabuki syndrome

G. S. Oliveira, *E. L. Sartorato, A. Marques-de-Faria*
Unicamp; Campinas, Brazil
giso@bestway.com.br

The Kabuki (Niikawa-Kuroki) syndrome (KS), first and independently described in Japan in 1981 by Niikawa et al. and Kuroki et al., is a multiple congenital anomalies/mental retardation syndrome characterized by a

peculiar facies which resembles the Kabuki theatre make-up, with long palpebral fissures and eversion of lower lids, arched eyebrows, depressed nasal tip and prominent ears, besides mild to moderate mental retardation, postnatal growth retardation, skeletal abnormalities and dermatoglyphic anomalies. The incidence in Japan was estimated around 1;32,000 newborns, and the frequency of non-Japanese patients is not available, although the number of cases has been increasing each year. The etiology remains undetermined and most cases have been sporadic. In Brazil, there are few reports of the condition, all of them corresponding to new case descriptions and not the result of a systematic survey. Therefore, we decided to elaborate the present study. Between June, 1998 and December, 1999, 18 patients were followed-up by a specific clinical and laboratory protocol, based upon the main characteristics of KS. Ten individuals (3 females and 7 males) were confirmed as having KS. Of the remaining 8 (4 females and 4 males), 3 had Noonan syndrome, 2 had Turner syndrome, 1 had monosomy 18p and the other 2 did not complete their clinical evaluation. Among the KS patients, 100% presented the peculiar facial aspect, and mental retardation in different degrees, 80% had dermatoglyphic abnormalities, 60% had vertebral anomalies and 50% presented with short stature. The present study was similar to several others in almost all aspects. Cases were sporadic, sex ratio was not deviated, and there were no references to parental consanguinity or familial recurrence, which supports the hypothesis of a mutation in a dominant inherited gene, the suggested etiology of KS. This protocol seemed useful by characterizing the studied population and should be applied to patients with clinical features of KS. It deserves mention that the facial characteristics may occur in diverse conditions, leading to a possible Kabuki phenotype determined by elongated palpebral fissures, eversion of the lower lid and arched eyebrow. The high degree of overlapping clinical features concerning Noonan and Turner syndrome, especially in patients with chromosome X rings, reinforces the necessity of a through evaluation when one of these conditions is considered present at initial diagnosis. More detailed studies concerning these issues and involving several genetic centers might contribute to a better characterization of KS.

P0417. Triopia; the second case in the literature.

D. Moretti-Ferreira[1], *R. L. F. Lima*[2], *J. Murray*[3], *A. Richieri-Costa*[4]
[1]*Dept of Genetics, IBB- UNESP; Botucatu, Brazil;* [2]*Dept. of Genetics, IBB/UNESP; Botucatu, Brazil;* [3]*Dept. of Pediatrics&Biological Sciences, University of Iowa; Iowa City, United States;* [4]*Dept. of Cinical Genetics-HRAC-USP; Bauru, Brazil*
sag@fmb.unesp.br

Triopia (three eyes) is a very rare condition. It was first reported by Stelnicki et al. (1995). These authors interpreted the presence of two globes in the left orbit as prosencephalon duplication. We have recently assessed a male patient, second child of a young, healthy, non-consanguineous couple. The patient presented a complex malformation involving the central nervous system, craniofacial structures and remarkedly the eyes. Main clinical findings included broad and backward sloping forehead, plagiobrachycephaly, wide skull sutures, a large facial cleft extending upward to the nose, a Tessier number 9 bilaterally, abnormally developed orbits with two hypoplastic and colobamatous globes at right and a hypoplastic and colobamatous eye at left (triopia), and low set and posteriorly rotated ears. G-banded chromosomes were normal. Molecular study of the gene PAX6 showed no mutation. MRI revealed no duplication of prosencephalie structures. The gestation was uneventful without any known ingestion of teratogens by the mother. Up to now, we have been seeking for an embryologic explanation for the ocular malformation presented by the patient. Supported by; Funda o Lucentis

P0418. Systemic Echovirus Infection in neonate mimicking a metabolic liver disease

D. Babovic-Vuksanovic, *M. El-Youssef*
Mayo Clinic; Rochester, MN United States
dbabovic@mayo.edu

We describe a neonate with systemic Echovirus Type 11 infection, who presented with an acute hyperammonemia, rapidly progressive multisystemic failure and fatal outcome. The patient presented at age 7 days with acute hyperammonemia, lethargy and hypothermia. Despite an aggressive therapy including inotropic support, ammonia conjugating therapy and hemodialysis, symptoms progressed and led to the fatal outcome at age 9 days. A dominant symptom of hyperammonemia in an otherwise normal neonate without known risk factors was suggestive of a metabolic decompensation. The patient underwent an extensive diagnostic evaluation. After exclusion of several metabolic disorders, the diagnosis of the infectious etiology was confirmed by Polymerase Chain Reaction (PCR) in liver and spleen tissue. Increased awareness for a number of metabolic disorders and improved diagnostic capabilities for detection of rare inherited conditions may bias toward neglecting other etiologies of neonatal liver failure. The possibility of perinatal infection as a cause of severe hyperammonemia and fulminant hepatic failure should be kept in mind when evaluating a sick neonate.

P0419. A new case of cranio- osteoarthropathy - clinical study

C. Rusu[1], *M. Volosciuc*[1], *A. Vlad*[2], *M. Covic*[1]
[1]*University of Medicine; Iasi, Romania;* [2]*Children s Hospital; Iasi, Romania*
abcrusu@yahoo.com

Cranio- osteoarthropathy is a rare AR disorder defined by hypertrophic osteoarthropathy, clubbed fingers, painful periarticular swelling, chronic effusion in large joints and eczematous skin lesions. We present a case in order to illustrate this rare disorder and to present some particular features recorded in this case. Our proband is the second child of a healthy, young, unrelated couple. Pregnancy and birth were uneventful. Postnatal development was normal, except the early tooth eruption and frequent chest infections. We have examined the child when he was 5 years old. He presented tall stature, astenic build with diminished muscle bulk, increased sweating, delayed closure of the anterior fontanelle (still present!), matinal pain and swelling of large joints and clubbing of fingers and toes. Radiographies showed mild bone resorbtion of distal phalanges and tubular coarse long bones. Lab investigations showed anemia and oxalic cristals in the urine. Based on the clinical and radiological features we have established the diagnosis of cranio- osteoarthropathy. We have done the differential diagnosis with infantile cortical hyperostosis and pachydermoperiostosis. In conclusion, we present a child with cranio- osteoarthropathy to illustrate this very rare disorder and to present the association of early tooth eruption, frequent chest infections, anemia and presence of urinary oxalic cristals, features not cited in the literature until now.

P0420. No evidence for mutations in the HLXB9 gene in the DNA of patients with anal sphincter dysplasia

M. Hergersberg[1], *J. Hirsig*[2], *A. Buchmann*[3], *V. Schneider*[1], *A. Schinzel*[1], *B. R thlisberger*[1]
[1]*Institut f r Medizinische Genetik; Z rich, Switzerland;* [2]*Kantonsspital; Winterthur, Switzerland;* [3]*Stadtspital Waid; Z rich, Switzerland*
hergie@medgen.unizh.ch

Anal sphincter dysplasia (ASDP) has been described as anteriorly or ventrally displaced anus (OMIM 105563). Absent or incomplete fixation of the sphincter to the coccyx are demonstratable by computer tomography as well as by intraoperative dissection of the sphincter muscles. The range of symptoms included chronic constipation, severe straining at defecation, encopresis, and chronic paradoxical diarrhea with fecal incontinence. This congenital malformation occurs often familial, and an autosomal dominant mode of inheritance with reduced penetrance has been suggested. Recently, mutations in the HLXB9 homeobox gene have been described in the DNA of patients with Currarino syndrome, an autosomal dominant inherited form of sacral agenesis. Because anorectal malformations are a component of the Currarino syndrome, the HLXB9 gene was analyzed in the DNA of 20 probands affected from ASDP with several affected family members. The three exons of the HLXB9 gene were amplified using nine different primer pairs, and the PCR products were analyzed by single strand conformation polymorphism (SSCP) analysis. There was no evidence for mutations in this gene in the analyzed DNA samples.

P0421. Achondrogenesis type IA; prenatal, clinical, radiological and pathological study of a new case

M. Covic[1], *C. Rusu*[1], *G. Iliev*[1], *D. Mihaila*[2]
[1]*University of Medicine; Iasi, Romania;* [2]*Children s Hospital; Iasi, Romania*
genetica_umfiasi2000@yahoo.com

Achondrogenesis is a lethal chondrodysplasia defined by short trunk, severe micromelia, normal head size and specific skeletal changes. We present a case of achondrogenesis type IA in order to underline the possibilities of prenatal diagnosis and also the importance of detailed radiologic and pathologic examination for the final diagnosis and genetic counselling. Our proband is the first child of a healthy, young, unrelated couple. Pregnancy evolved with threatened abortion; at 28 weeks gestation fetal ultrasound scan recorded polyhidramnios, very short limbs, hypoplastic lungs, hydronephrosis. Therapeutic termination of pregnancy was indicated. Physical examination of the fetus revealed; dysmorphic face, narrow chest, very short limbs, generalized edema. Radiologic investigation showed; poorly ossified skull, unossified vertebral bodies, crenate ilia, short square long bones. Pathologic examination also revealed hypoplastic lungs and hydronephrotic kidneys with dilated ureters. Histopathologic studies demonstrated hypercellular cartilage with clustered chondrocytes within a diffuse matrix and PAS positive, diastase resistant granules inside the

chondrocytes. Based on the clinical and paraclinical data, we have setablished the diagnosis of achondrogenesis type IA, AR. We have done differential diagnosis with other lethal chondrodystrophies (homozygote achondroplasia, thanatoforic dysplasia) and the other forms of achondrogenesis. In conclusion, we present this case to show the importance of prenatal and pathologic examination for the complete diagnosis and correct genetic counselling in lethat chondrodystrophies.

P0422. Sensorineural hearing loss and the incidence of connexin 26 mutations in Austria

A. R. Janecke[1], *D. Nekahm*[2], *A. Hirst-Stadlmann*[2], *J. L ffler*[1], *G. Utermann*[1]
[1]*Institute of Medical Biology and Human Genetics, University of Innsbruck; Innsbruck, Austria;* [2]*Department of Hearing, Speech and Voice Disorders, University Hospital of Innsbruck; Innsbruck, Austria*
Andreas.Janecke@uibk.ac.at

Hearing loss (HL) is the most common sensory deficit and is detected in about 1/1000 children before 1 year of age. HL has a genetic origin in up to 60% of cases and is of an unparalleled heterogeneity. A clinical evaluation and Cx26 mutation analysis were performed in 155 consecutive patients with sensorineural hearing loss in order to delineate the spectrum of genetically caused hearing loss. The aetiology of HL was determined to be hereditary in 47% of patients, either due to Cx26 mutations (18.7%), to genetically caused congenital syndromes (11.6%) or to the presence of a positive family history (16.8%). Cx26 mutations were found in 21.2% of patients with non-syndromic HL (31.5% of familial vs. 17% of sporadic cases). The mutation 35delG accounted for 46.6% of all presumed GJB2 disease alleles. The second most frequent mutation was L90P (10.3%) having been reported with a prevalence of 0,7-3,5% in other populations. Of three novel mutations, one (R143Q) was associated with dominant high-frequency hearing loss. A dominant de novo mutation, R75W, was observed in a patient with profound HL. Pseudodominant transmission of NSHL was seen in 4 families with Cx26 mutations. A mutation 35delG carrier rate of 0,9% was observed among 672 controls from Tirol (West-Austrian province). Cx26 mutations were found associated with mild to profound, and with asymmetric hearing impairment.

P0423. Genetic Studies of Couples with Recurrent Fetal Losses

S. Oztas
Ataturk University, Medical Faculty, Department of Medical Genetics; Erzurum, Turkey
sitkioztas@asteoclub.com

In this study, we aimed to determine the role of genetic factors in our region in the couples with habituel abortions and/or stillbirth to help couples with this kind of situation. In our study, we have a total of 419 people. Of this two hundred two couples (404 cases) are recurrent fetal losses, and 5 are cordocentesis materials from pregnant women with fetal anomalies, and finally we have 10 death babies with multiple malformations of the perinatal period. The distribution of the couples with fetal losses is as followed; 122 couples with habituel abortions whose abortion number vary for each case between 2 and 10, and 50 couples with up to 5 stillbirths, and 30 couples with both abortion and stillbirth. While the women s ages were varying between 18 and 42, men s ages were changing between 21 and 58 in all groups. Consanguinity rate among the parents was 25,2%. This ratio was 18,9% among the parents with habituel abortions, 36% among the parents with stillbirths and 33,3% among the parents with both habituel abortion and stillbirth. One hundred thirty five couples of 202 analysed for cytogenetics. While men did not show any chromosomal aberrations, a total of 6 women revealed various chromosomal aberration A total of 137 stillbirth cases or early death babies of perinatal period were recorded by 80 couples. Sixty eight of 137 cases have various anomalies (49,6%). Distribution of the etiology of malformations showed that mendelian disorders were 27,7% , multifactorial disorders were 16,1% and chromosomal disorders were 5,8%.

P0424. Expansion of Clinical Features in Ehlers-Danlos Syndrome type VIIA with a Newly Recognized COL1A1 Mutation.

T. Neumann[1], *A. Eigel*[1], *J. Horst*[1], *I. Kennerknecht*[1], *B. Steinmann*[2]
[1]*Institut f r Humangenetik, Westf lische Wilhelms-Universit t; M nster, Germany;* [2]*Division of Metabolism and Molecular Pediatrics, Kinderspital; Z rich, Switzerland*
thomas.neumann@uni-muenster.de

The Ehlers-Danlos syndrome (EDS) is a heterogenous group of inherited connective tissue disorders. EDS VII results from loss of the procollagen N-protease cleavage site by exon 6 skipping of COL1A1 (EDS VIIA) or COL1A2 (EDS VIIB), or by mutations in the pNPI gene itself (EDS VIIC). Distinct clinical features can be observed in EDS subtypes VIIA, VIIB (both arthrochalasia type) and VIIC (dermatosparaxis type). We present the clinical phenotype of a 12 year old boy with a newly recognized, heterozygous de novo mutation of COL1A1, i.e. IVS5-1G>T in the splice-acceptor site of IVS5/exon 6. cDNA analysis confirmed exon 6 skipping in COL1A1 mRNA. In addition to the cardinal features of EDS VIIA our patient had premature rupture of fetal membranes, dentinogenesis imperfecta and recurrent rectum prolapse, the last feature was so fare not described in EDS VIIA. Rectum prolaps is a sign of tissue extensibility seen for example in EDS I (classical type). Premature rupture of fetal membranes is described in EDS VIIC and EDS I, but our patient does not show sagging, redundant skin, a major criteria of EDS VIIC. Dentinogenesis imperfecta is a well known feature of osteogenesis imperfecta but until last year has not been described in EDS patients. This observation expands the clinical spectrum of EDS VIIA and shows overlapping clinical features with classical EDS, EDS VIIC, and osteogenesis imperfecta.

P0425. A novel mutation in exon V of the human neutrophil elastase gene; Association with Focal Segmental Glomerulosclerosis in Black South African paediatric patients.

P. Redelinghuys, *M. C. Gaillard, P. D. Thomson*
University of the Witwatersrand; Johannesburg, South Africa
pierre_r@mweb.co.za

Aims; Focal Segmental Glomerulosclerosis (FSGS) in its acute stages, is characterised by neutrophil-mediated inflammation and glomerular injury. The aim of our study was to investigate the presence of mutations in exon V of the human neutrophil elastase (HNE) gene in South-African paediatric renal patients presenting with Nephrotic Syndrome (NS). Methods;Forty-three Black(27 boys;16 girls) and 15 White (13 boys;2 girls) paediatric nephrotic patients were recruited from the Paediatric Nephrology Unit, Johannesburg Hospital, South-Africa. These included those presenting with FSGS, Minimal Change Disease (MCD), congenital NS and other renal conditions. HNE exon V was amplified from whole blood DNA. Amplicons were sequenced and screening extended by ARMS-PCR. Significance was assessed using two-sided Chi-squared and Fisher s Exact tests. Results; A novel GTC (Val190) to ATC (Ile190) transition was detected in HNE exon V in 4 of 18 (22%) Black patients with FSGS as compared with asymptomatic Black controls (0%)(p=0.008). The mutation was absent from Black patients with other renal diseases as well as from White patients.Protein modelling revealed the position of the mutation to be in the substrate binding pocket of HNE where the residue plays a role in substrate specificity. Conclusions; A novel mutation in the substrate binding pocket of HNE is associated with FSGS in Black paediatric nephrotics, which may explain the increased prevalence of FSGS in this group. Alterations in HNE substrate specificity may contribute to glomerular injury.

P0426. International Birth Defects Information System (IBIS) - A Prevention Tool

W. Wertelecki
University of South Alabama; Mobile, AL United States
wwertele@usamail.usouthal.edu

It is self evident that the prevention of birth defects (BD) is a complex task and that the World Wide Web (web) is a major communicaiton tool. Many web information providers are organizations with missions that are narrower than the full range of BD related issues. On the other hand, many international information consumers have limited access to and the needed skills necessary to expeditiously find scattered reliable BD web information sources. Furthermore, not-in-English materials are limited and many web sites offer incomplete, outdated or even slanted information. Such considerations prompted the development of IBIS, a web site for international information consumers and dedicated to the prevention and amelioration of BD. IBIS started by sharing resources of programs in Alabama, Ukraine, and Latin America. (http;//ibis-birthdefects.org/start/) IBIS offers access to carefully selected web sites and not-in-English sources. International contributors are urged to develop and post on IBIS, BD information sheets that adhere to published quality standards. IBIS versions in Ukrainian and Spanish are growing and versions in other languages are emerging. In a six-month period, and without promotion, consumers visited IBIS at a rate of 50000 per year. Some related satellite sites have even a greater number of visitors. Organizations can contribute to IBIS by concentrating on web site contents related to their mission and linking with IBIS regarding other matters relevant to the care, prevention, and amelioration of BD.

P0427. Terminal 22q13 deletion, recognizable phenotype or incidental finding?

G. S. Kupchik
M.M.C; Brooklyn, NY United States
gabriel@pol.net

Terminal 22q13 deletions are rare. Although a few reported cases have had variable clinical findings, it appears that a recognizable phenotype which includes mild facial dysmorphism, pervasive developmental disorder and hypotonia is emerging. We present a 2- year old female referred due to multiple congenital anomalies, dysmorphic features and unexplained hypotonia. After birth she was found to have an hypoplastic left pulmonary artery with anomalous aorto-pulmonary vessels that required surgical correction. A routine karyotype was initially reported as normal. A 22q13 deletion was detected incidentally when the control probe for the DiGeorge/VCFS region (ARSA) was found deleted. Parental karyotypes have been reported as normal. Interestingly, in a recent review, 3 out of 4 patients previously thought to have an isolated 22q13 terminal deletion were found to have a cryptic subtelomeric rearrangement instead. By presenting this case and reviewing the literature, we would like to emphasize the need for further studies and investigation on patients presenting with this phenotype.

P0428. The incidence of renal and pulmonary complications during pregnancy in women with tuberous sclerosis complex (TSC); a survey of female members of the National Tuberous Sclerosis Association (NTSA)

***A. L. Mitchell**, M. A. Parisi, V. P. Sybert*
University of Washington; Seattle, WA United States
almitch@u.washington.edu

Adults with TSC may develop renal and pulmonary complications. Approximately 70% have renal cysts and/or angiomyolipomas (RAML), which can lead to renal failure or hemorrhage. 2-3% develop pulmonary cysts or lymphangiomyomatosis, which can cause recurrent pneumothorax and/or progressive respiratory insufficiency. Whether pregnancy exacerbates these risks is unknown. 113 of 420 questionnaires sent to NTSA members for information regarding pregnancy were returned. Seven lacked sufficient information to confirm TSC; 3 were incomplete. 22 women had never been pregnant (11 with renal disease and 6 with lung involvement); 3 had RAML without TSC. 49 women with renal involvement had 122 pregnancies, and 29 without renal involvement had 58 pregnancies. Pregnancy complications included bleeding from RAML in two, renal pain in one, and kidney stones in two. One woman without kidney disease developed pregnancy-associated renal stones. A fifth of both groups developed toxemia. Pneumothorax occurred in 2 women. No maternal or fetal deaths occurred. Among women never pregnant, renal/pulmonary complications developed in 36% and 33%, respectively. Limitations of the study included inability to determine if renal involvement predated pregnancy (in many, diagnosis of TSC was made after birth of an affected infant); and validity of self-reports. While the literature reports a 21% incidence of renal hemorrhage during pregnancy (7 out of 33 pregnancies), our results suggest it is much lower. We recommend that women with TSC receive renal and pulmonary evaluation prior to pregnancy. Pregnancies should be closely monitored. The presence of either RAML or pulmonary cysts is not a contraindication to pregnancy.

Pregnancy outcomes in a survey of adult women with TSC

	N	G	P	SAb	TAb	On-going/ no info	ectopic	SB	renal compli-cations	Pulm. compli-cations	Post-partum depres-sion	toxemia/ HTN
pregnant + renal disease	49	122	94	19	3	2	1	3	5/49 (10%)	1/10 (10%)	2	11 (23%)
pregnant - renal disease	29	58	50	6	1	1	0	0	1/29 (3%)	1/4 (25%)	1	6 (20%)
RAML w/o TSC	3	6	5	1	0	0	0	0	1/3 (33%)	0	0	0

P0429. The 22q11 deletion syndrome ; Evaluation of thyroid dysfunction, from Hashimoto thyroiditis to and increased risk for familial form of Graves disease.

***B. Leheup**[1], F. Marcon[2], P. Bordigoni[3], P. Jonveaux[4]*
[1]Unit of Clinical Genetics CHU de Nancy-Brabois; Vandoeuvre, France; [2]Unit of Pediatric Cardiolology CHU de Nancy-Brabois; Vandoeuvre, France; [3]Department of Pediatric Hematology CHU de Nancy-Brabois; Vandoeuvre, France; [4]Department of Medical Genetics CHU de Nancy-Brabois; Vandoeuvre, France
leheup@facmed.u-nancy.fr

Thyroid abnormalities have been reported in association with the 22q11 deletion syndrome. One case of congenital hypothyroidism linked to thyroid dysgenesis has been reported by Scuccimarri (). We previously reported in two patients with the syndrome of 22q11 deletion the onset of Hashimoto type of auto-immune thyroiditis (ESHG Lisbon 1998). Recently a case of Graves disease has been reported in a 18 year old woman with evidence of DiGeorge syndrome related to a 22q11 deletion (Kawamura T 2000). Herewith we report on a patient with the 22q11 deletion syndrome in whom a Graves syndrome complicated the clinical evolution at the age of 18 years. He was born in 1980 from a 28 years old father and 24 years old mother who had been previously treated for a Graves disease. At birth the diagnosis of cardiac malformation (aortic arch kinking, retro-oesophageal right subclavian artery) and a posterior palatine cleft lead to the discussion of a mild form of DiGeorge syndrome. There was no evidence for immune deficiency or hypocalcemia. Later on developmental milestones were delayed and his school progression was delayed. His diagnosis was not reevaluated before 1998 at which time he developed evidence for Graces disease with high levels of antiTSH receptor and antithyroperoxydase antibodies. 22q11 deletion was demonstrated by FISH. His hematological and immunological evaluation show mild thrombopenia with increased mean volume, low level of IgM, decreased response to polysaccharide vaccination antigenes, mild lymphopenia and decreased T lymphocyte response. Compared to our two cases with Hashimoto thyroiditis and other 22q11 deleted patients, the immunological profile is similar. Thyroid dysfunction in 22q11 deleted patient is therefore quite frequent and requires regular evaluation of both the antibody profile and thyroid function during follow-up, especially if there is a familial predisposition for auto-immune disease.

P0430. Weaver Syndrome and Wilms Tumour in a child with an Unbalanced Cryptic Translocation . 7 Individuals with Mental Retardation in the One Family due to Translocation - t(12;22)(q24.33;q13.31).

***A. M. Turner**[1], M. Rosier[2], R. Cohn[1], G. Turner[3], K. Battese[4], M. Yip[1]*
[1]Sydney Children s Hospital; NSW, Australia; [2]Canberra Hospital; ACT, Australia; [3]Hunter Genetics; Newcastle, Australia; [4]Canberra Hospital; ACT, Australia
A.Turner@unsw.edu.au

A female child was diagnosed with Weaver syndrome based on typical dysmorphic features and growth pattern. She had developmental delay and overgrowth. She was hypotonic with delay in motor and other milestones obvious by 12 months. By 4 months height and weight >90th percentile and head large. By 7 months ht, wt >97th. Bone age was advanced - 18/12 at 11/12 and 3 yrs 6 mths at 1 yr 11mnths. At 3 years a Stage 1 left Wilms tumour was diagnosed and successfully treated with radiotherapy and chemotherapy. There was a history of mental retardation. Her brother was mild/mod delayed in development without dysmorphism. The mother had two normal looking brothers with moderate intellectual handicap. Karyotypes on all retarded individuals and parents normal. Fragile X testing of both children and uncles normal. 3rd child (female) born with very similar dysmorphic features to proband but without significant overgrowth. Repeat karyotypes and careful review showed mother has a balanced translocation (12;22) and both female offspring have unbalanced karyotype - Der 12 and male has Der 22. These results confirmed by FISH. Further family studies showed that both mother s brothers, uncle and cousin carry the Der 22 chromosome. This case illustrates that in retardation without dysmorphism, malformation or poor growth (non specific mental retardation) can be due to crytpic aneuploidy. It also shows that in a suspicious case a careful cytogenetic review can direct FISH studies appropriately. The question of whether some or all of the features of Weaver syndrome are due to monosomy of genes at 12q and/or trisomy of telomeric genes at 22q is raised.

P0431. Screening for Smith-Magenis Syndrome among a population of undiagnosed mental retardation/developmental delay

***M. M. Khalifa**, J. L. Struthers*
Queen s University; Kingston, ON Canada
mkhalifa@jhmi.edu

Smith-Magenis syndrome (SMS) is a MCA/MR syndrome caused by a microdeletion of chromosome 17p11.2. Individuals with SMS are typically characterized by minor craniofacial anomalies, short stature, brachydactyly, visual and auditory impairment, behaviour problems, sleep disturbance, and cardiac and renal malformations. The exact incidence of this condition is unknown, but it is believed that this condition is underdiagnosed because of its recent description and the lack of a pathognomonic phenotype. To estimate the frequency of SMS among patients with mental retardation/ developmental delay (MR/DD) we screened over 1600 DNA samples from patients with undiagnosed (MR/DD). Our molecular screening protocol included Southern blotting and co-hybridization with a SMS deletion specific probe and a control probe for dosages comparison. Samples suspected to have SMS deletion based on this screening were test further by either FISH -if fresh blood samples were obtained or by GeneScan on the DNA samples. Two cases of SMS have been identified among this pop-

ulation. Of a surprise we also identified several cases of previously undiagnosed sex chromosome aneuploidy in this population

P0432. X-linked Lissencephaly with Absent Corpus Callosum and Ambiguous Genitalia (XLAG). Clinical, MRI and neuropathological findings

D. Bonneau[1], *A. Gellot*[2], *S. Marret*[3], *A. Laqu rri re*[4], *A. Toutain*[5]
[1]*CHU de Poitiers; Poitiers, France;* [2]*Hopital Saint Vincent de Paul; Paris, France;* [3]*Neurologie p diatrique; CHU Rouen, France;* [4]*Anatomo-pathologie; CHU Rouen, France;* [5]*G n tique m dicale; Tours, France*
bonneau@chu-poitiers.fr

Lissencephaly (smooth brain) is a group of severe malformations of the brain cortex that results from abnormal neuronal migration during central nervous system embryogenesis. According to Dobbyns, lissencephaly syndromes are classified into four major categories depending clinical, radiological or pathological findings; 1) agyria-pachygyria spectrum and subcortical band heterotopia (classical lissencephaly), 2) syndromes with lissencephaly variants 3) syndromes with cobblestone lissencephaly and, 4) syndromes with lissencephaly and extreme microcephaly (microlissencephaly). In the second group of lissencephaly variants, the neuronal migration disorder which is close to the classical form of lissencephaly is associated with other brain anomalies such as microcephaly, atypical cortex aspect, absent corpus callosum or, cerebellar hypoplasia. Recently, a new malformation syndrome associating lissencephaly, absent corpus callosum and ambiguous genitalia has been recognized in the group of lissencepahly variants. As the initial report from Berry-Kravis clearly demonstrated an X-linked inheritance, the disorder was referred to as X-linked lissencephaly with ambiguous genitalia (XLAG). Dobyns et al. has recently refined the clinical and MRI scan criteria for XLAG including; a) lissencephaly with a posterior-to anterior gradient and intermediate (5-7 mm) increase in cortical thickness, b) agenesis of the corpus callosum, c) intractable epilepsy with onset prenatally or during the first hours of life, d) hypothalamic dysfunction manifest by poor temperature regulation, e) severe hypotonia from birth and f) ambiguous genitalia in genotypic male. The gene responsible for XLAG is not identified and the pathogenesis of the disease is currently unknown. We report here three families affected with XLAG, we present the neuro-pathological findings of XLAG in two affected males and we show that the gene is also responsible for partial or complete agenesis of the corpus callosum in carrier females.

P0433. The Phenotype of a Homozygous CADASIL Patient in Comparison with Nine Age-Matched Heterozygous Patients with the Same R133C Notch3 Mutation

M. P yh nen[1], *V. Juvonen*[2], *M. Savontaus*[2], *S. Tuominen*[3], *T. Kurki*[4], *J. Rinne*[5], *S. Tuisku*[6], *K. Amberla*[7], *M. Viitanen*[7], *H. Kalimo*[8]
[1]*Department of Medical Genetics, V est liitto, The Family Federation of Finland; Helsinki, Finland;* [2]*Dept of Medical Genetics, Turku University Hospital; Turku, Finland;* [3]*Dept of Neurology, Turku University Hospital; Turku, Finland;* [4]*Dept of Diagnostic Radiology, Turku University Hospital; Turku, Finland;* [5]*PET Centre and Dept of Neurology, Turku University Hospital; Turku, Finland;* [6]*Dept of Neurology, Keski-Pohjanmaa Central Hospital; Kokkola, Finland;* [7]*Karolinska Institutet, Division of Geriatric Medicine; Huddinge, Sweden;* [8]*Department of Patology, Turku University Hospital; Turku, Finland*
minna.poyhonen@vaestoliitto.fi

Background; In cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) multiple lacunar infarcts lead to cognitive decline and finally subcortical dementia. It is caused by different missense mutations in Notch3 gene, which encodes a transmembrane receptor protein Notch3. We describe the first homozygous CADASIL patient in comparison with nine age-matched (– 2 years) patients heterozygous for the same R133C mutation. Clinical details and pathology; A 52-year-old male, whose deceased father and two paternal uncles had a history of similar cerebrovascular disease. His mother had died at the age of 31 of tuberculosis without known neurological disease. His grandfather and one maternal aunt had died of stroke. The patient has not had migraine. He had his first stroke at 28 and a recurrence at 37 years of age. CT revealed periventricular white matter hypodensities. Neurological deficits and psychiatric symptoms progressed, and cognitive decline became manifest around the age of 45. Eight years later the patient was moderately demented scoring 19 in MMSE. CADASIL diagnosis at the age of 52 was based on characteristic MRI changes, granular osmiophilic material around the degenerative smooth muscle cells in dermal arteries and identification of the R133C mutation. Genetic analysis; Exon 4 of the Notch 3 gene was amplified by PCR. PCR product was digested with MspA1I restriction endonuclease to detect the C475T transversion mutation, which leads to replacement of arginine 133 with cysteine (R133C). To rule out the possibility that a primer binding site polymorphism prevented the amplification of a normal allele, internal primers were also used. The restriction pattern of this patient was consistent with a homozygous mutation, which was confirmed by DNA sequencing. The gene defect was also demonstrated in one living paternal relative. On the basis of the patient«s family history, supported by the fact that R133C is a common mutation in Finnish CADASIL families, the patient«s both parents must have been heterozygous carriers of the mutation. In comparison with nine age-matched (– 2 years) heterozygous CADASIL R133C patients, our homozygous patient has the earliest onset (stroke), most prominent MRI-changes and second most advanced cognitive decline (alcohol abuse possibly contributing to the rapid decline of the most severe affected heterozygote). The course of the homozygous patient«s disease was exceptionally protracted. Conclusion; In dominant diseases heterozygous and homozygous patients should in principle be equally affected, but this rule has several exceptions. Our patient«s disease represents the severe end of the clinical spectrum among the age-matched CADASIL-patients. However, because it is not an order of magnitude more severe, it indicates that the double dose of defective Notch3 gene causes at most a minor aggravation of the symptoms.

P0434. Metatropic dysplasia in three unrelated patients; review of familial and clinical data provides evidence for the existence of a single clinical entity

D. Horn[1], *E. Rupprecht*[2], *L. Neumann*[1], *J. Kunze*[1], *J. Pelz*[1]
[1]*Institute of Human Genetics; Berlin, Germany;* [2]*Department of Pediatric Radiology; Dresden, Germany*
denise.horn@charite.de

Metatropic dysplasia is a rare but well delineated skeletal dysplasia. Based on morphological criteria as well as life expectancy and rare familial recurrence the disorder is subdivided into several categories with a different mode of inheritance.We report here on three additional male patients representing this entity. Radiologically, patient 1 and 2 represent with a similar severity of skeletal changes, whereas the clinical course differs.Patient 3 shows relatively mild skeletal findings.The rarity of affected sibs and of consanguinity in the published pedigrees raises doubt about the assumed autosomal recessive mode of inheritance. A single clinical entity with a wide variability, ranging from mild to perinatal lethal phenotypes, and autosomal dominant transmission is to favor.

P0435. The combination of dyschondrosteosisi with malformations of genitalia.

E. Timoljanova[1], *S. Amelina*[1], *G. Baibikova*[2], *M. Lukashevich*[3], *A. Macionis*[1], *R. Shokarev*[1]
[1]*Research Institute of Obstetrics and Pediatrics; Rostov-on-Don, Russian Federation;* [2]*Resarch Institute of Obstetrics and Pediatrics; Rostov-on-Don, Russian Federation;* [3]*State Medical University; Rostov-on-Don, Russian Federation*
S.Amelina@rniiap.ru

Mesomelic limb shortening can be accompanied by malformations of others organs. Dyschondrosteosisi is the most frequent form of this skeleton anomaly. Reduction of stature, Madelung s deformity, mesomelia of limbs are clinically apparent. Bony exostosis, deformation of femoral and brachial bones and deafness are also possible. The combinations with malformations of genitalia are not described according to this patology. Five children (4 girls and 1 boy) from four unrelated families were observed. All these children had the signs of dyschondrosteosisi and hypoplasia of genitalia resulted in delayed puberty in females. All the children had short stature, mesomelic limb shortening, Madelung s deformity. Hypoplasia of genitalia in girls was demonstrated by hypoplastic uterus combined with gonadal dysgenesis in one case and with hypoplastic ovaries in another one. Two-sided cryptorchidism has been diagnosticated in a boy since birth. Karyotypes of children were normal. In four cases short stature, mesomelia and Madelung s deformity were inherited from mothers and in one case from father. Heredity of mesomelia and Madelung s deformity were corresponded to autosomal dominant type. The other types of malformation of genitalia were not observed in these families. These parents had healthy sibs; 2 girls and 2 boys didn t show any skeleton anomalies and malformations of genitalia. However we can t exclude an accidental combination of dyschondrosteosisi and malformation of genitalia. This finding shows the importance of detailed evaluation of genitalia in patients with dyschondrosteosisi in order to prevent disoders of sexual development.

P0436. Inherited constitutional translocation from a phenotypically normal mother to her son with Prader-Willi phenotype.

L. P. N. L. Neagu[1], *C. D. C. Georgmaneanu*[2], *E. M. E. Tomescu*[1], *A. G. A. Lungeanu*[2]
[1]*Institute A. Rusescu for Mother and Child Care; Bucharest, Romania;* [2]*National Institute V.Babes for Research in Pathology and Biomedical Field; Bucharest, United States*
genetica@vbabes.ro

In 1988, a male of 5 years old who is the second child of a familly was reffered for karyotype investigation, because of his phenotye suggesting of Prader-Willi syndrome. Verry suggestive features are;neonatale hypotonia with failure of thrive, hyperphagia and severe obesity, hypogonadism, short arms and feet, mental retardation. Ussing GTG-banding a translocation between 13-15 chromosomes was revealed, and so the karyotype was 45, XY,t(13;15). The same constitotional translocation was detected in mother s blood lymphocites, who is a phenotypically normal carrier. The proband s father seems to have a microdeletion 15q11-12 as could be evaluated by GTG-banding. The molecular mechanizme both for translocation and for microdeletion, have to be elucidated by FISH technique.

P0437. Correlation of morphological and molecular genetic findings in thanatophoric dysplasia

R. Okhowat[1], *R. Wieser*[1], *C. Fonatsch*[1], *H. Rehder*[2]
[1]*Institut f r Medizinische Biologie, Universit t Wien; Wien, Austria;* [2]*Institut f r Medizinische Biologie, Universit t Wien; Institut f r Klinische Genetik, Philipps-Universit t Marburg; Marburg, Germany*
Roberta.Okhowat@univie.ac.at

Thanatophoric dysplasia (TD) is the most common lethal skeletal dysplasia with an incidence of 1 in 20.000. Typical features are micromelic shortening of limbs, trident hands, platyspondyly, macrocephaly, short ribs with narrow thoracic cavity resulting in respiratory failure and characteristic X-ray findings with cupped spur-like irregular flaring of metaphyses. TD has been subdivided into two different subtypes. TD1 is characterized by bowed long bones with telephone receiver appearance of femurs and usually without craniosynostosis, whereas TD2 shows straight femurs and a clover leaf skull. In both types, mutations in the FGFR3 gene were identified. We present our radiological, morphological and molecular genetic findings in three fetuses with TD of 22, 27 and 24 gestational weeks. Two of them either showed typical TD1 or TD2 symptoms . The third fetus displayed curved femurs as well as a clover leaf skull with craniosynostoses of the lambdoid, sagittal and partially of the coronal sutures. Mutation analysis of the FGFR3 gene identified in the TD1 and TD2 cases the characteristical transitions, A1118G (exon 10) for TD1 and A1948G (exon 15) for TD2. In the third case, we identified an A to G transition at nt 1118 in exon 10, representing a point mutation characteristic of TD1. This result was confirmed by restriction analysis exploiting a Bsg I site created by this mutation. Our results are in agreement with the findings of Tavormina et al. (1995) who claim that the classification of thanatophoric dysplasia into TD1 and TD2 is dependent on the presence or abscence of curved femora rather than of craniosynostoses. Further cases will have to be examined in order to more closely define possible differences in the craniosynostosis patterns of the TD1 and TD2 clover leaf skulls.

P0438. A New Hamartomateous Syndrome Characterised By Facial-thoracal Asymmetry, Multiple Osteomas , Left Sided Hemihypertrophy, Caf Au Lait Spot ; a 22 Year Follow-up

S. Balci, *A. Kayikcioglu*
Hacettepe University; Ankara, Turkey
mehmet@gen.hun.edu.tr

We present a 24 year old girl with multiple hamartomateous lesions whom had been first admitted to the genetic department as an 18 months old child. The prominent features of the case were a macrodactylic left thumb and index finger, multiple cranial and facial osteomas, macrocephaly and a caf au lait spot of 1 cm on the neck. The left tonsil was found to be enlarged. Radiological examination revealed osteomas of the skull as well as advanced bone age of the left hand, enlargement of the left 11th rib, increased corpus hight on the left part of the 12th dorsal vertebra which caused scoliosis. Computerised tomography displayed dilated ventricles on the left side. The case had been followed up for 22years and during this period she underwent tonsillectomy, excisional biopsy of an osteoma and amputation of the index finger. Histopathologic examination of the left tonsil was consistent with hamartomateous hyperplasia, osteomas displayed roughened lamellae of bone and bone marrow which was rich in fat cells. The chromosome analysis from peripheral blood was 46, XX. This case had been previously presented in 1982 Firenze, Italy; Identification of Genetic Syndrome in Pediatrics, International postgraduate course as a new entity entitled Multiple exostoses in the skull , facial, ear and thoracal asymmetry, unilateral macrodactyly. Is it a new entity? . We believe that this case is different from the previously described patients of Proteus syndrome and other related syndromes. She displayed fully penetrant expression of congenital hamartomateous pathologies. Molecular studies of similar cases will clarify either the presented case is a separate entity or a genetic heterogeneity.

P0439. Dominantly inherited spontaneous pneumothorax is highly penetrant when studied with HRCT

K. Aittomaki[1], *M. Somer*[1], *P. Tukiainen*[2]
[1]*Dept. of Clinical Genetics; Helsinki, Finland;* [2]*Dept. of Pulmonary Medicine; Helsinki, Finland*
kristiina.aittomaki@hus.fi

Spontaneous pneumothorax occurs in 7.4;100 000 males and 1.2;100 000 females and predominantly affects young males. In addition to being sporadic, spontaneous pneumothorax is known to occur in patients with Marfan and Ehlers-Danlos syndrome type IV. However, there is also an inherited form of spontaneous pneumothorax without any other symptoms or signs of connective tissue disorder. This autosomal dominant form of spontaneous pneumothorax has a low penetrance both in males and females. It has been estimated that only 50% of male and 20-35% of female gene carriers actually develop pneumothorax. We have identified a large Finnish pedigree in which spontaneous pneumothorax occurs in three successive generations with several obligatory gene carriers who have not had any symptoms of lung disease. To study whether they actually have bullous lung lesions we have studied several family members with high resolution computerized tomography (HRCT). Of the 7 people studied so far, only one (a male aged 27 yrs) seems to be unaffected. All others (family members who have experienced pneumothorax or are obligatory carriers) have bullous lung lesions. These bullae are located anywhere in the lung and not particularly in the apical areas. We propose that the risk of having spontaneous pneumothorax is at least partly dependent on the location, that is, if the bullous lesions are inside the lung, the risk of rupture and pneumothorax is low. If, however, the bullae are on the surface of the lung, the risk is higher. So far the HRCT findings in this family are 100% penetrant. We conclude that spontaneous pneumothorax in some families is inherited as an autosomal dominant trait. Although the penetrance of actual spontaneous pneumothorax is low, particularly in women, the penetrance of bullous lung disease studied with HRCT is high, perhaps 100%.

P0440. MECP2 mutation in mild, non-specific X-linked mental retardation family

A. Verloes[1], *B. Cherif*[2], *S. Briault*[3], *J. Chelly*[2]
[1]*Sart Tilman University Hospital; Li ge, Belgium;* [2]*INSERM U129-ICGM. CHU Cochin; Paris, France;* [3]*CHU de Tours, Service de G n tique, Hopital Bretonneau; Tours, France*
alain.verloes@chu.ulg.ac.be

A wide spectrum of usually severe neurodevelopmental phenotypes has been observed in patients with mutations in the methyl-CpG binding protein 2 (MECP2) gene, located in Xq28; typical and milder/atypical Rett syndrome (in females and males), Angelman-like neurodevelopmental phenotype, and severe non specific mental retardation affecting male patients. We found an Arg to Trp substitution (R167W) in 4 retarded males of a 4 generation XLMR pedigree. The substituted residue, located 4 amino acids downstream to the methyl-CpG-binding domain, is conserved across species. The mutation occurred in a region where only nonsense mutations (leading most likely to a drastic loss of function) have been described, leading to a Rett phenotype. The four studied males present the same neurodevelopmental features ; mild mental delay (IQ > 50 ; 3/4 can read and write, albeit with difficulties), talkative, friendly personality (3/4), obesity (3/4), non progressive neurological anomalies consisting of brisk reflexes in the lower limbs (4/4) and essential tremor (3/4). One of the boys had severe behavioral problems. Among obligate carrier women, at least 2 present with essential tremor, and normal intelligence. The phenotype in this family probably represents the mildest expression of MECP2 mutation recorded in man. It clearly raises the hypothesis that MECP2 could play a major role in non specific MR, and could become the second gene (with FRAXA) that would be necessary to screen systematically in patients with unexplained, non syndromic MR.

P0441. Hypomelanosis of Ito - Natural History of 12 Patients

H.Santos, *A. Medeira, I. Cordeiro*
Servi o Gen tica, Hospital Santa Maria, Lisboa, Portugal
heloisa.santos@ip.pt

Hypomelanosis of Ito (HI) was first described by Ito in 1952 in a patient with

hypopigmented lesions in a zig-zag pattern on the trunk and linear pattern along the arms. Since then many cases are reported mostly presenting extracutaneous anomalies. High variable clinical manifestations, sporadic occorence and the frequent finding of chromossomal mosaicism suggested that HI is a cutaneous marker of genetic mosaicism. We report the clinical evolution of 12 HI sporadic patients seen in the last 17 years in our Clinics. Diagnosis done between 3 months and 17 years. All presented extracutaneous manifestations including (2 cases) exuberant fibromas on accidental scars. The typical hypopigmented lesions were present in all the patients but they faded with the age in the older. Chromossomal mosaicism was found on fibroblasts in 3 of 11 patients analysed. Comparing groups with and without mosaicism we found that all the mosaic patients present an evident and constant asymmetry but the mental development is not worst namely 2 of these 3 adults patients having a normal intelligence are attending the university. The importance of more clinical and cytogenetic data in the genetic counselling of patients with HI must be emphasized.

P0442. Clinical,cytogenetic and molecular analysis of two families with FRAXE mutation; implications for genetic counselling and prenatal diagnosis

M. I. Tejada[1], *P. Botella*[2], *M. A. L pez Ar ztegui*[3], *F. Mozo de Rosales*[4], *M. L pez Valverde*[4], *M. L. Onaindia*[1], *M. Molina*[1]
[1]*Genetic Unit, Basurto Hospital; Bilbao, Spain;* [2]*Dept. of Paediatrics, Txagorritxu Hospital; Vitoria, Spain;* [3]*Genetic Laboratory, Cruces Hospital; Baracaldo, Spain;* [4]*Dept. of Obstetrics and Gynaecology, Basurto Hospital; Bilbao, Spain*
itejada@hbas.osakidetza.net

FRAXE full mutations are rare and appear to be associated with mild mental retardation and with a fragile site in Xq28. This is due to an abnormal expanded GCC repeat, adjacent to a methylated CpG island proximal to the FMR2 gene. As part of a routine diagnosis, all individuals referred to us for Fragile X Syndrome (FXS) study, and tested FRAXA negative, were studied for FRAXE mutation. Among 485 index case patients, 2 were identified with the FRAXE full mutation, both with cytogenetic expression of the fragile site (29 and 23% respectively) and with no history of mental retardation. One of them has an autistic disorder with severe MR an a dysmorphic face similar to FXS. The second one is a nearly normal man with normal phenotype but who had learning difficulties at school when he was a child. Their families were also studied, with a total of 16 members at risk tested. We found 6 of them normal (3 men and 3 women) and 10 carrier women (4 premutated and 6 fully mutated). None of these women present any type of mental impairment. The last sample studied was a fully mutated female foetus that was spontaneously aborted after obtaining chorionic villi for prenatal diagnosis. Although the GCC expansion was greater than 0.8 kb, it was unmethylated in the majority of the tissues studied by us. So, even though molecular study of FRAXE mutation is now available, further studies are nedeed for the purpose of genetic counselling and prenatal diagnosis.

P0443. Eight years experience of direct molecular testing for Myotonic dystrophy in Wales

P. Harper[1], *S. Fokstuen*[1,2], *L. Meredith*[1], *L. Myring*[1], *D. Ravine*[1]
[1]*Institute of Medical Genetics, University of Wales College of Medicine; Cardiff, United Kingdom;* [2]*Current address; Division of Medical Genetics, University Hospital; Geneva, Switzerland*
SivFokstuen@gmx.ch

Since the identification of the specific gene defect in 1992 direct molecular testing for the Myotonic dystrophy mutation has developed as a widespread service in clinical practice. Between 1992 and 2000, the Molecular Diagnostic laboratory of the Institute of Medical Genetics, Cardiff has performed a total of 526 tests; 292 of these have been for the population of Wales (population 2,9 million). 213 of the samples from Wales were referred by clinical geneticists and 79 by other medical specialists, mainly paediatricians and neurologists. Data on the reason of referral, the pedigree and clinical status as well as the outcome of testing were available for 282. As no test result showed an equivocal repeat size, direct mutation analysis for myotonic dystrophy can be considered as a very accurate and specific test to differentiate normal and disease associated alleles. There were 200 diagnostic investigations on symptomatic patients (103 abnormal), 78 presymptomatic tests (16 abnormal, 62 normal) and 4 prenatal diagnosis (2 abnormal, 2 normal). In 117 symptomatic patients with a negative family history only 33 proved to have the mutation, reflecting the lack of specificity of clinical symptoms in myotonic dystrophy. A normal result was also found in 9 out of 77 symptomatic patients with a positive family history. Only 7 of the 16 individuals with an abnormal presymptomatic test result showed no clinical abnormality on examination, supporting the high penetrance of the myotonic dystrophy mutation by adult life and indicating the importance of careful clinical assessment in relation to presymptomatic testing.

P0444. The macrocephaly-hamartoma syndromes; A need for better diagnostic criteria in Bannayan-Riley-Ruvalcaba-Syndrome

M. Hoeltzenbein[1], *M. Poetsch*[2], *B. Kleist*[3], *S. Weigel*[4], *I. Weinke*[5]
[1]*Institut f r Humangenetik; Greifswald, Germany;* [2]*Institut f r Rechtsmedizin; Greifswald, Germany;* [3]*Institut f r Pathologie; Greifswald, Germany;* [4]*Universit tskinderklinik; Greifswald, Germany;* [5]*Sozialp diatrisches Zentrum; Greifswald, Germany*
Hoeltzen@uni-greifswald.de

Bannayan-Riley-Ruvalcaba-Syndrome (BRR) and Cowden Syndrome (CS) are rare autosomal-dominant diseases belonging to the group of macrocephaly-hamartoma syndromes. Germline mutations in the putative tumor supressor gene PTEN have been found in 80% of patients with CS, but only in 50-60% of patients with BRR. In few families CS and BRR are both observed, so that many features of BRR-Syndrome are also considered as major and minor criteria for CS. We describe a 6-year-old boy with mild mental retardation, who was seen for evaluation of muscular weakness. Because of the additional presence of lipomas and a mild macrocephaly BRR was suspected. Mutation analysis revealed a heterozygous missence mutation G389A in the PTEN-gene, thus confirming diagnosis of BRR. The mutation was found in DNA from leucocytes as well as in DNA isolated from a lipoma. The mutation G389A has already been described in a family with CS and is located in exon 5, a hotspot for CS mutations. Our patient does not fulfill the recently revised diagnostic criteria for CS (J. Med. Genet. 2000; 37;828-830) used for selecting patients suitable for molecular analysis. The low mutation detection rate in BRR patients could either be due to genetic heterogeneity or to the uncertainty concerning the exact phenotype and clinical spectrum of BRR. More patients with macrocephaly, muscular weakness and presence of lipomas should be screened for mutations in the PTEN-gene in order to determine the phenotypic variability and try to establish diagnostic criteria for BRR.

P0445. Syndrome of ectodermal dyplasia, agenesis of corpus callosum, primary hypothyreoidism and mental retardation; an entity?

I. Kennerknecht[1], *M. Herrmann*[1], *T. Neumann*[1], *H. St nder*[2], *J. Horst*[1], *H. Traupe*[2]
[1]*Institut f r Humangenetik; M nster, Germany;* [2]*Zentrum f r Dermatologie; M nster, Germany*
kennerk@uni-muenster.de

We present a 2 year old boy born at term by cesarean section after an uneventful pregnancy; birth weight was 3.080 g, length 52 cm, head circumference 37 cm. The non consanguineous parents, 32 and 36 years, are healthy and the family history is negative. The boy showed craniofacial dysmorphism (dolichocephaly, frontal bossing, low set ears, and retro-, microgenie), signs of ectodermal dysplasia (sparse and fine hair with microscopically kinky and irregular hair shaft partly curved by different thickness of the cuticula; late and irregular dentition with one splitted incisivus, clinically reduced sweating), hypoplasia of the corpus callosum, eye fundus with peripheral pigment anomalies, primary hypothyreoidism, and lateral fistula of the neck. At the age of 2 years severe psychomotoric and mental retardation was obvious. Other findings such as chromosome analysis (46,XY) and bone age were normal. Ectodermal dysplasias (ED) forms a large and complex nosologic group (Pinheiro and Freire-Maia (1994), AJMG 53;153ff). Reports of ED with CNS malformations and/or primary hypothyreoidism are rare. There is one other report with two unrelated patients showing the same symptoms as our patient (Silengo et al. (1998) AJMG 35;157ff). Together with our observation we suggest that this is one entity.

P0446. Phenotype of a terminal dup(4)(p15.3-16.3) with several facial features of MPS 1

M. Tschernigg, *E. Petek, P. M. Kroisel, W. Emberger, H. Zierler, K. Wagner*
Institute of Medical Biology and Human Genetics, University of Graz; Graz, Austria
michaela.tschernigg@kfunigraz.ac.at

We report on a 3 year old female patient with a de novo inverted duplication of the distal segment of the short arm of chromosome 4 corresponding to the following karyotype; 46,XX,dup(4)(p15.3-16.3). This leads to a partial trisomy 4p(15.3-16.3). Most striking clinical findings are; microcephaly, deep-set eyes, short pug nose, full cheeks, low-set hairline, bilateral simian crease as well as clinodactyly of upper and lower limbs and a psychomotor retardation (being able to sit with 12 months and to walk with 24

months) with no speech development so far. Facial appearance is somewhat coarse, as was already described for 4p duplications. The proposita described here shows some similarities to MPS I patients, resulting in a storage disease like facial expression. It is interesting in this context that the IDUA- (alpha-L-iduronidase) -gene is mapped to this duplicated region and could be responsible for some of those facial abnormalities. The development of this facial appearance will have to be documented for a long time evaluation and also biochemical studies of enzyme activity will be achieved. A precise breakpoint characterization by high resolution chromosome banding, whole chromosome painting as well as by using site specific YAC probes was performed. No deletion, but a duplication of the very distal Wolf-Hirschhorn-critical region was demonstrated. A phenotype-genotype correlation analysis in combination with an extensive literature review was undertaken to allow a further delineation of partial trisomy 4p.

P0447. The Mercedes Benz syndrome; variant of Crouzon syndrome?

Y. Hilhorst-Hofstee[1], *S. G. Kant*[1], *J. M. Vaandrager*[2], *E. A. J. Peeters*[3], *A. Clement-de Boers*[3], *M. H. Breuning*[1]
[1]*Leiden University Medical Center; Leiden, The Netherlands;* [2]*University Hospital Rotterdam; Rotterdam, The Netherlands;* [3]*Medical Center Haaglanden; Den Haag, The Netherlands*
y.hilhorst@kgc.azl.nl

A case is presented with the clinical picture of the Mercedes Benz syndrome, characterised by premature closure of the lambdoid sutures and the posterior part of the sagital suture. As far as we know no patients with Mercedes Benz syndrome are reported in the literature with a mutation in one of the fibroblast growth factor receptor genes causing craniosynostosis. Mutation analysis in our patient revealed a mutation in the FGFR2 gene previously described in 2 Crouzon families. The clinical phenotype of those patients was not described however the Crouzon syndrome is known to have a very variable expression. Our hypothesis is that the Mercedes Benz syndrome is a variant of Crouzon syndrome. Mutation analysis in other patients diagnosed as having the Mercedes Benz Syndrome is pending.

P0448. Translocation 2q;12q; A rare Chromosomal Aberration Associated to Turner Symdrome

A. C. Bertoncin[1], *J. I. F. Neri*[2]
[1]*Uniararas - Uni o das Faculdades da Funda o Herm nio Ometto; Araras, Brazil;* [2]*Ambulat rio de Gen tica Cl nica - Hospital e Maternidade Jundia ; Jundia , Brazil*
bertocin@yahoo.com.br

Excepting in the cases of hematologic diseases (e.g. leukemia), translocations involving chromosomes of the groups A and C are relatively uncommon, especially among the chromosomes 2 and 12. Turner syndrome (TS), not rarely, can be associated to others aberrations involving autosomal chromosomes or even situations of reciprocal translocation between the chromosome X and other autosomal. However until moment no case of TS was reported associated to a translocation 2;12. We report the case of a seven years old girl with severe mental retardation, short stature, microcephaly, up-slanting palpebral fissures, epicanthal folds, low-set and posteriorly rotated ears with mild microtia, anteverted nares, short and flat philtrum, narrow palate, pectus excavatum, hypoplastic nipples, short broad neck with low hairline, brachydactyly with short four to five metacarpals bilaterally, palmar and plantar hyperkeratosis, increased skin pigmentation in all lower part of the body, anteriorly placed anus and hypoplasia of genitals. Among others clinical and laboratorial investigations, we detach the normal hematologic evaluation and the karyotype 45,X,inv(9)(p11q12)t(2;12)(q23;q24). Parent s karyotypes were normal. As nor all the features observed in the patient can be referred to TS, a revision of the clinical picture and of the chromosomal aberrations involved was done and discussed.

P0449. Proteus syndrome; clinical manifestations in three individuals.

I. Naumchik, *N. Rumyntseva, R. Chmel*
Institute for Hereditary Diseases; Minsk, Belarus
lazjuk@bcsmi.minsk.by

Proteus syndrome (PS, MIM; 176920) is a rare congenital hamartomatous syndrome comprising hemihypertrophy, asymmetrical overgrowth of different parts of the body in association with various cutaneous abnormalities, and subcutaneous masses. We report the clinical data of three unrelated patients with normal intelligence. All parents were healthy and unrelated. Patient 1, a 28-year-old pregnant women, third child of 36 years old parents, at birth showed hypertrophy of right leg. Examination demonstrated rightsided hemihypertrophy, face asymmetry, gigantic right 2nd finger and left 2nd toe, broad foot with deep creases on the soles, soft tissue tumor on the right knee, varicose veins, cutis marmorata. Ultrasound examination at 26 weeks of gestation was normal. Patient 2 was first seen as infant because of macrocephaly, hemihypertrophy and cutaneous hemangiomas. At 9 years of age she showed macrocephaly (OFC 60.5), hyperostosis of the skull, severe asymmetry of the face, broad depressed nasal bridge, hypertrophy of the right half of the tongue, cutaneous hemangiomas, hepatosplenomegaly, rightsided hemihypertrophy, right arm and leg were longer than the left. Patient 3 at birth demonstrated hemihypertrophy. At 11 years of age he showed asymmetry of the face, mild hypertrophy of the right 3rd and 4th fingers and left 3rd and 4th toes, soft tissue tumors on the lumbar region and on the right femur, cutis marmorata, leftsided hemihypertrophy, his left arm and leg were longer than the right ones. We discuss wide spectrum of manifestations and severity of PS on the basis of our findings in comparison to the literature data.

P0450. Identification of a novel 14 bp deletion in Cx32

H. Weirich-Schwaiger[1], *S. Kiechl*[2], *A. R. Janecke*[1]
[1]*Institute of Medical Biology and Human Genetics, University of Innsbruck; Innsbruck, Austria;* [2]*Department of Neurology, University-Hospital of Innsbruck; Innsbruck, Austria*
Helga.Weirich@uibk.ac.at

CMTX is an X-linked dominant hereditary motor sensory neuropathy with slow nerve conduction velocity (NCV). Affected males exhibit a moderate to severe phenotype, whereas females usually have a mild neuropathy. The course of the disease is progressive over many years but does not decrease lifespan. CMTX is associated with Cx32 mutations in most cases. Cx32 maps to Chromosome Xq13 and codes for a Gap junction beta-1 protein which is found in central and peripheral myelin. We sequenced the whole Cx32 coding region contained in one single exon of a female index patient. Thereby we identified a novel deletion of 14 bp, comprising nucleotides 829 to 843. Family history revealed X-linked dominant inheritance in the three generation family with 5 affected males and 5 obligate carriers. Onset of clinical symptoms in affected persons was within the second decade of life with gradual progression. The mutation 829 del 14 bp is associated with a highly variable degree of clinical involvement in female carriers.

P0451. Prader - Willi syndrome - case report.

Z. Krumina, *B. Maliseva, I. Balode, R. Kamerade, I. Grivina, R. Lugovska*
State Medical Genetics center; Riga, Latvia
clinic@gc.bsg.lv

Prader-Willi syndrome (PWS) is a disorder of chromosome 15. The cardinal features are neonatal and infantile central hypotonia, improving with age, feeding problems in infancy, excessive weight gain between 1 and 6 years of age, generalised obesity, hypogonadism, hyperphagia, developmental delay. The most common serious complication is NIDDM. We present a patient with PWS, where diagnosis is confirmed by FISH technique first in Latvia. The proband is the second child in the family, born at 36 weeks of gestation (weight 2900g, height 50 cm). Till 3rd months of life he was tube-fed, had hypotonia and poor weight gain was till 1,5 years, afterwards rapid gain with excessive weight. At the age 11 appeared fatty liver symptoms. At the examination proband (13 years old) had many clinical findings characteristic for PWS; general obesity (106 kg), height 154 cm, hypogonadism, small hands and foot, strabismus, myopia, speech articulation defects, thick saliva. He had moderate mental retardation and sleep disturbances. Standard chromosome analysis on cultured lymphocytes (GTG banding) revealed changes of 15(q11q13), after with FISH technique the microdeletion was confirmed. It was the first time in Latvia when FISH is used to find out microdeletion.

P0452. Birth Defects Monitoring (BDM) in St.Petersburg; Reality or Rhetoric?

M. O. Mkheidze
The Medical Academy for Postgraduate Education (MAPE); St.Petersburg, Russian Federation
mkheidze@netscape.net ; mmkheidze@yahoo.com

To Frau Dorothea Buchberger and Dr. Robert Buchberger St.Petersburg is a city of about 4.5m of citizens. The number of babies with congenital anomalies was 123 - 150 per 10,000 births (1986,1997). To identify all cases of malformed infants and fetuses is complex, intricate, long-term process. Data observed are very responsible and BDM must be done correctly. Are our physicians well educated in this field? This question is the aim of this study. Materials, methods. 194 of the physicians (gynecologists, obstetricians-50%, micropediatricians-12%, pediatricians-32%, different specialists-6%) were interviewed with the help of 3 questionnaires. The first questionnaire contains 4 positions focused on monitoring. There are

12 specific terms for phenotype description in the second. The third questionnaire includes 50 questions associated closely with medical genetics and dysmorphology. Results. The first and the second questionnaires demonstrate unsatisfactory knowledge of human genetics and dysmorphology. The mean marks of the both tests were below 50 %. 4 out of 12 terms are absolutely unknown. All doctors think that BDM is very important project and deals with medical genetics. Nobody says that he (she) is well educated in these medical fields. All doctors consider that it is necessary to have special short training course before their participation in BDM. The genetic basis of BDM , the intensive free fortnight s course, has been organized by our Department, but only 19 doctors decided to use this lucky chance. The main conclusion of this investigation consists in the fact that BDM has weak basis for successful realization in St.Petersburg.

P0453. Acrofacial dysostosis and midline defects in two male foetuses ; a new X-linked recessive syndrome ?

O. Boute[1], *A. Moerman*[1], *A. Coeslier*[1], *L. Devisme*[2], *S. Manouvrier*[1]
[1]*Hopital Jeanne de Flandre; Lille, France;* [2]*H pital Calmette; Lille, France*
oboute@lille-chru.fr

We report on two male foetuses, whose mothers were sisters. Both pregnancies were medically terminated because of ultrasonographic detection of similar multiple malformations including severe limb defects. The foetuses aged 20 (case 1) and 24 weeks (case 2) gestation respectively presented with; 1) severe intra-uterine growth retardation, 2) abnormal face reminiscent of mandibulofacial dysostosis, 3) transposition of great vessels with pulmonary atresia and ventricular septal defect, 4) bifid thymus, 5) single median kidney and absent gall-bladder, 6) scoliosis, 7) bilateral asymmetric radial aplasia and oligodactyly. Additional findings were in case 1; omphalocele, absent diaphragm and abnormal larynx with tracheo-esophageal fistula; and in case 2; humeral and fibular aplasia. No cerebral abnormality was observed in both foetuses, in particular they had no hydrocephaly. The chromosomes were normal in both foetuses and their parents. Therefore the patients, cousins through their healthy mothers, had a lethal acrofacial dysostosis with midline defects. Several diagnoses could be discussed; a severe form of NAGER acrofacial dysostosis, another case of the lethal acrofacial dysostosis described by Rodriguez et al [1990], or the association of NAGER and VACTERL syndromes. However none of these hypothesis could explain the X-linked recessive inheritance. Furthermore absence of cerebral malformation in both patients allows excluding a VACTERL + hydrocephaly association even if this association has been described in association with branchial arch defects. Then we suggest that our patients present with a new form of X-linked recessive inherited acrofacial dysostosis.

P0454. A newborn Down syndrome baby-boy with a partial isochromosome 21q

N. Horelli-Kuitunen[1], *A. Niss n*[1], *A. Slunga-Tallberg*[1], *M. Gr sbeck*[2], *H. Koillinen*[3], *R. Salonen*[4]
[1]*Medix Laboratory services Ltd; Espoo, Finland;* [2]*Kymenlaakso Central Hospital; Kotka, Finland;* [3]*Family Federation of Finland; Helsinki, Finland;* [4]*Helsinki University Central Hospital; Helsinki, Finland*
nina.horelli-kuitunen@medix.fi

We report a newborn baby with Down syndrome caused by partial isochromosome of chromosome 21q. The chromosomes of the baby were studied based on clinical findings - such as low nasal bridge, palmar simian creases, upslanting palpebral fissures, loose skin of the neck and muscular hypotonia - typical for Down syndrome. The karyotype of the baby turned out to include a normal chromosome number, but a partial isochromosome 21q; the karyotype was 46,XY,i(21)(q22). FISH procedure using locus specific probe for band region 21q22.13-22.2 revealed one signal in the normal chromosome 21 and two signals in the isochromosome 21. Therefore, the so far known critical region for Down syndrome was trisomic in the genome of this baby. The chromosome analysis of the parents showed a normal karyotype for the father, but an abnormal karyotype for the mother containing an inverted chromosome 21; the karyotype was 46,XX,inv(21)(p11.2q21). FISH procedure with the same locus specific probe as mentioned above revealed only one specific signal in each chromosome 21. The partial isochromosome 21q of this baby must have resulted through crossing over in the meiosis during gamete formation. This case shows an elevated risk for Down syndrome for a carrier of an inverted chromosome 21. In the near future the specific genes causing the phenotype of Down syndrome will be revealed and gene tests will help to confirm diagnosis also in these rare cases.

P0455. Goldberg — Shprintzen Syndrome In Three Children Of Consanguineous Italian Parents

M. Giovannucci Uzielli, *E. Lapi, A. Cecconi, G. Ricotti, L. Ombroni, E. Andreucci, S. Guarducci, U. Ricci, L. Giunti*
Genetics and Molecular Medicine Unit, Department of Paediatrics, University of Florence, A.Meyer Children Hospital; Florence, Italy
uzielli_ml@unifi.it

Goldberg-Shprintzen syndrome (OMIM 235730) is a very rare genetic disorder; an Autosomal Recessive mode of inheritance was suggested, based upon two sibs in one pedigree with unaffected parents, and two consanguineous pedigrees. Common clinical features in reported subjects were severe mental retardation, microcephaly, distinctive face, and Hirschprung disease (HSCR). Coloboma of the iris and neurological abnormalities were not always present; submucous cleft palate was only reported by Goldberg and Shprintzen in the first description. We report the clinical data observed in three children (two females and one male) of consanguineous parents. The similarity of some clinical aspect to the phenotype of the Cornelia de Lange syndrome seems very interesting. One further child is presently being studied; a young girl, born to non-consanguineous parents of unrelated families, referred to our Centre for the molecular analysis of MECP2 gene on the basis of a natural history apparently suggestive for Rett syndrome; in this patient we didn t find mutations of the MECP2 coding region. We recently started a wide molecular genome screening in the first family, for the identification of a Goldberg-Shprintzen locus, and we will present the preliminary results.

P0456. Evidence for autosomal recessive inheritance in the infantile spinal muscular atrophy variant with congenital fractures.

W. Courtens[1], *A. Johansson*[2], *B. Dachy*[3], *F. Avni*[4], *N. Telerman-Toppet*[3], *E. Vamos*[1], *H. Scheffer*[5]
[1]*Department of Medical Genetics, Brugmann University Hospital; Brussels, Belgium;* [2]*Department of Neonatology, Children s University Hospital Queen Fabiola; Brussels, Belgium;* [3]*Department of Neurology, University Hospital Brugmann; Brussels, Belgium;* [4]*Department of Paediatric Radiology, Children s University Hospital Queen Fabiola; Brussels, Belgium;* [5]*Department of Medical Genetics, University of Groningen; Groningen, The Netherlands*
wcourten@ulb.ac.be

We report on a female newborn with a severe acute form of SMA, congenital bone fractures, camptodactyly of fingers and toes, bilateral hip dislocation and congenital heart defect, with early lethal outcome. DNA studies showed the absence of homozygosity for a deletion of exons 7 and 8 of the SMNt gene. A new lethal syndrome consisting of infantile spinal muscular atrophy (SMA) and multiple congenital bone fractures in 2 sibs has been suggested in 1991. Recently, another infant with a form of SMA and congenital fractures, was reported, thus validating the suggestion of a distinct and rare form of SMA associated with congenital bone fractures. Autosomal recessive inheritance was suggested in the original report, but no history of consanguinity was noted in the second. X-linked inheritance could however not be excluded since those three affected infants were male. Since our case is a female, an X-linked inheritance can be excluded. Since she was furthermore born to first cousin parents, it suggests an autosomal recessive inheritance in this rare variant of SMA type 1 with congenital fractures. We further conclude that this SMA variant, with early lethal outcome, is probably not linked to 5q.

P0457. Robinow Syndrome; Report of a case with bilateral, postaxial hexadactyly and review

B. Weissenrieder[1], *D. Schmitz*[1], *R. Sauter*[2], *G. Wolff*[1]
[1]*Institut f r Humangenetik; Freiburg, Germany;* [2]*Kinderabteilung St. Hedwig am St. Josefskrankenhaus; Freiburg, Germany*
weissenr@humangenetik.ukl.uni-freiburg.de

Introduction; Robinow syndrome is a short stature syndrome with characteristic facies, mesomelic limb shortening and hypoplastic genitalia. Both autosomal dominant and autosomal recessive inheritance have been reported. The autosomal recessive cases seem to have more severe brachymelia, and in addition digital anomalies and costovertebral segmentation defect. The gene mutated in the autosomal recessive form has been mapped to chromosome 9q22, a region that overlaps the locus for autosomal dominant brachydactyly type B. Recently, the ROR2 gene has being identified as mutated in brachydactylia type B and is regarded as a candidate gene for recessive Robinow syndrome. Reported mutations suggest that a loss of activity of ROR2 causes recessive Robinow syndrome. Case report; We report on a 15-month-old girl with classical Robinow Syndrome. The girl is the first child of healthy, non-consanguineous parents. The family history is inconspicuous. Clinical examination of the father revealed a

notched tongue, dental anomalies, a preauricular tag on the left side, clinodactyly II and V, and a double mammilla on both sides. Clinical symptoms in the child include short arms and legs, characteristic face with hypertelorism, frontal bossing, triangular mouth, midface hypoplasia, dental abnormalities, notched maxilla, micrognathia and macrocephalus. In addition, the girl had bilateral postaxial hexadactyly, a symptom which has not been described in Robinow syndrome before. An X-ray excluded a costovertebral segmentation defect. A chromosomal analysis showed a normal female karyotype. Mutation analysis of the ROR2 gene is in progress. Conclusions; A literature review shows that this is the first case of Robinow Syndrome with hexadactyly. Microsymptoms of this syndrome in first degree relatives have not yet been described. Therefore, we cannot decide whether the polydactyly is indicative of dominant or recessive inheritance. It is not clear whether the symptoms in the father can be interpreted as microsymptoms of autosomal dominant Robinow syndrome. The lack of costovertebral segmentation defects in the child argues against autosomal recessive inheritance. These questions will only be resolved by further molecular analysis of this syndrome.

P0458. Patients with Turner syndrome (TS); point of view of a policlinic endocrinologist

N. Gladkova[1], *M. O. Mkheidze*[2]
[1]*Children s out-patient Dept. N 38; St.Petersburg, Russian Federation;* [2]*The Medical Academy for Postgraduate Education (MAPE); St.Petersburg, Russian Federation*
mkheidze@netscape.net

TS is a sex chromosome aneuploidy with its incidence of about 1; 3000 live female births in St.Petersburg. All of them are registered by Municipal Center of Medical Genetics (MCMG), but they have constant health service at their district out-patient Department. The objectives of this study were (1) to summarize our patients data and (2) to determine their necessities. We used self-report survey methodology. The questionnaire was designed to solicit information about the proband - parents health and lifestyle habits. There are 69000 children, aged from some days to 16 years, under observation of policlinic endocrinologist. Eight of persons, aged from 6 to 16 years, have TS. It is only the one case of TS recognized at the maternity hospital. The rest were not diagnosed till 6-13 years old. The parents of our probands are young in years (at the moment to conceive the mothers were 22- 25 years old and the fathers were 23-27). 50% of the couples reported that they had been exposed to environmental hazards. Data of the Cytogenetic Laboratory of MCMG are following;45,?{4}; 45,?(30)/46,??(8),9ph; 47,???(60)/45,?(40); 46,X,del (X)(p11); 46,X,del(X)(q10).The main problems of our respondents are financial difficulties, problems of social rehabilitation and interpersonal communication. Sometimes they feel themselves like children neglected and forgotten. We suppose that it is necessary to join hands with these families and organize special center for them. The best health service and social support of TS patients and their families must become the principal purpose of this center

P0460. Some family cases of ophthalmic pathology

O. K. Janvareva, *M. O. Mkheidze*
The Medical Academy for Postgraduate Education; St.Petersburg, Russian Federation
mkheidze@netscape.net

Modern medical genetics has begun to make a significant contribution to the diagnosis of ophthalmology. This study accentuates the great importance of cooperation between an ophthalmologist and a medical geneticist to diagnose hereditary diseases. Four families with different rare hereditary diseases are under our observation. Goldmann -Favre disorder (MIM 268100) was diagnosed in 2 sisters, aged 10 and 11 years, and their mother, aged 32.This disorder is characterized by hyaloideoretinal degeneration with retinoschisis and early hemeralopia. Stargardt disease, juvenile macular degeneration, (MIM 2482000) was observed in 2 unrelated families. There are 2 affected brothers with onset of this disease at 7 and 8 years in the first family. We revealed a brother and a sister suffered from the same disorder in another family. All parents aren t affected. Crouzon disease, craniofacial dysostosis, (MIM123500) was diagnosed in a father, aged 30 years, and his daughter, aged 3 years. The disease is characterized by cranial synostosis, hypertelorism, bilateral exophthalmos, external strabismus and some other facial dysmorphic features. Both father and his daughter suffer from partial optic atrophy. To make a diagnosis and genetic prognosis and to use an adequate treatment we apply special methods of investigation suitable for ophthalmology and medical genetics. Now we continue to work on the pedigrees of our clients to reveal new cases or mild features of the diseases among their relatives. Our cooperation improves quality of health service and enriches our experience and erudition.

P0461. Progeroid disorders -two new unrelated cases

N. Rumyantseva, *I. Naumchik, V. Kulak*
Institute for Hereditary Diseases; Minsk, Belarus
lazjuk@bcsmi.minsk.by

Progeroid syndromes is a heterogeneouse group of rare genetic disorders, common characterized by premature senility signs. Growth failure, mental retardation, malformations, clinical course, vital prognosis are variable. We present clinical data of two new patients with normal intelligence and progeroid pictures. Patient 1; a 43-year-old female looked healthy till the age of 38 years. She was married, had a healthy son. Examination showed scoliosis, reduction of subcutaneous tissue, marked wrinkling skin of face and neck, thinning greying hair, heart failure. No cataracts, diabetes mellitus were found. Decreased replicative life-span of cultured skin fibroblasts were revealed. Werner syndrome was suspected. Patient 2; a 18-year-old girl (W=42kg, H=155cm, OFC=54cm) was born at term (G2; P2) with BW 3500g, BL 52cm to unrelated healthy young parents. Her elder brother was healthy. She looked normal at birth till the age of 6 years. Senility signs were noted in early childhood, showing progressing, but not Hutchinson-Gilford syndrome appearance. Short stature, failure to thrive, hypotrichosis, bird face, protruding eyes, beaked nose, progressive loss of subcutaneous tissue, atrophic changes of skin and muscles, thin limbs with scleroderma-like lesions, prominent joints, flexion contractures, hyperkeratosis on the soles, heart failure, large abdomen, hepatosplenomegaly, diabetes mellitus, diminished sexual development were observed at follow up examinations. No cataracts were recorded yet. Laboratory and skin biopsy results will be presented. Is it Werner syndrome with early onset or new juvenile form of progeroid disorder? Wide variability of Werner syndrome s phenotype and differential diagnosis will be discussed.

P0462. Williams syndrome unmasking autosomal recessive Chronic Granulomatous Disease

D. Donnai[1], *K. A. Metcalfe*[1], *P. Bolton-Maggs*[2], *T. Segal*[3], *M. Tassabehji*[4]
[1]*St Mary s hospital; Manchester, United Kingdom;* [2]*Alder Hey Childrens Hospital; Liverpool, United Kingdom;* [3]*University College Hospital; London, United Kingdom;* [4]*University of Manchester; Manchester, United Kingdom*
kaym@central.cmht.nwest.nhs.uk

Williams-Beuren syndrome (WS) is a developmental disorder caused by a hemizygous microdeletion of ~1.5Mb at chromosomal location 7q11.23. Chronic granulomatous disease (CGD) is a rare inherited condition disorder characterised by chronic and recurrent infection. In CGD the failure of phagocytic cells to produce superoxide upon the ingestion of microorganisms, due to a lesion in a membrane-associated NADPH-oxidase, leads to an enhanced susceptibility to bacterial and fungal infections. Although defects in the X chromosome-linked cytochrome account for the majority of CGD patients, up to 35% are due to an autosomal recessive disease. Of these, greater than 90% have been shown to be defective in the synthesis of a 47-kDa cytosolic component of the oxidase (p47-phox/NCF1 gene) mapping to the WS critical region at 7q11.23. We have identified a patient with WS and CGD who has a molecular lesion in both copies of the NCF1 gene. Molecular analysis of somatic hybrids from this patient showed that the NCF1 gene is encompassed within the WS deletion on one chromosome 7 homologue. PCR analysis of the genomic DNA using ARMS primers specific for a common dinucleotide deletion (detected in ~90% of p47-phox deficient CGD cases) at a GTGT tandem repeat in exon 2 identified this mutation in the NCF1 gene on the other chromosome 7 homologue. The resulting frameshift leads to a premature stop codon further downstream. This study describes the first case of a WS patient with CGD and provides an easy method for the rapid detection of CGD patients homozygous for this common NCF1 mutation.

P0463. An apparently balanced 2;16 translocation co-segregating with macrocephaly and autistic like features and some phenotypic overlap with Goldenhar Syndrome

T. R. P. Cole[1], *R. Negrycz*[2], *S. Larkins*[3]
[1]*Clinical Genetics Unit; Birmingham, United Kingdom;* [2]*Child Health Directorate North Staffordshire Hospital NHS Trust; Stoke on Trent, United Kingdom;* [3]*West Midlands Regional Genetics Service; Birmingham, United Kingdom*
trevor.cole@bham-womens.thenhs.com

We report a father (AB) and son (MB) with a balanced translocation 46,XY,t(2;16)(q21;q13) and distinctive features. Neither the translocation, nor the phenotype is present in AB s parents. At birth, MB had an imperforate anus, talipes, an ASD, a limbic dermoid, dysplastic ears with a flat top to the helix and prominent lobes, and pre auricular tags. In childhood, a pervasive developmental disorder with specific communication and behavioural difficulties was noted and a diagnosis of Aspergers syndrome (AS) was subsequently made. He has relative macrocephaly, (OFC 97th centile,

height 10th centile). AB has similarly shaped ears, anal atresia, short stature and relative macrocephaly. Although never diagnosed with Aspergers, AB recalled childhood behavioural similarities to his son. The phenotype overlaps with a branchial arch syndrome (BAS) and the additional features, including the limbic dermoid are similar to Goldenhar syndrome. Goldenhar is not typically associated with macrocephaly or AS and therefore this could be a separate association with the alternate breakpoint. The macrocephaly/ autistism association has been recognised in recent years, including autosomal dominant macrocephaly (ADM). Literature review did not identify reports of BAS in association with these candidate loci and indeed chromosome 22 abnormalities are described with Goldenhar like phenotypes. However cases with anal and cardiac abnormalities and 16q deletions have been reported. ADM has been reported with PTEN mutations but not with linkage or chromosome abnormalities at 2q or 16q, however, sib pair analysis has suggested that proximal 2q could be a candidate loci for an autism susceptibility gene.

P0464. The Meier-Gorlin syndrome; Report of eight additional cases and review

E. M. H. F. Bongers[1], *J. M. Opitz*[2], *A. Fryer*[3], *P. Sarda*[4], *R. C. M. Hennekam*[5], *H. van Bokhoven*[6], *B. C. J. Hamel*[6], *N. V. A. Knoers*[6]
[1]*Department of Human Genetics University Medical Center Nijmegen; Nijmegen, The Netherlands;* [2]*Department of Pediatrics, Division of Medical Genetics; Salt Lake City, United States;* [3]*Mersey Regional Clinical Genetics Service; Liverpool, United Kingdom;* [4]*Department of Medical Genetics; Montpellier, France;* [5]*Department of Pediatrics and department of Clinical Genetics, Academic Medical Centre; Amsterdam, The Netherlands;* [6]*Department of Human Genetics, University Medical Centre Nijmegen; Nijmegen, The Netherlands*
e.bongers@antrg.azn.nl

The Meier-Gorlin Syndrome (MGS) or Ear, Patella, Short stature syndrome (EPS;MIM 224690) is a rare autosomal recessive disorder, characterized by the association of bilateral microtia, a/hypoplasia of the patellae, and severe pre- and postnatal growth retardation. Twenty-one cases have been reported in literature thus far. Here, we report on eight patients and compare them with previously described cases. One of the present cases had previously undescribed genital anomalies. There is a difference in facial characteristics between patients reported in early infancy and those described at older age; follow-up of patients is needed to substantiate this changing facial phenotype. We recommend radiographic survey of the patellae in patients at older age to investigate the weight of absent or hypoplastic patellae in the diagnosis of the syndrome. Based on the striking similarities of specific morphological skeletal findings, Lacombe et al. (Ann Genet, 1994;37;184-191) suggested that the MGS might be a human equivalent to the short ear (se) murine disorder. The murine se phenotype is caused by homozygous mutations in Bone Morphogenetic Protein 5 (BMP 5). Molecular genetic studies including testing of candidate genes in MGS are presently being performed. e.bongers@antrg.azn.nl

P0465. Considerations on comorbidity in neurofibromatosis

O. Brumariu, *I. Miron, C. Rusu, M. Munteanu*
Children s Hospital; Iasi, Romania
brumariu@mail.dntis.ro

We present ten cases of neurofibromatosis type 1 diagnosed in the Nephrology and Oncology Clinic of Iasi Children s Hospital in order to discuss different types of complications of this disorder. All the cases accomplished the diagnostic criteria and were confirmed by the geneticist. Most complications were oncologic, nephrologic, ophtalmologic and dermatologic, but some other rare complications are discussed. Familial cases are presented, with a comparison of the clinical expression in different affected personswithin the same family. A protocol for the management of the affected families is presented. In conclusion, we underline the importance of the complete examination and investigation of the patients with NF1 and their families, many of them presenting complicated forms of the disorder.

P0466. Septo-optic dysplasia and digital anomalies; Another observation

M. Ouach e-Chardin[1], *V. Cormier-Daire*[1], *J. Amiel*[1], *F. Monceaux*[2], *D. Genevi ve*[1], *D. Teillac*[3], *A. Munnich*[1], *L. Faivre*[1]
[1]*D partement de G n tique; Paris, France;* [2]*Service d Endocrinologie P diatrique; Paris, France;* [3]*Service de Dermatologie P diatrique; Paris, France*
lfaivre@hotmail.com

Septo-optic dysplasia (SOD) is defined by a variable combination of absence of the septum pellucidum, optic nerve hypoplasia, and pituitary anomalies. SOD is extremely rare in syndromes. Interestingly, SOD has been reported three times with digital anomalies suggesting amniotic bands. We report on another case with SOD, similar digital anomalies and multiple angiomas. This girl was the first child of young healthy parents. Clinical examination at birth at 37 weeks of pregnancy (length 46 cm, weight 2740g, OFC 32 cm) revealed multiple angiomas, plagiocephaly, high forehead, strabismus, and horizontal nystagmus. Hands abnormalities included bilateral hypoplasia of the fifth finger affecting the second and third phalanx, bilateral syndactyly of the fourth and fifth finger, and a constriction on the first phalanx of the second left finger. She sat at 9 months of age and could not walk at 17 months of age. Height and weight were — 2 standard deviations. Investigations revealed SOD, optic nerve hypoplasia, normal chromosomes, and isolated growth hormone deficiency. The pathogenesis of SOD with digital anomalies is unknown. It is unlikely that random phenomena could produce similar defects in all 4 patients. All cases were sporadic and no parental consanguinity was known. Further reports will help delineating this association as a new entity.

P0467. Tetrasomy 18p; Syndrome definition based on 6 new cases and literature review

T. Eggermann[1], *E. Prott*[2], *K. Eggermann*[1], *M. Cohen*[3], *S. Stengel-Rutkowski*[4], *U. A. Mau*[5]
[1]*Institute of Human Genetics; Aachen, Germany;* [2]*Institute of Human Genetics; Essen, Germany;* [3]*Kinderzentrum; M nchen, Germany;* [4]*Institut f r Medizinische Genetik; M nchen, Germany;* [5]*Department of Medical Genetics; T bingen, Germany*
teggermann@post.klinikum.rwth-aachen.de

Isochromosomes of the short arm of chromosome 18 [i(18p)] are the most frequent isochromosomes in humans. They can be detected in a non-mosaic state by conventional cytogenetic analysis in cells of different tissues, confirmation is carried out by FISH. The majority of i(18p)s originates from maternal meiosis II, followed by a postzygotic transverse centromere misdivision. Children with tetrasomy 18p show consistent features suggesting a characteristic phenotype. However, the aberration is rare and the clinical picture remains unprecise and not well known. Published reports mention psychomotor and mental retardation, muscular hypertonia, microcephaly, facial asymmetry, high-arched eyebrows, epicanthus, strabism, short palpebral fissures, small pinched nose, prominent upper lip, microgenia, low-set/malformed ears, scoliosis/kyphosis, long fingers with contractures and feet malformations. To define the tetrasomy 18p phenotype more precisely, we collected data of 6 children with supernumerary i(18p) investigated in our institutions as well as 34 literature cases for which clinical data were available. A frequency based spectrum of clinical and anthropological features is elaborated. Regarding the developmental and behavioural phenotype, the results of video analyses performed in three of the 6 children will be given.

P0468. Clinico-genetic markers of the bronchial asthma formation

N. Kovalchuk
Ivano-Frankivsk State Medical Academy; Ivano-Frankivsk, Ukraine
sluchyk@il.if.ua

The study is aimed to evaluate the cytogenetic markers and the life quality in patients suffered from bronchial asthma (BA). 154 patients were enrolled in the study; 73 of them had non-allergic asthma, 81 — mixed formed; 74 — the disease of average severity and 80 — severe course of bronchial asthma. The control group included 40 healthy persons of the same age. Clinical symptoms, the data of spirometry and pykflowmetry were studied. The specific AQ20 questionnaire was used for life quality evaluation.

The object of the cytogenetic research were epitheliocytes of mucous membrane of the oral walls and peripheral blood limphocytes. Somatic cells kariogram indice changes were revealed in patients with BA (chromatization index, sex chromatin, nucleolar and pathologically altered nuclei) testifying to violation of genome functional status. Decrease of chromatin transcription activity by 18 — 23%, increase of pathological nuclei number by 1.5 — 2.1 times, presence of heteropicnotic X-chromosome in males in 14.8 — 18.3% of cells have been determined. Variations of indexes depended on the severity of the disease.

While making metaphase analysis of perypheral blood lymphocytes we have determined that in patients with hereditary tendency to BA the level of chromosome abberations prevailed over those in the control group by 1.4 — 2.1 times.

Positive correlation between life quality improvement, indices of external respiration and cytogenetic markers have been revealed.

P0469. The Description Of A Case Of A Chromosome Translocation

A. Turitsa[1], *T. Kulakova*[1], *I. Laputin*[2], *G. Stefanenko*[2], *E. Grusdeva*[2], *E. Litvinovich*[1], *S. Tokarev*[1]
[1]*State Medical Academy; Omsk, Russian Federation;* [2]*Regional Genetic Center; Omsk, Russian Federation*
pinicola@mailru.com

The balanced and unbalanced translocation can pass from parents to children and be a cause of a family accumulation of multiple developmental defects because the chromosome apparatus is damaged. It leads to the violation of gene distribution to embryonic cells. Child T., was born in Omsk, by the 1st pregnancy. Mother is 20, father is 21. Genealogy is not complicated. Ultrasound on 34 week of pregnancy shows the indirect signs of chromosome violation in fetus. The delivery was artificial on 39 week. The child had the signs of an arrested prenatal development; the mass is 2000 g, the body length is 43 cm, the chest circumference is 28 cm, the head circumference is 32 cm. Phenotype; the parietal-occipital part of a head is plate, the frontal tubercles are protruded, the face cranial part prevails brain one. Slight prognathy, the nose is beak-shaped, its end is forked. The bridge of the nose is thick. The filter is short. Eye abnormalities; microophtalmia and cataract. Mouth is small and round, lips are thin, palate is of an arch-form, ears are low-set. The left ear is of a cup-form. Neck is short with pterygoideae plicae. There is also right ren hypoplasia; sacral sinus. Neurosonography showed agenesis of a corpus callosum. Karyotype of a child; 46, XX, der (13) t (6;13) (p21;q34) mat.

P0470. Waardenburg Syndrome Type 2 in a Turkish Family. Implications for the Importance of the Pattern of Fundus Pigmentation

A. Muellner-Eidenboeck[1], *E. Moser*[1], *H. Frisch*[2], *A. Read*[3]
[1]*Department of Ophthalmology, University Hospital; Vienna, Austria;* [2]*Paediatric Department, University Hospital; Vienna, Austria;* [3]*University Department of Medical Genetics, St.Mary Hosp.; Manchester, United Kingdom*
andrea.muellner-eindenboeck@akh-wien.ac.at

Waardenburg syndrome type 2 (WS2) is a heterogenous disorder, in about 10% of patients MITF gene mutations could be found, but for most cases the genetic basis is as yet unknown. The diagnostic criteria for WS2 include congenital sensorineural hearing loss, pigmentary disturbances of the hair and of the iris, but pigmentary anomalies of the fundus are not considered. A Turkish family with 2 of 3 sons showing clinical symptoms of WS2 was investigated both clinically and genetically. Special attention was paid to the pattern of fundus pigmentation in relation to the severity of sensorineural hearing loss. Genomic DNA samples were tested for mutations in the PAX3 and MITF genes. None of the individuals had dystopia canthorum, which is consistent with the clinical picture of WS2. One boy had complete iris heterochromia with a brilliant blue iris on the left side. He and his brother had severe bilateral sensorineural hearing loss and fundus pigmentary anomalies with areas of hypopigmentation next to areas of dense hyperpigmentation. The mother showed pigmentary mottling in the periphery of the fundus only. Ophthalmological and audiological evaluations of the other family members were within normal limits. Single strand conformation polymorphism analysis of the PAX3 and MITF genes showed no anomalies. Due to the conspicuous picture of pigment distribution one might suggest that the clinical symptoms in WS2 could be the consequence of a disorder in melanocyte distribution in the respective target tissue. The genetic basis, as yet unknown in most cases of WS2, might be found in a very late step of the pigmentation pathway.

P0471. Proteus syndrome ; A frequently misdiagnosed condition.

D. A. Teixeira, *G. S. Oliveira, A. P. Marques-de-Faria*
Departamento de Gen tica M dica - Universidade Estadual de Campinas; Campinas, Brazil
artedan@yahoo.com

Proteus Syndrome (PS) is a rare and complex hamartomatous condition, characterized by overgrowth of multiple tissues with partial gigantism and asymmetry of the limbs and viscera, connective tissue nevi, hemangiomata, lipomata, lymphangiomata, epidermal nevi and hyperostoses. It is highly variable and appears to affect patients in a mosaic manner. This could explain the significative variability and misdiagnosis. Usually it is confused with the Klippel-Trenaunay syndrome, hemihyperplasia with multiple lipomas and other conditions. Herein, we describe 3 girls, all sporadic cases, with the initial diagnosis of PS, which were reviewed taking into account the diagnostic criteria proposed by Biesecker et al.[Am J Med Genet 84;389, 1999]. A routine clinical evaluation, including dermatologic assessment, imaging studies with ultrasound, plain skeletal radiographs and computed tomography were performed. The first case was characterized by renal and lower limbs asymmetry, which became worse with the evolution; a lipoma and hyperchromic lesions appeared. Although she had not filled the main diagnostic criteria and had lost the follow-up, the hypothesis of PS was kept. The second case presented progressive overgrowth of the feet, hemangioma, lipoma, skin thickness and hyperpigmentation, with cerebriform appearance of the soles, confirming the diagnosis of PS; feet amputation was required. The third patient presented overgrowth of the foot comprising metatarsal region; no other lesions were observed during a 5 years follow-up; PS diagnosis was disregarded and the hypothesis of isolated gigantism of the foot was considered. This report reinforces the need of a multidisciplinary approach, criterious evaluation and follow-up on patients with the initial diagnosis of PS.

P0472. Clinical and genetical aspects of Williams-Beuren syndrome

G. von Beust[1], *A. Wessel*[2]
[1]*Institute of Human Genetics, University of Goettingen; Goettingen, Germany;* [2]*University of Goettingen / Cardiology; Goettingen, Germany*
h.woehler@t-online.deWas

The suspected diagnosis of Williams-Beuren syndrome, which is a retardation syndrome with great clinical variability, was cause for comparison of molecular genetic, molecular cytogenetic analysis to clinical symptoms. The results of the genetical analysis of a microdeletion of the elastin gene region on chromosome 7 were compared to the clinical symptoms. Are there any differences between symptoms in case of deletion or non-deletion? How informative are the molecular genetic, molecular cytogenetic analysis? 44 patients with suspected diagnosis of WBS were examined using molecular and molecular cytogenetic methods. The clinical symptoms as general symptoms, heart anomaly, dysmorphic signs and unusual neurobehavioural features were reported during clinical investigation in standardized questionnaires. The genomic DNA of the patients and their parents was analyzed using microsatellite markers. In some cases (e. g. uninformative microsatellite studies) we also used fluorescence in situ hybridization (FISH) with an elastin gene probe and performed a conventional chromosome banding analysis. 15 patients had a microdeletion. 4 patients had a deletion of the paternal allel and 7 patients showed the deletion of the maternal allel. The polymorphisms were of limited informativeness. In 2 cases microsatellite analysis was not able to determine whether the paternal or the maternal allel had been lost. In 2 cases the microsatellite analysis was uninformative so that FISH analysis was performed. All FISH analysis performed had an informative result. 80% of the children with a microdeletion of chromosome 7q11.23 showed the typical dysmorphic signs, 70% exhibited the typical WBS behaviour pattern, 50% had a specific heart anomaly. In contrast, in the group of children without a chromsomal microdeletion only 30-40% showed typical dysmorphic signs, only 10% had a typical heart anomaly and none of them showed specific behavioural changes. We found no indication to association of specific symptoms with paternal versus maternal origin of the deletion. The FISH analysis combined with a conventional chromosome banding analysis is very informative for diagnostic values. The results are compared to data of literature. Children with developmental retardation and WBS dysmorphic signs and an unusual behaviour should be examined by molecular cytognetic FISH analysis. If a microdeletion of band 7q11.23 is found a special cardiologic examination should be offered.

P0473. Promotion of Birth Defects Prevention Alliances

I. Baryliak[1], *I. Kharytonova*[1], *W. Wertelecki*[2]
[1]*Ukrainian Birth Defects Center; Kyiv, Ukraine;* [2]*University of South Alabama; Mobile, AL United States*
bariliak@usch.kiev.ua.

Birth Defects (BD) Prevention Alliances at regional (Alabama), National (Ukraine), and International levels (World Alliance of Non-Governmental Organizations for the Prevention of Birth Defects or WANGOPBD) provide a basis for two generalizations and a recommendation. A broad definition of BD as any structural or functional anomaly caused by genetic or environmental factors that impact embryonal, fetal, and child development facilitates communications with healthcare planners and providers. In Ukraine, such risk factors as chronic low-dose ionizing radiation from the Chornobyl disaster, iodine poor soils, low consumption of folic acid, and high consumption of alcohol are then perceived as causes of BD. Emphasizing that mental retardation, malformations, congenital syphilis, and childhood cancers ofter are BD, stresses the crucial role genetics and teratology in public health. The idea that every child has the right to be born free from preventable BD generates strong empathy. This goal helped to forge BD Prevention Alliances linking a variety of parental support groups with a variety of professionals and opinion makers. An Alliance confers a

stronger voice to what my have been a number of small advocacy groups. BD Alliances are strengthened by resource centers we call Omni BD Centers . The Centers offer a common ground shared by lay and professional members. The main components are information materials, technical and staff assistance, conference facilities, and access to the Web.

P0474. Discordance of dental anomalies in monozygotic twins with beta-thalasemia

***E. Severin**, I. Ioachim, S. Colceriu*
Carol Davila University of Medicine and Pharmacy; Bucharest, Romania
severin@rnc.ro

A pair of 16 years old monozygotic twins (MZ) with beta-thalasemia was studied.The male twins were discordant for several dental anomalies of permanent dentition and concordant for a few genetic traits; ABo blood group system, Rh blood group, HLA haplotype and beta-thalasemia. One twin showed a supernumerary tooth known as paramolar on the right maxillary molar region and delayed eruption of permanent teeth;the co-twin has normal number of permanent teeth and timing of eruption. In their family the anomalies of permanent teeth have been established only in the proband. Comparison between monozygotic twins have provided strong support for the idea that MZ do not share all their alleles;they are genetically similar, but not identical.Discordance of anomalies may reflect differences in developmental timing, differences in susceptibility to one or more teratogens, or random events occuring within very complex programs of teeth development.

P0475. Complex Phenotype of a Patient with an appearently balanced de novo Translocation Y;22 - A new Syndrome?

***P. M. Kroisel**[1], E. Petek[1], U. Binder[2], K. Wagner[1], H. Zierler[1]*
[1]Institute of Medical Biology & Human Genetics; Graz, Austria; [2]St. Anna Children Hospital; Vienna, Austria
peter.kroisel@kfunigraz.ac.at

Here we report on a 20 year old male patient with an complex combination of phenotypic anomalies. He was born at term as the first child of a healthy unrelated couple following an uneventfull pregnancy by caesarian section because of macrocephaly OFC 37.5 cm (> 90 centile), birth weight 3.6 kg and length 52 cm. Facial dysmorphic findings as macrocephaly, hypertelorism, antimongoloid slant, malformed protruding ears of normal size, a short nose with broad nasal bridge and full lips were noticed. He shows several additional anomalies like mental and growth retardation and anaemia. He currently is 156 cm tall (<< 3 centile), weight 52 kg (< 3 centile) and OFD of 62.5 cm (>> 97 centile). Cytogenetic analysis revealed a Y;22 translocation with breakpoints on the short arm of both chromosomes with no further anomalies. The karyotype 46,X,(Y;22) (p11.2;p11.2) de novo was also confirmed by FISH analysis using whole chromosome painting and locus specific probes. Parental karyotypes are normal and paternity was confirmed by microsatellite analysis. He does not show anomalies of inner organs, except a moderate hepatomegaly. Most of the standard laboratory parameters are normal, but anaemia is treated with EPO since a few month successfully. Because no gene related to the phenotype is mapped to the chromosomal breakpoint at Yp11.2 and involvement of a genein22p11.2ishighlyunlikely,we are now focussing on cloning the breakpoint in Yp11.2 in an effort to approach the complex phenotype of the patient that well may represent a new syndrome.

P0476. Williams Syndrome. The Greek experience

***H. Fryssira**[1], S. Amenta[1], L. Thomaidis[2], A. Kolialexi[1], A. Mavrou[1], C. Metaxotou[1]*
[1]Medical Genetics, Athens University, School of Medicine; Athens, Greece; [2]1st Dept. of Pediatrics, Athens University; Athens, Greece
efrysira@cc.uoa.gr

Williams syndrome is a well recognized but not fully understood neurodevelopmental disorder, involving both connective and central nervous systems. The distinctive cognitive profile may represent a prototype to unravel the relationship between the genes and the behavior.
In the present study we collected blood from 25 W.S children,aged from 4 months to 21 years. All cases were non familial while two patients were monozygotic twins. All children underwent detailed clinical, developmental, cardiological, neurological and laboratory investigation. Diagnosis was confirmed by FISH. Their follow up continued for five years. Molecular analysis for the detection and the origin of deletions in all children and their parents was performed using the intragenic polymorphic markers D7S613, D7S489B and D7S1870 .Our results showed a higher incidence of pulmonary stenosis than what appears in the literature. Severe hypertension was noticed in more than half of our cases and caused selective abortion in a first trimester pregnancy.Another patient had severe hypertension due to renal artery stenosis which led to brain hemorrhage and death. Hyperacousis was a consistent finding in our series of patients.According to the molecular analysis the pair of twins and 6 other cases had maternal deletion and 2 cases had paternal deletion while the rest were either normal or non informative.We could not demonstrate any relationship between the size of the deletion and the phenotype. The absence of genes other than elastin which probably contribute to the variability of the phenotype remains to be determined.

P0477. Variable expression of Microcephaly, Colobomatous Microphtalmia, Short Stature and Psychomotor Retardation Syndrome in consanguineous Omani family.

***A. Rajab**[1], H. Hamamy[2], S. Antonorakis[3], K. Sperling[4]*
[1]Ministry of Health, Sultanate of Oman; Muscat, Oman; [2]WHO; Geneva, Switzerland; [3]Geneva Univercity; Geneva, Switzerland; [4]Charitee; Berlin, Germany
drarajab@omantel.net.om

Colobomatous Microphtalmia is a common malformation, is anatomically, clinically and etiologically a heterogenous disorder. Microphtalmia has been reported in more than 150 syndromes with overlapping clinical findings in association with chromosomal and monogenic traits. We report five girls from consanguinous Omani family, with variable expression of multiple congenital anomaly syndrome of Microcephaly, Colobomatous Microphtalmia, Short Stature, Severe Psychomotor Retardation, Congenital Heart Defects and 46 XX karyotype. Molecular analysis is currently in progress for better understanding this Syndrome. Detailed clinical findings and literature review will be presented.

P0478. Congenital generalized fibromatosis associated with birth defects; a third patient

***A. Bottani**[1], V. Desmangles[2], J. F. Egger[1], M. A. Brundler[3], A. M. Calza[4], R. Pfister[2]*
[1]Division of Medical Genetics; Geneva, Switzerland; [2]Department of Pediatrics; Geneva, Switzerland; [3]Division of Clinical Pathology; Geneva, Switzerland; [4]Department of Neurosciences & Dermatology; Geneva, Switzerland
bottani@cmu.unige.ch

Congenital generalized myofibromatosis (CGF) is a rare condition characterized by widespread firm nodules in the skin, muscles, bones and internal organs. Histologically, the lesions consist of cells which have both muscle and fibroblast characteristics. Etiology of CGF is unknown. Most cases are sporadic and are not associated with other malformations and/or mental retardation. Exceptional familial cases compatible with autosomal recessive or dominant inheritance have been reported. Only two patients have been described with CGF and associated birth defects (Spraker et al., J Am Acad Dermatol 1984; 10; 365-371; Michel et al., Eur J Pediatr 1990; 149;251-252). We report a third patient with CGF and congenital malformations. The newborn female was the only child of non-consanguineous parents. The mother had congenital unilateral cataract. Delivery occurred at 41 weeks of gestation after an uneventful pregnancy. Birth measurements were in the normal range. Examination in the neonatal period revealed facial dysmorphism (coarse face, epicanthic folds, long philtrum, macrostomia), a posterior cleft palate and a malplaced anus. In addition, numerous (approx. 50) subcutaneous firm nodules, varying in diameter from 0.2-4 centimeters, were found scattered over the whole body. Histopathological examination of one nodule allowed the diagnosis of CGF. Echocardiography showed multiple intramyocardial round structures, as well as a small perimembranous ventricular septal defect. Xrays revealed a clavicular pseudarthrosis and multiple lytic lesions of long bones. Blood and skin karyotypes were 46,XX. Despite treatment with interferon, vincristine and actinomycine, the child died at the age of 3 months of respiratory failure. Autopsy confirmed the widespread involvement of muscles, bones and internal organs. Given the probably non-random recurring association of CGF with various congenital malformations and the familial occurence of some cases, one can postulate that this condition is most likely caused by a mutation in a (yet unknown) gene acting very early in development and cell differentiation.

P0479. Fibrodysplasia ossificans progressiva - a report of two cases

***E. Sukarova-Angelovska**, M. Kocova, J. Anastasovska*
University Childen Hospital; Skopje, The former Yugoslav Republic of Macedonia
valid@mt.net.mk

Fibrodysplasia ossificans progressiva FOP) is a condition described centuries ago, but exact pathogenetic mechanism is still not well understood. FOP is characterized by ectopic soft tissue calcification that progresses always in the same direction; from cranial o caudal. Neck, spine and shoul-

ders are described as most common sites for this heterotopic ossification. The second feature of FOP is malformed great toe, which leads to proper diagnosis early after appearance of the first swellings. Most of the cases are sporadic, but in some families autosomal dominant inheritance is described. Some authors suggest that mutations in the bone morphogenetic protein 4 gene or it s promotor are responsible for ossification in the abnormal tissues. The first case is a girl, which is the first child of unrelated parents. Swellings, 4 at a time appeared at the age of 2 months at the dorsal occipital region and neck. The size of these swellings was different;1-3cm. The child had typical short and malformed great toes; also minor coxal congenital dysplasia. Biopsy of the tissue was performed to exclude malignancy, and the presence of normal fibroblasts, fibrocytes and collagen fibers were found. These swellings started to resolve several months later, with just a slight reminiscence of them. The time will show whether it is a phase of remissions, or a different condition. The second patient is a 7-year-old boy, with ectopic calcifications first found in the shoulders, and consequently the disease proceeded along the spine and the arms. The appearances of the swellings were painful, and the child developed restricted mobility of the arms and spine in a period of a few years. Additional malformations were present; one-phalangeal short great thumb, and fused cervical vertebrae. Both of the children had no positive family history. None of the family members in the two families had abnormal or short thumbs. We can conclude that two of the cases were sporadic, representing a fresh mutation of the disease.

P0480. Microdeletion of chromosomal region 22q11 and dysmorphic features in children with conotruncal heart defects

M. Derbent, *Z. Yilmaz, V. Baltaci, A. Saygili, B. Varan, K. Tokel*
Baskent University, Faculty of Medicine; Ankara, Turkey
muratder@superonline.com

Chromosome 22q11 deletion causes DiGeorge syndrome, velocardiofacial syndrome (VCFS) and conotruncal anomaly face syndrome. Cardiac outflow malformations are frequently main findings of these syndromes. The aim of this study was to determine the incidence of 22q11 deletion in children with conotruncal heart defects and to evaluate additional dysmorphic findings in these patients. Cardiac catheterization had been performed in 30 patients (age; 3 days-9 years) with conotruncal heart defects. The patients were also examined by a pediatrician and clinical geneticist. Their blood samples were obtained for fluorescence in situ hybridization (FISH) analysis with LSI DiGeorge/VCFS region probe. Cardiologic diagnosis of them were as follows; tetralogy of Fallot; 7 cases, double outlet right ventricle; 8 cases, transposition of the great arteries; 8 cases, truncus arteriosus; 3 cases, other conotruncal defects with malalignment ventricular septal defect; 4 cases. Twenty children were found to be dysmorphic. FISH analysis of these 20 patients demonstrated 22q11 deletion in 7 cases. Only two patients without dysmorphic features had 22q11 deletion. In this study, the details of conotruncal heart defects and dysmorphic findings of our patients were presented.

P0481. Neurocognitive Profile of Young Children with XXY (Klinefleter syndrome)

C. A. Samango-Sprouse[1], *P. Law*[2]
[1]*George Washington University; Washington, DC United States;* [2]*Johns Hopkins University; Baltimore, MD United States*
cssprouse@aol.com

XXY occurs 1;500 & the facets of the neurodevelopmental(ND)profile of the young child with XXY have not been well desribed in a large sample. There were 52 subjects with XXY prenatally diagnosed. Comprehensive ND assessments were completed using standardized tools to determine the level of functioning in the five areas of development. The mean age was 13m(r=2-48m). Parents were well educated with 25% having postgraduate degrees. Data was segregated and analyzed using Hottellings t-test at p<.05 level. Speech(EL=85.7[sd=12.8],p<.001)& motor(PDI= 88.46[sd=12.39],p<.001)delays were significant with normal cognition(MDI= 97.6[sd=12.8]). 50% of the boys had abnormal muscle tonus in the trunk & extremeties. This profile is highly suggestive of an infantile presentation of developmental dyspraxia(IDD),a motor planning disorder with speech, motor and attention conmpromised. This large study on XXY who were prenatally diagnosed reveals an infantile presentation of a learning disorder which may be an predictive of an increased risk for educational difficulties. Further follow-up will determine the incidence of learning disablilties and the predictive factors from these early findings. Prenatal counseling with families should discuus the possibility IDD, normal cognition and need for EI evaluation that are focused on specific weaknesses. This study further defines the natural history of a sex chromosome disorder in early childhood years. Further studies are warranted to investigate if there are any correlations between ND performance and parental origin of the additional X. Studies investigating the relationship between skewed X-inactivation and the ND profile of boys with XXY may further clarify the variability of function in boys with XXY.

P0482. Evaluation Of Clinical And Cftr Status Of Vas Deferens Agenesis In Infertile Men

S. Murat[1], *E. Yilmaz*[2], *H. Samli*[3], *N. Imirzalioglu*[4], *M. Bahce*[4], *S. Kahraman*[5]
[1]*Afyon Kocatepe University; Afyon, Turkey;* [2]*Hacettepe University, Department of Medical Genetics; Ankara, Turkey;* [3]*Afyon Kocatepe University, Faculty of Medicine, Department of Medical Genetics; Afyon, Turkey;* [4]*GATA, Faculty of Medicine, Department of Medical Genetics; Ankara, Turkey;* [5]*Memorial Hospital, IVF Center; Istanbul, Turkey*
msamli@tr.net

Congenital absence of the vas deferens is an uncommon anomaly that may effect male fertility. About 1,5% of infertile men have bilateral congenital absence of vas deferens. Advances in genetics and the advent of reproductive techniques have revolutionized the aspect of the reproductive potential of these men. Polymerase chain reaction amplifications of genomic DNA was used to amplify each of the CFTR exons and their flanking regions. Four common CFTR gene mutations were assayed (˜F508, 1677-D TA, Poly-T, M470V). To elucidate other urogenital anomalies and CFTR mutations that accompany the absent of vas deferens, we report 60 azoospermic men and 1 asthenoteratospermic man with unilateral or bilateral absence of vas deferens. Sixty-one patients with unilateral or bilateral absence of vas deferens applied to our IVF clinic due to male factor infertility between 1996-1999. Mean age of the patients were 34.4–5,8 (S.D.), and mean infertility time was 8.5–5.3 years. Major urogenital anomalies of vas deferens, seminal vesicle and epididiymal anomalies were detected. No DTA nad Poly-T mutations were detected in our patient population. ˜F508 mutation was detected in 4% and M470V mutation was detected in 13% of patients. Twenty-four patients underwent sperm retrieval procedure for intracytoplasmic sperm injection (ICSI); 7 MESA, 12 PESA, 3 TESA and 2 TESE procedure were carried out. The number of sperm retrieved with these procedures were between 20.000/mL and 15million/mL differentiating according to the type of procedure. Thirty-four patients have a history of previous scrotal exploration and testicular biopsy. Reevaluation of these preparations revealed 14 normospermatogenesis, 7 hipospermatogenezis, 9 complete or incomplete maturation arrest and 1 tubular hyalinization. Other urogenital anomalies are common for men with unilateral or bilateral absence of vas deferens. CFTR gene mutations which are known to be frequent among these patients could not be detected in our patient group. Vasal agenezis can occur without evidence of CFTR defects. Sperm retrieval from these patients is almost always possible for assisted reproductive techniques.

P0483. Rett Syndrome; Phenotype Expansion and Broadening Parameters for Diagnosis and DNA Testing Resulting From the Availability of Mutation Analysis.

L. R. Shapiro[1,2], *H. G. Taska*[2], *R. V. Scott*[2], *D. F. Kronn*[1,2]
[1]*New York Medical College; Valhalla, NY United States;* [2]*Westchester Medical Center; Valhalla, NY United States*
lrskicker@msn.com

The diagnosis of Rett Syndrome prior to the availability of mutation analysis by sequencing the MECP2 gene was based on rigid clinical criteria; females who had normal development until 6-18 months of age with subsequent regression characterized by loss of speech and loss of purposeful use of hands, microcephaly, seizures, autistic behavior, ataxia, hyperventilation and stereotypic hand movements. 4 patients (16, 4, 3.5, 3 years old) who were found to have a MECP2 mutation exhibited an inconstant phenotype with incomplete clinical criteria; all (4/4) had regression following normal initial development with loss of speech and stereotypic hand movements; (2/4) seizures; (2/4) loss of walking;(0/4) microcephaly; (2/4) autistic behavior;(2/4) ataxia and (1/4) hyperventilation. The absence of microcephaly resulted in delay of diagnosis in 3/4 of the patients because a number of physicians felt that microcephaly was a requisite criterion. While additional patients will be necessary to fully appreciate the changing phenotype of Rett Syndrome, the diagnosis should be considered and MECP2 gene sequencing for mutation analysis done when a developmentally delayed female had normal early development followed by regression with the loss of speech, especially in the presence of stereotypic hand movements.

P0484. Acromegaly in a patient with Turner syndrome, a rare disorder.

G. Casti eyra, *M. Guitelman, J. Herrera, S. Copelli, S. Fiszlejder, M. Zeller, A. Oneto*
Division of Endocrinology, Hospital Durand, Cedie, Buenos Aires; Argentina
castineyra_gra@yahoo.com

Acromegaly and Turner syndrome is not a frecuent clinical finding. We present a case of 23 years old, is the first daughter of non consanguineous parents, family history is unremarkable. Clinical endocrinological cytogenetics and molecular studies are analyzied. Physical examination show height 153(pc10) weight66(pc75), HC 61(+4SD). The phenotype is Turner Syndrome excluding facial features like overbite and nasal bone hypertrophy and prognatism. Acanthosis nigrans and hirsutism are clearly evident and stature is in the 90pc for TS. Hormonal levels (IRMA;RIA);GH 26ng/ml, IGF1 400ng/m, insulin 200ugU/ml, FSH68mUI/ml, E2 15pg/ml, Androstened. 2,9ng/ml, DHEAs 3000ng/ml, To 0,85ng/ml, PRL10ng/ml, 17OHP4 1,2-ng/ml, Dynamic test OGGTshow not inhibition of GH.ACTH plus DXM8 inhibit the adrenal steroids. MR; an intrasuprasellar tumor with bilateral cavernous sinus expantion. US; absent gonads and hypoplasic uterus. RX; Cervical fusion (C1-2-3). Karyotype (lymphocytes) ; 45X/46,X,del (X) (q22-q ter)in 40% and 60% of cells respectively. DNA (leukocytes); any sequence of SRY gene, centromere and heterochromatic region of Y was detected by PCR. To our knowledge is the second case reported. Clinical findings, indicate the prevalence of the 45,X line, stature may be influence by the presence of some genes in the Xq and GH excess. The metabolic efects are potencialy altered, insulism resistance, and the elevated levels of GH and IGF1 may act in the adrenal receptor producing an increment of adrenal androgens. The understanding of genetic factors would be of great value and is our next step. Clinical course and dificulties in clinical managment will be present.

P0485. Homozygosity for a Novel MSH2 Mutation in a Child with Acute Lymphoblastic Leukemia and Multiple Caf -au-lait Spots.

G. E. Graham[1], *D. Whiteside*[2], *S. E. Andrew*[2], *K. Booth*[1], *D. R. McLeod*[1]
[1]*Alberta Children s Hospital and University of Calgary; Calgary, AB Canada;* [2]*University of Alberta; Edmonton, AB Canada*
gail.graham@crha-health.ab.ca

MLH1 and MSH2 heterozygotes develop HNPCC when somatic loss of the wild-type allele produces faulty mismatch repair (MMR). Until recently, the consequences of homozygosity for germline mutations in human HNPCC genes were unknown. In 1999, Ricciardone et al. and Wang et al. reported a total of five offspring from two consanguineous matings whose parents were MLH1 mutation carriers. DNA was available from four of the five children. Two were homozygous for a nonsense MLH1 mutation and the remaining two were homozygous for a missense MLH1 mutation. Four of the five met the clinical criteria for a diagnosis of Neurofibromatosis type I. All five had been diagnosed with leukemia or lymphoma, four below the age of 3, and one also developed a medulloblastoma. Since hematological malignancies can occur in the setting of NF type 1, the authors speculated that the NF1 gene was particularly susceptible to mutations resulting from faulty MMR. We now report the first instance of germline homozygosity for a truncating MSH2 mutation in a 2-year-old boy of East Indian/Fijian parents who has multiple caf -au-lait spots and acute lymphoblastic leukemia. The mutation is a novel G->A transition in a splice site that is predicted to result in exon 11 skipping, a frameshift and a premature stop codon in exon 12. Our patient s parents are 22 and 27 years of age and are not knowingly related. Neither have been diagnosed with malignancies in the HNPCC spectrum and neither has features of NF1.

P0486. A family with the association of the Goldenhar syndrome and X-linked ichthyosis

R. Rivera[1], *E. Due as*[1], *S. Kofman*[1], *L. M. Gonzalez*[1], *M. Valdes*[2], *S. Cuevas*[1]
[1]*Hospital General de Mexico; Mexico DF, Mexico;* [2]*Instituto Nacional de Ortopedia; Mexico DF, Mexico*
sergioa@servidor.unam.mx

Goldenhar syndrome is an entity classified into the facio-auriculo-vertebral (FAV) spectrum. Although the inherited pattern is uncertain, there are several reports with dominant and recessive autosomal traits. X-linked ichthyosis (XLI) is an entity characterized by dark, regular, adherent scales of skin on abdomen, neck, back and extremities. XLI is due to the deficiency of the steroid sulfatase enzyme (STS). The STS gene is located on Xp22.3. Most XLI patients present large deletions of the STS gene and flanking sequences. We report a 25 year female with clinical characteristics of the FAV spectrum and her son of 7 years of age with XLI. To establish XLI diagnosis, STS activity was determined in leukocytes using 7-[3H]-dehydroepiandrosterone sulfate as a substrate. It was also performed amplification of both extremes of the STS gene by PCR and FISH analysis of the STS gene. STS activity was undetectable in the patient (0.00 pmol/mg protein/h) and very low in the mother (0.35 pmol/mg protein/h vs 0.79 pmol/mg protein/h of the normal control). No amplification of the 5 and 3 ends of the STS gene were observed in the patient. FISH analysis was positive for XLI in the patient and for XLI-carrier in the mother. FAV spectrum has been associated with several entities but this is the first report in the literature in which FAV spectrum and XLI are present in the same family

P0487. Clinical Variability in Neonatal Progeroid Disorders; More Than One Disease

J. Hou
Chang Gung Children s Hospital; Taoyuan, Taiwan Republic of China
houjw741@adm.cgmh.com.tw

Several progeroid disorders of childhood onset have been reported. Among them, a specific type presenting as an old-man appearance since birth was named as neonatal progeroid syndrome(NPS). Since the first case of NPS(Am J Med Genet 1995;69;195-6), another 4 neonates with similar features were found in Taiwan. They all have progeroid face(pinched appearance with hypoplasia of the lower half of the face), prominent scalp veins, frontal bossing, small beaked nose, and severe growth retardation. Thorough endocrinological and metabolic investigations were performed in those patients and the results disclosed; hypothyroidism or hyperthyrotrophinemia(4/5), very low level of insulin-like growth factor I(3/5), hypolipidemia(4/5), and a specific organic aciduria(2/5). The associated anomalies included; laryngomalacia(4/5),cardiac defects(2/5), neonatal teeth(2/5), congenital glaucoma and achondroplasia(1/5), and contractural arachnodactyly in all patients. The karyotyes wre all normal. However, chromosomal breakage test showed markedly increased breakage rates in 3 patients, suggesting the role of DNA repair defects in such premature ageing disorders. More than one disease may account for the clinical variability of neonatal progeroid disorders.

P0488. Heterozygous and homozygous inheritance of the Y90H mutation in the PAX3 gene in a Turkish family with Waardenburg syndrome (WS-I) and Klein-Waardenburg syndrome (WS-III)

M. Y ksel-Apak[1], *T. T kel*[2], *A. Ghanbari*[1], *H. Kayserili*[1], *M. Emiroglu*[3], *G. Hafiz*[3], *O. Uyguner*[1], *B. Wollnik*[1]
[1]*Division of Medical Genetics, Child Health Institute, Istanbul University; Istanbul, Turkey;* [2]*Division of Medical Genetics, Department of Pediatrics, Istanbul Medical Faculty, Istanbul University; Istanbul, Turkey;* [3]*Ear, Nose, and Throat Department, Istanbul Medical Faculty, Istanbul University; Istanbul, Turkey*
wollnik@superonline.com

Auditory-pigmentary syndromes are caused by the absence of melanocytes from the skin, hair, eyes, or stria vascularis of the cochlea. Examples with patchy depigmentation are Waardenburg syndromes (WS) which are usually inherited in an autosomal dominant pattern. Type 1 Waardenburg syndrome (WS-I) is caused by heterozygous loss of function mutations in the PAX3 gene. Klein-Waardenburg syndrome (WS-III) is a very rare condition and represents an extreme presentation of WS-1, with additional extremity abnormalities. We present a 4-year old Turkish child with typical features of Klein-Waardenburg syndrome including dystopia canthorum, partial albinism, hearing impairment, and upper-limb defects. The child was born to a consanguineous marriage and both parents were affected with WS-I. We screened the entire coding region of the PAX3 gene for mutations and identified a novel missense mutation, Y90H, in exon 2 of PAX3 gene within the paired box domain. Both parents with WS-I were heterozygous for the mutation and the child with WS-III was homozygous. This is the second report of a homozygous PAX3 mutation causing the WS-III phenotype. Homozygous PAX3 mutations in the Splotch mouse, a mouse model of Waardenburg syndrome, have lethal neural tubes defects, but we could not observe any neural tube abnormality in our patient. Clinical and molecular analysis in 4 additional Turkish families with WS-1 showed the clinical variability of the WS-I phenotype among and within WS-I families. Further, we have identified 4 novel mutations in the PAX3 gene in these families.

P0489. Clinical description of a new patient with de novo partial trisomy 7q identified by SKY

A. B. Caliebe[1], *M. Kautza*[1], *J. I. Martin-Sobero*[1], *A. Holthausen*[2], *R. Siebert*[1], *W. Grote*[1]
[1]*Department of Human Genetics; Kiel, Germany;* [2]*Neuropediatric Hospital; Kiel, Germany*
caliebe@medgen.uni-kiel.de

The male proband was first seen at the age of six months because of developmental delay and facial dysmorphism. He had progressive macrocephaly, dysplastic deep set ears, depressed nasal root, and anteverted nares. There were genital anomalies (undescended right testis, hypoplastic scrotum, hypospadia glandis, and a micropenis). The boy exhibited central strabism. Head control had not been achieved at that time, he smiled since the eighth week and had just learned to transfer objects from one hand to the other. The propositus is the second child of healthy non-consanguineous parents, born at term after an uncomplicated pregnancy. Chromosome analysis on peripheral blood lymphocytes revealed additional chromosome material on the distal part of the long arm of one chromosome 11 in 20 metaphases analysed. The parents‘ karyotypes were normal. Spectral karyotyping (SKY) suggested the material of unknown origin attached to chromosome 11 to be derived from chromosome 7. FISH with whole chromosome painting probes for chromosomes 7 and 11 confirmed addition of chromosome 7 material to chromosome 11 due to an unbalanced translocation t(7;11). According to the G-banding pattern, the fragment of chromosome 7 translocated to chromosome 11 comprised the distal part of the long arm, from 7q34 to 7qter. Pure partial trisomy 7q has so far rarely been described. Macrocephaly, dysplastic pinnae, micropenis, and undescended testis in boys, and moderate till profound retardation seem to be features of this syndrome. When re-evaluated at the age of thirteen months head control had been achieved and the propositus could roll over.

P0490. Split hand/split foot, iris coloboma, hypospadias and subfertility; a new developmental malformation complex?

J. Giltay[1], *D. Wittebol-Post*[2], *H. VanBokhoven*[3], *P. Kastrop*[4], *T. Lock*[5]
[1]*University Medical Center Utrecht; 3508 AB utrecht, The Netherlands;* [2]*FCDonders Institute of Ophthalmology; Utrecht, The Netherlands;* [3]*Department of Human Genetics, University Medical Center Nijmegen; Nijmegen, The Netherlands;* [4]*University Medical Center Utrecht, Division of Obstetrics, Neonatology and Gynaecology; Utrecht, The Netherlands;* [5]*Dept of Urology; Utrecht, The Netherlands*
j.c.giltay@dmg.azu.nl

A 35-year-old male was referred for fertility treatment. Semen analysis revealed a teratozoospermia. Apart from the wish for fertility treatment the patient wanted to be informed on the risk of transmitting his hand and foot abnormalities to his possible progeny. The abnormalities consisted of split hand/split foot on the left side, a hypoplastic 5th ray of the right hand and a hypoplastic 1st ray of the right foot with a small cleft between the first and second ray. His medical record further revealed several surgical corrections of a glandular hypospadias and eye abnormalities which consisted of a complete iriscoloboma of the left eye in an atypical position (cranio-temporal) and a coloboma of the papilla in the right eye. So the hand, foot and eye abnormalities appear to be more severe on the left side. Family history revealed no abnormalities which could be related to the findings in our patient. The symptoms appear different from those described in Hand-Foot-Genital Syndrome, moreover an iriscoloboma would not fit in this diagnosis. Since split hand/split foot can be caused by mutations in the p63 gene, mutation analysis of this gene was performed. However, sequencing of the majority of the exons did not reveal a mutation. So far, we do not have a diagnosis or an explanation for this malformation complex. In counselling this patient we felt we could not exclude a recurrence risk of 50% for his progeny.

P0491. Malpuech syndrome; case report and review

W. S. Kerstjens-Frederikse, *C. M. L. van Dael, A. J. van Essen*
University Hospital Groningen; Groningen, The Netherlands
w.s.kerstjens@medgen.azg.nl

Malpuech syndrome is a rare autosomal recessive syndrome with growth retardation, mental retardation, cleft lip and palate, hypertelorism, ptosis of the eyelids, urogenital anomalies including renal agenesis, undescended testes and micropenis and caudal appendage and/or sacral increased skin pigmentation. We report an 8 month old boy, adopted from Brasil, with bilateral cleft lip and palate. His length was 58 cm (<<-2SD), weight 5.5 kg(<<-2SD), OFC 42 cm (normal if corrected for length). His hair was sparse and wooly . He had a broad forehead, downslanting palpebral fissures, ptosis and telecanthus, ICD 3.2 cm (> P97); OCD 6.9 cm (< families. 6 from cases reported 11 the review We syndrome. Malpuech of that matches clearly patient our phenotype The pending). are probes subtelomeric and 4p- (FISH 46,XY. male, normal, was Karyotype year. 1 age at valve urethral a because operated He heterotopy. crossed with ectopic, kidney right normal. examination Opthalmological dysfunction. ear middle loss hearing mild had delayed. severely development Motor absent. other canal, inguinal in high palpable testis One thin. short very penis hypoplastic scrotum sparsness. flaring lateral arched were eyebrows>

P0492. The Medical Genetic Examination Of Children With Congenital Laryngeal Patology.

I. L. Soldatski[1], *V. G. Solonichenko*[2]
[1]*Moscow Medical Academy; Moscow, Russian Federation;* [2]*N.F.Filatov Children Hospital; Moscow, Russian Federation*
ysoldatsky@mailru.com

The genetic factors can play important role in the forming pathology of breathing system, but hereditary diseases of larynx is a very seldom pathology. We performed medical-genetic examination in 54 children s families with congenital stridor in the Clinic of Reconstruction Surgery of Larynx and Trachea for Children of Sechenov s Moscow Medical Academy with the aim of specification of hereditary background and its influence on the clinical picture and on the results of the treatment. The age of the children at the moment of entering to the clinic was from 21 days to 3years. We performed to every child the examination of larynx during direct microlaryngoscopy and tracheoscopy. The diagnosis of laryngomalacia was determined in 30 patients, the paralysis of vocal folds - in 10, laryngeal cyst - in 5, congenital laryngeal membrane - in 3, tracheomalacia — in 6. Syndromological diagnosis (Aarskog, Ehlers-Danlos, Pierre Robin, Noonan, Weaver and Down syndromes) was found in 7 (13.0 %) patients. The isolated defect of larynx without manifestation from other organs and systems were found in 12 (22.2 %) patients. The rest 35 patients (64.8 %) had the combination of development organ s defects in several systems, not inducted by one another, which are possible to mark as multitude congenital defects of development. So, the medical - genetic examination is may be necessary part of the preoperative preparation of children with congenital stridor.

P0493. Bilateral anophtalmia and esophageal atresia

B. Gilbert[1], *C. Menetrey*[1], *S. Odent*[2], *L. De Lumley*[1], *V. Belin*[1]
[1]*CHRU Dupuytren; Limoges, France;* [2]*CHU Pontchaillou; Rennes, France*
gilbert@unilim.fr

Only few cases of associations of esophageal atresia with anophtalmia have been observed since Rogers in 1988. We report on the seventh case. At birth, this girl presented with these two malformations. Her face looked dysmorphic, pear-shaped, with a large forehead and a small chin. In profile, her nasal-root was not very well-marked, and her nucha was smooth. The ears seemed to be low implanted, and her front fontanel was very small with overlapping sutures. Her chromosome testing on leukocytes and fibroblasts was normal. On the fourth day of her life she died of general failure. Post-mortem necropsy confirmed complete bilateral anophtalmia and type III esophageal. There were no other malformations. To our knowledge, this observation is the 7th case of anophtalmia and esophageal atresia association. Moreover among males, external-genitals abnormalities are described. SHAH proposed to call this association Anophtalmia-Esophageal-Genital syndrome (AEG syndrome). All of the cases whose pictures has been published, presented a similar dysmorphic face. As regards embryology, those two malformations appear at the same moment of embryonic life. There are, as in hypospadias, a midline abnormality. The homogeneous character of those seven reported cases and the fact that they are midline abnormalities, plead in favor of a genetic origin. Some genes are known to be responsible for anophtalmia in animals; Eyeless 1 and 2 in the mouse, and ANOP 1 and 2 in the rat. In our patient, no mutation of SIX 3 gene (the human equivalent of the animal Eyeless) was found.

P0494. Clinical Spectrum And Natural History Of Adams- Oliver Syndrome; Report Of Two New Cases

E. Lapi, *A. Cecconi, L. Ombroni, E. Andreucci, G. Ricotti, M. Giovannucci Uzielli*
Genetics and Molecular Medicine Unit, Department of Paediatrics, University of Florence, A.Meyer Children Hospital; Florence, Italy
uzielli_ml@unifi.it

Adams-Oliver Syndrome (OMIM 100300), also reported as Congenital Scalp Defects with distal limb reduction anomalies, is a rare genetic disorder, transmitted as Autosomal Dominant Trait. We report two new unrelated cases, both males; a newborn and a young boy aged 8 years, referred to our Centre for a genetic evaluation. Two major features suggested us the diagnosis; scalp and limbs defects. Scalp defect, represented by

extensive aplasia cutis of the scalp affecting the cranial vault without an underlying defect of skull bone, was present at birth in both patients, with progressive spontaneous healing. The degree of limbs anomalies is different in the two childrens. Duplication of first finger with very short phalanges and small or absence of fingers and toes nails were obvious in the newborn; short distal phalanges and small toenails represent the limb defects in the young boy. Other clinical features, characteristic of Adams-Oiliver syndrome were also present in both patients, mild mental retardation included in the eight years old boy. We consider important to report these new cases of Adams-Oliver Syndrome, because it is a rare genetic disorder, with variable clinical expression, but well distinct from other syndromes sharing some of the clinical features; first of all scalp and limbs defects, associated with chromosomal abnormalities, transmitted as Mendelian traits, or apparently caused by non-genetic factors.

P0495. Pitfalls in genetic counseling; Identification of large repeat expansions in SCA7, responsible for the fatal infantile SCA7 phenotype in two sibs.

T. Kleefstra[1], *C. Frenken*[2], *H. Kremer*[3], *R. Sinke*[4], *M. Ausems*[4], *B. v/d Warrenburg*[3], *N. Knoers*[1]
[1]*Human Genetics, UMC St Radboud; Nijmegen, The Netherlands;* [2]*Neurology, CWZ; Nijmegen, The Netherlands;* [3]*Neurology, UMC St radboud; Nijmegen, The Netherlands;* [4]*Medical Genetics, UMC Utrecht; Utrecht, The Netherlands*
t.kleefstra@antrg.azn.nl

Autosomal dominant cerebellar ataxias (ADCAs) are clinically characterised by progressive cerebellar ataxia, with an onset usually after childhood. Based on additional clinical characteristics and age of onset, Harding suggested a classification of ADCAs in type I, II and III.Genetic mapping studies in ADCA families have detected fourteen genetic loci (SCA 1-8 and SCA 10-15) and nine of the corresponding genes have already been cloned. ADCA type II is characterised by progressive pigmentary macular degeneration in association with cerebellar ataxia and is caused by patological repeat expansions (37 to 306 repeats) in the SCA7 gene. The rarely documented infantile SCA7 phenotype, characterised by additional extraneurological manifestations and a rapid progressive course, is due to enormous repeat expansions on paternal disease transmission. We report upon a family, in which the onset of ataxia and subsequent detection of the SCA7 muation in the proband retrospectively explained the fatal illness resulting in early death in two children. Post-mortem analysis of the SCA7 gene in the offspring revealed repeat expansions of 325 and 460 repeats, the largest ever reported. We discuss the infantile SCA7 phenotype and comment on the problems encoutered in differential diagnosis and genetic counseling.

P0496. Heart Congenital Malformations In Children Genetic Diseases Clinical And Epidemiologycal Aspects

M. Porumb[1], *M. Bembea*[1], *R. Spineanu*[1], *S. Porumb*[2], *C. Skrypnyk*[1]
[1]*Clinical Children Hospital; Oradea, Romania;* [2]*Clinical CountyHospital; Oradea, Romania*
mporumb@usa.net

The goal of our study was to follow the prevalence of the heart diseases, the type of heart malformations in genetic diseases and their influence on the infantile morbidity and mortality in Bihor County-Romania. Material and Method; The study included 361 patients with an average age of 8.80+(-) 5.30 with limits 1-day-20 years presenting genetic disorders with possible heart involvement. Results; Heart involvement of the genetic diseases was found in 134 (37.11%) patients, from which 44 (32.84%) cases survived and 90(67.16%) cases died, 123(91.7%) patients presented congenital heart diseases with left-right shunt, in 10 (2.77%) patients progressive heart involvement being present. Heart abnormalities with left-right shunt were more frequent and severe in chromosomal abnormalities (104 cases-76.61%). They represented determinant cause for decease in 36 (26.86%) cases by irreducible heart failure and favoring cause for decease in 30 (23.38%) cases. Conclusions; The vital prognosis of the chromosomal genetic diseases is influenced by the presence of congenital heart diseases, especially by the lesions on left-right shunt type. They represent determinant and favoring cause of the infants‘ decease.

P0497. Heart malformations in developmental field defects; a teratological series

H. Metzke[1], *U. Kohl*[2], *W. Meerbach*[3]
[1]*Institute of Human Genetics and Anthropology; Jena, Germany;* [2]*Catholic Hospital St. Nepomuk; Erfurt, Germany;* [3]*Institute of Pathology, Klinikum Erfurt GmbH; Erfurt, Germany*
hmet@mti-n.uni-jena.de

Congenital heart defects are frequently observed in syndromes and malformation associations. For better understanding their correlation to developmental field defects the data from a teratological series of heart malformations have been analysed in detail. For that, 563 postmortem examinations of children with at least two malformations were used, which have been performed in the Institute of Pathology of the former Medical School of Erfurt. 250 (44,4%) of them had congenital heart defects. The necropsy reports of 202 with selected heart malformations have been analysed for the presence of associated defects. Statistical calculations were performed using the Fisher exact test. The following results could be ascertained; - Truncus arteriosus communis, transposition of the great vessels, double outlet right ventricle and cor bi- or triloculare, particularly in combination, are significantly more frequent than other heart defects associated with asplenia, trilobed left lung, situs inversus or ambiguus and malrotation. - Aortic coarctation in our study is associated with diaphragmatic hernia and (not significant) polysplenia. - Ventricular septal defect is significantly associated with esophageal atresia and CNS- malformations - The pattern of associated malformations in Fallot s tetralogy is similar to that of ventricular septum defects, but the number of cases is to small to draw final conclusions. The results show, that the analysis of the pattern of associated malformations of teratological series are more useful for the investigation of malformation associations than single marker malformations.

P0498. Molecular characterisation of a cryptic 2q37 deletion in a patient with Albright hereditary osteodystrophy-like phenotype.

N. Chassaing[1], *P. De Mas*[1], *M. Tauber*[2], *G. Bourrouillou*[1], *P. Calvas*[1], *E. Bieth*[1]
[1]*Purpan hospital, department of genetic; Toulouse, France;* [2]*Children hospital, department of endocrinology; Toulouse, France*
bieth.e@chu-toulouse.fr

The Albright hereditary osteodystrophy(AHO)-like syndrome has been recently defined as a rare dysmorphic syndrome including brachymetaphalangism and mental retardation. This phenotype occurs in Albright hereditary osteodystrophy but unlike it, the level of the Gs alpha protein is not reduced in AHO-like syndrome. To date as few as 20 patients with these clinical and biochemical features have been published, and for the majority of them a cytogenetically visible deletion of chromosome 2q37 has been observed. We report a new case of typical AHO-like syndrome with normal karyotype. Using the polymorphic marker D2S125 we found in this patient loss of heterozygosity suggestive of a de novo deletion of maternal origin. This hypothesis was confirmed by FISH analysis with a telomeric 2q probe. Genetic analysis using a series of microsatellite markers of the 2q37 region allowed us to map the centromeric end of the deletion within an interval of about o,5 megabase delimited by D2S2338(present) and D2S2253(deleted). This is to date the smallest 2q subtelomeric deletion observed in association with a typical AHO-like phenotype. These data contribute to refine the AHO-like critical region and prompt to study positional candidate genes linked with the expression of this phenotype (in progress).

P0499. Adducted thumb-clubfoot syndrome in sibs of a consanguineous Austrian family

T. M ller[1], *K. Unsinn*[1], *A. Kreczy*[2], *I. Baldissera*[3], *I. Ga§ner*[1], *N. Neu*[1], *G. Utermann*[4], *A. R. Janecke*[4]
[1]*Department of Paediatrics, University-Hospital; Innsbruck, Austria;* [2]*Department of Pathology, University-Hospital; Innsbruck, Austria;* [3]*Department of Ophthalmology, University-Hospital; Innsbruck, Austria;* [4]*Institute of Medical Biology and Human Genetics, University; Innsbruck, Austria*
th.mueller@tirol.com

Recently, a dysmorphogenetic syndrome featuring adducted thumbs, clubfeet, and distinctive facial dysmorphism was first distinguished in two cousins of a large consanguineous Turkish family. We report two male sibs with the same condition, the product of a fourth cousin marriage of an Austrian family. The patients presented with facial dysmorphism comprising broad and bossed forehead, widely patent anterior fontanel, telecanthus, downslanting palpebral fissures, deep-set ears, arachnodactyly, severely adducted thumbs, and clubfeet. Our observations confirm that the adducted thumb-clubfoot syndrome most likely represents a distinct autosomal recessively inherited phenotype.

P0500. Caudal Regression And Holoprosencephaly Recurring Among Sibs

D. P. Cavalcanti[1], *R. Barini*[2], *F. Luiz*[2], *K. Andrade*[2]
[1]*UNICAMP; Campinas - SP, Brazil;* [2]*UNICAMP; Campinas, Brazil*
denisepc@unicamp.br

Although autosomal recessive inheritance has been suggested as the origin of caudal regression, most cases are sporadic or associated with

maternal diabetes. Classically, the spectrum includes imperforate anus, sacral agenesis, and sirenomelia. To the best of our knowledge the association of holoprosencephaly and caudal dysgenesis in a child who does not have a well-known syndrome has been described only in two unrelated children. In this work, we describe a family in which holoprosencephaly associated with caudal dysgenesis in the first child is recurring in the fetus of the present gestation. The propositus, a male infant was born of young, healthy, and non-consanguineous parents, with no family history of similar birth defects. The pregnancy was complicated by influenza in the first trimester. The baby weighed 1050 g and died after 15 minutes. He presented alobar holoprosencephaly, severe ocular hypotelorism, proboscide, microstomia, solitary left renal cyst, anus imperforate, hemivertebra in L2, hypoplasia of iliac, irregularities in all middle phalanges of the fingers, and sacral agenesis. His karyotype was normal (46,XY). Six months after propositus s birth the mother became pregnant. A 20 weeks prenatal ecography also showed holoprosencephaly and sacral agenesis. The recurrence of the same pattern in sibs in the present family suggests a syndromic picture, probably from autosomal recessive origin.

P0501. Smith-Magenis syndrome - early diagnosis in Slovak origin patient

D. Durovc kov
Slovak Postgraduate Medical Academycademy; Bratislava, Slovakia
darina.durovcikova@faneba.sk

During the past few years in several forms of behavioral phenotypes such as Prader-Willi, Williams and Smith - Magenis syndrome (S-M sy) chromosomal microdeletion has been confirmed by FISH method in our department. Only one of them Smith-Magenis syndrome (deletion 17p11.2) has been shown to be associated with self injurious behavior, mild dysmorphism and various neurologic signs. According to this characteristics in our patient the first investigation was aimed to metabolic disease exlusion. The male child ,aged 2 ,was reffered to geneticist because of psychomotoric retardation and stereotypy in behaviour of unclear etiology. Hypotonia, failure to thrive, sleeping problems and delopmental delay was evident from the beginning. The self-injurious behaviour was manifested at the age of 20 months.Other features included plagiocephaly, cerebral cortex atrophy, coloboma of optic disk, telecanthus, protruding tongue, poor dentition, thin marmorate skin with ligament laxity and decreased sensitivity to pain. The recognition patterns of behavioral phenotypes in different age categories enabled us to confirm the diagnosis in early infancy.

P0502. A Case Of Spondylometaphyseal Dysplasia — Sedaghatian Type

V. Christophidou Anastasiadou[1,2], *K. Aristidou*[1], *G. Stylianidou*[1], *E. Spanou Aristidou*[2]
[1]*Arch. Makarios III Pediatric Hospital; Nicosia, Cyprus;* [2]*The Cyprus Institute of Neurology and Genetics; Nicosia, Cyprus*
vanast@spidernet.com.cy

We report a girl with congenital skeletal changes, severe neurological deficit and dysmorphic features. At birth she was noted to be hypotonic and have dysmorphic features (midface hypoplasia, hypertelorism, long palpebral fissures and low posteriorly rotated ears among others). Her radiological findings were characteristically metaphyseal widening, cupping, flaring and splaying, rhizomelic shortening of both upper and lower limbs and abnormally shaped talus and calcaneous. These features were consistent with spondylometaphyseal dysplasia — Sedaghatian type. Spondylometaphyseal dysplasia — Sedaghatian type is a lethal skeletal dysplasia and there are no cases reported in the literature that have survived beyond the age of 160 days. Our patient survived to the age of four years. During her lifetime she was severely developmentally delayed and over time developed seizures and other neurological sequelae. We believe this case is of interest as it charts the natural history of this rare disorder.

P0503. New reccurent lethal chondrodysplasia with microcephaly and associated anomalies in two distinct sibships.

A. Co slier[1], *S. Manouvrier*[1], *O. Boute*[1], *A. Moerman*[1], *G. Gr n*[2], *A. L. Delezoide*[3], *L. Dewisme*[4], *M. Le Merrer*[5]
[1]*G n tique Clinique, H pital Jeanne de Flandre, CHRU; Lille, France;* [2]*Service de Gyn cologie-Obst trique, CH de Troyes; Troyes, France;* [3]*Unit de Foetoplacentologie, H pital Robert Debr ; Paris, France;* [4]*Service d Anatomie et cytologie pathologiques, H pital Calmette, CHRU; Lille, France;* [5]*D partement de G n tique, Unit de G n tique clinique, H pital Necker; Paris, France*
acoeslier@yahoo.com

We present an undescribed reccurent lethal chondrodysplasia associated with microcephaly, distal arthrogryposis, biliary anomalies and midline defects in four patients belonging to two unrelated kindred. The four cases presented short limbs with hands and feet malposition, narrow thorax, microcephaly, retrognathism and similar visceral anomalies. Skeletal radiological anomalies were similar ; shortness of long bones with wide metaphyses; skull, ischio-pubic rami and vertebrae s lack of ossification; rounded aspect of iliac wings; flare clavicles; costal anomalies and short first metacarpal. In family 1, they were two girls of non consanguineous parents. Case one was diagnosed as a unclassified chondrodysplasia punctata because of carpal and tarsal punctuations and died at 2 days of life of heart failure. For case 2, the pregnancy was interrupted at 25 gestational weeks in front of prenatal findings suggesting a recurrence. Autopsy revealed corpus callosum agenesis, pulmonary malsegmentation, interventricular septal defect, biliary fibroadenomatous and didelphis uterus. Standard karyotype were normal. In family 2, they were two female f˚tuses, for whom pregnancy was interrupted at 26 and 25 gestational weeks. Parents were consanguineous. Prenatal ultrasonography revealed short limbs, malposition of hands and feet, narrow thorax, microcephaly and corpus callosum anomalies. Autopsy confirm these findings and revealed liver and biliary anomalies for both. To our knowledge this type of lethal chondrodysplasia had never been described, and we suggest that these patients present a new lethal polymalformative chondrodysplasia with autosomal recessive inheritance according to familial genealogy.

P0504. Poland - Moebius Syndrome

M. Kuklik
Charles University Prague 2nf Faculty of Medicine, Czech Republic; Prague 5 - Motol, Czech Republic
jan.handzel@lf1.cuni.cz

The author summarizes hitherto experience with the clinical and genetic characteristics of Poland«s and Moebius syndrome. Poland — Moebius syndrome is an overlapping disruption spectrum of inborn defects affecting the face and extremities. Five selected case-records with this disease and the sequence of the Poland — Moebius are presented. For establishment of a more accurate symptomatology, and irreplaceable is held by anthropometric examination; for objectifying the assymetry of the chest the so called cyrtogram, the chest circumference recorded by means of a wire, is valuable. From the aspect of genetic counselling preconception care is always provided to mothers from families with reproductive intentions, as well as ultrasonographic examination of the fetus in area of assumed acral symptomatology (signaling phenotype). In two families ultrasonography was used for prenatal diagnosis. Invasive prenatal diagnosis by amniocentesis was employed in a family with Moebius syndrome. In these families dermatoglyphs have certain common characteristics, such a tendency towards simple patterns. In the wider family of one of our patients we detected in a cousin Parkes — Weber — Klippel — Trenaunay«s syndrome, which may indicate common vascular predisposing factors.

P0505. Mutation analysis in a large cohort of Rett patients and genotype-phenotype correlation.

F. Laccone[1], *P. Huppke*[2], *M. Meins*[1], *M. Held*[2], *F. Hanefeld*[2], *W. Engel*[1]
[1]*Institute of Human Genetics, University of Goettingen; Goettingen, Germany;* [2]*Neuropediatric Department, University of Goettingen; Goettingen, Germany*
flaccon@gwdg.de

Mutations in the MECP2 gene (Methyl-CpG-binding protein) have recently been reported causing Rett syndrome, an X-linked dominant neurodevelopmental disease. We investigated 302 sporadic cases of Rett syndrome by direct sequencing. 55 different mutations were found in 201 patients. We provide evidence for the existence of several hot spot regions and of a deletion prone region located at the 3« most region of the gene. Nine of 10 recurrent mutations were located in either the methyl CpG binding domain (MBD) or in the transcriptional repression domain (TRD) and all missense mutations were located in one of these functionally important domains. With more than 60% there was a high frequency of truncating mutations (nonsense mutations along with frame shift mutations). We are comparing the genotype to the phenotype of our collective. First results seem to show that truncating mutations cause a more severe phenotype than the missense ones, which on turn are more severe than deletion mutations. Comparing the mutation rate in the group of children until the age of 4 years, there is a predominance of children without mutations (36 out of 101) versus children carrying a mutation (31 out of 201) ($p < 0.01$). These findings mirror most probably the difficulties of an early correct diagnosis. The detection rate in our collective was 66,6%. However in classical cases the detection rate is > 80%. Our findings show that the majority of German Rett patients carry mutations in the MECP2 gene confirming the suggested locus homogeneity for the disease.

P0506. Prevention of Orofacial Clefts with high doses of Folic Acid

L. Timar[1], *A. Sarkozi*[2], *A. Czeizel*[3]
[1]*National Centre of Public Health; Budapest, Hungary;* [2]*Founnd. of Comm. Contr. Hered. Dis.; Budapest, Hungary;* [3]*Found. of Comm. Contr. of Hered. Dis.; Budapest, Hungary*
saljot@mail.datanet.hu

In 1982, Tolarova found a reduction in the recurrence rate of isolated cleft lip (CL) with or without cleft palate (CP; CL + CP) after periconceptional supplementation with a multivitamin including a very high dose (10 mg) of folic acid. The Hungarian randomized, double-blind, controlled trial of periconceptional supplementation with a multivitamin including a physiologic dose (0.8 mg) of folic acid did not show any preventive effect on the first occurrence of isolated CL + CP and CP. However, the general evaluation of congenital abnormalities in the Hungarian Case-Control Surveillance of Congenital Abnormalities indicated, among others, a reduction of isolated CL + CP and CP after the use of high dose of folic acid in the critical period for the development of these congenital abnormalities in the 12-year dataset between 1980 and 1991. We hypothesized that the prevention of orofacial clefts by folic acid has a dose-dependent effect, and this hypothesis was tested in 2 recent Hungarian datasets, in a prospective cohort study, and in contrast, in the 17 years dataset of the Case-Control Surveillence of Congenital Abnormalities, between 1980 and 1996.

P0507. Restricted expression of T cell receptor gamma chain gene rearrangement in multiple sclerosis patients

D. Januszkiewicz[1], *M. Pernak*[1], *J. Rembowska*[1], *H. Hertmanowska*[2], *J. Lossy*[3], *M. Wender*[4], *J. S. Nowak*[1], *J. S. Nowak*[1]
[1]*Institute of Human Genetics Polish Academy of Sciences; Poznan, Poland;* [2]*Department of Neurology; Poznan, Poland;* [3]*Academy of Medical Sciences; Poznan, Poland;* [4]*Neuroimmunological Unit; Poznan, Poland*
janusz@man.poznan.pl

Presence of alfa/beta and gamma/delta T lymphocytes in demyelinating lesions in multiple sclerosis (MS)suggests, that they are responsible for local destruction of nervous tissue. This T cells may have autoimmune potential and are thought to play important role in progression of MS. Limited junctional diversity of TCR gamma gene may indicate on in vivo clonal expansion of selected gamma/delta T lymhocytes, which can be considered as antigen driven (auto)immune response.Our previous work showed restricted TCR delta chain gene repertoire on the DNA and RNA level in peripheral blood of MS patients. The aim of this study was to analyze the TCR gamma chain gene rearrangement on the RNA level and to compare to that on the DNA.TCR gamma gene rearrangement has been analyzed in MS patients and healthy individuals using primers specific for V gamma 1-4 subfamilies and J gamma 1/2 genes(DNA level)and specific for V gamma 1-4 and C gamma genes (RNA level). In almost all MS patients and healthy individuals poli- or oligoclonal V1-, V2-, V3-, and V4-J gamma gene rearrangements have been observed. Comparison of TCR gamma chain gene rearrangements on DNA and RNA level showed, that the majority of TCR gamma rearrangements are not expressed. The V2-C gamma gene rearrangement was predominantly observed in MS as well in healthy individuals.The rearrangements of V1-, V3-, and V4-C gamma have been seen only in individual cases. Contrary to normal individuals, in MS patients the TCR gamma rearrangements on the RNA level were mostly oligoclonal.It could be concluded tha oligoclonal pattern of TCR V2-C gamma gene rearrangement at the RNA level along with increase of activated gamma/delta T cells, i.e. expressing HLA-DR and CD25 strongly argue for significant role of gamma/delta lymphocytes in the pathogenesis of MS.

P0508. Orofacial findings in chromosomal breakage diseases

J. Handzel[1], *M. Kuklik*[2]
[1]*Charles University Prague; Prague 2, Czech Republic;* [2]*Charles University; Prague, Czech Republic*
jan.handzel@lf1.cuni.cz

Chromosomal breakages are caused by inborn insufficiency of enzymatic systems repairing mutations of chromosomal DNA. These diseases represent a serious precancerous state and in patients (and also in carriers) of these genetically determined diseases there is a greater incidence of oncological cases. Following immunodeficiencies are associated with chromosomal instability; Ataxia teleangiectatica, Bloom syndrome, xeroderma pigmentosum, incontinentia pigmenti, Nijmegen breakage syndrome, Jadassohn — Lewandowski syndrome etc.. This group of pathological conditions could be already described as chromosomal breakage diseases. The authors are describing two cases where chromosomal instability were accompanied either by bizzare odontodysplasias and incontinentia pigmenti or by severe oligodontia, dyskeratosis congenita and onychogryphoses. The acquired chromosomal abberations were caused in our patients probably by essential exposures to environmental influences. The most important tasks in patients with chromosomal breakages are; early diagnosis and special oncological preventive program.

P0509. About Different Phenotypes and Similar Genotypes in the CBFA1-Gene

U. Baumert[1], *I. Golan*[1], *M. Preising*[2], *D. M §ig*[3]
[1]*Department of Orthodontics, Center for Craniofacial Genetics, University of Regensburg; Regensburg, Germany;* [2]*Department of Paediatric Ophthalmology, Strabismology, and Ophthalmogenetics, University of Regensburg; Regensburg, Germany;* [3]*Department of Orthodontics, University of Regensburg; Regensburg, Germany*
uwe.baumert@klinik.uni-regensburg.de

Cleidocranial dysplasia (CCD) is a rare autosomal dominant generalized bone dysplasia characterized by high penetrance and variable expressivity. Mutations in the alpha 1 subunit of the *core-binding factor* (CBFA1; now RUNX2) have been correlated with this disorder. Each of our CCD-patients was examined genetically and phenotypically in order to perform genotype-phenotype correlations. We identified two heterozygous mutations in two index cases; R190Q and G146R with the latter shown to segregate in a family with variable expressivity. While the father showed all classical symptoms, the son exhibited a less severe manifestation of the phenotype that was exemplified by dental anomalies only. The R190Q mutation was found in a patient previously misdiagnosed based on clinical evidence as having Rubinstein-Taybi-Syndrome (RTS). However, clinical reevaluation showed a phenotypical variety of CCD. Zhou et al. (*Hum Mol Genet* 8; 2311-2316, 1999) originally correlated the mutations R190Q and G146X with the classical phenotype due to haploinsufficiency. Both mutations affected the runt domain of the protein and both were shown to completely abolish DNA binding. Our cases show that, due to the variable expressivity of certain mutations of the CBFA1 gene, CCD cannot be explained by haploinsufficiency alone as exemplified by the T200A mutation reported by Zhou et al. This mutation was correlated with variable expressivity, but in contrast to our findings, the manifestation of CCD was intensified in passing from father to son. In our patient cohort, the mutations R190Q and G146R were both correlated with non-classical phenotypes and/or variable expressivity. Therefore, we agree with Zhou et al. that the clinical expressivity of these mutations is altered due to hypomorphic effects or genetic modifiers.

P0510. Craniofacial Treatment Management of Cleidocranial Dysplasia (CCD)

I. Golan[1], *U. Baumert*[1], *H. Wagener*[2], *H. Niederdellmann*[3], *D. M §ig*[4]
[1]*Department of Orthodontics, Center for Craniofacial Genetics, University of Regensburg; Regensburg, Germany;* [2]*Department for Oral and Maxillofacial Surgery, Center for Craniofacial Genetics, University of Regensburg; Regensburg, Germany;* [3]*Department for Oral and Maxillofacial Surgery, University of Regensburg; Regensburg, Germany;* [4]*Department of Orthodontics, University of Regensburg; Regensburg, Germany*
ilan.golan@klinik.uni-regensburg.de

Cleidocranial dysplasia (CCD) is a rare autosomal dominant generalized bone dysplasia characterized by a widely variable expressivity. Anomalies of the membranous and enchondral bone as well as dental characteristics have been reported. Homozygous mice mutants show a complete lack of bone development. Mutations in CBFA1 (now RUNX2), a master gene of the runt-domain family, were identified as the etiological factor of this disorder. While the dental system shows an almost constant affection in all CCD cases, major skeletal symptoms like clavicular hypoplasia are not always expressed. The reason for dental affection cannot be explained by mutations in the CBFA1 gene. Based on 15 CCD-Patients treated in our clinic and over 200 reviewed publications, we describe our treatment approaches for various dentofacial symptoms. These include supernumerary teeth, persistence of deciduous teeth, retained tooth germs leading to a reduced chewing ability, cysts, and the risk of pathological mandible fracture. The craniofacial expressions are, according to the patient s point of view, the main cause for the reduction in quality of life. By interdisciplinary treatment involving an orthodontist, dentist and maxillofacial surgeon, a well-functioning permanent dentition as well as an aesthetically satisfying facial appearance can be achieved. Due to the rareness of the disease, general treatment guidelines are difficult to define. Most of the reports published in the literature are single case reports, not allowing statistically significant conclusions. Therefore, CCD-patients should be advised as soon as possible by a qualified orthodontist to determine the optimal starting time to achieve the best individual treatment.

P0511. Spinal neurofibromatosis caused by a point mutation in exon 33 of the Neurofibromatotis type 1 gene

D. Kaufmann[1], *G. Assum*[1], *B. Bartelt*[1], *R. M ller*[1], *B. Schmoll*[1], *S. Wieland-Lange*[1], *H. G tz*[1], *M. Wolf*[1], *K. Kunzi-Rapp*[1], *R. Fahsold*[2], *C. Hein*[1], *W. Vogel*[1]
[1]*University of Ulm; Ulm, Germany;* [2]*Gemeinschaftspraxis Prager & Junge; Dresden, Germany*
dieter.kaufmann@medizin.uni-ulm.de

We describe a 32 old female with numerous paraspinal tumors. The tumors were detected in routine NMR looking for the reason for a lumbago. Histological investigation of one of these paraspinal tumors excised showed a schwannoma. Clinical examination showed no other symptoms related to Neurofibromatosis type 1 (NF1) or type 2. In her family no other patients are known. Investigation of DNA derived from a skin excision for a NF1 mutation by EMD and sequencing revealed in a point mutation in NF1 exon 33 (T6200C, L2067P). This alteration does not seems to be not a polymorphism as shown by investigation of about 1000 other NF1 patients. The mutated allele was expressed equally on mRNA level as shown by quantitative competitive RT-PCR. In contrast the amount of neurofibromin was reduced clearly in cytoplasmic lysates of the fibroblasts of this patient as shown by immunoprecipitation and western blotting. To our knowledge, this is the first case of a patient with spinal neurofibromatosis without other NF1 or NF2 symptoms carrying a NF1 mutation.

P0512. Rett Syndrome; clinical manifestations in males with MECP2 mutations, germline mosaicism and implications for genetic counseling and prenatal diagnosis

A. Orr-Urtreger[1], *B. Ben Zeev*[2], *C. N. Schanen*[3], *H. Wolf*[1], *N. Brandt*[2], *N. Ginot*[4], *R. Shomrat*[1], *Y. Yaron*[1]
[1]*Tel Aviv Sourasky Medical Center; Tel Aviv, Israel;* [2]*Sheba Medical Center; Ramat Gan, Israel;* [3]*UCLA School of Medicine; Los Angeles, CA United States;* [4]*Meir General Hospital; Kfar Saba, Israel*
aviorr@tasmc.health.gov.il

Rett syndrome (RTT) is a neurodevelopmental disorder characterized by cognitive and adaptive regression with autistic features, loss of acquired skills like speech and hand usage, stereotypic hand movements, epilepsy, ataxia and deceleration of head growth that almost exclusively affects females. Even before the causative gene for this syndrome was identified , pedigree studies indicated that it was likely to be an X-linked dominant disorder, with presumed lethality in males. Nonetheless, there were a few descriptions of males suspected of having Rett syndrome. These include sporadic cases meeting the clinical inclusion criteria, sporadic males with Klinefelter syndrome (47,XXY), and males with severe neonatal encephalopathy in known Rett syndrome families. With the recent discovery that the MECP2 gene on Xq28 is the gene responsible for most cases of Rett syndrome, it is possible to molecularly assess cases suspected of having male Rett syndrome by direct sequencing analysis. We describe the first 4 cases of molecularly confirmed cases of Rett syndrome in Israel. One of the families presented here consists of a female having classical Rett syndrome and a male sibling with severe neonatal encephalopathy. Molecular analysis revealed that both sister and brother have the same MECP2 gene mutation, however their mother does not, suggesting germline mosaicism. The case emphasizes the point that maternal germline mosaicism may lead to affected female or male offspring, which should be taken into consideration during genetic counseling regarding recurrence risks, and suggests that prenatal diagnosis may be warranted in siblings of apparently sporadic cases of Rett syndrome. It also highlights the importance of molecular diagnosis in every case of idiopathic neonatal encephalopathy in males.

P0513. Linkage of the Gene for Otopalatodigital Syndrome Type 2 (OPD2) to Distal Xq28; Support for Allelism with OPD1

S. P. Robertson, *M. Oldridge, S. Walsh, A. O. M. Wilkie*
Institute of Molecular Medicine; Oxford, United Kingdom
steve.robertson@imm.ox.ac.uk

Otopalatodigital syndrome type 2 (OPD2) is an X-linked dominant condition characterized by campomelia, cleft palate, craniofacial dysmorphism and variable combinations of solid organ malformations. Carrier females can have skeletal anomalies, cleft palate and deafness. OPD2 is hypothesised to be allelic to OPD1, based on clinical similarities and segregation pattern. The gene for OPD1 has been linked to a >10cM region within Xq28, but no linkage analysis of OPD2 has previously been reported. We have performed a linkage study on three generation Maori family segregating OPD2 in an effort to localize a candidate interval for the responsible gene. The distal Xq28 markers DXS1073 and DXS1108 showed perfect segregation with the disease allele, with two point lod scores of 2.41 and 2.71 respectively, and a multipoint lod score of 3.31. Studies of X-inactivation in this family showed that the degree of skewing of the inactivation pattern correlated with carriage of the disease allele and also the severity of the phenotype in carrier females (1 moderately skewed and 4 extremely skewed patterns in carriers, 2 random patterns in non-carriers, 4 uninformative results). An unaffected male was also shown to be recombinant at the BGN locus narrowing the candidate interval to a 3.1 cM region within Xq28. This region contains in excess of 60 described genes. Identification of the disease gene will allow direct examination of the hypothesis of allelism, not only with regard to OPD1, but also other phenotypically related syndromes such as Melnick-Needles syndrome and frontometaphyseal dysplasia.

P0514. Natural History of Spondylothoracic Dysplasia

A. Santiago-Cornier[1], *N. Ram rez-Lluch*[2], *J. R. Acevedo-Ramos*[3], *S. Arroyo*[4], *A. M rquez*[5], *E. Rosado*[6], *M. Warman*[7]
[1]*Ponce School of Medicine, Genetics Division; Ponce, PR United States;* [2]*Department of Ortopedics, Ponce School of Medicine; Ponce, PR United States;* [3]*University of Puerto Rico-Arecibo, Dept. of Nursing; Arecibo, PR United States;* [4]*Mayaguez Medical Center, dept. of Radiology; Mayaguez, PR United States;* [5]*Ponce School of Medicine, Dept. of Radiology; Mayaguez, PR United States;* [6]*Ponce School of Meidicne, Genetics Division; Ponce, PR United States;* [7]*Case Western Reserve University; Cleveland, OH United States*
scornier@PSM.edu

Spondylothoracic dysplasia (MIM#277300) is an autosomal recessive disorder with high prevalence in Puerto Rican population that has been described as a lethal condition. Since Jarcho and Levin described it in 1938 has been referred to as Jarcho-Levin syndrome (JLS), costovertebral dysplasia and spondylothoracic dysostosis among other terms. We have prospectively characterized 20 patients with spondylothoracic dysplasia, taken detail medical histories, performed physical examinations, pedigree analysis, spine x-rays, spinal 3-D reconstructive CT scans, virtual bronchoscopies and pulmonary function tests (PFT s). Numerous vertebral segmentation and formation defects were found. Characteristic vertebral shapes that has not been described in the medical literature before was also found. Eight out of these 20 patients has been born during our study and four have survived to 3,18,24,38 months respectively. The cause of death in disease patients was respiratory insufficiency secondary to restrictive lung problems and pneumonia. Age of the remaining patients ranges from 8 to 41 years. PFT s performed on individuals older than 6 years showed a restrictive pattern that do not seems to exacerbate as patient progresses in age. Mortality in our kindred was 50% of the identified newborns with JLS. This is an important finding since the vast majority of the JLS patients cited in the medical literature died in early newborn or childhood periods. Their intellectual capacity and development is normal in all cases. This is the largest kindred of patients with JLS described in the medical literature and so forth has allowed us to established a detail phenotype and natural progression of the disease. A genome-wide linkage analysis is currently undergoing to determine the locus (i) of JLS in Puerto Rican population.

P0515. Trisomy 13, analysis of 4 cases

K. Kaczanowska, *J. R. Kowalczyk, M. Babicz, A. Gaworczyk, M. Lejman*
Dept of Pediatric Hematology and Oncology, Cytogenetic Laboratory; Lublin, Poland
agaworczyk@poczta.onet.pl

Among the chromosomal abnormalities that can be differentiated by their spectra of clinical findings — trisomy 13 (incidence 1; 5000) is associated with a very high risk of spontaneus death in utero and early death in infancy. The presence of an additional genetic material derrived from an extra copy of chromosome 13 determines characteristic phenotypical features usually found in children with this syndrome. Purpose; We report on 4 cases of trisomy 13. All children were diagnosed at the birth. Clinical examination; clinical examination revealed the presence of craniofacial dysmophism as well as hexadactyly in all four patients. Additionally anophthalmos was observed in two patients. Another two had occipital region skin depletion. Cytogenetic study; cytogenetic evaluation revealed simple trisomy 13 in two cases and Robertsonian translocations [t(13;13) and t(13;14)] in another two. Outcome; both newborns with simple trisomy (patient 1 and 2)died within 2 months . Children with translocation trisomies (patient 3 and 4) are still alive at the age of 2 months and 3 years respectively. This work was supported by the Polish Committee of Scientific Research — KBN4P05E 081 18

P0516. Impact of full and Partial blindness (Genetically and Non-Genetically) on academic performance of the visually handicapped students

D. Choudhury, *S. K. Bandyopadhyay*
Research and Development Wing, Calcutta Blind School; Calcutta, India
sde4701@vsnl.com

A study was undertaken to find out the effect of genetically and non-genetically groups of full and partial blindness of the visually handicapped students in West Bengal with regard to their academic performance. The data contained 47 students of partial blindness and 78 students of full blindness from Calcutta Blind School and also 17 students with normal vision form Behala High School in the region of East Calcutta of West Bengal . All of the students were followed up from the Class-1 (1985) to the Class-X (1995) according to their academic (educational) performance. The educational qualification of the Teaching Staffs and the Syllabus of the Class of the School are similar in both of Calcutta Blind school. Pedigree, family income, family size, mother s education and father s education are alos taken for the purpose of the study. Among the partial blindness of the students, 85% students belonged to geneticalluy group and rest of the students are non-genetically group. Similarly 77% and 23% students have no vision (full blind) as causes of genetically and non-genetically factors respectively.

Genetically group indicates blindness either partial or full by congenital or other causes of genetics. Non-genetically group means partial or full blindness due to causes of accident encephalitis, typhoid, dipthria etc. The statistical analysis revealed that no significant difference are to be found between the students of genetically group and non-genetically group of the partial blindness with regard to their academic achievement. According to educational performance of the students the students of genetically group of full blindness were found to be statistically non significant with the students of non-genetically group of full blindness.

In addition, the partial and full blind students have not significant differences with that of the normal vision of students in respect of their educational presentation.

P0517. Molecular genetic of Neurofibromatosis 1

J. Kadlecov [1], *B. Rav ukov* [1], *P. Kroupov* [2], *A. Vesel* [2], *I. Val ?kov* [1], *R. Gaillyov* [1]
[1]*University of Children«s Hospital; Brno, Czech Republic;* [2]*Faculty of Science, Masaryk University; Brno, Czech Republic*
jkadl@atlas.cz

Neurofibromatosis type 1 (NF1) of von Recklinghausen is a common autosomal dominant disorder, characterized by peripheral neurofibromas, cafe au lait spots and Lish nodules of the iris. The high mutation rate at the NF1 locus results in a wide range of molecular abnormalities. We have scaned 7 different exons of the NF1 gene using DNA single strand conformation polymorphism (DNA-SSCP) and denaturing/temperature gradient gel electrophoresis (DGGE/TGGE) methods (Gasparini P. et al., 1996) in series 60 NF1 patients. Six different mutations have been detected (one in the exons 6, 12b, 31, 37 and two in the exon 29). To date, majority of the reported NF1 mutations are predicted to resulted in protein truncation, but very few studies have correlated the causative NF1 mutation with the effect at the mRNA level. We have applied a whole NF1 cDNA screening methodology in our study and we have used a rapid and efficient strategy to screen the entire NF1 coding region by cDNA-SSCP analysis, followed by DNA sequencing.The identification of mutations in our NF1 patients using the cDNA-SSCP method indicates that this approach is very powerful in the search for mutations in the NF1 gene and is extremly useful for the molecular diagnosis of NF1, especially for sporadic cases as have been predicted by Ars et al., 2000. References; Gasparini P. et al., Hum Genet 1996, 97;492 - 495 Ars E. et al., Hum Molecular Genetics 2000, Vol. 9, No. 2; 237 - 247

P0518. Clinical, cytogenetic and molecular cytogenetic findings in a patient with 8p-syndrome.

B. Zoll[1], *I. Bartels*[1], *S. Langer*[2], *M. R. Speicher*[2], *R. Boehm*[3]
[1]*Institute of Human Genetics, University of Goettingen; Goettingen, Germany;* [2]*Institute of Anthropology and Human Genetics, University of Munich; Munich, Germany;* [3]*Neuropediatrics Department, University of Goettingen; Goettingen, Germany*
bzoll1@gwdg.de

Molecular cytogenetic methods have assisted in characterizing critical regions in chromosomal deletion syndromes. Deletions of the distal part of chromosome 8p (del 8p) are suspected to have a strong association with congenital heart defects, microcephaly and unusual behavior. A critical region for heart defects was tentatively assigned to 8p23.1. Here we report on a female child with facial dysmorphisms, mild motor and moderate mental retardation born after an uneventful pregnancy with normal birth measurements, Apgar score 10/10. At the age of 8 month she developed an oligoepilepsy. Speech development was severely retarded. There was no malformation of inner organs. Conventional cytogenetic analysis revealed a terminal deletion of chromosome 8p , 46,XX,del(8)(p22) or a putative derivative translocation chromosome, 46,XX,der(8;?)(p22;?), respectively. 24 colour multiplex-FISH analysis demonstrated the loss of chromosome 8p material, without any hint of interchromosomal exchanges. FISH with subtelomeric probes, a microdissected subtelomere-probe (generously provided by Dr. J. Trent, NIH), a subtelomeric PAC- and cosmid-probe, revealed the loss of telomeric material for chromosome 8p and confirmed a terminal deletion in this child. The parents had normal karyotypes, 46,XX and 46,XY. Thus, in contrast to earlier reports, this case does not give evidence of genes in 8p23.1 responsible for heart defects.

P0519. Non 5q-linked variants of infantile spinal muscular atrophy (SMA)

K. Zerres[1], *B. Wirth*[2], *S. Rudnik-Sch neborn*[3]
[1]*Insitut f r Humangenetik; Aachen, Germany;* [2]*Institut f r Humangenetik; Bonn, Germany;* [3]*Institut f r Humangenetik; Aachen, Germany*
kzerres@post.klinikum.rwth-aachen.de

With the localization and identification of the SMN-gene in proximal SMA the definition of non-5q linked SMA variants became possible. At least three variants have been identified so far. Their genetic basis is still unknown for the majority of cases. Among more than 1000 cases analyzed for diagnostic purposes, the following cases could be identified; SMA with (olivo)pontocerebellar hypoplasia; The phenotype known as PCH-1 is characterized by severe muscle weakness and hypotonia starting in many cases prenatally or at birth with a shortened life span often not exceeding a few months. We report on 5 families with a severe phenotype, however, 3 patients had a milder disease course with first symptoms around the first birthday. In 3 families linkage with 5q-markers could be excluded, none showed the typical deletion of the SMN1 gene. Diaphragmatic SMA; This entity is usually characterized by initial respiratory insufficiency due to diaphragmatic palsy often followed by a distally pronounced weakness and wasting. We report 10 families without SMN1 deletion. One responsible gene (SMARD) could recently be localized on chromosome 11. SMA and neurogenic arthogryposis; This is a heterogeneous group including non progressive and non hereditary forms, while in other patients congenital contractures are a consequence of anterior horn cell loss leading to progressive weakness. We report on at least 15 cases of different phenotypes without SMN1 deletion. With the exception of a severe X-linked type, the genetic localization is still unknown. The definition of distinct clinical phenotypes is a prerequisite for the disclosure of the underlying genetic defects. Knowledge of further genes causing neurogenic muscular atrophy is of great interest for elucidating the complex pathogenesis of anterior horn cell disease. Therefore, we are interested in DNA studies of further families with different SMA variants.

P0520. Facial Dysmorphism and quasidominant inheritance in Cenani-Lenz syndrome

S. A. Temtamy
National Research Centre; Cairo, Egypt
stemtamy@intouch.com

Cenani-Lenz syndrome is a rare specific syndactyly/synostosis syndrome(MIM 212780).While most reports described autosomal recessive inheritance,one report described affected father &daughter.No facial dysmorphism was previously noted.We studied 2 families; in the 1st an affected female had a previously affected brother & her father was said to have been similarly affected.Extensive inbreeding in this family suggests quasidominant inheritance.In the 2nd family there was history of a similarly affected sib who in addition had genital anomalies and cleft palate.Parents were normal first cousins.Both probands had similar facial dysmorphism;high,broad,bulging forhead,hypertelorism depressed nasal bridge,downslanting palpebral fissures,short nose,short&prominent philtrumand malar hypoplasia.The present report suggests for the first time facial dysmorphism & quasidominant inheritance in Cenani-Lenz syndrome

P0521. Methylenetetrahydrofolate Reductase (MTHFR) Genetic Polymorphism; A Possible Restrictive Relationship Between C677T Mutation With Lower Neural Tube Defects (NTD)

J. I. C. F. Neri[1], *V. R. Arruda*[2], *D. P. Cavalcanti*[3]
[1]*Universidade Estadual de Campinas; Campinas, Brazil;* [2]*Hemocentro - UNICAMP; Campinas, Brazil;* [3]*Dpto de Gen tica M dica - FCM - UNICAMP; Campinas, Brazil*
jineri@uol.com.br

A number of studies have demonstrated two common polymorphisms (C677T and A1298C) in the gene encoding MTHFR associated with NTD. The majority of the papers showing a link between a mutation in the MTHFR gene and NTD deal only with spina bifida. However, besides spina bifida, NTD includes anencephaly and encephalocele, all considered being consequence of an incomplete closure of the neural tube. It has been suggested an alternate hypothesis; NTD of the upper type (anencephaly, encephalocele and thoracic spina bifida) may have different pathogenesis from those of the lower type (lumbosacral spina bifida). In this work we describe a study of mutations C677T and A1298C in MTHFR gene in a total of 119 families with at least one fetus/child affected by NTD and 302 normal controls. The results show that 15.1% of probands are homozigotes 677TT. There was no significant difference among parents and controls ($c^2_{(3)}$=1.59; p=0.66). The frequency of 4.2% of the 1298CC genotype among probands was no different of parents and controls ($c^2_{(3)}$=1.20; p=0.75). A significant difference in the frequency of homozigote 677TT was observed between upper and lower NTD ($c^2_{(1)}$=6.53; p=0.01) and between lower defects and controls ($c^2_{(1)}$=8.05; p= 0.004). There was more alleles 677T among lower NTD than upper NTD ($c^2_{(1)}$=4.84; p=0.02) and controls ($c^2_{(1)}$=4.57; p=0.03). Combined heterozygosity 677CT/1298AC was not found as a risk factor in the present study. However, combined homozygosity 677TT/1298CC was closely inexistent. These results suggest that the polimorphism 677TT in the MTHFR gene seems to be associated only with lower NTD.

P0522. Multiple Malformations including short Stature, Anal Atresia, upper Limb Defect, cardiac Malformation, Hearing Impairment and mild mental Retardation in Combination with primary Immunodeficiency in a 31 year old Patient

M. Krei§-Nachtsheim[1], *D. von Bubnoff*[2], *T. Bieber*[2], *M. M. N then*[1]
[1]*Institute of Human Genetics; Bonn, Germany;* [2]*Department of Dermatology; Bonn, Germany*
kreiss@mailer.meb.uni-bonn.de

We report on a 31 year old male patient with multiple anomalies and an increased susceptibility to infections. Malformations include right upper limb defect (peromelia), congenital heart defect (ventricular septal defect), imperforate anus, short stature (1.55 m), strabismus divergens and hearing impairment (neurosensory). Minor anomalies are hypospadia glandis, cryptorchidism, scoliosis and inguinal hernia. There was mild developmental delay and mild mental retardation. No similar anomalies were reported in the patient s family, except for one brother, who had an atrial septal defect that had been corrected in childhood. Cellular anomalies include functional impairment and deficiency of T-helper cells, low serum IgM-level and mild thrombocytopenia. Specific laboratory investigations showed no evidence of Fanconi anemia, DiGeorge-Syndrome, Townes-Brocks-Syndrome and Wiskott-Aldrich-Syndrome. Some of the manifestations seen in our patient are part of the VACTERL-association and the Oculo-Oto-Radial-(IVIC-)Syndrome. However none of these syndromes can explain the spectrum of anomalies seen in our patient suggesting a new syndrome.

P0523. Reevaluation of the nosological standing of the C syndrome.

A. Bohring[1], *J. M. Opitz*[2]
[1]*Dept. of Pediatrics, Ostholstein Kliniken Eutin; Eutin, Germany;* [2]*Dept. of Pediatrics and Human Genetics, University of Utah; Salt Lake City, UT United States*
Abohring@aol.com

Nearly 30 years ago (1969) Opitz et al. reported on 2 sibs with a new, previously apparently undescribed syndrome of multiple congenital anomalies. These sibs had a characteristic appearance with trigonocephaly, flat nose, strabismus, epicanthal folds, multiple bucco-labial frenula, short stature, postaxial hexadactyly, joint contracture/dislocation/crepitation, and loose skin. The syndrome was named the C syndrome. To date, about 40 published and about 30 unpublished cases are known to us for consultation. A gene causing C syndrome is not known. Defining syndromes on the basis of propositi is fraught with many problems and frequently leads to truncation of the phenotype to its severest form. Moreover, more than 70 conditions are described with trigonocephaly, of which the C syndrome is only one; phenotypes overlap widely. Thus, we reviewed all of these cases of (presumed) C syndrome to reevaluate their nosological standing. Paying tribute to causes and symptoms, we present a decision tree of how to make the diagnosis, which may help to distinguish between the C syndrome and other entities with trigonocephaly. In the remaining cases of the so-called C phenotype (Reynolds, 1989), further heterogeneity is suggested on the basis of several sets of individuals sufficiently similar to each other and sufficiently different from the C-propositi. Thus, for clinical and methodological reasons it makes sense to create several C syndrome-like groups to test the hypothesis of heterogeneity. The recently published severe cases (Bohring et al., 1999) which may turn out now as a 3p subtelomeric duplication (McGaughran et al., 2000) are a striking but not the only example.

P0524. Different localisation of point mutations in the ligand-binding domain of androgen receptor allows differentiation between Complete and Partial Androgen Insensitivity Syndrome

M. Ignacak[1], *M. Niedziela*[1], *S. Zajaczek*[2], *W. H. Trzeciak*[1]
[1]*University of Medical Sciences; Poznan, Poland;* [2]*Pomeranian Medical University; Szczecin, Poland*
mignacak@eucalyptus.usoms.poznan.pl

The androgen receptor (AR) is a transcription factor that binds ligands and regulates expression of genes involved in sexual differentiation and maintenance of the male phenotype. Different mutations in the AR gene are responsible for a wide spectrum of phenotypic abnormalities in 46XY individuals, resulting in a. clinical condition called androgen insensitivity syndrome (AIS). We studied the AR gene in two 46XY phenotypic females. One exhibited typical symptoms of complete form of AIS (CAIS), while the symptoms in the second girl were milder. Direct sequencing of the amplification products in first patient revealed C2718T transition in exon 6, creating a stop codon in position 786, in place of Arg codon. It has been previously established that this mutation results in complete loss of ligand binding ability and is responsible for clinical symptoms of CAIS. The examination of exon 6 in the second girl revealed G2754C transversion causing Gln798Glu substitution and might trigger partial form of AIS (PAIS). It was found that mutation in the AR gene localised less than 40 nt downstream from nt 2718, might affect ligand binding to a lesser extent and cause change in the patient s phenotype from CAIS to PAIS. Supported by grant No 4 PO5E 058 18 form the Polish Committee for Scientific Research.

P0525. Esophageal atresia and amniotic band-like hypoplasia of fingers in Ehlers-Danlos syndrome type IV

H. Y. Kroes[1], *G. Pals*[2], *A. J. van Essen*[1]
[1]*University Hospital Groningen; Groningen, The Netherlands;* [2]*Free University Medical Centre; Amsterdam, The Netherlands*
h.y.kroes@medgen.azg.nl

EDS IV, or the vascular type of Ehlers-Danlos syndrome, is a connective tissue disease predominated by vascular phenomena, the most serious complications being rupture of large arteries or bowels. Congenital anomalies are rare in EDS IV. Mutations in the COL3A gene, resulting in an abnormal structure of collagen III, are the cause of EDS IV.

We describe a mother and son with EDS IV. The mother was born with hypoplasia of digiti II-V with amniotic band-like circular constrictions on the right hand, a clubfoot on the right, and macrocephaly, according to the neurologist as a result of normal pressure hydrocephaly. After the delivery of her son she had severe spontaneous haemorhage from a ruptured left subclavial artery. She died two months later of massive dissection of the thoracic and abdominal aorta. Her son was born with a esophageal atresia, and macrocephaly due to communicating hydrocephaly.

Protein analysis in fibroblast of the mother for collagen III abnormalities was normal.

However, DNA-analysis of the COL3A gene revealed a pathogenic mutation (388G T, Gly130Arg) in the mother, that was also found in the son. We will discuss the possible relationship between the congenital anomalies in our patients and EDS IV. Besides, we want to stress that normal collagen III protein analysis does not exclude EDS IV.

P0526. Netherton Syndrome; Report of One Case

S. Lin, *M. Tu, C. Chuang, B. Chen*
MacKay Memorial Hospital; Taipei, Taiwan Republic of China
zsplin@ms2.mmh.org.tw

Netherton syndrome (NS, MIM 256500) is a rare autosomal recessive disorder characterized by congenital ichthyosis, a specific hair-shaft defect (trichorrhexis invaginata), and atopic manifestations. Infants with this syndrome often fail to thrive. The clinical features may be variable and they change gradually from birth on. As a results, diagnosis of this disorder in

early childhood is difficult, and the first tentative diagnosis may mistake it for other congenital ichthyosiform erythroderma. We report on a 2 years old Taiwanese girl who was diagnosed with NS at age of 15 months. She had generalized erythroderma with desquamation and intermittent oozing since birth, and it changed to ichthyosis linearis circumflexa in late infancy. Atopic manifestations are present, including eczema-like rashes, allergic conjunctivitis, urticaria, allergic rhinitis, high serum Ig E (422 IU/mL) level and hypereosinophilia (1080/cmm). Skin biopsy showed parakeratosis of the epidermis and a dense lymphohistiocytic infiltrate in the upper dermis. Her scalp hair is sparse and brittle, and showed characteristic bamboo hair picture by microscopy. Her body weight gain is poor (< 3 rd %ile). Medical treatment only alleviates her clinical symptoms slightly. Since NS gene was mapped to 5q32 by Chavanas, et al. recently, linkage analysis is performed on the family. We will show our preliminary data at the conference.

P0527. Molecular characterisation of a ring 22 chromosome in a patient with severe language delay. A contribution to the refinement of the telomeric 22q deletion syndrome.

P. De Mas[1], *N. Chassaing*[1], *Y. Chaix*[2], *G. Bourrouillou*[1], *P. Calvas*[1], *E. Bieth*[1]
[1]*Purpan Hospital, department of Genetic; Toulouse, France;* [2]*Children Hospital, department of Neurology; Toulouse, France*
bieth.e@chu-toulouse.fr

Several studies have reported cases of ring 22 chromosome in patients with mental retardation characterised by severe language delay and some dysmorphic features. Here, we report the case of a 7 years old boy with these phenotypic and chromosomic conditions. The propositus was the unique affected case of a non-consanguineous family. A mild motor developpement delay was observed from 12 months (first steps at 17 months). In contrast, the speech developpement was more severely affected, with by the age of 7, language limited to only few words. Behavioral problems were also noted as hyperactivity, short attention span and aggressive outbursts. The only minor dysmorphic findings were a broad nasal bridge, long lashes, a dental malocclusion and a hight arched palate. The cytogenetic analysis revealed a ring 22 chromosome with normal parental karyotypes. Using a combination of fluorescent in situ hybridization (FISH) and genotypic analysis, we were able to detect a telomeric deletion on the ring 22 chromosome occuring beyong the mapped marker D22S39. Further molecular investigations are in progress to map more precisely this deletion and to attempt to characterise the 22q critical region involved in language developpment. This is, to date, the first characterised deletion of a ring 22 chromosome in a patient with mental retardation. Similar phenotype being previously described in patients with telomeric 22q deletion, we considere, on the basis of our data, that ring 22 chromosome may be a contributive approache to the 22q telomeric deletion syndrome.

P0528. The Clinical, Cytogenetical and Molecular Studies in Polish Families with Sterility or Repeated Early Pregnancy Loss.

J. Kocki, *S. Bakalczuk, G. Jakiel, L. Putowski, I. Korszen-Pilecka, B. Kwiatkowska-Drabik, J. Wojcierowski*
Medical University of Lublin; Lublin, Poland
kocki@hipokrates.am.lublin.pl

Female fertility depends upon; normal anatomic development permitting coitus, cervical mucus favorable for sperm transport, normal ovarian function, lack of tubal obstruction, normal tubal function and normal hormones function. The selected causes of infertility in males there are; abnormal gamete formation - spermatozoon abnormalities or chromosomal abnormalities, cryptorchidism, androgen insensivity syndromes, hypo- or hypergonadotropic disorders, oligospermia or azoospermia and environmental factors. Cytogenetic studies were performed in 72 maried couples with sterility or repeated early pregnancy loss. Peripheral blood lymphocytes were stimulated by phytohemagglutynine for 72 hours. After 2 hours colcemid exposure, harvesting, slide preparation and G- or Q-banding were performed by standard procedure. Ten patients showed chromosome changed; 1; 46,Xi(X)/45,X; 2; 47,XXY; 3; 46,XY,t(2;16)(q13;p12); 4; 46,XY,t(2;16)(q13;p13); 5; 46,XX/46,XXt(1;X)(q41;q28); 6; 46,XY,der(13q;14q); 7; 46,XY,r(22); 8; 46,XX/47,XXX; 9; 46,XY,t(2;15)(q22;q26); 10. 47,XXY. We examined CFTR gene mutation in a group of men with oligo- or azoospermia by INNO-LiPA Cystic Fibrosis procedure, there was based on the reverse-hybridization pronciple. In this group of patients we diagnosed 4 carriers of cystic fibrosis. The good genetic counseling has practical importance for the affected families with sterility or repeated early pregnancy loss. This paper was supported by Polish Commitee of Scientific Research. Grant no 4 P05E 06416.

P0529. Identification of 6 new mutation in the fibrillin-1 gene of 31 unrelated patients with Marfan syndrome

K. Rommel, *M. Arslan-Kirchner, J. Schmitdke*
Institut f r Humangenetik, Medizinische Hochschule; Hannover, Germany
rommel.kathrin@mh-hannover.de

The Marfan syndrome (MFS) is an inherited autosomal dominant disorder, characterised by phenotypically variable manifestations in skeletal, ocular, and cardiovascular systems. To date, over 190 Mutations have been identified in the fibrillin-1 (FBN 1) gene, the gene implicated in the aetiology of MFS. (Human Gene Mutation Database;
http;//www.uwcm.ac.uk/uwcm/mg/hgmd0.html) Here we report 3 missense mutations, one splice site alteration and two mutations causing frameshifts; a 16-bp deletion and a single nucleotide insertion. Two of the missense mutations occur in calcium-binding epidermal growth factor like (EGF) domains, resulting in the substitution of tyrosine by cysteine (Y1101C) and threonine by isoleucine (T1908I) in the exons 26 and 46 respectively. One missense mutation (V449I) replaces a valine residue by isoleucine in the non-calcium binding epidermal growth factor like domain of exon 11. The frameshift mutation 1903del16bp leads to a premature termination codon (PTC) in exon 17, while the frameshift mutation 8025insC is causing a PTC in exon 64. Finally, we identified a splice site alteration; 4817-1G/T changes the splice acceptor consensus sequence in intron 38, most probably leading to the skipping of exon 39.

P0530. Otocephaly with multiple malformations without situs inversus or holoprosencephaly

C. Schiffer[1], *C. Sergi*[2], *M. Schiesser*[3], *J. Zschocke*[1], *G. Tariverdian*[1]
[1]*Institute of Human Genetics; Heidelberg, Germany;* [2]*Institute of Pathology; Heidelberg, Germany;* [3]*Dept. of Obstetrics and Gynaecology; Heidelberg, Germany*
christiane_schiffer@med.uni-heidelberg.de

Otocephaly (agnathia) is a rare developmental field complex with structural defects limited to the craniofacial region. It can occur alone or in association with a variety of malformations, most frequently holoprosencephaly and situs inversus. We report of a fetus with otocephaly and a previously undescribed malformation pattern lacking both holoprosencephaly and situs inversus. The parents were non consanguineous, and there were no evidence of infection or exposure to teratogenic factors during pregnancy. Ultrasound examination at 30 weeks gestation revealed polyhydramnion and fetal malformations. Amniocentesis was performed; spontaneous fetal death occurred five days later. Subsequent examination (length 34 cm, 3rd centile, weight 810 g, 3-10th centile) showed otocephaly with very deep set, dysmorphic ears, nearly complete absence of the mandible, microstomia, hypoplasia of the tongue, and blind ends of nasal and oral cavities. There was no holoprosencephaly or situs inversus. Both kidneys and ureters were absent, and there was anal atresia. Skeletal malformations included bilateral club feet, multiple rib fusions with dysgenesis of the thoracic spine and os sacrum. The umbilical cord contained one artery and one vein. Laboratory investigations showed normal chromosomes and excluded microdeletion 22q11.2. The pathogenesis of otocephaly and the associated malformation pattern is as yet unknown but is thought to represent a developmental field defect involving the first branchial arch. Evidence indicates that it may be due to a range of chromosomal, monogenic and teratogenic factors.

P0531. Neurofibromatosis-Noonan Syndrome (NFNS)- Case reports and review of the literature

A. Buske[1], *K. Lehmann*[2], *D. Kaufmann*[3], *S. Rudnik-Sch neborn*[4], *S. Tinschert*[5]
[1]*Institut f r Medizinische Genetik, Charit , Humboldt-Universit t Berlin, Germany; Berlin, Germany;* [2]*Institut f r Medizinische Genetik, Charit , Humboldt-Universit t; Berlin, Germany;* [3]*Abteilung f r Humangenetik, Universit t Ulm; Ulm, Germany;* [4]*Institut f r Humangenetik, Universit t Aachen; Aachen, Germany;* [5]*Institut f r Medizinische Genetik, Charit , Humboldt-Universtit t; Berlin, Germany*
annegret.buske@charite.de

Since Allanson et al. (1) described four individuals with the co-occurrence of the Noonan syndrome and manifestations of neurofibromatosis type 1 (NF1), more than 30 patients are reported in the literature. As discussed from Carey (2) it is still debated if the Neurofibromatosis-Noonan syndrom (NFNS) represents a true syndrome or simply a variable manifestation of NF1. We want to present two patients with the Neurofibromatosis-Noonan phenotype. The first patient is a 22-year-old woman with fully expressed NF1 and with the additional features; pulmonary stenosis, ptosis of the eyelids, short stature and mental retardation. The underlying mutation in the NF1-gene is identified; a stop-mutation in exon 13; C2041T. The second patient is a 18-year-old woman. She has multiple caf -au-lait-spots

and a pulmonary stenosis, a short stature and facial features typical of Noonan syndrome. She has neither Lisch nodules nor neurofibroma. However, in the MRI there was a lesion indicative of a glioma of the medulla oblongata. Her mental development is normal. A mutation in the NF1-gene is not yet identified, but the analyses are still in progress. A FISH analysis could exclude a large deletion of the NF1-gene. In both cases there is no family history of NF1 or Noonan syndrome. We want to discuss the clinical and molecular findings in these patients. Reference; 1 Allanson JE, Hall JG, Van Allen MI (1985); Noonan phenotype associated with neurofibromatosis. Am J Med Genet 21; 457—462. 2 Carey JC (1998) Neurofibromatosis-Noonan Syndrome — editorial comment. Am J Med Genet 75; Am J Med Genet 21; 263-264.

P0532. Determination of microdeletions in AZF regions on Y chromosome by PCR in infertile man

C. F. Sargin[1], *S. Berker Karauzum*[1], *A. Manguoglu*[1], *G. Luleci*[1], *S. Karaveli*[2], *H. G Ikesen*[2], *T. Erdogru*[3]
[1]*Akdeniz University, Faculty of Medicine, Department of Medical Biology and Genetics; Antalya, Turkey;* [2]*Akdeniz University, Faculty of Medicine, Department of Pathology; Antalya, Turkey;* [3]*Akdeniz University, Faculty of Medicine, Department of Urology; Antalya, Turkey*
toraman@hipokrat.med.akdeniz.edu.tr

Cytogenetic studies on idiopathic infertile men have shown structural defects on Y chromosome. These findings suggest that Y chromosome contains the genes regulating spermatogenesis. Recently the genes responsible for spermatogenesis which are located at intervals V and VI of Yq.11.23 regions have been cloned and named as Azoospermia Factor Lokus (AZF) by the use of molecular techniques. In this study, we studied 38 infertile men. The infertility etiology of twenty three of these patients had been defined as idiopathic, while varicocele, infection, obstruction, and toxic agent exposure was given as the reason for infertility in seven, three, four and one patients respectively. Twenty six patients had azoospermia and twelve had oligospermia. SY84 and SY86 primers of AZFa zone, SY127 and SY134 primers of AZFb zone, SY255 and SY254 primers of AZFc zone and SY14 primers of SRY gene were used as internal controls and target DNA samples were amplified by multiplex PCR method to determine the presence of microdeletions in q11.23 region of Y chromosome of 38 infertile men whose karyotypes were cytogenetically normal (46,XY). Amplification by SY254 and SY127 has not revealed any visible bands in one of the cases (2.6%), indicating deletions in AZFb and AZFc (DAZ gene) regions.

P0533. Microcephalic primordial dwarfism and brain perisylvian dysgenesis ; a unique association.

S. Andries[1], *W. Dobyns*[2], *E. Rebuffat*[3], *F. Christiaens*[3], *L. Van Maldergem*[1]
[1]*Centre de Genetique Humaine, Institut de Pathologie et de Genetique; Loverval, Belgium;* [2]*Dpt of Pediatric Neurol, University of Chicago; Chicago, IL United States;* [3]*Dept of Pediatrics, Centre Hospitalier du Val de Sambre; Auvelais, Belgium*
secret.genet@ipg.be

Microcephalic osteodysplastic primordial dwarfism is a heterogeneous group of very rare skeletal dysplasias determining severe growth retardation of prenatal onset. It may be associated with brain dysgenesis such as neuronal heterotopias. Perisylvian dysgenesis is a rare cortical dysplasia with variable neurological dysfunction. We describe a 3 yr-old female child born to unrelated Caucasian parents. Severe intrauterine growth retardation was detected by ultrasound during the pregnancy. Birthweight was 1,645 g (- 4 SD) with a length of 41 cm (- 4 SD) and head circumference of 28 cm (- 8 SD). Placental infarcts and fibrosis were observed. Proportionate severe global short stature, microcephaly and mild dysmorphic signs were noted. There was left arm hemiparesis. Karyotype was 46,XX. Clinical course was relatively mild with respect to severe (-8 SD) microcephaly, but there was major failure to thrive and delayed milestones (sat at 12 months, walked at 24 months, said a few words at 20 months). Growth parameters are still at - 6 SD for weight and length. A skeletal survey indicated several of the signs described by Majewski in the so called primordial osteodyplastic dwarfism type II such as plastyspondyly and mild vertebral clefting, narrow pelvis with dysplastic iliac wings, short humeri and femora with metaphyseal flairing. MRI showed asymetric perisylvian polymicrogyria involving most of the right hemisphere which was most severe in the right perisylvian region and sparing the right mesial occipital lobe. We suggest the patient has a hitherto undescribed association of osteodysplastic primordial dwarfism and perisylvian polymicrogyria.

P0534. Familial Form Of Dystrophic Epidermolysis Bullosa Of Hands And Feet Type Cockayne- Touraine, Associated With Translocation 5;13

B. Dimitrov[1], *E. Simeonov*[1], *A. Durmishev*[2], *A. Andreev*[1], *I. Boneva*[1]
[1]*Section of Clinical Genetics, University Pediatric Hospital; Sofia, Bulgaria;* [2]*Department of Dermatology and Venerology, Medical Faculty; Sofia, Bulgaria*
emil@medfac.acad.bg

Epidermolysis bullosa is a group of hereditary dermatoses in which mild mechanical trauma or other causes lead to blistering of the skin. First Cockayne (1938) described the type with bullose eruption of the feet and Readett (1961) reported a family in which 14 members in 5 generations were known to have localized epidermolysis bullosa similar to those observed by Cockayne. Cartledge and Myers (1943) reported an affected family from West Virginia where blistering occured only on the hands and feet and mainly in warm weather after continuous walking or labor with hand tools. The clinical findings varied widely in severity from severe generalized blistering from birth to temporary blistering of feet in summer. Bouwes Bavinck et al. (1987) reviewed the clinical features of the Cockayne-Touraine form of dystrophic epidermolysis bullosa. The clinical features showed considerable interfamilial variation. They described an extensively affected family with autosomal dominant dystrophic EB; at least 4 members of the family also had congenital localized absence of skin as seen in Bart syndrome. The autors describe an affected family with dystrophic epidermolysis bullosa on the hands and feet in 4 generations. Two members of the family, father and son, have been investigated personally. The blistering started immediately after birth and progressed slowly to a phase of dystrophic scars and areas of atrophy. The bullae were localized on hands and feet, showing worsening in summer. In addition to the skin lesions, the nails were dystrophic too. Cytogenetic analysis showed translocation (5;13) (q13; q3.34) in affected persons. Clinical and genetic heterogeneity of the disease will be discussed.

P0535. Paucity of cancer among Down syndrome-associated deaths in the United States

Q. H. Yang[1], *S. A. Rasmussen*[1], *J. M. Friedman*[2]
[1]*Birth Defects and Pediatric Genetics Branch, CDC; Atlanta, GA United States;* [2]*Department of Medical Genetics, University of British Columbia; Vancouver, Canada*
qyang@cdc.gov

Recent studies have shown that the risk of many solid tumors is decreased among people with Down syndrome. We used Multiple-Cause Mortality Files compiled from U.S. death certificates for 1983 to 1997 to examine the occurrence of major categories of malignant neoplasms among more than 17,800 Down syndrome-associated deaths. Proportionate mortality ratios, which measure whether a specific medical condition is more or less likely to be listed on death certificates on which Down syndrome is also listed than in all deaths, was used to test for associations. We found that persons with Down syndrome were 1.5 times (95% CI 1.4-1.7) more likely to have leukemia listed on their death certificates than persons without Down syndrome, confirming this well-recognized association. However, death certificates from persons with Down syndrome were much less likely to list malignant neoplasms other than leukemia (PMR = 0.09, 95% CI 0.08-0.10). PMRs for almost all major classes of malignant neoplasms were significantly lower than expected. For example, the PMRs for prostate cancer, ovarian cancer, breast cancer in females, cancers of the lung and airways, and cervical cancer were all less than 0.1. Strikingly low PMRs for malignancy were associated with Down syndrome at all ages and in both genders. Possible explanations for the paucity of cancers among Down syndrome-associated deaths include decreased susceptibility of cells from people with Down syndrome to tumorigenesis and/or reduced exposure to environmental risk factors such as tobacco and certain occupational exposures.

P0536. Styckler Syndrome

M. Lima, *L. Ramos, M. Martins, C. Mota, A. Fortuna*
Instituto Genetica Medica Jacinto de Magalh es; Porto, Portugal
linil@esoterica.pt

Stickler syndrome (MIM108300) is a autosomal dominant disorder with characteristic ophthalmological and orofacial features, deafness and arthritis. Very similar features are also found in Marshall syndrome (MIM 154780) but these patients more often have short stature and abnormalities in cranial ossification and retinal detachment occurs less frequently. High myopia, retinal detachment and abnormalities of vitreous gel architecture are a pathgnomonic feature of the Stickler syndrome. The majority of families with This syndrome have mutations in the COL2A1 gene. One mutation causing Stickler syndrome and one causing Marshall syndrome

have been detected in the COL11A1. A null-allele mutation in the COL2A1 gene led to a typical phenotype of Stickler syndrome. Clinical details of our patients (11) and families are discussed with emphasis on differential diagnosis.

P0537. Moebius Syndrome;clinical And Genetic Evaluation

C. S. Canton[1], *R. I. Ortiz de Luna*[2], *C. Garcia Delgado*[2], *F. Flores*[2]
[1]*Servicio De Genetica Complejo Hospitalario Metropolitano De La Caja De Seguro Social; Panam , Panama;* [2]*Servicio De Genetica Hospital Infantil De M xico, Federico G mez; Mexico, Mexico*
celiacanton@hotmail.com

We report the most frequently anomalies was found in 77 patients with Moebius Syndrome, studied in the period of January 1970 to october 1998 in Hospital Infatil de Mexico, Federico Gomez . The patients were found limbs anomalies in 80.52%, orofacial in 58.44% and ophtalmological in 44.16%.
In 53.25 % we noted exposition to external chemical agent, in this case very interesting Montanoa tomentosa in 9.09% of the total. (Zoapantle of the Mexican Herbolaria).
Cytogenetic studies in 24 patients found 8.33% abnormal and the blink reflex were abnormal in all the patients studied.

P0538. Mt 3298 A -> G mutation in a patient with diabetes, deafness, mental retardation and seizures.

L. Van Maldergem[1], *P. Hilbert*[1], *N. Ribai*[2], *S. Noel*[2], *Y. Gillerot*[1]
[1]*Centre de Genetique Humaine, Institut de Pathologie et de Genetique; Loverval, Belgium;* [2]*Dept of Neurology, Centre Hospitalier Universitaire A. Vesale; Montigny-le-Tilleul, Belgium*
vmald@skypro.be

Mitochondrial diabetes encompasses the previously described spectrum of 3243 and 1555 mutations of the Mt tRNA genes. Recently a Japanese study identified an additional tRNALeu mutation at position 3298 in a subset of diabetic patients. We describe a patient with a combination of insulin dependent diabetes mellitus, late onset deafness, epilepsy, a porencephalic cyst and mental retardation. He also exhibited some widespread, bluish skin lesions. A history of neonatal asphyxia was recorded. Since there was an association of diabetes and deafness a search for tRNA mutations was performed. A heteroplasmic 3298 A -> G transition was identified in more than 80 % of DNA extracted from peripheral lymphocytes. This result confirms that mutation 3298 determines mitochondrial diabetes. However if we assume that mental retardation and porencephalic cyst are not only due to perinatal brain insult, this report may also expand the spectrum of clinical signs and symptoms associated with this mutation. This would be similar to the 3243 mutation which can determine mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes in some cases, deafness only or deafness and diabetes in others. This finding indicates that the 3298 mutation may represent another example of mitochondrial unpredictable clinical expression.

P0539. Non random X chromosome inactivation in CHILD syndrome

P. Vabres[1], *P. Amati-Bonneau*[2], *D. Hamel-Teillac*[3], *Y. De Prost*[3], *M. Larregue*[2], *D. Bonneau*[2]
[1]*Centre Hospitalier Universitaire de Poitiers; Poitiers, France;* [2]*CHU; Poitiers, France;* [3]*Hopital Necker Enfants Malades; Paris, France*
p.vabres@chu-poitiers.fr

CHILD syndrome is an X-linked male-lethal condition with congenital limb hemidysplasia and ichtyosiform nevus, in which mutations of the 3 beta hydroxysteroid dehydrogenase gene have been reported. Functional mosaicism due to X chromosome inactivation has been suggested as an explanation to unilateral limb hypoplasia and patchy skin involvement. We studied X inactivation in two patients with CHILD syndrome. One patient, aged 14, had a mild phenotype with keratosis palmoplantaris, ichtyosiform epidermal nevus of the neck and inguinal folds (ptychotropism), and complete agenesia of one finger. Both parents were unaffected. The other patient, aged 22, had a more severe phenotype with hypoplastic right superior and inferior limbs, extensive ichtyosiform nevi with axillae and groin involvement, scarring alopecia and linear keratosis palmoplantaris. Polymorphism at the HUMARA locus was studied on DNA from blood lymphocytes and affected keratinocytes. Non random inactivation of the same X chromosome (paternal X in the first patient) was found on affected skin and blood. Skewed X inactivation in peripheral blood lymphocytes is known to occur in other X-linked male lethal disorders. In incontinentia pigmenti, it is a consequence of selection against cells expressing the lethal allele. In CHILD syndrome, the cause for skewed X inactivation is unknown. Interestingly, X inactivation in blood lymphocytes is not correlated with disease severity, as complete skewing was found in both cases.

P0540. Prader-Willi Syndrome with a partial deletion 3q and 15q due to a de novo t(3;15)(q28;q12) in a patient with 45 chromosomes.

C. Windpassinger, *E. Petek, W. Emberger, H. Zierler, K. Wagner, P. M. Kroisel*
Institute of Medical Biology and Human Genetics; Graz, Austria
windpass@kfunigraz.ac.at

Here we report on a 6 month old male patient with a rare variant of Prader-Willi Syndrome (PWS). Birth weight 3,2 kg, length 50 cm were within the normal range but due to his head circumference (OFC) of 37 cm a caesarian section was performed. In most cases PWS is caused by an interstitial deletion of the proximal segment of chromosome 15q, a maternal disomy 15q11-13 or more rarely due to an imprinting centre mutation. In addition structural chromosome aberrations, like translocations, inversions, isochromosomes or rarely ring chromosomes that lead to a deletion or a duplication of the PWS critical region were described in PWS patients. An unbalanced translocation 3;15 was found by high resolution banding and FISH analysis leading to the following karyotype; 45,XY,-15,der(3)t(3;15)(q28;q12) de novo. Since this findings suggested the presence of PWS in the patient, molecular analysis was performed. Diagnosis was confirmed by methylation-specific PCR, Southern Blot and FISH analysis using the D15S10 probe. The patient thus represents a rare case of PWS combined with an additional deletion of the terminal segment of chromosome 3. This certainly is an unique observation, but is of general interest to study combinatorial effects of PWS with autosomal deletions others than of chromosome 15. The most striking phenotypic findings of the patient at an age of 6 month were hypotonia, macrocephaly (>90 centile) with frontal bossing, retrognatia, preauricular tags, strabismus divergens, flexions anomalies of the upper limbs, and bilateral club feet, however a more detailed description will be presented.

P0541. Autosomal Recessive Duodenal Atresia Of Membranous Type

T. Krustev, *I. Iazova, M. Stefanova*
Medical University; Plovdiv, Bulgaria
brigite@plovdiv.techno-link.com

Congenital duodenal membrane (CDM) has been classified as a type of duodenal atresia. It occurred mainly sporadically and several cases in family have been observed. Here we describe a membranous type of duodenal atresia in two sibs, a sister and a brother, following third and fourth pregnancy of consanguineous parents. The first pregnancy in the family ended with a spontaneous abortion in a first trimester and the second resulted in a healthy girl. Both affected children showed isolated duodenal atresia having normal karyotype. They were successfully operated using duodenojejunostomy. An autosomal recessive type of inheritance was suggested.

P0542. Private Syndromes In Genetic Counselling

E. Simeonov, *B. Dimitrov*
Section of Clinical Genetics, University Pediatric Hospital; Sofia, Bulgaria
emil@medfac.acad.bg

Genetic counseling in pediatric genetics is involved mainly in providing genetic information to families with children affected of mental retardation, neurologic deterioration and multiple congenital anomalies, caused by chromosomal aberrations, single gene defects and dysmorphic syndromes of other etiology. Four undescribed up to now nonchromosomal MCA syndromes have been observed over the last several years. 1. 7 year old brother and 4 year old sister with dysmorphic face, Dandy-Walker malformation, pronounced muscular hypotonia, severe, progressive mental retardation and chronic renal insufficiency. 2. Newborn girl, who died at 3 months with dysmorphic face, hydrocephaly, bilateral cataract, nail hypoplasia, ambiguous genitalia and severe developmental delay. 3. Two sisters of 10 and 8 with slightly dysmorphic face, mild mental retardation /IQ 60 and77/, growth retardation and hypo/aplasia of lachrymal /no tears/ and salivary /dry mouth/ glands. 4. Brother of 8 and sister of 10 months with growth retardation, progressive mental retardation, dry, coarse, brittle hair and renal tubular acidosis. The observed disease states are discussed from the point of view of differential diagnosis and whether or not they represent new unreported entities.

P0543. Radiological manifestations of SHFM

P. Tsipouras[1], S. Sifakis[1], M. Arici[2], D. Viljoen[3], P. Beighton[4]
[1]Dept Pediatrics, Div Human Genetics, University of Connecticut Health Center; Farmington, CT United States; [2]Dept of Radiology, University of Connecticut Health Center; Farmington, CT United States; [3]Dept Human Genetics, University of Witwatersrand; Johannesburg, South Africa; [4]Dept of Human Genetics, University of CapeTown; Cape Town, South Africa
sifakis@excite.com

Aim; Split Hand Foot Malformation (SHFM) is characterized by genetic heterogeneity and phenotypic variability, while frequently is a syndromic manifestation. Although SHFM implies the existence of a cleft in an affected limb, numerous other abnormalities are considered under this term. Our objective was to describe the phenotypic spectrum of SHFM for a more accurate delineation of this condition. Subjects-Methods; We reviewed 31 limb radiographs of 17 individuals with non-syndromic SHFM. Totally 12/17 individuals had some sort of kinship, and 6 of them were members of the Wadoma tribe in which high frequency of SHFM has been reported. Results; Eleven individuals displayed SHFM anomalies in 20 affected hands, with bilateral involvement in 9 cases. Hand abnormalities included; hypoplastic, dysplastic, absent phalanges (#11) or metacarpals (#10), triphalangeal thumb (#5), articulation between metacarpal heads (#4), abnormal growth plates (#4), bone or soft tissue syndactyly (#3), clinodactyly (#2), transversely grown bones (#2). Twelve individuals had SHFM in 22 affected feet, with bilateral involvement in 10 cases. They included; aplastic, hypoplastic, dysplastic metatarsal heads (#22) or phalanges (#22), mid tarsal row dysplasias (#10), clinodactyly or toes abnormal angulation (#9), articulation between metatarsal heads (#3). In 4/17 cases there was a single limb involvement, while in 6/17 of the both hands or feet. In 5/17 cases all limbs were affected. Conclusion; Our results may assist clinicians and radiologists in the accurate identification of SHFM cases, avoiding the nosological confusion that frequently exists. These observations could also be useful in the elucidation of the underlying developmental mechanisms.

P0544. Congenital Laryngotracheal Webs and Early Onset Colorectal Carcinoma in a Family with Three-Generation Transmission of Del(22q11.2)

J. Larsen Haidle, K. Keppler-Noureil, A. B. Kanis, A. Muilenburg, J. Welch, Q. Qian, L. Yang, S. Patil
University of Iowa; Iowa City, IA United States
joy-haidle@uiowa.edu

Microdeletion of chromosome 22q11.2 has a wide phenotypic spectrum with variable associated anomalies. Familial transmission of this deletion in two-generations has been reported previously. To our knowledge, we report the first three-generation transmission of del(22q11.2) in one family who had unique findings of congenital laryngotracheal webs (CLW) and early onset colorectal carcinoma. The proband, a 53 year-old white female, presented with psychiatric illness and mild mental retardation. As a newborn, she was diagnosed with a ventriculoseptal defect and severe respiratory problems secondary to a CLW. She was diagnosed with adenocarcinoma of the sigmoid colon at 41 years. Her daughter presented with apparent hypertelorism, while her grandson had a CLW, hoarse voice, hypertelorism, ventriculoseptal defect, and anal stenosis. All three individuals had del(22q11.2). CLW are rare defects that may occur alone or in association with other anomalies. Two families with CLW, congenital heart disease, and short stature have been reported. However, analysis for del(22q11.2) was not performed. These findings suggest that CLW may be caused by the deletion. The early onset of colorectal malignancy in our case is of interest. Recently, an unidentified putative tumor suppressor gene involved in colorectal carcinoma has been localized to 22q. The loss of heterozygosity for alleles on certain chromosomes, including 22q, has been identified in nealy 50% of colorectal tumors. Further studies are needed to assess whether individuals with del(22q11.2) are at increased risk for developing this malignancy.

P0545. Trisomy 13/trisomy 21 Mosaicism In A Girl Child

B. Peterlin[1], J. Babnik[2], K. Writzl[1], M. Debevec[1]
[1]Division of Medical Genetics, Department of Obstetrics and Gynecology, UMC Ljubljana; Ljubljana, Slovenia; [2]Department of Obstetrics and Gynecology, UMC Ljubljana; Ljubljana, Slovenia
borut.peterlin@guest.arnes.si

Background; Trisomy 13 is in 90% a lethal condition in the first year of life. The clinical outcome associated with mosaic trisomy 13 is less clear and can range from severe mental retardation and birth defects to normal inteligence. Chromosomal mosaicisms with two autosomal trisomies are exceedingly rare. Double non-disjunction or anaphase lag or non-disjunction and anaphase lag during early embryonic mitotic division could be the cause. Case report; We present a 3-year-old girl, the second child of healthy, non-consanguinal parents. At birth physical examination showed dysmorphic features; low-set dysplastic ears, microphthalmia, slightly upsalnted palpebral fissures, bulbous nose, small mouth and micrognathia. Cardiac ultrasound scan showed atrial septal defect, ventricular septal defect and open ductus Botally. Motor development was slightly retarded. At the age of 3 years her Denver developmental screening test result approached the developmental level deemed appropriate for her chronological age. GTG banded preparations of the patient obtained from cultures of peripheral blood samples showed 47 chromosomes in all the spreads. The patient s karyotype was 47, XX,+13/47,XX,+21. A total of 75 metaphase spreads was analysed out of which 65 cells were 47,XX,+21 and 10 were 47,XX,+13. Conclusions; A girl child with facial dysmorphism, congenital heart disease and mosaicism of two trisomic cell lines, trisomy 13(13%) and trisomy 21(87%) is reported. To our knowledge, trisomy 13/trisomy 21 mosaicism has not been previously reported.

P0546. Genotype-Phenotype Correlation in Croatian Cystic Fibrosis Patients

R. Gjergja[1], I. Barisic[1], S. Hecimovic[2], G. Tanackovic[2], J. Sertic[3], J. Knezevic[2], A. Milic[2], K. Pavelic[2]
[1]University Children s Hospital Zagreb; Zagreb, Croatia; [2]Institute Rudjer Boskovic ; Zagreb, Croatia; [3]University Hospital Rebro ; Zagreb, Croatia
romana.gjergja@zg.hinet.hr

BACKGROUND; Cystic fibrosis (CF) is the most common recessive disorder caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator Gene (CFTR). The distribution of over 800 different CFTR mutations depends on the ethnic and geographic origin. OBJECTIVE; To determine genotype-phenotype correlation in Croatian CF patients in order to establish weather patients with different genotypes showed differences of phenotypic expression in pancreatic and pulmonary disease. METHODS; The clinical diagnosis od CF was made according to the high sweat chloride values,chronic pulmonary disease, pancreatic insufficiency and Pseudomoans colonisation. The molecular analysis of the 16 most common CFTR mutations (according to the data from Cystic Fibrosis Genetic Analysis Consortium) was performed. RESULTS; The analysis of 98 chromosomes revealed the frequency of deltaF508 in 59% of Croatian patients. R117H was present in 4%,G542X in 3%, 1717-1G-A (3%), N1303K (2%), G85E (1%), and R1162X (1%). Further analysis of remaining CF alleles is planned. All deltaF508 homozygous patients had a severe phenotype; an early age at diagnosis, high sweat chloride values, pancreatic insufficiency, similar severity of lung disease. Severity of the clinical course in deltaF508 heterozygous patients depended on another mutation present in CFTR gene; heterozygotes with N1303K, G542X, 17171G-A, R1162X and G85E had severe CF phenotype with pancreas insufficiency, but variable pulmonary disease. The heterozygous patients with R117H mutation revealed mild or severe clinical course with variable pancreatic phenotype but consistent mild pulmonary disease.

P0547. Congenital malformations of the central nervous system in spontaneous abortion

I. Dimofte[1], T. Poalelungi[1], A. Popa[1], D. Balaban[1], L. Enache[2], V. Broasca[1], S. Nedelcovici[1]
[1]Faculty of Medicine; Constanta, Romania; [2]Faculty of Medicine; Iasi, Romania
revarsarea@lycos.com

Sumary; A study of 2620 pregnancies ending in spontaneous abortion revealed a CNS defect in 3.6% of embryos an fetuses, and 3% of all complete conceptuses. The type of malformation observed varied with the gestational age at expulsion, encephaloceles being predominant in earlier specimens, while more typical anencephalus and spina bifida were more common among later abortions. Chromosome abnormalities were found in 40% of abortuses with CNS defects, but were almost entirely confined to those which were still at the embryonic stage of development. 53 % of the later were chromosomally abnormal, which is the same as the proportion found among embryos without a CNC malformation. Using published life-tables of recognised pregnancies it was estimated that the prevalence of anencephalus, spina bifida or related malformation (other than hydrocephalus), without a chromosome anomaly is 5.3 per thousand conceptuses at the begining of the eight week of gestation. By comparing this with the prevalence in total births, it was further estimated that only 24% of these are born alive, with 54% aborting spontaneously and 22% being stillborn.

P0548. A mosaic ring chromosome 4 in a fetus with Wolf-Hirschhorn phenotype - molecular and cytogenetic analysis

A. Kocks[1], *S. Endele*[1], *B. Schr der*[1], *R. Heller*[1], *M. Makrigeorgi-Butera*[2], *C. Staedler*[3], *H. Sch fer*[3], *A. Winterpacht*[1]
[1]*Institute of Human Genetics, University of Hamburg; Hamburg, Germany;* [2]*Department of Neuropathology, University of Hamburg; Hamburg, Germany;* [3]*Institute of Pathology, University of Hamburg; Hamburg, Germany*
endele@uke.uni-hamburg.de

The molecular basis of Wolf-Hirschhorn syndrome (WHS), also deletion 4p syndrome, has not yet been elucidated. However, on the basis of genotype-phenotype correlation studies and through molecular analysis of chromosomal breakpoints on chromosome 4p the critical region has been narrowed to an interval of 165 kb between markers D4S43 and FGFR3. Several candidate genes are being tested for their role in WHS by us and others using cloning techniques and mouse knock-out models. The study of dysmorphological and histopathological features in affected human fetuses with well characterized chromosome 4p deletions is an additional strategy to gain insight into the developmental pathology of WHS.
To this purpose, we present the case of an aborted fetus (17th gestational week) with Wolf-Hirschhorn phenotype; pronounced growth deficiency, complex cardiac defect, leftsided diaphragmatic hernia, aplasia of the gallbladder, and typical craniofacial manifestations (bilateral cleft lip and palate, ocular hypertelorism with broad base of nose, low-set ears). Conventional karyotyping revealed a mosaic ring-chromosome 4 with breakpoints tentatively assigned to r(4)(p16q33). We report on the breakpoint mapping using FISH probes from subtelomeric regions 4p and 4q, probes for the WHS critical region and for 4q33. An attempt was made to correlate the cytogenetic and molecular data with the histopathological findings. We propose that a thorough work up of human aborted fetuses with WHS is an important strategy to complement data obtained from other approaches (including mouse knock-out models) in order to understand the molecular basis of this syndrome.

P0549. Woodhouse-Sakati syndrome; report of a patient

I. Medica[1], *N. Teran*[2], *J. Sep?i?*[3], *S. Risti?*[4], *B. Brajenovi?*[4], *B. Peterlin*[2]
[1]*Outpatient Paediatric Dept; Pula, Croatia;* [2]*Division of Medical Genetics, Dept of Obstetrics and Gynecology, Univ Medical Centre Ljubljana; Ljubljana, Slovenia;* [3]*Dept of Neurology, Univ Medical Centre Rijeka; Rijeka, Croatia;* [4]*Dept of Biology, School of Medicine, Univ Rijeka; Rijeka, Croatia*
igor.medica@pu.tel.hr

Background; Woodhouse-Sakati syndrome is an autosomal recessive disorder characterized by hypogonadism, alopecia, diabetes mellitus, mental retardation and electrocardiographic abnormalities. The combination of endocrine and neuroectodermal abnormalities has been reported as distinctive familial syndromes. The number of variants of the alopecia-mental retardation-hypogonadism syndromes represent a clinical problem in differential diagnosis. Case report; In a 52-year-old woman from a consanguine pedigree we observed mental retardation, short stature, alopecia with absence of eyebrows, hypogonadism, primary amenorrhea and diabetes mellitus. Other manifestations include problems of limited mobility of upper extremities. A deceased cousin also had a similar phenotype. The diagnosis of Turner syndrome and congenital myotonic dystrophy were suggested by referring physicians, however clinical, neurophysiological, cytogenetic analysis and molecular genetic testing for myotonic dystrophy excluded the two syndromes. Conclusion; Comparing the phenotype of our patient to similar cases in the literature, the features suggest that she has Woodhouse-Sakati syndrome. Other manifestations, such as problems of limited mobility of upper extremities, are previously undescribed as symptomatic of the syndrome. The report of this patient with Woodhouse-Sakati syndrome, a rare but distinctive disorder, is to our knowledge the third described in the literature.

P0550. Unravelling Genetic Conditions Associated To Malignant Hyperthermia; A Study Of 25 Families

A.A.P.S. Santa Rosa[1], *R.T. Sudo*[2], *J. C. Cabral de Almeida*[1], *J.C. Llerena*[1]
[1]*Instituto Fernandes Figueira/FIOCRUZ; Rio de Janeiro, Brazil;* [2]*Depto. de Farmacologia - Universidade Federal do Rio de Janeiro; Rio de Janeiro, Brazil*
dandara@msm.com.br

Since 1993 the Malignant Hyperthermia Center of the Department of Pharmacology has received 121 patients to perform the caffeine/halothane test according to the American Protocol (Larach, 1994). 11 patients were sent to evaluation for having clinical/genetic conditions associated to a greater risk for developing malignant hyperthermia (MH). Among the other 110 patients, 32 had presented a suspected MH crisis themselves and 78 had a positive familial history. The familial cases were arranged in 25 families. Among the 66 positive patients, there were 2 with Noonan phenotype, 9 with palpebral ptosis, 1 central core disease, 1 enchondromatosis, 2 with high CPK, and 1 had Kabuki Make-up Syndrome, a previously undescribed association. Upon a thorough clinical review by a clinical geneticist, including complete pedigrees from all families, we found, among the non-syndromic patients on admission, the Kabuki patient, a Noonan family and a probable myotonic dystrophy patient, mother of two affected siblings. We state that clinical geneticists working together with pharmacologists can be of great help to diagnose previously unnoticed genetic conditions in families with known pharmacogenetic diseases like MH and also to select within those families the best subjects for further mutation studies on the RYR1 gene and others involved on the malignant hyperthermia phenotype.

P0551. Craniofacial,orodental and neurological findings in a syndromic case with Hypomelanosis of Ito.

C. F. Salinas, *G. S. Pai*
Medical University of South Carolina; Charleston, SC United States
salinasc@musc.edu

Hypomelanosis of Ito (HI) is a pigmentary dysplasia characterized by linear streaks and swirls of depigmented skin. In many cases of HI, skin depigmentation is the only abnormality, but in 70% of reported HI cases there are other associated anomalies. These include mental retardation, neural, skeletal and ocular anomalies. The status of orodental abnormalities in the syndromic cases of HI has not been adequately described. A 29 year old male followed since he was 10 years of age is presented. He shows a constellation of abnormalities, some of them have not been previously reported. The clinical findings include short stature, asymmetric face and body, right microcornea, bilateral juvenile cataracts, hearing loss, localized alopecia, hypodontia, hypoplastic teeth, retention of primary teeth, migratory glossitis, mal-occlusion, prognathism, prominent chin, and linear streaks and swirls of depigmented skin involving the arms, trunk, and face. Recently, he developed glaucoma of the left eye and his vision has deteriorated rapidly. At 27 years of age he has developed seizures and the MRI shows structural brain anomalies. He is of normal intelligence. Independent chromosome studies of 20 and 100 cells from peripheral blood and of 30 and 100 cells from biopsies of hypomelanotic and normal skin showed a normal 46,XY male karyotype. Cytogentic analysis of cultured corneal cells showed significant karyotypic mosaicism;
45,X[7/30]/46,X,Y,+mar[3/30]/47,XY,+20[4/30]/47,XY,+5[2/30]/46,XY[14/30].
It is no clear if this corneal mosaicism is associated with the observed clinical findings or,if it is a coincidental finding as it has been reported by Pettenati MJ et al., 1996. It should be noted that the lack of mosaicism in the skin biopsy has been reported in about 25% of HI cases. The presence of depigmentation of the skin and the lack of hair follicles in the depigmented skin of the chin sugguests that this is due to genetic alterations expressed in the dermal cells. To our knowledge, this is a new finding that supports the hypothesis of dermal changes for HI. Although a specific syndrome has not yet been recognized for MCA associated with HI, the severity and wide spectrum of the findings in our case suggest the existence of such a syndrome. The natural history of this case demonstrates the late onset of several clinical manifestations, such as seizures, glaucoma, loss of vision, brain anomalies, and the progressive nature of the disorder.

P0552. Joubert Syndrome; Genetic Studies in Families of French-Canadian Ancestry with a Common Founder

A. Badhwar[1,2], *F. Andermann*[3], *S. Mercho*[1], *M. Labuda*[2], *I. Blair*[4], *B. Rosenblatt*[5], *M. Shevell*[5], *P. Chance*[4], *M. Pandolfo*[2], *E. Andermann*[1]
[1]*Neurogenetics Unit, Montreal Neurological Hospital and Institute; Montreal, PQ Canada;* [2]*Centre Hospitalier de l Universit de Montr al, Notre Dame Hospital; Montreal, PQ Canada;* [3]*Department of Neurology and Neurosurgery, Montreal Neurological Hospital and Institute; Montreal, PQ Canada;* [4]*Department of Pediatrics, University of Washington; Seattle, WA United States;* [5]*Montreal Children s Hospital; Montreal, PQ Canada*
abadhw@po-box.mcgill.ca

Joubert syndrome (JS) is an autosomal-recessive disorder involving agenesis or dysgenesis of the cerebellar vermis and brain stem abnormalities. Characteristic clinical features include facial dysmorphia, episodic hyperpnea alternating with apnea, ataxia, early developmental delay, mental retardation, and oculomotor abnormalities. The molecular basis of JS is unknown. Linkage to chromosome 9q34.3 demonstrated in one affected Omani family (Saar et al. Am J Hum Genet 1999;65;1666-1671) was recently excluded in our cohort of 26 North American pedigrees. To date, it appears that the broad phenotypic variability present in JS, combined with suggested genetic heterogeneity, has greatly compromised the genome-wide scan for a gene(s). Establishment of a genetically homogeneous population may help to identify one of the genes for this disorder. In the present study we have identified seven French-Canadian families with JS and carried out a genealogical study. We have constructed a pedigree of 400

people spanning 11 generations, linking the original family described by Joubert et al. in 1969 with two additional families of French-Canadian origin. We were able to establish that all three families trace back 9 generations to the same founder who immigrated to Quebec, Canada from the province of Perche, France in 1634. Based on this information we expect to find all or most of our affected individuals to be homozygous around the disease locus. We genotyped our families with polymorphic markers (D9S164, D9S1818, D9S114, D9S1826, D9S158, and D9S1838) on 9q34.3. Results indicated that they were not linked to the chromosome 9q34.3 locus. This indicates that one, or more major loci for JS are yet to be identified. A genome-wide scan is underway. Clinical heterogeneity is characteristic and broad in JS and evident within Joubert sibships and sporadic cases. Identifying a gene(s) involved in JS would allow conclusive diagnosis using genetic markers, thereby differentiating it from other congenital malformation syndromes of similar phenotype but varied and differing etiology. Characterization of the underlying mutations would lead to genotype-phenotype correlations and the eventual development of therapies.

P0553. Unusually mild tuberous sclerosis in a large French-Canadian kindred

D. M. D Agostino[1], *F. Dubeau*[1], *A. Al-Asmi*[1], *S. Bourgoin*[2], *M. Labuda*[2], *F. Andermann*[1], *D. J. Kwiatkowski*[3], *M. Pandolfo*[2], *E. Andermann*[1]
[1]*Montreal Neurological Institute and Hospital; Montreal, PQ Canada;* [2]*Centre Hospitalier de l Universit de Montr al; Montreal, PQ Canada;* [3]*Brigham and Women s Hospital; Boston, MA United States*
dagoust@hotmail.com

Tuberous sclerosis (TS) is an autosomal dominant disorder characterized by development of hamartomata in a variety of tissues and organs. Intellectual handicap, epilepsy and abnormal behavioral phenotypes derive from hamartomatous brain involvement. Other involved organs are skin, kidneys and heart. The disease is caused by a mutation in either TSC1 (9q34) or TSC2 (16p13.3). The two gene products probably interact as tumor suppressors. One third of TS cases are familial equally represented between TSC1 and TSC2. The majority of sporadic cases are found to have TSC2 mutations. Although the disease phenotype appears to be milder in TSC1 mutations than in TSC2, there is a great deal of overlap. The phenotypic variability between sporadic and familial cases has not been clearly defined. We describe a large 4 generation family from Mont Laurier, Quebec, showing an exceptionally mild form of TS. The proband was ascertained at the Montreal Neurological Institute and a detailed family history obtained. Two field trips were carried out on the extended family to examine the relatives and to collect blood samples for molecular genetic studies. All available family members underwent a structured questionnaire and a thorough clinical examination including fundoscopy and Wood s lamp test. Previous medical information was reviewed. Sixty individuals were evaluated. These included 15/25 living family members with epilepsy. In most cases, this was well controlled by medication and became milder or resolved at older ages. All assessed individuals with epilepsy had skin lesions such as hypopigmented macules (more than three) and/or ash-leaf shaped spots on the limbs and trunk. Additional 12 family members only had skin lesions. No facial angiofibromas or periungual fibromas were noted. Fundoscopy was normal in all. Avilable neuroimaging studies showed no lesion in 7 individuals, subependymal gian cell astrocytoma in one and subependymal nodules in another. This family contributes to define the spectrum of the TS complex. Molecular genetic studies are underway to assess the chromosomal assignment of the phenotype and to perform mutation analysis

P0554. Is Schinzel-Giedion Syndrome a Metabolic Disorder - Investigations in a Newly Diagnosed Patient.

H. M. Bedford[1], *A. Feigenbaum*[1], *B. Ngan*[1], *L. Seargeant*[2], *M. M. Nezarati*[1]
[1]*Hospital for Sick Children, University of Toronto; Toronto, ON Canada;* [2]*Health Sciences Centre; Winnipeg, MB Canada*
melanie.bedford@utoronto.ca

Schinzel-Giedion Syndrome (SGS) is a rare, presumed autosomal recessive condition, characterized by midface hypoplasia, coarse features, as well as multiple skeletal, cardiac and genitourinary abnormalities. Those surviving beyond the newborn period develop seizures, spasticity, and are severely developmentally delayed. An underlying metabolic condition has been hypothesized given the clinical features, in combination with the demonstration by serial magnetic resonance brain imaging of a progressive neurodegenerative process in one patient. Investigations to date have not detected an inborn error of metabolism. We report a male patient with a pattern of malformations consistent with SGS. Features included microcephaly, large anterior fontanelle, splayed sutures, midface hypoplasia, depressed nasal bridge, prominent ear lobules, short neck with redundant nuchal fold, deep-set hyperconvex nails, rocker-bottom feet, small penis and generalized hirsutism. Pelvicaliectasis was present with vesico-ureteral reflux. Skeletal radiographs showed broad ribs and scoliosis. Cranial MRI demonstrated agenesis of the corpus callosum, enlarged ventricles and loss of convolutions. Along with coarsening features, he developed spasticity, progressive contractures and global delay. Seizures developed at 6 weeks, with a markedly abnormal EEG. A metabolic work up was performed, including skin, bone marrow and liver biopsies. The latter demonstrated a dilatation of the cisternae of the rough endoplasmic reticulum with no evidence of storage material. Isoelectric focusing of transferrins was suggestive of an abnormal glycosylation pattern, however the proportions were not typical of that seen in Congenital Disorders of Glycosylation (CDG). Further investigations are underway to elucidate the significance of these results.

P0555. Clinical characteristics in the congenital familial long QT syndrome. The value of genetics in prognosis evaluation

S. Chuprova[1], *M. Shkolnikova*[1], *E. Zaklyazminskaya*[2], *A. Polyakov*[2], *O. Evgrafov*[2]
[1]*1- Federal Center of Children s Arrhythmias; Moscow, Russian Federation;* [2]*Research Center for Medical Genetics; Moscow, Russian Federation*
stver@online.ru

The long QT syndrome (LQTS) is a cardiac disorder characterized by a prolonged QT interval and life-threatening tachyarrhythmias, which may give rise to recurrent syncope or sudden cardiac death. The long QT syndrome is a heterogenic clinical and genetic entity. Five genes have been identified for cardiac potassium (KCNQ1-LQT1, HERG-LQT2, KCNE1-LQT5, KCNE2-LQT6) and sodium (SCN5A-LQT3). Sixty unrelated Russian families (122 patients with Romano -Ward syndrome and 3 patients with Jervell and Lange-Nielsen syndrome) were clinically evaluated using medical histories, ECGs, Holter recordings with the heart rate variability (HRV). An exercise test (treadmill) was performed in 52 long QT syndrome patients. We have tested all the patients for mutation in 4 genes; KCNQ1 (exons 2,6,7), HERG (exons 6,7), KCNE1 and KCNE2 using PCR-SSCP analysis. A single mutation was found in KCNQ1 in 6 families (R174H in one family-2 patients, G314S in two families-7 patients and A341V in three families-7 patients).
Clinical and ECG Characteristic in 6 families with LQTS

	R174H 2 pts	G314S 3 pts	G314S 4 pts	A341V 1pt	A341V 2 pts	A341V 4 pts
Age,yrs	25±21	24±13	24±14	16	24±13	17±16
Sex (M/F),n	0/2	2/1	2/2	0/1	0/2	3/1
Pts with syncope	2 (100%)	2 (67%)	3 (75%)	0	2 (100%)	3 (75%)
sudden death, pts	0	0	0	0	0	1 (25%)
Pts with LCTS	0	0	0	0	1 (50%)	1 (25%)
QTc, ms(lead II)	555±18,4	482±40,0	448±38,2	547	540±11,3	497±23,9
Pts with n QTc	0	1 (33%)	2 (50%)	0	0	0
Pts with syncopeon propr.	0	0	0	0	2(100%)	2 (50%)

LCTS = left cervico-thoracic sympathectomy propr.-propranolol n QTc = QTc <440 ms The phenotype was less severe in G314S mutation than A341V. The phenotype probably varies by specific mutation. Phenotypic heterogeneity is also caused by variable penetrance and expressivity.

P0556. Using OMIM as a decision support system in the diagnostic process of malformation syndromes

F. von Luebken, *J. Kunze, J. Pelz*
Institut fuer Humangenetik; Berlin, Germany
joerg.pelz@charite.de

An increasing number of malformation syndromes makes it difficult for the clinician to reach a diagnosis. OMIM (Online Mendelian Inheritance in Man) was tested as a decision support system for the diagnostic process of patients with malformations. We used the data of 99 patients with different clinically confirmed syndromes and searched in OMIM for differential diagnoses by using the signs of the respective patient in all possible combinations in the search string. Two different search strategies were tested; (1) utilisation of all signs of the patient, (2) usage of those signs, that a clinical expert considered to be important for diagnosis. All 99 syndromes were described in and presented by OMIM. With one sign used for a search the number of differential diagnoses varied from no diagnosis at all to 1512. With strategy (1) 29% of all signs were not used for the description of the respective syndrome in OMIM, while this was 7% with strategy (2). The number of differential diagnosis decreased with the number of signs used in combinations using the AND-junction. By utilising combinations of two

signs the list of differential diagnoses was reduced to an average of 10 for the 1st strategy. Parallel the number of tests yielding no results increased to 21 percent. For the 2nd strategy 3% of the searches using two signs gave no results. OMIM was an acceptable tool in both strategies although it has not been developed as a decision support system in the realm of malformation syndromes. Our results show, that the application of OMIM is sometimes tedious; a consultation of OMIM seems to be useful in the diagnostic process of malformation syndromes, however.

P0557. Angelman syndrome - impact of a genetic disorder on patients and their families

S. Holz, *J. Kunze, J. Pelz*
Institut fuer Humangenetik; Berlin, Germany
joerg.pelz@charite.de

A study was conducted to gather information about the physical, mental and social health of patients with Angelman syndrome (AS) and about their influence on the family life focussing on social aspects. Personal or telephone interviews were conducted using a structured questionnaire. 203 parents of 127 children (60 girls,67 boys) with AS were contacted with the help of the Angelman e.V and participated in the study. Average age of the patients was 9 years (range 3 to 25). - The parents of 93 patients reported a variation of the reaction to pain in their children; for 96 children, especially the older ones a good local orientation was described, which proofed to be much better than had been anticipated due to the mental retardation of the children. - More than 90% of the families disclosed a high impact of the AS children on the family life. In about 70% of all cases at least one of the parents had to stop working or could not work as much as they had liked to do because of the caring for the diseased child. In about half of all cases friendships were lost. 25% of the families were not satisfied with the financial situation, nearly 40% reported problems with the health insurance, about half of all interviewees informed about problems with the administration. All had to change furnishing of their homes to build up a safe environment for their AS child. The care for a chronically ill child deserves high efforts on part of the parents, therefore an urgent demand exists for a better social support of the families caring for an AS child.

P0558. Three Unrelated Cases With 5 Alpha-reductase Deficiency

M. Stefanova, *L. Grozdanova*
Medical University; Plovdiv, Bulgaria
margarit@plovdiv.techno-link.com

Three unrelated cases with 5 alpha-reductase deficiency, a very rare form of male pseudohermaphroditism are reported. By the time of their puberty they appeared girls although their genitalia showed clitoromegaly and their behaviour were estimated as masculine by their parents. All three were identified during puberty, when their sexual development evolved in a male direction. Male body structure and skin hair pattern as well as voice changes and male-like genitalia were formed. Hormonal tests revealed a very low level of dihydrotestosterone with abnormaly high level of follicle-stimulating hormone whereas serum testosterone and luteinizing hormone levels were in a normal male range. Both of the patients have chosen to change their formal gender whereas the third decided not to.

P0559. Gastric Dilatation and Gastric Necrosis - Health Risks to Individuals with Prader-Willi syndrome

A. Schulze[1], *O. Gr¿nne*[2], *A. Vesterby Charles*[3], *H. Ki r*[4], *K. Br¿ndum-Nielsen*[5]
[1]*Odense University Hospital; Odense, Denmark;* [2]*Ringk¿bing Hospital; Ringk¿bing, Denmark;* [3]*Department of Forensic Medicine, Aarhus University; Aarhus, Denmark;* [4]*Department of Pathology, Svendborg Hospital; Svendborg, Denmark;* [5]*Department of Medical Genetics, The John F. Kennedy Institute; Glostrup, Denmark*
ahmschulze@mail.tele.dk

Two individuals with Prader-Willi syndrome (PWS) due to deletion of 15q11-q13 died after a short period of minor symptoms of gastroenteritis. The first case is a 26-year old female who experienced loss of appetite and feelings of discomfort prior to an episode of unexpected vomiting and subsequent collapse. Laparatomy revealed gastric necrosis and rupture. The second case is a 32- year old male who complained of feeling unwell with loss of appetite for a period of 48 hours. He suffered diarrhoea and vomiting and subsequently collapsed. A dilated stomach with necrotic patches were noted at post mortem. Four other cases of PWS (all female) have been reported with symptoms of gastroenteritis associated with gastric rupture and/or gastric necrosis. A diagnosis of PWS was made at the molecular level in each case (Wharton RH et al. Am J Med Genet (1997) 73;437-41). Two of these individuals survived after a subtotal gastrectomy, the other two died. These findings illustrate that minor abdominal symptoms and/or slightly altered behaviour may be indicative of a life threatening disease in PWS individuals. On this basis, we suggest that parents and health professionals inform the Hospital staff nearby about the features of reduced pain perception and of lack of vomiting in PWS. Post mortem investigation and molecular confirmation of diagnosis should be provided. Future work to investigate the physiology of pain perception and gastric function in PWS is recommended.

P0560. Cri du Chat National Association - Report of a Brazilian experience

P. Ramos[1], *M. Ramos*[1], *H. Buchm ller*[2], *R. L. Chaves*[3]
[1]*Associa o Nacional Cri du Chat; Rio de Janeiro, Brazil;* [2]*Funda o Oswaldo Cruz; Rio de Janeiro, Brazil;* [3]*Hospital Universit rio Pedro Ernesto - Universidade Estadual do Rio de Janeiro; Rio de Janeiro, Brazil*
paularam@ig.com.br

Cri du chat (CDC) is a genetic disorder caused by the lost of genetic material of chromosome s 5 short arm (5p-). The syndrome affects 1;50.000 children born and takes place at the moment of conception. The objective of this work is to relate the experience of a group of parents and professionals who joined themselves to create a national association, whose goal is to find as much CDC patients as possible. In three months since the foundation, we have promising results. We have already a home page (www.criduchat.com.br) that has been working as a reference to families and professionals interested in CDC. We have localized 30 patients within Brazil with different ages. All these people are registered and their characteristics filed. Empirically, we can already ascertain that there are too many differences between those older and the younger, due to the investment made in early stimulation. Indeed, among the younger the information plays a special role in the search of the best therapy. We also could observe that the association, with its role of centralizing and propagating information, has been contributing to the recovery in the treatment of the older. As a conclusion, we can say that the formation of such association is crucial to aid the parents, to increase CDC people s quality of life and represents a concrete option to change the history of CDC patients in Brazil.

P0561. Chromosome abnormalities in children with mental retardation associated with dysmorphia

O. Miljanovic[1], *M. Kaludjerovic*[2]
[1]*Instutute for childrens diseases; 81000 Podgorica - Montenegro, Yugoslavia;* [2]*Milmedica; Budva, Yugoslavia*
oljam@cg.yu

About 15% of children with mental retardation (MR) have pathological changes in chromosomes, associated with expressive dysmorphic signs in their phenotype. The aim of study was to assess the frequency of chromosome abnormalities in children with MR and expressive dysmorphic sings. The cytogenetic analyses were done for children with MR and high dysmorphic score (equal or > 7). The Waldrop protocol for the assessment of dysmorphology was used. A sample of 217 children with MR was examined. Chromosome abnormalities were found in 59,5% of children with expressive dysmorphic signs and MR, predominantly in those from the group with severe and deep MR. Only 4,3% of the group with easier forms of MR, had chromosome abnormalities (autosome and gonosome abnosrmalities were found in equal number). In the group with severe and deep MR, all pathological changes were found on autosomal chromosomes (16,5% children from this group). Leading chromosome abnormality was trisomy of 21 chromosome, present in 6,9% children with MR, with reference to 15,2% of children with severe and deep MR. Ethiologic basis of MR in children with expressive dysmorphic signs, are mostly chromosomal abnormalities, specialy in the group with severe and deep MR. Assessment of dysmorphic score by Waldrop protocol is simple, quick and aplicative method useful as a criteria for cytogenetic analyses and revealing of MR ethiology.

P0562. Hypertelorism in clinical diagnosis - an example of pattern recognition in clinical genetics

A. von Schwerin, *J. Kunze, J. Pelz*
Institut fuer Humangenetik; Berlin, Germany
evaparadies2000@yahoo.co.uk

The diagnosis in dysmorphology is to a large extent not in the realm of molecular genetics or cytogenetics. The clinical practice requires experience and observation competence by the practitioners. They are confronted with quantitative morphological variation and clinical signs, which together result in a complex phenotype. There are no simple (mechanical) relations between signs and diagnosis. We examined the components of the visual diagnostic act choosing hypertelorism as an example. The eye distances in the portraits of 20 adults were manipulated with the help of

computer programs. For each face we produced a series of 20 pictures with a systematically increasing eye distance. The variant pictures of all faces were arranged in a randomised order into 4 catalogues. 70 students, 10 advanced students in paediatrics and 12 clinical geneticists had to decide for seven traits whether these seemed to be normal or not. The judgements on traits which were not modified vary for each face unspecificly and to a small degree. Those on the eye distance show an overall characteristic relation to the degree of the modification of the individual face, whereas the judgements on the identical eye distance in different faces are diverse. Additionally we found only a small concurrence between the judgements of the probands and the objective measurements of the eye distance which are used in anthropometrics. By multivariate analysis we showed that different parts of the individual faces influence the judgements to different degrees. In particular the form of the midface is strongly related to the process of making up someones mind about the eye distance. Finally there are no striking differences between the judgements of the different groups of probands. Diagnosis of hypertelorism is a process of pattern recognition. Nevertheless (or just therefore) the clinical judgement of the practitioner works well. All attempts to make diagnosis more intersubjective will have to take these results into account.

P0563. Use of new modified PCR-based methods for rapid differential diagnostics of myotonic dystrophy

***M. Falk**, R. Gaillyova, M. Vojtiskova*
Department of Human Genetics, University Hospital; Brno, Czech Republic
9835@mail.muni.cz

In present time neurology recognized more than 300 neuromuscular disorders. Their fenotypical manifestation is extremly variable and clinical features may overlap among them. So there are a strong interest for developping molecular methods which facilitate unequivocal differentional diagnostics. Recently we have focused on myotonic dystrophy (MD), one of the most frequent neuromuscular disease of adult age. The molecular base of MD is the expansion of CTG trinucleotide repeat in dystrophia myotonica protein kinase gene (DMPK) which is located on the 19q13.3. Clinical picture of MD is very variable including all the spectra of forms, from the very mild old-age form to the very serious and often fatal congenital one. To improve the diagnostics we have introduced and modified PCR methods for precise measurement of fyziological and premutation range allels and for rapid detection of expanded pathological alleles [1]. Reliability and fidelity of three PCR methods were demonstrated on the group of MD-positive control samples provided us by reference laboratories. Using suggested PCR protocols we have shown the allelic frequency of the CTG repeats on the group of MD patients in correlation with control one. In MD patients amplified alleles range from about 100 CTG to more (unlimited) CTG repeats. The 5 CTG allele was presented only in 5%, still much lower than for control individuals (about 40%). Clinically affected subjects were correctly identified by introduced PCR methods and confirmed by Southern blot analysis as recommended [2].

[1] Falk M. et al.; J. Biomol. Struct. and Dyn. 17 (2000), pp. 1161-1162
[2] Mol. Gen. Service Guidelines, CMGS (1997) (UK MG NEQAS SC ed. D. Barton)

P0564. A new approach for a rapid and unequivocal identification of marker chromosomes

***S. Langer**[1], C. Fauth[2], S. Uhrig[2], C. Maierhofer[2], M. Speicher[2]*
[1]Institute of Anthropology and Human Genetics LMU Muenchen; Muenchen, Germany; [2]Institute of Anthropology and Human Genetics; Muenchen, Germany
langer@fish.med.uni-muenchen.de

Supernumerary marker chromosomes are small extra chromosomes (ESACS) that are unidentifiable by cytogenetic banding analyses alone due to their small size and lack of distinct banding pattern. The incidence of marker chromosomes has been estimated in different studies to range between 0.4/1000 and 1.5/1000. Precise identification leads to a better understanding of the origin of these chromosomes and their effect on the phenotype. However, a detailed characterization of ESACs is a diagnostic challenge. The majority of marker chromosomes is derived from acrocentric chromosomes and consists merely of heterochromatin. In these cases, 24-color karyotyping techniques, such as M-FISH, are not helpful as hybridisation to ESACs composed of only heterochromatin would be expected to be completely suppressed, because of the addition of Cot1-DNA. However, a diagnosis based on a missing signal is problematic in cases of a poor hybridization efficiency. Thus, we generated a special hybridization mix, termed acro-mix . We developed a strategy for the simultaneous hybridization of whole chromosome painting probes and repetitive DNA probes. The acro-mix consists of painting probes for chromosomes 13,14,15,21, and 22, of centromere probes for chromosomes 13/21, 14/22 and 15 and a probe for rDNA, all labelled in different color combinations. Until now we analyzed eight marker chromosomes with the acro-mix. Half of them were unidentifiable with M-FISH because there was no staining with whole chromosome painting probes. The advantage of this new hybridization mix is a very rapid and precise identification of ESACS even if the metaphase quality is poor. For a rapid work-up in prenatal diagnosis we propose to use a combination of both M-FISH and the acro-mix, to decipher the composition of an ESAC.

P0565. Study on phacomatoses in St. Petersburg; looking for co-operation

T. A. Ledatscheva
St. Petersburg Centre for Medical Genetics; St. Petersburg, Russian Federation
petergen@peterlink.ru

We have initiated studies on incidence rate and clinical heterogeneity of the syndrome in 5 million population of St. Petersburg. CT, MRT, EEG, and chromosome analysis have been used. The most frequent form was Recklinghausen neurofibromatosis; 94 patients from 71 families. CT with subsequent histology revealed optical nerve gliomas (2 cases), astrocytomas (2 cases), epindimoma of CNS (1 case), acoustic nerve neurinoma (1 case) and some another tumours. Ataxia-teleangiectasia (Louis-Bar) was in 10 patients without family traits of the disease. One of them had a chromosomal aberration (48,XX,+6,+8,t(7;14)(q36;q11). Klippel-Trenaunay-Weber syndrome was revealed in 7 patients (3 of them were from one family). Typical encephalotrigeminal angiomatosis (Sturge-Weber syndrome) was in 22 patients (from 19 families). We observed a family with 5 female patients of pigment incontinence (Bloch-Sulzberger syndrome). The cutaneous changes were localized on lateral surfaces of the trunk and extremities. The disease had X-linked dominant type with lethal effect for the male fetuses. The analysis of the main clinical symptoms showed early manifestations of different forms of phacomatoses, high frequency of family cases, increased risk of the neoplastic processes. The early diagnosis provides an opportune medical genetic family counseling, improves recognaizing of tumours, and follow up of patients. Proposals of joint investigations of clinical heterogeneity and clinico-molecular correlation in the syndrome are warmly welcome.

P0566. Duyggve-Melchior-Clausen Disease and Smith-McCort Dysplasia - Report of five affected children

***L. Neumann**[1], J. Pelz[1], H. Weber[2], E. Fastnacht[2], A. Leenen[3], S. Lyding[4], F. Majewski[5]*
[1]Institut fuer Humangenetik; Berlin, Germany; [2]Zentrum fuer Kinder und Jugendmedizin; Luedenscheid, Germany; [3]Pediatric Radiologie, Charite Campus Virchow; Berlin, Germany; [4]University Children s Hospital; Duesseldorf, Germany; [5]Institute of Human Genetics; Duesseldorf, Germany
luitgard.neumann@charite.de

In 1962 Dyggve, Melchior and Clausen published 3 mentally retarded sibs with a peculiar bone dysplasia resembling Morquio s disease. Smith and McCort, in 1958 were the first to describe patients with this pattern of bone changes and normal intelligence. Both conditions share identical radiological signs Spinal cord compression due to atlantoaxial instability is a serious and preventable complication of both disorders. We provide the first report of a Turkish patient with the rare skeletal dysplasia, Smith-McCort Dysplasia (SMC), and the first report of a Georgian (Russia) sibpair with the related Dyggve-Melchior-Clausen disease (DMC). Early detection in a 14 month old Lebanese girl of DMC with mild skeletal findings is demonstrated. We found some further features in this condition, such as delayed puberty in a 15 year old patient. Anal prolapse, observed for the third time, in one of our patients seems to be a feature associated with the condition. Different degrees of mental retardation within one family were noted. Hyperactive behavior in one patient with DMC is reported here for the second time. Morphological criteria such as phenotype, radiological and electron microscopical findings do not allow differentiation of DMC from SMC. The presence of mental retardation in all cases of DMC, sometimes accompanied by microcephaly, and its absence in all cases of SMC is a discriminating factor. There is no overlap of both conditions in any family of the literature nor in our familial patients. This finding together with the geographic distribution, the uniform autosomal recessive mode of inheritance in DMC and the heterogeneous situation in SMC, leads us to assume that these conditions are separate entities.

P0567. Clinical outcome of BRCA1 and BRCA2 testing

A. Kempe[1], *K. Rhiem*[1], *K. Bosse*[2], *T. Bauknecht*[1], *B. Wappenschmidt*[1], *J. Schmolling*[1], *R. K. Schmutzler*[1]
[1]*University of Bonn, Dep. ObGyn; Bonn, Germany;* [2]*University of Bonn, Inst. of Human Genetics; Bonn, Germany*
andrea.kempe@uni-bonn.de

Introduction; Genetic predisposition is responsible for 5% of all breast cancer cases. Mutation carriers of BRCA1 and BRCA2 (breast cancer susceptibility genes) have a life time risk of 80-90% to develop breast cancer and of 20-40% to develop ovarian cancer. The last 3? years we counseled 615 women from 453 families with a family history of breast and ovarian cancer in our breast cancer clinic. Material and Method; We analyzed the BRCA1 and BRCA2 genes in 119 families by direct sequencing and DHPLC. All individuals were counseled by an gynecologist, psychologist and geneticist. All women were offered a intensive breast and ovarian cancer screening program (self-examination, clinical examination, mammography, MRI, ultrasound of breast, vaginal ultrasound and serum-CA125 starting at age 25). Prophylactic surgery was discussed as an option depending on the individual situation. Results; 569 women participated in the screening program (compliance 92%). We detected 30 breast cancers cases. All incident cases were diagnosed in an early stage of cancer before spread (pTis or pT1, pN0, M0). Four women requested prophylactic bilateral mastectomy and 20 prophylactic oophorectomy. We identified BRCA1 or BRCA2 mutations in 39 families (19 of them being unclassified variants). A predictive genetic testing was performed in 34 unaffected women; 28 women were tested negative (proven noncarriers) and could be reassured. Six women were tested positive (proven carriers). In 80 families BRCA1 or BRCA2 mutations were excluded. Conclusions; Our preliminary results indicate that women from high risk families may benefit from increased surveillance and prophylactic therapy. A special screening program helps to identify early stages of breast cancer. Further studies are needed to prove a reduction of mortality rates. Acknowledgement; This work was supported by the Deutsche Krebshilfe.

P0568. Artificial neural networks and diagnosis of malformation syndromes

G. Graetschel*, J. Pelz*
Institut fuer Humangenetik; Berlin, Germany
evaparadies2000@yahoo.co.uk

Computer programs which can be used as an aid to diagnose multiple congenital malformation syndromes have been used for many years. These programs are based either on algorithms which define a diagnosis by a set of phenotypic components all having the same weight or on algorithms based on a concept of Bayesian statistics. A third and new approach for this field are artificial neuronal networks (ANNs), which are popular as classification mechanisms in medical decision support systems. As a software tool we took the commercially available shell ORKA, which is suitable for building up feedforeward ANNs trained using backpropagation of errors. We used data from 234 patients representing individual examples of 21 different established syndromes. For the description of the cases the numbers of symptoms were limited to 55. After the export of the patient data to ORKA the data of 161 patients were used for the training of the ANN, the remaining 73 patient data-sets were used to test the diagnostic abilities of the different ANNs. A diagnosis was yielded in 96% of all tests. The percentage of correct diagnoses suggested by the ANNs without concurrent differential diagnoses was 23%, taking all tests during which the correct diagnosis was produced among other diagnoses amounted to 68% - only in 11% of all tests the correct diagnosis was calculated with a small probability. The application of ANNs during the diagnostic process of malformation syndromes is promising, also one shortcoming is that different sets of training data produce models with different generalisation accuracies. The value of a ANN is to a great extent influenced by luck, instinct and experience of the developer.

P0569. Shprintzen-goldberg Syndrome Followed Up During 24 Years

Y. Alembik[1], *C. Stoll*[2]
[1]*Medical Genetics; Strasbourg, France;* [2]*H pital de Hautepierre; Strasbourg, France*
Claude.Stoll@chru-strasbourg.fr

Several patients with marfanoid features and cranio-stenosis have been reported. Shprintzen and Goldberg put these features together and named the syndrome which bears their names. Natural history of this syndrome is unknown. We had the opportunity to follow up a patient with this syndrome during 24 years. Laurent was born after a normal pregnancy and a normal delivery. Father (27 years old) and mother (24 years old) were normal and unrelated. At birth weight (3.300 g), height (50 cm) and OFD (34 cm) were normal. Walk was delayed, first words appeared at 3 years. Facial dysmorphia was present at birth ; brachycephaly, flat occiput, ears floppy, large and posteriorly angulated, hypertelorism, ocular proptosis, strabismus, hypoplasia of the middle part of the face, flat palate, pectus excavatum, scoliosis, bilateral dislocation of the radial heads, hammer toes and hallux valgus, pes planus, arachnodactyly, camptodactyly and Steinberg sign. Skin was thin without scars but with numerous sub-cutaneous hematomas. Laurent was operated on twice for bilateral inguinal and crural hernia. He had a mitral valve proplase. CT scan showed ventricular enlargment. In the following years Laurent was operated on for his feet deformities several times. Mental retardation improved dramatically. Puberty was delayed until 18 years. At 24 years of age he was still thin, dolichocephalic, scalp hair were fine and sparse, the facial dysmorphic features were still obvious. Pectus excavatum was still severe and thoracolumbar scoliosis mild. Feet deformities improved, joints were less hypermobile. Psychomotor development is quite normal. His main concern was the minimal subcutaneous fat and fragility of skin. Echocardiography was normal. He is working as a cuniculiculturer. He has a driver licence and he is now engaged. A normal brother and sister were born. Etiology of the Shprintzen — Goldberg syndrome is unknown, all cases were sporadic but the family reported by Ades et al. (1995). No defect in the fibrillin gene was found in this patient.

P0570. Hemoglobin D Los Angeles in a family from Brazil

C. Bonini-Domingos[1], *G. Leoneli*[2], *W. Silva Jr*[3]
[1]*UNESP; S o Jos do Rio Preto, S o Paulo, Brazil;* [2]*UNESP; S o Jos do Rio Preto, SP, Brazil;* [3]*HEMOCENTRO; Ribeir o Preto, SP, Brazil*
bonini@bio.ibilce.unesp.br

Many beta globin gene defects leading to hemoglobinopathies are known. Most of these hemoglobin variants have been identifies at the protein level by alkaline electrophoresis, but the underlying molecular defect has not been determined for many hemoglobinopathies. Advanced molecular genetic techniques offer the possibility of identifying the under lying mutations in these variants. Hemoglobin D - Los Angeles is the most common D - hemoglobin. We have analyzed A family with a beta thalassemia suspect and founded a hemoglobin variant similar to hemoglobin S at alkaline electrophoresis. In the analyses by HPLC on the Bio - Rad variant automated analyzer we found a sample whit retention time similar to hemoglobin D - Los Angeles, confirmed by DNA analysis.

P0571. Differential gene expression analysis in Normal vs. Trisomy 21 cells with identical genetic background.

S. Deutsch[1], *L. Bartoloni*[1], *V. Doucas*[1], *M. Vasquez*[1], *S. Daoun*[1], *D. Bricarelli*[2], *R. Lyle*[1], *S. Antonarakis*[1]
[1]*University of Geneva; Geneva, Switzerland;* [2]*Istituto Gaslini; Genova, Italy*
samuel.deutsch@medecine.unige.ch

Down syndrome (DS), the most common cause of mental retardation, is caused by the presence of three copies of chromosome 21 (HC21). DS individuals suffer a wide range of complex phenotypes most of which are variably expressed and incompletely penetrant. It is not clear how the presence of a third copy of HC21 leads to DS, and the number of genes and pathways actually involved in DS phenotypes is unknown, in spite of the recent determination of the entire sequence of HC21. In order to explore further this question we are studying the global gene expression differences between normal and trisomic cells by macroarray filter hybridisation. There are several problems associated with this type of approach, the most challenging of which is that patients and controls show inherent (polymorphic) variation in gene expression which is unrelated to their disease status. In order to overcome this problem, investigators have traditionally pooled several samples or used inbred animal models to reduce polymorphic variation. In our study we have separated normal vs. trisomic 21 fibroblasts from a mosaic DS patient, having thus isolated cell lines that are 100% (46, XY) and 100% (47, XY, +21) but are otherwise genetically identical. RNA from these cell lines has been hybridised to filters of human cDNAs and gene expression differences are currently being analysed. These results may provide further insight on how this common chromosomal imbalance affects the transcriptome, and which pathways and genes are likely to be involved in the development of DS phenotypes.

P0572. Patient with polymalformation syndrome diagnosed as Smith-Lemli-Opitz syndrome

V. Brysova[1], *D. Valik*[2], *A. Mikuskova*[2], *J. Uteseny*[2]
[1]*Children s hospital Brno; Brno, Czech Republic;* [2]*Children s hospital; Brno, Czech Republic*
vbrys@med.muni.cz

Background; Smith-Lemli-Opitz syndrome is a syndrome of multiple congenital malformations with growth retardation, severe mental retardation, microcephalia, genital anomaly and unfavorable life outcome. Biochemical basis of the syndrome is deficient cholesterol synthesis caused by malfunction of 7-dehydrocholesterol reductase, which was mapped to the chromosome 11q12-q13. This leads to accumulation of a toxic metabolite 7-dehydrocholesterol, which subsequently associates with the family of *sonic hedgehogs* signaling proteins important during embyogenesis.
Case report; the index case described was a full-term boy of physiological pregnancy. Because of perinatal asphyxia he was one week in incubator. Hypotonia and degenerative sings were observed early after birth and his psychomotorical development soon became delayed. A clinical diagnosis of SLO syndrome was made.
Methods; HPTLC was developed for identification of 7-DHC. Serum was deproteinized with acetone, 25 l put of Merck Kieselgel 60 plate and chromatographed twice in hexane+ethylacetate 10+1.5. A blue fluorescent product visible at 360nm of 7-DHC was developed after derivatization with sulphuric acid. Subsequent DNA analysis of mutations in 7-DHCR gene (W151X, V326L, IVS8nt1g-c, L109P and R446Q) was done.
Results and Conclusions; Diagnosis of SLO was established by identification of 7-DHC in serum of the index case by a new HPTLC method and confirmed by DNA analysis with following results; proband V326L/V326L, father V326L, mother V326L At the age of 6 years, patient clinical conditions appear stable. Currently, high-cholesterol therapy is being administered.

P0573. Clinical features of Waardenburg syndrome type I (WSI) in a family with missense mutation in PAX3 gene

T. Markova
ENT and Speech Research Institute; St Petersburg, Russian Federation
hospDA@hospDA.yar.ru

WSI is a well recognizable owing to facial features and pigmentary anomalies of hair, eye and skin. The freguency of sensorineural hearing loss is only 20% of cases.
We have consulted 9-year old girl with unusual appearance for WSI. Girl arrived from the area to the cochlea implantation. She initially showed broad nasal root and congenital profound hearing loss. We have suspected the dystopia canthorum which was confirmed by index W calculation (index W= 2.42). Girl was only with her mother who was healthy. Mother just said that the child looks like her father and his mother who had normal hearing. After detailed clinical examination we have found small depigmented spots on the fingers of girl s right hand. Neither heterochromia irides nor white forelock nor other skin pigmentary changes was found. Proband s DNA sample was tested in Molecular Genetic Laboratory in Manchester (professor A. Read) and a missense mutation in the paired domain in exon 2 of PAX3, G48R, was found. Futher we have had an opportunity to examine proband s father who had only broad nasal root, dystopia canthorum (index W=2.46) and unilateral hearing loss which he had hidden. His family had a history of typical facial features in three generations without hearing loss and pigmentary anomalies. This case confirm the importance of the clinical examination of each family members and DNA-diagnosis in such case.
According this observation and our previous results each family with WSI has individual characteristics with variable expressivity of clinical features, but facial features such as dystopia canthorum, broad nasal root are always appear in WSI patients.

P0574. CHILD syndrome; molecular analysis

K. Grzeschik, *D. Bornholdt, A. Koenig, H. Engel, R. Happle*
Philipps Universitaet Marburg; Marburg, Germany
grzeschi@mailer.uni-marburg.de

CHILD syndrome (Congenital Hemidysplasia with Ichthyosiform nevus and Limb Defects) is an X-linked dominant male-lethal trait characterized by an inflammatory nevus affecting usually one side of the body with strict midline demarcation, associated with ipsilateral hypoplasia of the brain, lung, kidney, heart, limbs, and skeletal structures. Recently, we found CHILD syndrome to be caused by mutations in the gene NSDHL [NAD(P)H steroid dehydrogenase like protein] encoding a putative 3§-hydroxysteroid dehydrogenase that functions in a late step of cholesterol biosynthesis. This human gene maps to Xq28. Two murine X-linked dominant male-lethal traits, bare patches (Bpa) and striated (Str) are associated with mutations in the homologous mouse gene Nsdhl. We describe NSDHL-mutants observed in 16 CHILD patients from 12 families (15 females and one male). None of the changes in NSDHL was detected in 100 control persons. Thus CHILD syndrome is added to the list of developmental defects caused by an enzymatic deficiency in cholesterol biosynthesis. In spite of the fact that the murine Nsdhl mutations affect similar protein regions as the human ones, one mutation is even completely identical, the striking lateralization of the skin defect occurs in humans only. The lateralized hypoplasia might result from the absence of NSDHL function in organizer cells determining one side of the body. We conclude that CHILD syndrome identifies a gene that plays a pivotal role at an early stage of human development when the formation of the primitive streak confers bilateral symmetry on the embryonic disk and left right asymmetry is determined.

P0575. Nonsyndromic childhood hearing loss; mutation analysis of GJB2 and audiological findings

L. Sbaiz, *A. Sedita, A. Gomez, E. Cocco, S. Bosso, L. Leone, G. Restagno*
Molecular Genetics Laboratory, Children s Hospital Regina Margherita; Torino, Italy
restagno@tin.it

Mutations in the gene for connexin 26 (CX26), GJB2, are a major cause of autosomal recessive inherited and apparently sporadic non-syndromic congenital hearing loss (locus DNFB1). The frameshift mutation 35delG is the most represented in Caucasoid populations, with a variable relative frequency. We have analyzed 88 unrelated patients affected by sensorineural deafness with various degrees of hearing loss. Audiological characteristics were investigated and the entire coding region of GJB2 was sequenced. GJB2 mutations were present in 37 (56%) of 66 cases of severe/profound deafness, while three GJB2 mutations were found in 22 patients with moderate deafness (13%). The most common mutation is 35delG, accounting for 66% of all deafness alleles. The mutation V95M and E47X were detected four and two times,with a relative frequency of 5 and 2.5% respectively. Other mutations (L90P, 290-291 insA, 334 delAA, W24X, E119del) were detected once. We also found six allele variants of unknown significance (V37I, F83L, R127H, V153I and the novel alleles Q80P and M162V) all in heterozygosity in deaf patients but also in normal-hearing subjects. While the search of mutations in CX26 is fundamental for genetic counselling, the goal is now to clarify the role of the missense mutations of unknown significance in the pathogenesis of hearing loss. This will allow to appropriate genetic counselling with valuable prognostic and therapeutic informations.

P0576. The Laboratory Diagnosis Of Rare Hemoglobinopathies In Brazil

A. R. Chinelato[1], *M. G. Ginabreda*[2], *C. R. Bonini-Domingos*[3]
[1]*UNESP - Sao Jose do Rio Preto; Sao Jose do Rio Preto, Brazil;* [2]*DLE; Rio de Janeiro, Brazil;* [3]*UNESP; Sao Jose do Rio Preto, Brazil*
archinelato@ig.com.br

The diagnosis of hemoglobinopathies is of growing importance, particulary because of an increasing requirement for antenatal diagnosis of significant disorders of globin chain synthesis. The identification of hemoglobins is often presumptive, based on a characteristic electrophoretic mobility or other characteristics in an individual of appropriate ethnic origin. Brazil is a country with a special ethnic characteristic. The hemoglobinopathies diagnosis has a considerable importance, and reflects racial composition and geographic origin. Scientific, sanitary, social and cultural factors explain a quantitative and qualitative subestimation of the structural variants of hemoglobin. The cellulose acetate electrophoresis is an easy, fast and reproductible method to identify abnormal hemoglobins. We have analyzed blood samples with mobility as hemoglobin S detected on cellulose acetate, by HPLC on the Bio- Rad variant automated analyzer methodology and Isoelectric focusing. Applications of these methods greatly aided in defining the different hemoglobinopathies. like hemoglobin Korle-Bu, G-Philadelphia and D- Los Angeles. Accurate identification of hemoglobin variants is important not only to understand the clinical course, but also, in some cases for proper clinical management, especially in those cases involving hemoglobin S. The determination of rare variants may also assist in family studies or genetic counseling.

P0577. Elevate Fetal Hemoglobin In Blood Donors.

P. J. A. Zamaro[1], *W. A. Silva-Jr*[2], *C. R. Bonini-Domingos*[3]
[1]*UNESP- S o Jos do Rio Preto; S o Jos do Rio Preto, Brazil;* [2]*Hemocentro; Ribeirao Preto, Brazil;* [3]*UNESP; S o Jos do Rio Preto, Brazil*
Paula.z@lycos.com

Fetal hemoglobin is produced in a sub population of erythrocytes termed F-Cells and is the predominant hemoglobin in fetal life and early infancy.

During the first year of life, fetal hemoglobin is gradually replaced by hemoglobin A, and only small amounts of fetal hemoglobin can be found in adult life; fetal hemoglobins levels were shown to be increasing in some subjects with hereditary persistence of fetal hemoglobin and beta thalassemia. We found blood donors whit high levels of fetal hemoglobin on cellulose acetate electrophoresis screening and confirmed by the alkali denaturation method and HPLC on the Bio- Rad variant automated analyzer. The family studies and DNA analysis will be made, to evaluate which molecular mechanism are envolved in these cases.

P0578. Y chromosome microdeletions and cystic fibrosis mutations in infertile men

***R. Gaillyov** , J. Kadlecov , B. Rav ukov , I. Val skov , J. Sojkov , I. Crha*
University Hospital; Brno, Czech Republic
gaillyova@fnbrno.cz

Our collaborative study with the Centre of Assistant Reproduction starting from the 1998 year was focused on the group of 159 infertile men as candidates for ICSI and IVF programme. Cytogenetic examinations have detected chromosomal abnormalities in 16 infertile men. Chromosomal abnormalities have represented 10% of pathological cytogenetic findings. Recently, the number of reports documented the prevalence of Yq microdeletions in infertile men. We have examined the group of 143 infertile men with karyotype 46,XY on the microdeletion DNA test in locus Yq11,23 for DAZ (deleted in azoospermia) gene and found microdeletions in specific fragments sY277, sY283 (AZFc region) with prevalence of 18% in 44 azoospermic or severe oligospermic series patients from our tested group. Recently DNA analyse of 12 cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations in group 159 of infertile men demonstrated genotype ˇF508/nonˇF508 in 6 patients (4 were included in the azoospermic series). Also it was analysed a DNA variant (the 5T alele) in a noncoding region of intron 8 CFTR gene that caused reduced levels of the normal CFTR protein. It has detected the 13 men carrying 5T allelic variant, one man was homozygote for genotype 5T/5T, two men with genotype ˇF508/5T in relation to the congenital absence of the vas deferens. Our results of the complex screening programme in infertile men from the Moravia region demonstrate in 27% causes that chromosomal and gene disorders make a significant contribution to spermatogenic imparment.

P0579. Haemophilia; Assessment of the carrier status in Indian females by pedigree analyses.

***M. Singh**, P. P. Singh, H. Kaur*
Department of Human Biology, Punjabi University; Patiala, India
s_puneet@angelfire.com

Eighty five families of haemophiliac patients of India (Haemophilia A=75, Haemophilia B=10) were examined to assess the carrier status of the females in this pedigree dataset. The probability values of the females for being carrier were calculated by the Bayesian method. It was observed that 45 mothers were proven carriers and 40 probable .The transmission was of familial nature in 45 families with 37 families having well established history of the disorder whereas, in the remaining 8 the trait showed up in propositus and his brothers i.e. only in one generation. The remaining 40 were categorised as isolated cases where the propositus was the only haemophiliac affected. On the basis of type of mutation, 22 cases were observed to be due to de novo mutations in haemophiliac and the rest 18 were the cases where there was an equal chance of mutation taking place either in the mother or in the propositus itself.The probability values of 425 females in 37 familial cases were calculated and a wide range of probability values fluctuating from 0.0295 to 1 was observed.

P0580. Early radiologic attributes of metaphysaric chondrodysplasia

Y. Ignatyev
Omsk State Medical Academy; Omsk, Russian Federation
rozhdestvensky@omsknet.ru

Late diagnostics of hereditary diseases of a skeleton results in delayed and wrong treatment and is the reason for invalidized complications.
The roentgenograms of the lower extremities of 22 children 2 and 3 years old are examined with the purpose of revealing early radiology differential attributes of the metaphysaric chondrodysplasia of the Shmid type. Roentgenogrammetric parameters of were offered for the objectivization of the research data; a cervicodiaphysial index (the relation of a neck diameter of a femoral bone to of a diaphysis diameter of a bone), a metaphysaric index (relation of width distal metaphysis of a femoral bone to a diameter of its diaphysis). The cervicodiaphysial index on the average was equaled to 1,38–0,035 and authentically was not differed from that parameter of a control group. The metaphysaric index was on the average 3,37–0,079 and was authentically differed from a control group (3,15–0,019; t-5,23; p<0,01). The height of a distalsprout zone of a femoral bone from the internal and outside parties was accordingly 6,62–0,38 and 3,32–0,21. The varus deformation of the lower extremities changed from 150 up to 162 degrees. 12 men had the structure of necks changed, and 17 patients had the structure of internal departments of distal metaphyses of a femoral bone changed.
Thus, the characteristic picture of the metaphysaric chondrodysplasia of the Shmid type was characterized with the varus deformation of the lower extremities, change of structure of necks and distal metaphyses of a femoral bone, presence of the metaphysaric index of a femoral bone within the limits of 3,37–0,079 and almost double excess of height of a sprout zone from the inside in the area of the distal end of a femoral bone.

P0581. Machado-Joseph Disease in the Azores Islands (Portugal). Clinical features and CAG repeat length

***T. Kay**[1], M. Lima[2], J. Vasconcelos[1], C. Silva[2], J. Santos[1]*
[1]Hospital of Divino Espirito Santo; Ponta Delgada, Portugal; [2]University of the Azores; Ponta Delgada, Portugal
neurazor@mail.telepac.pt

Machado-Joseph disease (MJD) is a neurodegerative disorder associated with a CAG repeat expansion in a gene or chromosome 14q32.1. A recent survey of MJD in the 9 islands of the Azores (Portugal) identified 103 cases, which represents a prevalence of 1 in 2309 individuals. In the Azorean island of Flores, the disease has its highest world-wide prevalence (1 in 103 people affected). To evaluate the clinical characteristics, onset and disease duration with reference to CAG repeat length, we performed a detailed clinical analysis of 20 Azorean patients. The age of onset was inversely correlated with the repeat length, confirming previous findings. Repeat length in the expanded allele did not overlap that of normal allele. The relationship between the number of CAG repeat units and several clinical finding was extensively studied. The influence of the normal allele on disease presentation was also analysed. The results obtained further document the importance of the repeat length in the phenotypic variation of MJD.

P0582. A novel mutation in the EDAR gene in an autosomal form of anhydrotic ectodermal dysplasia

***S. A. Wisniewski**, K. Kobielak, A. Kobielak, W. H. Trzeciak*
University of Medical Sciences; Poznan, Poland
whatsupdoc@go2.pl

The most common variant of ectodermal dysplasia is its anhydrotic form (EDA) mostly caused by mutations in two genes; EDA gene, localised on the X chromosome, and EDAR gene localised on chromosome 2. The affected individuals exhibit symptoms of anodontia or oligodontia, deficiency of sweat glands and hypotrichosis. EDA is caused by improper interactions between the cells of ectodermal and mesenchymal origin during embryogenesis. This interaction depends on protein products of EDA and EDAR genes, belonging to TNF family of ligands and receptors respectively. DNA was isolated from peripheral blood lymphocytes of 16 individuals. Fragments of DNA corresponding to exons 1 through 9 of EDA gene and exons 1 through 12 of EDAR gene were amplified by PCR using specific primers. The fragments that exhibited aberrant banding patterns in SSCP analysis were sequenced using an automated DNA sequencer. In 5 patients mutations in the EDA gene were detected. In one of the remaining patients sequence analysis demonstrated a novel T1109C transition in exon 12 of EDAR gene, resulting in Val370Ala substitution in the death domain of EDAR protein. However, in 10 affected individuals sequence analysis demonstrated no mutations in both EDA and EDAR genes. It was postulated that about 30% of the cases of anhydrotic ectodermal dysplasia results form mutations in the gene encoding the ligand (ectodysplasin A), and the receptor (EDAR). The remaining 70% of the cases were probably due to mutations of the other recently described genes; XEDAR and NFkB essential modulator (NEMO). Supported by KBN grant 4PO5A05417

P0583. First Molecular Analysis of a Family with Spinocerebellar ataxia type 7 in Iran

***S. Khazaeli**[1], A. Lofgren[2], M. A. Molavi[3], Z. Noorparast[1], K. Karhrizi[3], Y. Shafeghati[3], C. Van Broeckhoren[2], H. Najmabadi[3]*
[1]Rasht Genetic Counseling Center, East; Tehran, Islamic Republic of Iran [2]Dept of Biochemistry, University of Antwerp; Antwerp, Belgium; [3]University of Welfare and Rehabilitation; Tehran, Islamic Republic of Iran
maryamneishabury@yahoo.co.uk

The dominant autosomal ataxias are a group of neurodegenerative disorders caused by expanssion of a CAG triplet repeates in different genes. Spinocerebellar ataxia type 7(SCA7) is a degenerative disorder which affects the retina. The trinucleotide repeates in normal individual ranges

from 7 to 16, in affected persons the repeats can exceed over three hundred. Here we are reporting the first family of SCA7 in Iran. Their mother has mild syptoms including mild ataxia with bilateral macular degenerative changes and abnormal cerebellar atrophy in MRI. Her three children are affected, one of them is a 39 year old man who has PMD (pigmentary macular dystrophy) with cerebellar ataxia and his children died due to rapid fatal course of early onset disease. Another is a 37 year old man who has cerebellar ataxia and bilateral optic atrophy with blindness. The last one is a 25 year old woman who has PMD and cerebellar ataxia. The others are normal. Eight members of the family were analysed for the repeats. 6 out 8 had the expanded repeats ranges from 40 to 50 in one of the alleles. 2 members of the family had normal range of repeats for both alleles.

P0584. Mutation analysis in families with X-linked adrenoleukodystrophy (X-ALD) and ornithinkarbamoyltrasferase (OTC) deficiency

L. Dvorakova, *G. Storkanova, R. Dobrovolny, M. Hrebicek, S. Kmoch, E. Hruba, E. Kostalova, J. Zeman, M. Elleder*
Institute of Inherited Metabolic Disorders, , 1stFaculty of Medicine and General Faculty Hospital; Prague, Czech Republic
ldvor@lf1.cuni.cz

In families with gonosomal recessive disorders a reliable detection of carriers is crucial. Mutation analysis is widely accepted to be a most effective diagnostic approach for identification of heterozygotes. We have set up methods for mutation analysis of X-ALD and OTC deficiency. As the mutations are usually private, we start the analysis by direct sequencing of PCR or RT/PCR products amplified from probands gDNA or cDNA. Subsequently, family members are genotyped by specific PCR-RFLP assays. X-ALD is characterized by impaired peroxisomal very long chain fatty acids beta-oxidation. We have analysed 12 X-ALD patients from 11 unrelated families and found eight novel mutations in ALD gene. In one family mutation analysiswas used for prenatal diagnosis. OTC deficiency is the most common inborn disorder of the urea cycle. In three patients with neonatal form of OTC deficiency we have identified two novel nonsense mutations and one published missense mutation. Mutation R277W, which was reported previously, was found in four unrelated patients with late onset form of OTC deficiency. We screened 55 family members for the mutations found in probands and identified three presymptomatic boys and 24 heterozygotes. In three cases the mutations found in probands were not detected in DNA isolated from leukocytes of their mothers - obligatory heterozygotes. In the case of these mothers we strongly recommend genetic counseling and prenatal diagnosis in future pregnancies as the discrepancies observed, cannot be explained only by mutation de novo but, more likely, by gonadal mosaicism .

P0585. Effects of cytoplasmic DNA on the men infertility

M. Houshmand[1,2], *F. Sharifpanah*[1,3], *A. Saremi*[2]
[1]*National Research Centre for Genetic Engineering and Biotechnology; Tehran, Islamic Republic of Iran;* [2]*Sarem Medical Centre; Tehran, Islamic Republic of Iran;* [3]*Islamic Azad University Research and Science Branch; Tehran, Islamic Republic of Iran*
massoudh@nrcgeb.ac.ir

Mitochondrion is one of the intracytoplasmic organells that it is called as cell energy generator. Mitochondrion contains circular double-stranded DNA (mtDNA) with 16,569 bp in length. mtDNA is very condense and it contains 37 genes (2 genes for mt-rRNA and 22 genes for mt-tRNA and 13 genes encoding oxidative phosphorylation proteins, that need for most important biochemical role of mitochondrion). Every mitochondrion approximatly has 2-10 copies of mtDNA. There is 100 to 1000 mitochondria in every cell, depending on to cell function. Sperm contains between 70 to 100 mitochondria which take plase in midpiece region. Mitochondria are major source of energy production for mobility of sperm. By reason of mitochondrial sensitivity role for ATP production and extremely mobility and fertility of sperm, every defect on mtDNA can cause diseases such as Neurospermia and Asthenozoospermia. To date, has recognized different deletions in the mtDNA such as 7599 bp and 7345 bp and 4977 bp deletions that later deletion indicate most frequencies in the low-mobility and immobility of sperm and infertility in men. The purpose of this study are detection of deletions in the mtDNA of sperm and correlation between deletions and infertility in man. Sperm semens was used for DNA and Multiple PCR method was used to extraction detect deletions in infertile men as well as healthy controls. 5 Kb deletion was found in one infertile man. 5 Kb deletion (common deletion) was reported in aging, age of patients is important to detect this deletion.

P0586. Congenital leukaemia or transient myeloproliferative disease (TMD) in a neonate with mosaicism for trisomy 21 ; a case report

C. Walczak[1], *I. M ller-Hansen*[2], *P. Lang*[2], *P. Schlegel*[2], *H. Kendziorra*[3], *H. Enders*[1], *A. Dufke*[1]
[1]*Department of Medical Genetics; Tuebingen, Germany;* [2]*University Children s Hospital; Tuebingen, Germany;* [3]*Institute of Pathology; Tuebingen, Germany*
andreas.dufke@uni-tuebingen.de

The prognosis of congenital leukaemia is poor even with chemotherapeutical treatment. Transient myeloproliferative disease (TMD) is characterised by spontaneous remission with complete clinical recovery. TMD is often associated with Down syndrome or a trisomy 21 cell line. At the initial stage TMD is clinically indistinguishable from congenital leukaemia. We report on a girl born at 30 weeks gestation to a 36-year-old mother. Caesarean section was performed because of fetal distress and pre-eclampsia. The neonate presented with hydrops, anaemia and a high white blood cell count consisting mainly of CD34+/CD56+ blasts. Without the use of chemotherapy complete clinical recovery was observed within the first few months of life. Chromosome analysis of cultivated peripheral blood lymphocytes revealed 80% of cells with trisomy 21. The ratio of trisomic cells in peripheral blood decreased as the frequency of blasts went down. Retrospective analysis on archived tissue sections from placenta, fetal cord and fetal membrane respectively, showed disomy for chromosome 21 in connective tissue with infiltration of trisomy 21 cells. Cytogenetic and molecularcytogenetic investigation of cultivated skin fibroblasts revealed a small proportion of trisomy 21 cells, confirming constitutional mosaicism for trisomy 21. The girl shows no phenotypical symptoms of Down syndrome. Our case supports the reserved use of chemotherapeutical agents in congenital leukaemia with the presence of a constitutional trisomy 21 cell line despite the lack of phenotypic evidence for Down syndrome. We discuss our case with respect to comparable cases in the literature.

P0587. Autosomal Dominant Interstitial Pulmonary Fibrosis

E. G. Lemire[1], *R. Chibbar*[2], *K. Ramlall*[1], *R. P. Skomro*[3], *E. Cutz*[4]
[1]*Dept. of Pediatrics, University of Saskatchewan; Saskatoon, SK Canada;* [2]*Dept. of Pathology, University of Saskatchewan; Saskatoon, SK Canada;* [3]*Dept. of Medicine, University of Saskatchewan; Saskatoon, SK Canada;* [4]*Division of Pathology, The Hospital for Sick Children; Toronto, ON Canada*
lemiree@sdh.sk.ca

Familial cases of interstitial pulmonary fibrosis (IPF) were first described in the article by Donohue et al. (Pediatrics 24;(1959);786-813). They suggested that there was a genetic basis to familial IPF and proposed an autosomal dominant mode of inheritance with reduced penetrance. We have identified three descendants with pulmonary disease from one of the original IPF families reported by Donohue et al., (1959). The proband is a 3-year-old female who presented at age 17 months with failure to thrive, chronic respiratory symptoms and hypoxemia. A lung biopsy revealed a picture of cellular/desquamative interstitial pneumonitis. Her younger brother also presented in infancy with persistent respiratory distress. A lung biopsy taken from him showed similar histological findings to his sister. The children have a 45-year-old maternal great uncle with chronic interstitial lung disease secondary to presumed IPF. This individual has recently undergone a lung transplant and the pathological findings are pending. The family history was extensively reviewed and has uncovered 8 additional confirmed or presumed cases of IPF affecting descendants in four different generations. The pattern of inheritance is consistent with autosomal dominant inheritance with variable expressivity. A second autosomal dominant IPF family has been identified through a search of the hospital records. The pedigrees on these two families will be presented and the pathological findings will be reviewed and compared.

P0588. Autosomal dominant porencephaly with cerebral palsy

B. G. Kousseff
University Of South Florida; Tampa, FL United States
bkoussef@hsc.usf.edu

As many as 60% of the children with cerebral palsy (CP) do not have a documented pre-. peri- or neonatal cause. We report a three-generation family with CP (see pedigree). The proband III-3 was born by uneventful vertex delivery with a birth weight of 3010g, length 51 cm and head circumference 32 cm. Episodes of apnea and reduced right extremities movements led to brain CT and MRI. They showed asymmetric lateral ventricles due to porencephaly of the left periatrial area. EEG was normal. III-1 was diagnosed as having right spastic hemiparesis at age three months. CT showed similar porencephaly. Subsequently, mild microcephaly devel-

oped and at age 5 years, she has limited speech. Her chromosomes were normal 46, XX. I-1 had right spastic hemiparesis diagnosed during infancy. He is of normal intelligence and his MRI showed left porencephaly, compromising the frontoparietal white matter. This pedigree suggests a hereditary developmental porencephaly due to faulty neuron migration and leading to CP. The trait appears to be autosomal dominant with reduced penetrance; II-1 and II-2 are asymptomatic with pending MRI. There are a few reports of similar families. Seizures and bilateral porencephaly were additional manifestations in some of the affected individuals. By adding this family to the reports, a trait of proencephaly with CP is established.

P0589. Differential radiodiagnosis of rachitislike deseases of children

Y. Ignatyev, *T. Mulik*
Omsk State Medical Academy; Omsk, Russian Federation
rozhdestvensky@omsknet.ru

The analysis of an X-ray research of bones of 152 children aged from 2 to 16 years with rachitislike diseases; a phosphatic diabetes (84 men), a renal tubular acidosis (36), an illness of DeTony-Debre-Franckony (25), a vitamin D-dependent rachitis (7) was done.
All patients were divided into three groups depending on the hardness of disease display. There were 101 (66,4 %) child with moderately expressed displays in the first group, 4 patients (27 %) with the average hardness of display in the second group and 10 men (16,6 %) with serious changes were referred to the third group.
The varus deformation of the lower extremities, osteoporosis of the hypertrophic type, advancing of an osteal age on 2-3 years were defined in the first and second groups of the patients with the phosphatic diabetes.
Patients from all three groups with the renal tubular acidosis were distinguished by valgus deformation of the lower extremities, lagging of the osteal age and atrophic osteoporosis.
The first and the second groups of children with the illness of DeTony-Debre-Franckony were characterised with valgus deformation of the lower extremities, atrophic osteoporosis and moderate lagging of the osteal age. The resorption of a cortical layer of a diaphysis of tubular bones was determined in the third group side by side the expressed osteoporosis.
In the first and the second groups of patients with the vitamin D-dependent rachitis were defined varus deformations, hypertrophic osteoporosis, conforming of osteal age to Passport age, and there was a tendency to lagging of the osteal age in the third group.
Thus, the realization of differential diagnostics is possible at mild and average hardness of displays of rachitislike diseases. At a serious degree of disease, authentic differential radiologic attributes of various forms of rachitislike diseases are absent.

P0590. A Study on the Clinical Variation of Bilateral Childhood Cataracts in South India

S. T. Santhiya[1], *D. Rawlley*[1], *S. Manohar*[1], *A. Ramesh*[1], *P. Vijayalakshmi*[2], *P. Namperumalsamy*[2], *J. Graw*[3], *P. M. Gopinath*[1], *J. Loster*[3]
[1]*Dr. ALMPGIBMS, Univ. of Madras; Chennai, India;* [2]*Aravind Eye Hospital and PG Inst. of Ophthalmology; Madurai, India;* [3]*Institut f r S ugetiergenetik, GSF; Neuherberg, Germany*
drawlley@yahoo.com

A total of 3244 children of age group 0-15 yrs presenting severe visual impairment or blindness were registered at Aravind Eye Hospital, S. India during November, 1995 through July, 1996. Of these, Bilateral Childhood Cataracts (BCCs) accounted for 19% (611/3244) representing nonsyndromic (N=468) and syndromic (N=143) cases. Of the eleven phenotypes registered, lamellar, total and nuclear were the most frequently encountered clinical entities. Age at appearance amongst non-syndromic BCCs were mostly developmental betweeen the age group 1-15 yrs, while it was congenital/infantile (0-1 yr) in phenotypes such as total, nuclear and anterior polar BCCs. Lamellar and posterior polar were most often developmental. About 70% (426/611) of the BCCs reveal a probable genetic basis and in 28% (171/611) the aetiology was undetermined. Fifty two percent of the syndromic BCCs were associated with ocular anomalies with microcornea being the most frequent anomaly followed by microphthalmos and buphthalmos/glaucoma. Among nonocular associations delayed milestones/mental retardation was most common. Both ocular and nonocular malformations were present in about 26% of the BCC probands. Few of the syndromal cases include those of Marfan (N=6), Down (N=1), Hallermann-Strieff (N=1) and Bardet Biedl (N=1) syndromes. Thus the clinical profile of the BCC phenotypes documents clinical variation and the under lying genetic heterogeneity in this ethnic group.

P0591. Heteroplasmy for the A1555G mutation in mitochondrial DNA in Spanish families with non-syndromic sensorineural hearing loss

I. del Castillo[1], *M. Villamar*[1], *M. Moreno-Pelayo*[1], *L. Romero*[1], *C. Mart n*[1], *B. Arellano*[2], *R. Ram rez-Camacho*[2], *C. Morales*[3], *J. Solanellas*[4], *F. Moreno*[1]
[1]*Hospital Ram n y Cajal; Madrid, Spain;* [2]*Hospital Puerta de Hierro; Madrid, Spain;* [3]*Hospital Sierrallana; Torrelavega, Spain;* [4]*Hospital de Valme; Sevilla, Spain*
idelcastillo@hrc.insalud.es

Several mutations in the human mitochondrial genome result in non-syndromic sensorineural hearing loss. Among them, the A1555G mutation in the gene encoding the mitochondrial 12S ribosomal RNA confers susceptibility to the ototoxicity of aminoglycoside antibiotics, but it also causes non-syndromic hearing impairment in absence of exposure to these antibiotics. In this work, we have found the A1555G mutation in 64 out of 345 families with non-syndromic hearing loss, so confirming the high frequency of this mutation in the Spanish population. In most of the cases, the mutation was found in homoplasmy, but in five families heteroplasmy was detected. The percentage of mutant copies was determined in a total of 16 heteroplasmic individuals, and it ranges from 4% to 97%. In 14 of these individuals the hearing level was normal for middle frequencies (500 to 2000 Hz), but a significant loss in high frequencies (4000 and 8000 Hz) was observed in individuals with a percentage of mutant copies higher than 70%. In some but not all of the families the percentage of mutant copies correlates with the severity of the hearing loss. Our results indicate that other factors (age and genetic background, i.e. putative nuclear modifier genes) may also modulate the phenotype associated to the A1555G mutation.

P0592. Clinical benefits of identification of a novel mitochondrial DNA mutation in a patient with focal cryptogenic epilepsy.

M. Diegoli[1], *I. Sartori*[2], *M. Grasso*[3], *A. Pilotto*[3], *E. Porcu*[3], *L. Pillitteri*[3], *M. Versino*[2], *A. Tartara*[2], *E. Arbustini*[3]
[1]*Institute of Pathology, University of Pavia; Pavia, Italy;* [2]*IRCCS Institute of Neurology, C.Mondino; Pavia, Italy;* [3]*Cardiovascular Pathology, Molecular Diagnostic and Research Transplantation Laboratories, IRCCS Policlinico San Matteo; Pavia, Italy*
diagnostica.molecolare@smatteo.pv.it

A 58 year old female comes to our attention for focal cryptogenic epilepsy. She was treated with valproic acid and during this therapy she developed mental slowing and stroke-like episodes, characterized by ataxia, nystagmus and cephalea; an increase of rest lactacidemia was recorded. She was addressed to the molecular analysis with the clinical hypothesis of a Melas syndrome. We identify a novel heteroplasmic mutation C8393T in ND5 gene (CCC->TCC; Pro->Ser). Heteroplasmia was assessed with RFLP Ava II enzyme on DNA from blood and skeletal muscle; the quantitative densitometric evaluation showed 85% and 95% mutant in blood and muscle DNA respectively. Three unaffected siblings carried the mutation, tested in blood DNA. The mutation was absent in 150 consecutive normal controls and present in one of 160 patients screened for dilated cardiomyopathy. One homoplasmic polymorphism (G1719A in 16s rRNA) was also identified in the same patient. Given that the mutation is heteroplasmic, absent from normal controls, only found in 1 disease control, and the amount of mutant segregates with affected tissue, we suspected that this mutation is likely to play some pathogenetic role in the disease. After the identification of the mutation, the patient withdrew valproic acid with an immediate and persistent (6 months) clinical benefit.

P0593. Folate pathway gene alteration in patients with leber optic nerve syndrome (LHON)

S. Aleyasin, *M. Ghazanfari*
National Research Center for Genetic Engineering and Biotechnology; Tehran, Islamic Republic of Iran
sogand@nrcgeb.ac.ir

Leber optic atrophy is due to atrophy of the retinal ganglion cell and nerve fiber layers as well as of the optic nerve. The penetrance of the disease is estimated to be 20% for men and 4% for women. Many characteristic features of LHON, such as the long latency to onset, the acute nature of the vission loss and clinical involment confined to the optic nerve are not explained adequately. The effect of common mutation (677 C to T) of the methylenetetahydrofolate reductase (MTHFR) gene have been studied in five unrelated leber patients live in different regions in IRAN. The pathogenicity of any mtDNA mutaions have been fairly known but a definitive cause and effect relationship between an mtDNA mutation and a clinical phenotype can be difficult to establish. Different mutations can be associated with the same phenotype, the same mutation can be associated with

different phenotypes. Both LHON disease and folic acid methabolism problem can be involved in common neurodegenrative symptomes such as Alzhimer disease but the relationship of mtDNA disease such as LHON and MTHFR mutation has not been studied before. This study is uniqe in studing this mutation amoung LHON patients and showing the pervalence of 100% of heterozyous mutation of 677 C to T substitution in five studied LHON patients. The mechanisms underlying this phenomenon in these patients are at the present time not very well known. However, these observations may be useful for study of LHON pathogenesis.

P0594. Fetal cells in maternal blood as a screening test for fetal aneuploidies

A. Mavrou[1], *A. Kolialexi*[2], *A. Antsaklis*[3], *A. Koratzis*[4], *C. Metaxotou*[5]
[1]*Medical Genetics Athens University School of Medicine; Athens, Greece;* [2]*Medical Genetics, Athens University, School of Medicine; Athens, Greece;* [3]*Ist Dep of Obstetrics & Gynecology, Athens University; Athens, Greece;* [4]*Dep. of Obstetrics & Gynecology,Patras Universitya; Patras, Greece;* [5]*Medical Genetics, Athens University school of Medicine; Athens, Greece*
ariamav@hol.gr

Anti ? hemoglobin chain antibody has been used to detect fetal nucleated red blood cells (NRBCs) entering maternal circulation during pregnancy. In a previous study we demonstrated, however, that women carriers of ?-thalassemia produce themselves during pregnancy an increased number of ?+ NRBC making it difficult to distinguish between fetal and maternal NRBCs. Use of Ab against embryonic hemoglobin ? may increase specificity for fetal NRBCs. In the present study NRBCs were isolated by MACS from 20ml peripheral blood of 50 pregnant women carriers of ? thalassemia trait, 27 in the first and 23 in the second trimester of pregnancy. NRBCs were next identified immunocytochemically using anti ? or anti ? monoclonal antibodies (MoAb). FISH was performed in 22 cases known to carry male fetuses with X/Y chromosome specific probes. The mean number of NRBCs isolated with anti ? was 51 (range 14-158) in the 1st trimester and 52 (range 22-153) in the 2nd. Using anti ? an average of 12 (range 3-23) NRBC were identfied in the 1st and 5 (range 3-10) in the 2nd trimester of pregnancy. When anti ? was used all cells identified were XY. In contrast when anti ? was used FISH identified both XX and XY NRBCs. Our results demonstrate that since anti ?MoAb shows increased specificity for fetal NRBC it should be preferentially used in the first trimester of pregnancy to imrove prenatal diagnosis from fetal cells isolated from maternal circulation and facilitate distinction between fetal and maternal NRBCs, especially in women carriers of ? thalassemia.

P0595. Detection of fetal apoptotic cells in maternal peripheral blood during pregnancy

A. Kolialexi[1], *G. Tsangaris*[2], *A. Mavrou*[1], *A. Antsaklis*[3], *F. Tzortzatou*[2], *V. Touliatou*[1], *C. Metaxotou*[1]
[1]*Medical Genetics, Athens University School of Medicine; Athens, Greece;* [2]*1st Dep. of Pediatrics, Athens Univesity School of Medicine; Athens, Greece;* [3]*1st Dep. of Obstetrics & Gynecology, Athens University School of Medicine; Athens, Greece*
ariamav@hol.gr

In a previous study we demonstrated that apoptosis increased according to gestational age accounting partly for the presence of free fetal DNA in maternal plasma and serum. Using simultaneously TUNEL assay and FISH analysis we identified the fetal origin of part of the apoptotic cell population but very few TUNEL+ cells showed hybridization signals since they were in a late apoptosis stage and nuclei were distorted. In the present study apoptotic cell population was identified immunocytochemically using Annexin V, a marker of cells in an early stage of apoptosis. Apoptosis was determined in mononuclear cells isolated from the peripheral blood of 29 pregnant women in the 16th-19th week of pregnancy. The mean apoptosis rate using Annexin V was 6.8–0.5% (range; 4.2-8.1%) vs 6.14–0.5% (range; 3.7-6.9) obtained using the ethidium bromide staining. FISH using X and Y chromosome specific probes was applied in 17 cases known to carry male fetuses. The proportion of apoptotic cells showing X/Y signals was 7.8% (range; 5-12%) while 75% of Annexin V + cells showed hybridization signals. In 12 samples from women with female fetuses no X/Y cells were recorded. Although our results are still preliminary, it seems that use of Annexin V antibody to detect apoptotic cell population improves FISH analysis and allows a more accurate determination of the proportion of fetal cells among the apptotic cell population.

P0596. Evaluation Of Prenatal Diagnosis Of Congenital Anomalies By Fetal Ultrasonographic Examination In Europe.

C. G. Stoll[1], *M. Clementi*[2]
[1]*Medical Genetics; Strasbourg, France;* [2]*The EUROSCAN Working Group; Strasbourg, France*
Claude.Stoll@chru-strasbourg.fr

Ultrasound scans in the midtrimester of pregnancy are now a routine part of antenatal care in most European countries. Thanks to registries of congenital anomalies, a study was undertaken in Europe. The objective of the study was to evaluate prenatal detection of congenital anomalies by routine ultrasonographic examination of the fetus. All congenital malformations suspected prenatally and all congenital malformations, including chromosome anomalies, confirmed at birth were identified from the Congenital Malformation Registers, including 20 registers from the following European countries ; Austria, Croatia, Denmark, France, Germany, Italy, Lithuania, Spain, Switzerland, The Netherlands, UK and Ukrainia. These registries are following the same methodology. The study was performed between July 1996 and December 1998, including 709,030 pregnancies. At delivery, 8126 babies/fetuses were diagnosed with abnormalities. The percentage of detection was variable for the diverse categories of congenital anomalies; it was high for neural tube defects (NTD) 96.4% and 68.6 for spina bifida, but low for ventricular septal defect and for atrial septal defect (ASD), 6.7% and 7.9%, respectively. The detection rate was higher for multiply malformed children (for example four times higher for ASD). Detection rate varied between European countries according to the policies used ; no routine scan, 1, 2 or 3 routine scans varying from 17.9% (no routine scan) to 55.6% (3 routine scans). The rate of pregnancy termination was high for central nervous system anomalies (54.5%) and chromosomal anomalies (53.1%) and low for renal anomalies (23.5%) and congenital heart defects (11.9%). Overall 25.0% of all pregnancies were terminated after prenatal detection of congenital anomalies. This study showed that many fetuses with major malformations can be identified prenatally in routine practice. Because policies, methods and techniques continually change, ongoing surveillance of prenatal diagnostic services is vital.

P0597. Prenatal Diagnosis Of Limb Reduction Deficiencies By Fetal Ultrasonographic Examination In Europe

A. Wiesel[1], *C. Stoll*[2], *A. Queisser-Luft*[3], *U. Froster*[3]
[1]*Medical Genetics; Strasbourg, France;* [2]*Service de G n tique M dicale, Hautepierre; Strasbourg, France;* [3]*The EUROSCAN Working Group; Strasbourg, France*
Claude.Stoll@chru-strasbourg.fr

Ultrasound scans in the midtrimester of pregnancy are now a routine part of antenatal care in most European countries. Using data from registries of congenital anomalies a study was undertaken in Europe. The objective of the study was to evaluate prenatal detection of limb reduction deficiencies (LRD) by routine ultrasonographic examination of the fetus. All LRDs suspected prenatally and all LRDs, including chromosome anomalies, confirmed at birth were identified from 20 Registries of Congenital Malformation from the following 12 European countries ; Austria, Croatia, Denmark, France, Germany, Italy, Lithuania, Spain, Switzerland, The Netherlands, UK, Ukrainia; these registries are following the same methodology. During the study period (1996-98) there were 709.030 births, including 7758 cases with congenital malformations. Two hundred fifty cases of LRDs with 63 (25,2%) termination of pregnancies were identified including 138 cases with isolated LRD, 112 cases with associated malformations, including 16 cases with chromosomal anomalies and 38 cases with non chromosomal recognized syndromes. If more than one LRD was present the case was coded as complex LRD. The prenatal detection rate of isolated terminal transverse LRD was 22,7% (22 out of 97), 50% (3 out of 6) for proximal intercalary LRD, 8,3% (1 out of 12) for longitudinal LRD and 0 for split hand/foot; for multiply malformed children with LRD those percentages were 46,1 (30 out of 65), 66,6 (6 out of 9), 57,1 (8 out of 14) and 0 (0 out of 2), respectively. The prenatal detection rate of LRDs varied in relation with the fetal ultrasound screening policies (no routine scan, 1, 2 or 3 routine scans) from 20,0% to 64,0%.

P0598. Prenatal Diagnosis Of Dysmorphic Syndromes By Routine Fetal Ultrasonographic Examination Across Europe.

M. Hasanhodzic[1], *C. Stoll*[2]
[1]*Medical Genetics; Strasbourg, France;* [2]*H pital de Hautepierre; Strasbourg, France*
Claude.Stoll@chru-strasbourg.fr

Ultrasound scan in the midtrimester of pregnancy is now a routine part of antenatal care in most European countries. The objectives of this study was to evaluate the prenatal diagnosis of dysmorphic syndromes by fetal ultrasonographic examination. Data from 20 registries of congenital malformations in 12 European countries were included in the study. At least one routine anomalies scan was offered to the pregnant women participating to this study. There were 8127 cases with congenital anomalies including 1866 cases with multiple malformations ; recognized syndromes, chro-

mosomal or non chromosomal and multiple malformed, non syndromic. Only recognized syndromes were included in this study. There were 2454 cases with congenital heart diseases, 479 out of them were recognized syndromes including 375 chromosomal anomalies and 104 non chromosomal syndromes including 28 deletions 22q11 (44,4% were prenatally detected), 17 heterotaxy sequences (64,7% were prenatally detected), 15 VATER association (46,6% were prenatally diagnosed) and 4 Noonan, 4 fetal alcoholism, 3 CHARGE association and 33 others (47,7% of them were detected prenatally). Hundred ninety two out of 1130 cases with renal anomalies were recognized syndromes, 161 out of them (83,3%) were diagnosed prenatally including 107 chromosomal aberrations and 54 non chromosomal syndromes (15 VATER, 13 Meckel Gruber, 12 osteochondrodysplasias, 5 caudal regression, 3 megacystis-microcolon, 2 Di George, 2 fetal alcoholism and 12 others). Fifty four out of the 250 cases with limb reduction deficiencies were recognized syndromes, including 16 chromosomal syndromes and 38 non chromosomal syndromes (7 amniotic bands, 6 VATER, 3 limb body wall complexe, 3 caudal regression, 2 each Poland, Hanhart, de Lange and TAR and 11 others); 20 of them were diagnosed prenatally (37,0%) including 9 chromosomal syndromes. There were 243 cases of abdominal wall defects including 57 recognizable syndromes, 48 with omphalocele (27 chromosomal, 5 OEIS, 2 caudal regression, 2 osteochondrodysplasia and 5 others) and 9 with gastroschisis (4 limb body wall complexe, 2 megacystis-microcolon, 2 chromosomal aberrations and 1 OEIS), 47 out of them were diagnosed prenatally (82,5%). Hundred fifty 2 out of 809 cases with intestinal anomalies were recognized syndromes, 46 were diagnosed prenatally (30.2%). There were 549 cleft lip and palate (CL(P)) and 197 cleft palate (CP) including 74 chromosomal aberrations and 73 recognised syndromes (23 Pierre Robin, 11 holoprosencephaly, 7 amniotic bands, 5 skeletal dysplasia, 4 Meckel Gruber, 2 OFD, 1 van der Woude and 20 others). Prenatal diagnosis was done in 51 CL(P) (53.1%) and 7 CP (13.7%). Few anencephalic cases were syndromic. Out of 290 cases with spina bifida, 28 were recognized syndromes, 20 of them (71.5%) were diagnosed prenatally. Six out of the 9 syndromic encephaloceles were diagnosed prenatally. Prenatal detection rates of syndromes varied significantly between European countries regarding to the screening policy. In conclusion this study showed that around 50% of the recognized syndromes can be detected prenatally by the anomaly scans. However the detection rate varied with the type of syndromes and with the policy of prenatal screening between countries.

P0599. Evaluation Of Prenatal Diagnosis Of Congenital Heart Disease By Ultrasound ; Experience From 20 European Registries Of Congenital Anomalies.

E. Garne[1], *M. Clementi*[2], *C. Stoll*[2]
[1]*Medical Genetics; Strasbourg, France;* [2]*Service de G n tique M dicale, Hautepierre; Strasbourg, France*
Claude.Stoll@chru-strasbourg.fr

Ultrasound scans in the midtrimester of pregnancy are now a routine part of antenatal care in most European countries. Using data from registries of congenital anomalies a study was undertaken in Europe. The objective of the study was to evaluate prenatal detection of Congenital Heart Defect (CHD) by routine ultrasonographic examination of the fetus. There were 2456 cases with CHD with an overall prenatal detection rate of 25%. Termination of pregnancy was performed in 293 cases (12%). There was significant variation in prenatal detection rate between regions with the lowest detection rate in countries without ultrasound screening (11%) and the highest detection rate in the countries with 3 fetal scans, 40 to 48% (p<0,01). There were 1696 cases with isolated CHD of which 16% were diagnosed prenataly and 5% of the pregnancies were terminated (TOP); 45% of the 761 CHD with of least one other major extra cardiac malformation (associated cases) were detected prenataly and 21,5% were TOP (p<0,01). Prenatal detection rate and TOP were 40,3% and 22,9 for chromosomal anomalies, 51,9% and 30,8% for non chromosomal recognized syndromes and 48,6% and 16,3% for multiply malformed with non chromosomal and non other recognized syndromes, respectively. Only 69 cases (2,8% of the total) were fetal deaths. Prenatal detection rate of CHD varied significantly between countries in relation with fetal ultrasound screening policies and also between countries even with the same screening recommendations. Risk of fetal death is low, which is important to know when counselling parents after prenatal diagnosis of CHD in order to provide ideal care for the patient.

P0600. Evaluation Of The Prenatal Diagnosis Of Neural Tube Defects By Fetal Ultrasonographic Examination In Different Centres Across Europe.

T. Boyd[1], *D. Wellesley*[2], *H. De Walle*[2], *R. Tenconi*[2], *S. Garcia Minaur*[2], *G. Zandwijken*[2], *C. Stoll*[2], *M. Clementi*[2]
[1]*Medical Genetics; Strasbourg, France;* [2]*Service de G n tique M dicale, Hautepierre; Strasbourg, France*
Claude.Stoll@chru-strasbourg.fr

Ultrasound scans in the midtrimester of pregnancy are now a routine part of antenatal care in most European countries. Using data from registries of congenital anomalies a study was undertaken in Europe. The objective of the study was to evaluate prenatal detection of Neural Tube Defects (NTD) by routine ultrasonographic examination of the fetus. All NTDs suspected prenatally and all NTDs, including chromosomes anomalies, confirmed at birth were identified from 20 Registries of Congenital Malformation from 12 European countries; the registries are following the same methodology. During the study period (1996-98) there were 670.766 births. A NTD was diagnosed at delivery in 542 cases. In 453/542 (84%) the lesion was isolated (166 anencephaly; 252 spina bifida; 35 encephalocele). Of the 166 isolated cases with anencephaly, 159 (96%) were correctly identified prenatally; one was missed to scan, two were wrongly diagnosed, four were not scanned (sensitivity 98%). 87% of the prenatal diagnoses were made before 24 weeks gestation; 142 (86%) of isolated anencephalic pregnancies were terminated, 11% of these after 24 weeks. Of the 252 cases of isolated spina bifida 171 (68%) were correctly identified prenatally, 112 (65%) before 24 weeks gestation. The diagnosis was missed on scan in 60 cases and 21 were not scanned (sensitivity 75%). The mean reduction in birth prevalence because of termination of pregnancy for spina bifida was 49% (range 6-100%). There was a wide variation between centres in prenatal detection rate (33-100%), termination of pregnancy of prenatally diagnosed cases (17-100%) and gestation both at diagnosis and termination of pregnancy. In conclusion a high prenatal detection rate for anencephaly was reported by all registers although some diagnoses were made late in gestation. There is a large variation in prenatal detection and termination rates for spina-bifida between centres, reflecting differences both in policy and culture.

P0601. Familial Orofaciodigital syndrome type I revealed by ultrasound diagnosis of porencephaly.

C. Thauvin-Robinet[1], *L. Faivre*[1], *T. Rousseau*[2], *C. Durand*[2], *N. Laurent*[2], *C. Maingueneau*[2], *P. Sagot*[2], *A. Nivelon-Chevallier*[1]
[1]*Service de G n tique M dicale; Dijon, France;* [2]*Centre pluridisciplinaire de diagnostic pr natal, Maternit ; Dijon, France*
christelthauvin@hotmail.com

Porencephaly is a rare central nervous system abnormality and is rarely associated with syndromes. Here, we report on a prenatal diagnosis of complex central nervous system abnormalities including agenesis of the corpus callosum, agenesis of the cerebellar vermis, bilateral hydrocephaly, and multiple cystic porencephaly in a 33 WG foetus. Termination of pregnancy was proposed after genetic counselling. Autopsy showed a female foetus with macrocephaly (W 1615g; L 43cm and OFC 32cm), unilateral postaxial polydactyly on the hand, and unilateral preaxial polydactyly on feet. Facial dysmorphism included hypertelorism, epicanthus, hypoplastic nasal alae, left choanal atresia, midline pseudocleft of upper lip, multilobulated tongue with oral hamartoma and multiple gingival frenulae. Clinical examination of the family revealed that the mother, sister and maternal grandmother of the proband were affected, and family history argued in favor of an X linked dominant inheritance pattern. This is the fourth report of porencephaly in association with orofaciodigital syndrome type I and is another example of a large intrafamilial variability. This report raise two issues; i) the difficulties in genetic counselling of orofaciodigital syndrome type I families due to the great variability of the disease, and ii) the importance of a detailed ultrasound examination after a prenatal diagnosis of porencephaly.

P0602. Firsttrimester ultrasound screening; Ethical aspects of pregnancy terminations with life compatibility fetal pathology.

A. S. Latypov, *L. E. Teregoulova*
Republican clinical hospital, Tatarstan; Kazan, Russian Federation
kazanMGC@mail.ru

Carried out during 2000 ultrasonic screening for nuchal translucency in Kazan, Republic Tatarstan, Russia, has allowed to reveal significant amount cases of expressed fetal pathology, owing to what the women have selected interruption of pregnancy.
1680 pregnant women are inspected for the first half-year of 2000.
11 large defects are detected (0.65%);

Abnormality	Cases
Body stalk anomaly	1
Holoprosencephaly	1
Encephalocele	1
Acrania	3
Anencephaly	5

Introduction measurement nuchal translucency into the screening protocol has resulted the appearance the group of the pregnant women with a fetuses without visible defects, but requiring invasive diagnostics procedures for exception chromosome pathology.
From 26 cases thickness nuchal translucency (1.55%) invasive procedure was carried out in 14 cases, the chromosome pathology was detected in 7 cases.
Thus, in spite of the fact that the detected pathology in 4 cases was life compatible (various variants of Turner syndrome) , the women have selected interruption of pregnancy. At the same time there was showed incorrect tendency at the practical gynecologists to recommend interruption of pregnancy at revealing markers without realization of confirming diagnostics.
Conclusion: The introduction of the high resolution ultrasonic equipment allow to detect a significant amount of fetal anomalies. Obviously, the final solution concerning of pregnancy determination remains for family. But it is the physician s responsibility to give objective information to family about all kinds of fetal abnormality and its developing.

P0603. A case of ultrasound and molecular diagnosis of thanatophoric dysplasia type I

G. Ginocchio[1], *P. De Biasio*[2], *F. Prefumo*[2], *P. Marchini*[2], *I. Bruzzone*[2], *M. Baffico*[3], *M. Lerone*[4], *M. Vignolo*[5], *P. L. Venturini*[1]
[1]*Department of Obstetrics and Gynaecology, Institute; Genova, Italy;* [2]*Department of Obstetrics and Gynaecology, Institute G. Gaslini , University of Genova; Genova, Italy;* [3]*Laboratory of Human Genetics, Ospedale Galliera; Genova, Italy;* [4]*Department of Molecular Genetics, Institute G. Gaslini ; Genova, Italy;* [5]*Department of Pediatrics, Institute G. Gaslini , University of Genova; Genova, Italy*
gmginocchio@yahoo.it
Thanatophoric dysplasia (TD) is the most common type of lethal skeletal dysplasia (LSD). Most of the prenatally diagnosed cases show abnormal ultrasound findings in the second and third trimester of pregnancy. There are reports of cases of TD diagnosed during the early second trimester, as well as cases of normal sonograms at 12-13 weeks of fetuses subsequently shown to be affected. Moreover, while a general diagnosis of LSD can be reliably made by ultrasound, a specific disease diagnosis remains often difficult. We observed a case of TD type I suspected at 18 weeks of gestation during a routine ultrasound scan. The main findings were abnormally short and bowed long bones, extremely small thoracic circumference and a typical telephone receiver appearance of the femurs. We were able to analyse genomic DNA obtained by chorionic villus sampling, identifying a C to G substitution at position 746 in the fibroblast growth factor receptor (FGFR) 3 gene, resulting in a Ser249Cys substitution already known to be associated with TD type I disease. While ultrasound scans remain the most important clue to suspect the disease, genetic molecular analysis of TD-associated mutations on CVS samples can be of great help in early definition of the diagnosis and in genetic counselling.

P0604. Ductus venosus doppler flow in the first trimester of pregnancy; repeatability of an adjunctive marker for Down s syndrome screening

F. Prefumo[1], *P. De Biasio*[1], *G. Ginocchio*[1], *I. Bruzzone*[1], *S. Ferrero*[1], *P. Marchini*[1], *P. L. Venturini*[2]
[1]*Department of Obstetrics and Gynaecology, Institute G. Gaslini , University of Genova; Genova, Italy;* [2]*Department of Obstetrics and Gynaecology, Institute; Genova, Italy*
prefumo@ermes.cba.unige.it
The analysis of Doppler waveforms of fetal ductus venosus flow has been proposed as a second level screening test for Down s syndrome in fetuses with increased nuchal translucency thickness. Limiting invasive karyotyping to fetuses with abnormal flow could dramatically reduce the number of procedures, minimally affecting the detection rate. However, since no data are available on the reproducibility of this technique, we assessed the intra- and inter-observer repeatability of fetal ductus venosus Doppler measurements at 11-14 weeks of gestation. Waveforms were recorded transabdominally. Intra-observer repeatability was studied in 22 fetuses in whom four repeated measurements were performed by the same observer. Inter-observer repeatability was assessed in 54 fetuses in each of whom two observers performed two repeated measurements. The pulsatility index for veins (PIV), peak systolic velocity (S wave), peak velocity during atrial contraction (A wave) and time-averaged maximum velocity (TAMV) were recorded. The coefficient of variation (CV) and intraclass correlation coefficient (ICC) were calculated. Cohen s k-coefficient was used for categorical data. Both intra-observer and inter-observer variations were low (intra-observer; CV of 10, 13, 22 and 13% and ICC of 0.85, 0.94, 0.94 and 0.95; inter-observer; CV of 8.8, 14, 22 and 13% and an ICC of 0.86, 0.84, 0.87 and 0.84 respectively for PIV, S wave, A wave and TAMV). There was 100% agreement for the detection of normal/abnormal blood flow. In summary, intra- and inter-observer repeatability of all parameters was acceptable, suggesting that the test could be reliably employed in clinical practice after further validation in larger series.

P0605. Chromosomal aberrations detected with amniocentesis in the fetuses with abnormal ultrasound

K. Crkvenac, *D. Muzinic, D. Begovic*
Division of Medical Genetics and Metabolism, Dept. of Paediatrics, University Hospital Center; Zagreb, Croatia
kriste2604@yahoo.com
Most fetuses with cytogenetic abnormalities have structural anomalies, which can be recognized by detailed ultrasound examination. Objective; During 1978-1999, amniocentesis was performed on 16 604 pregnant women. Retrospective study of routine ultrasound was performed to establish correlation between cytogenetic karyotyping and ultrasound in the detection of chromosomal abnormalities.The main indications for karyotyping are ultrasound suspection of fetal malformations, ultrasound suspection of growth retardation, hydramnios and olygohydramnios. Results; 360 medical record charts of women with ultrasound as a major indication for prenatal diagnosis were reviewed. Karyotypes were available in all cases. Of the 360 documented karyotypes, 27 chromosomal abnormalities were discovered (7.5%). Detected chromosomal abnormalities included one triploidy 69,XXX, one triploidy 69,XXY, ten trisomy 18, seven trisomy 21, one trisomy 9, six monosomy 45X and one balanced translocation 46,XX, t(10;11) (q24;q13). Conclusion; A ultrasound detectable fetal defect is a useful marker for detection of chromosomal abnormality.

P0606. Prenatal diagnosis of limb malformations;a retrospective study in 135 pregnancies

S. Manouvrier-Hanu[1], *O. Boute-Benejean*[1], *D. Thomas*[2], *L. Devisme*[3], *A. Moerman*[1], *A. Coeslier*[1], *F. Puech*[4], *M. Holder-Espinasse*[1]
[1]*Service de g n tique Clinique; Lille, France;* [2]*P diatrie en maternit ; Lille, France;* [3]*Laboratoire de Foetopathologie; Lille, France;* [4]*Centre de diagnostic pr natal; Lille, France*
smanouvrier@chru-lille.fr
Limb malformations (LM) have a prevalence of 1 in 1000. They can either be isolated or occur in association with other malformations as part of chromosomal abnormalities or multiple congenital anomalies syndromes (MCAS). The ultrasonographic prenatal detection of LM is difficult depending on the severity of the malformation and additional findings. We performed a retrospective study of 135 pregnancies with LM referred to the prenatal diagnostic centre of Lille (France) between January 1997 and December 1999. 22 cases were excluded because of incomplete data. The LM had been diagnosed thanks to the ultrasonographic survey in 74/107 cases (69%). 49 were isolated (66%), 25 (34%) had an identified MCAS, 3 (4%) had isolated amelia. 54 (73%) gestations were terminated, 5(6%) presented with spontaneous in utero death, 15 (20%) were continued. The LM had not been prenatally diagnosed in 33 cases (31%). 25 (76%) were alive, 4 were foetuses whose pregnancy had been terminated because of the diagnosis of another malformation (2 had abnormal chromosomes), 3 were stillborn. Among the alive neonates (40 cases), 27 (67.5%) had isolated LM, 13 (32.5%) had a MCAS with a known diagnosis in 10 cases. Genetic counseling was performed in 7 instances (17.5%). Among the dead cases (67), 3 (4.5%) had a severe isolated LM (amelia), 64 (95.5%) had associated findings (23% chromosomal abnormality, 77% MCAS with a known diagnosis in 2/3 cases). Genetic counseling was performed in 27 instances (40%).

P0607. Fusion 3 D Image as a tool for early diagnosis of congenital defects in the I. and II. trimester of pregnancy.

J. Santavy[1], *P. Polak*[2], *A. Santava*[1], *J. Hyjanek*[3], *I. Dhaifalah*[4]
[1]*Palacky University; Olomouc, Czech Republic;* [2]*University Hospital Olomouc; Olomouc, Czech Republic;* [3]*University Hospital; Olomouc, Czech Republic;* [4]*Palacky University; Olomouc, Yemen*
santavy@aix.upol.cz
Contemporary options of the foetal ultrasonographical diagnostics are per-

manently broadening. In the case of congenital defects new methods enable their depiction in earlier stages of pregnancy.They are a base for more detail differential diagnosis of found pathological features. At our department we have received an opportunity to manage with quit new ultrasonographical method which facilitates a fusion of Color Angio Doppler and Tissue Imaging pictures both in plane and space imagination. Method; Fusion 3 D ultrasonography Image in the pregnancy. Collection; Pregnant women send to our Foetal Medicine Department with suspicion for congenital defects of the foetus durin III. 2000 — I. 2001. Results; A new method iextends medical possibilities in the sphere of some types of congenital defects, where a space display of vessels bed plays an important role in differential diagnosis (rens, hepar, lung, CNS etc.). It has a meaningful role at a consideration about foetal postnatal prognosis.

P0608. Smith-Lemli-Opitz syndrome presenting as hydrops foetalis.

A. T. J. M. Helderman-van den Enden[1], *S. G. Kant*[1], *C. A. G. Holleboom*[2], *C. J. Tinga*[3], *H. H. H. Kanhai*[4], *J. E. Groener*[1], *J. A. Bakker*[5], *H. R. Waterham*[6], *M. H. Breuning*[1]
[1]*Department of Human and Clinical Genetics, Leiden University Medical Center; Leiden, The Netherlands;* [2]*Department of Gynaecology, Diaconessen Ziekenhuis Bronovo; The Hague, The Netherlands;* [3]*Department of Pathology, Diaconessen Ziekenhuis Bronovo; The Hague, The Netherlands;* [4]*Department of Obstetrics, Leiden University Medical Center; Leiden, The Netherlands;* [5]*Laboratory for Metabolic Diseases, University Hospital Maastricht; Maastricht, The Netherlands;* [6]*Laboratory of Genetic Metabolic Diseases, Academic Medical Center; Amsterdam, The Netherlands*
A.T.J.M.Helderman@KGC.AZL.NL

The Smith-Lemli-Opitz syndrome (SLO) is an autosomal recessive multiple congenital anomaly syndrome caused by a deficiency of the enzyme 7-dehydrocholesterol (7-DHC)reductase. The incidence is around 1 in 60,000-80,000 births. The presence of non-immune hydrops foetalis (NIHF) has been described twice as an atypical finding in SLO (Angle et al 1998, Maymon et al, 1999). We present in this abstract a third case of SLO with NIHF. The pregnancy was normal till 27 weeks, then IUGR was noted. At 29 weeks hydrops was present, karyotyping was normal 46,XY. At 30 weeks intrauterine death had occurred. Post mortem examination showed ambiguous external genitalia, postaxial polydactyly, syndactyly of the 2nd and 3rd toes, abnormal lung segmentation and right renal agenesis, signs suspect for SLO. Low cholesterol and a high 7- and 8-DHC concentrations in prenatal collected blood from the umbilical cord were found. The parents were both carrying a point mutation in the 7-DHC reductase gene. Chromosomal, metabolic, syndromic and infectious causes are found in NIHF. Metabolic diseases are found in 1-10%. It is to be expected that this percentage will increase because metabolic diseases like SLO can now be confirmed by laboratory testing. It is important to diagnose SLO because of the possibility for early postnatal treatment. Furthermore it allows for accurate genetic counseling and management of subsequent pregnancies. References; Angle B et al. Am J Med Genet. 1998 Dec 4;80(4);322-6. Maymon R et al. Prenat Diagn. 1999 Feb;19(2);105-7.

P0609. Prenatal Diagnosis In A Fetus With Holoprosencephaly

R. Pinto Leite[1], *A. Carvalho*[2], *C. Candeias*[1], *E. Ribeiro*[1]
[1]*Servi o de Gen tica-Hospital Vila Real; Vila Real, Portugal;* [2]*Servi o de Obstetr cia- Hospital de Chaves; Chaves, Portugal*
genetica@hvreal.min-saude.pt

Holoprosencephaly (HPE) is the most common developmental defect of the forebrain and midface in humans, occurs with a frequency of about 1 in 16,000 live births and about 1 in 200 spontaneous abortion. Although the majority cases of HPE exhibit normal karyotypes, chromosomal disorders are not uncommon. At least 12 different loci have been associated with HPE and now several distinct human genes for holoprosencephaly have been localized (21q22.3, on 2p21, on 7q36, on 13q32 and on 18p). We report a case of a 30-year-old pregnant woman with an abnormal scan at 19 weeks of gestation. She had a normal obstetric history with a phenotipically normal son. In the present pregnancy the fetus presented holoprosencephaly. Amniocentesis for cytogenetic evaluation was performed. Chromosome analysis of cultured amniocytes with GTG banding showed a apparently balance reciprocal translocation 46,XX,t(1q15q)(q32q26.1). Karyotype of parents were referred and demonstrated to be normal. To our knowledge this is the first case of holoprosencephaly and a chromosome anomaly involving these chromosomes. A bibliographic review is presented where association of holoprosencephaly and chromosome anomalies are discussed.

P0610. A case report of Desbuquois Syndrome by prenatal diagnosis

S. Subha Reddy[1], *S. Srinivas Reddy*[2], *S. Bali Reddy*[1]
[1]*National Medical Diagnostic Lab; Southfield, MI United States;* [2]*M.G.R. Medical University and Research Institute; Chennai, India*
subhareddy@hotmail.com

A case of Desbuquiois syndrome diagnosed prenatally is reported. A 32-year-old Asian Indian, gravida 3 and para 2 was referred for evaluation after a routine ultrasound examination at 22 weeks of gestation. The ultrasound revealed skeletal dysplasia with bilateral clubbed feet. The consanguinity was involved and the husband is patient s first cousin. There was no history of skeletal dysplasia or any other genetic disease in the family. The cytogenetic analysis was 46,XX karyotype. All long bones measure <10%ile. The ribs measure at the 45%ile for 22 weeks of gestation. The chest circumference was at 15th-30th%ile. Bilateral syndactyly of second, third digits of right hand and third, fourth and fifth digits of left hand was noted. The toes of both feet seemed unusually long. After gentic counseling the couple chose for the termination of pregnancy. Autopsy findings showed the long bones of lower and upper extremities were short; narrow face with high forehead; arched confluent eyebrows; posteriorly rotated prominent and dysplastic ears; proximal syndactyly of toes 2 and 3; micrognathia. X-ray examination revealed supernumerary ossification centers between proximal phalanx and second metacarpal of the index and ring fingers. The diagnostic patterns based on the supernumerary ossification and extreme shortening of the long bones in this autosomal recessive skeletal dysplasia are discussed.

P0611. Prenatal diagnosis in Slovakia in 1991-1999.

F. Cisarik
The Hospital of Zilina; Zilina, Slovakia
foxi@mail.viapvt.sk

Prenatal Genetic Diagnostics / PGD / was practised in seven Clinical Genetic Departements of Slovakia in 1991. Nowadays, twelve Clinical Genetic Departments perform PGD in Slovakia, two of which provide DNA analysis. Fetal sonographic examinations are routinely performed three times during gravidity of every mother, in 10th, 20th and 30th week of gestation. Sonographic identification of fetal aneuploidy markers is currently not regularly used. Standard procedure of obstetric care provides screening test of maternal serum alpha-fetoprotein, which we offer to all pregnant women between 15 and 20 weeks of gestation. We also offer the triple-marker screening for aneuploidy (including software analysis) only to part of population of pregnant women. In Slovakia, 16,051 of prenatal genetic analyses were done between 1991-1999. There were following indications for PGD; 44.7% maternal age of 35 and higher, 29.8% positive maternal serum screening, 11.2% high genetic risk, 2.5 % sonographic identification of fetal anomaly or positive sonographic markers for aneuploidy. There were found 319 /i.e. 2%/ of chromosomal anomalies. We determined pathological mutations in 27 fetuses from 116 prenatal DNA analyses performed. Statistics of the birth defects in newborns did not reveal a decrease of birth defects frequency calculated per 10,000 newborns. This frequency fluctuated around 230-240 per 10,000 newborns in period from 1991 to 1999. However, frequency of newborns with central nervous system anomalies substantially decreased. Annual number of newborns with Down syndrome and other chromosomal anomalies did not decrease though. From sum of prenatal and postnatal cases of chromosomal anomalies, we were able to find 44.9 % by prenatal chromosomal analysis.

P0612. Risk factors for birth defects in a developing country.

M. Thong, *C. Lim*
University of Malaya; Kuala Lumpur, Malaysia
thongmk@medicine.med.um.edu.my

A major birth defect is defined as an abnormality of prenatal origin that if uncorrected or uncorrectable, significantly impairs normal physical or social function or reduces normal life expectancy. Whilst the infant mortality rate in Malaysia has decreased the past 30 years as a result of better obstetric and neonatal services, the percentage of all medically certified infant deaths related to birth defects had risen steadily from 6.2% (1970) to 20.8% (1990). Information on risk factors associated with birth defects are not well studied in developing countries. We postulated that a hospital-based birth defect registry might be able to provide some information on the magnitude of the problem and identify at-risk factors for birth defects in our population. We ascertained these risk factors associated with major birth defects in Malaysian births of 24 weeks gestation and above, using a case-controlled study from a hospital-based birth defect registry over a 12-month period. The results showed the incidence of major birth defects to be 1.8% amongst all births above 24 weeks of gestation and established the baseline incidence of major birth defects in the University of Malaya

Medical Centre in Kuala Lumpur. The majority of major birth defects in Malaysian newborns are potentially detectable antenatally by ultrasonography and karyotyping studies during the first and second trimesters. At-risk factors identified for major birth defects were family history of birth defects, adverse past obstetric events, low intake of folate / haematinics, maternal diabetes, poly or oligohydramnios and late or unavailability of ultrasonography in pregnancy. We concluded that the application of appropriate preconceptional care, genetic counselling and other preventive strategies might reduce the incidence of major birth defects and infant mortality in Malaysia. Larger population-based study for birth defects may be able to confirm these observations.

P0613. Chorion and decidual morphology in spontaneous abortuses with normal and pathologic karyotypes

***J. Gorbatcheva**, I. Voloschuk, N. Dyscheva, E. Sidneva*
I.M.Sechenov s Moscow Medical Academy; Moscow, Russian Federation
nik.bochkov@mtu-net.ru

The morphological investigation of spontaneous abortuses (A) with normal and abnormal karyotype were (67 specimens of first trimester A) was carried out. It included a histologic pattern and proliferative activity. The last one was determinated as the rate of Ki-67-positive cytotrophoblast cells. The karyotyping was made on chromosome preparations of chorion villi prepared by direct method with routine and G-staining. Chromosomal aberrations were estimated in 37,3%; 9 trisomies, 3 monosomies, 3 triploidies, 1 tetraploidy, 9 mosaics. The partial moles were diagnosed in triploidy, as it was expected. Identical morphological signs of the development retardation of villi were diagnosed among abortuses with normal karyotype and with aneuploidies. The diameter of villi was bigger in aneuploid A than in A with normal karyotype ($p<0.05$). Changes in the decidua, which reflected mechanism of realisation of abortion (hemorrheologic disturbances, necrosis, etc.) were the same in A with normal and abnormal karyotype. The trophoblast proliferative activity was high in A with abnormal karyotype and low or completely absent in A with normal karyotype. The erythrocytes in the intervillous space were detected in all specimens with chromosomal aberrations. Our results indicate that the blood flow in the intervillous space appears prematurely as a consequence of the placentation defect in the aneuploid conceptuses. It leads to the abruptio of the ovum.

P0614. Amniocentesis for II and III trimester cytogenetic prenatal diagnosis in Costa Rica

***I. Castro Volio**[1], K. Sander Mangel[2], M. Vargas Prado[3], L. S nchez Ch ves[2], G. Escalante L pez[2]*
[1]Instituto de Investigaciones en Salud (INISA), Universidad de Costa Rica; San Jos , Costa Rica; [2]Unidad de Perinatolog a, Servicio de Obstetricia, Hospital R. A. Calder n Guardia, Caja Costarricense del Seguro Social (C.C.S.S.); San Jos , Costa Rica; [3]Unidad de Perinatolog a, Servicio de Obstetricia, Hospital M xico, C.C.S.S.; San Jos , Costa Rica
icastro@cariari.ucr.ac.cr

The identification of fetal abnormal chromosomes in high risk pregnancies, allows proper pediatric and obstetric management of the cases as well as genetic counseling. The results of 842 genetic amniocentesis from 1986 to 1999, are reported. All procedures were performed transabdominally and under ultrasound guidance, in hospitals of the social security system and in private facilities. There were two main reasons for referral; abnormal ultrasound assessment (48% of cases) and advanced maternal age (35%). Most procedures (66%) were performed during the second trimester of pregnancy and 34% during the third trimester. Fetal cells were closed cultured and suspension harvested. Turn around time was 14 days median. In 217 amniotic fluid samples no diagnosis could be obtained, mainly due to absence of cell growth in late gestation samples and in blood contaminated ones. In 625 fetal karyotypes obtained, 55 (9%) were abnormal, due to 33 trisomies (including a robertsonian translocation trisomy 13), eight cases of monosomy X, three mosaics (including a mosaic trisomy 22), balanced and unbalanced translocations, extra structurally abnormal chromosomes and other defects. Pseudomosaicism was detected in five cases. Taking into account the reason for referral, cases studied as a result of abnormal ultrasound assessment exhibited 17% abnormal karyotypes, in contrast to 2.5% cytogenetic defects in pregnancies of women 35 years or older. Prenatal cytogenetic and sonographic findings correlated with the phenotype of the newborn in 211 cases available for follow-up. Prenatal diagnosis of fetal defects allowed genetic counseling as well as better obstetric management and pediatric care. Normal results of both tests provided reassurance to prospective parents.

P0615. Evaluation of the hereditary components of intrahepatic choleostasis of pregnancy.

***S. Turyk**[1], M. Salvo[2], M. Palermo[2]*
[1]Hospital Brit nico; Buenos Aires, Argentina; [2]Hospital Nacional Prof. Dr. A. Posadas; Buenos Aires, Argentina
sagm@drwebsa.com.ar

Choleostasis of pregnancy is a disease that occurs during the second half of pregnancy and regresses in puerperium, characterised by pruritus and elevated serum bile acid levels. Its etiology remains unknown, but probably involves the interaction of abnormalities in the metabolism of estrogens and progesterone.Its incidence varies according to geographical area. I.Ch.P. is associated with substantially increased mother morbidity and fetal morbidity and mortality. We report here a detailed clinical history, pedigree and examination, obtained from 22 pregnant woman with choleostasis. In all the cases maternal serum transaminase and bile acid levels were done and viral and drug induced hepatitis and gallbladder disease were ruled. Ten pedigrees contained two or more cases of I.Ch.P. and six suggested autosomal recessive inheritance.The incidence of spontaneous abortion and fetal death in the 22 pedigrees was high. The mean maternal age was 32,5 years and the mean gestacional age at the moment of the diagnosis was 32,3 weeks. To reduce the risk of this pathological condition, it should be very important that patients have genetic counselling before conception, in order to plan the pregnancy.

P0616. Organisation and Practice of Prenatal Diagnostics in Estonia. Summary of ten years work.

***M. Sitska**[1], T. Ilus[1], P. Ilisson[1], K. Kuuse[1], P. Tammur[1], A. Ehrenberg[2]*
[1]Medical Genetics Center of the Clinicum of the University of Tartu; Tartu, Estonia; [2]Women's Clinic of the Clinicum of the University of Tartu; Tartu, Estonia
Mari.Sitska@kliinikum.ee

In Estonia (pop. 1.46 million, birth rate 13185) prenatal diagnosis of genetic disorders was introduced into clinical practice in 1990 and is to a great extent (95%) performed in one center - Medical Genetics Center (MGC) of the Clinicum of Tartu University . The uptake of invasive prenatal studies is not high in Estonia. In 1999only 5.5% of pregnancies were monitored by AC or CVS; The funding arrangement for PND is the national health insurance system. Sources of information. Exact records about prenatal invasive testing in Estonia are kept at the MGC of the Clinicum of Tartu University . Invasive PND (amniocentesis, CVS, cordocentesis). Altogether 2767 procedures have been done. Abnormalities were detected in 87 (3.1 %) cases. For fetal chromosomal analyses we mostly use amniocentesis (97 %). Alltogether 2700 amniocenteses have been done. The main indication (69%) has been maternal age. Chromosomal disorder was diagnosed in 71 (2.7%) cases. Chromosome anomalies have been screened for advanced maternal age since 1993. In 1999, 35 % of women >35 had fetal karyotypes. Transabdominal CVS we have used only in highrisk pregnancies in 44 cases. Abnormalities were detected in 13 cases (30%). Cordocentesis has been done in highrisk pregnancies in 23 cases. Chromosomal abnormalties were detected in 3 cases (13%) . Maternal serum screening has been routinely offered since 1998 only in Women s Clinic of Tartu and since 2000 in Southern Estonia. The impact of PND of chromosomal is increasing every year. During the 1999 year 50% of the trisomy 21 cases were detected prenatally .

P0617. Somatic microsatellite DNA mutations in spontaneous abortions with normal karyotype

***T. V. Nikitina**, S. A. Nazarenko, N. N. Sukhanova*
Institute of Medical Genetics; Tomsk, Russian Federation
nikita@img.tsu.ru

The causes of the loss of considerable proportion of human embryos with normal karyotype remains obscure. Kiaris et al. (1996)reported high level of somatic microsatellite instability in spontaneous abortions; 40% revealed discrepancy of PCR alleles between tissue sections from different parts of embryos. To clarify the frequency of somatic microsatellite mutations in spontaneous abortions we have analyzed 55 missed abortions and anembryonic pregnancies with normal karyotype. DNA was extracted from fetal membranes or chorionic villi, which originated from two different embryo determinants; extra-embryonic mesoderm and cytotrophoblast. These tissues differentiates during the first several days of ontogenesis and such approach allows to reveal mutations which took place at early stages of embryonic development. We used polyacrylamide gel electrophoresis following PCR amplification for 21 tetranucleotide high-polymorphous loci located on seven autosomes. The isolated STR mismatches were evaluated in parent/abortus allelic transfers. An undetected twin demise (chimerizm) and non-paternity were excluded with high probability (more then 99.9%) by using of the other loci. We found four cases of third

allele appearance. In two cases all three alleles were analogous with parent s ones, what is a result of cytogenetically unrecognized mosaic trisomy. The presence of somatic mutation was detected by appearing in gel analysis a new (third) allele which can be distinguished from the parents ones. We observed two (3.6%) unambiguous events of somatic microsatellite mutations only, that is by order of magnitude smaller than was found by Kiaris et al. (1996). A comprehensive analysis is in progress to confirm these results.

P0618. The Early Genetic Amniocentesis (ea) - Risks, Complications And Use In Prenatal Diagnosis

S. Brankovic-Niksic[1], *G. Radivojevic-Ilic*[1], *S. Nikolic*[1], *D. Plecas*[2]
[1]*Institute of Mental Health; Belgrade, Yugoslavia;* [2]*Institute of Obstetric and Gynecology; Belgrade, Yugoslavia*
nbrankov@eunet.yu

Amniocentesis is the most commonly performed during the midsecond trimester (16 - 18 week). We have reported the results of our groups of patients who had early amniocentesis (EA) before 16th week of gestation. This paper presents the results of amniocentesis performed between 13 - 15th week of gestation and its complications. We have also followed the offspring at birth and again by the first year of life.

P0619. Minimal volume of amniotic fluid for reliabe prenatal cytogenetic diagnosis

B. Sikkema- Raddatz*, J. van Echten, J. van der Vlag, C. H. C. Buys, G. J. te Meerman*
University of Groningen, Department of Medical Genetics; Groningen, The Netherlands
B.Sikkema-Raddatz@medgen.azg.nl

In current amniocentesis practice, 20 ml amniotic fluid (AF) is drawn for prenatal cytogenetic diagnosis. When, as is often the case in a not negligible proportion of cases, only a smaller volume is obtained, repeated amniocentesis is recommended. This prompted us to investigate the minimal volume of AF required for a reliable cytogenetic diagnosis (14 clones). Based upon the number of clones per fl35 mm petri dish in which amniocytes were cultured, the first possible day for in situ cytogenetic analyses was determined for 12 samples, taken at 14- 16 weeks of gestation. Cultures were started from a range of small volumes of AF. It appeared that volumes smaller than 0.8ml yielded too few clones for a reliable diagnosis. The 12 cultures which started from 0.8 ml AF yielded 14–7 clones at day 12. Controls, started with approximately 5 ml AF; 18 – 5 clones at day 9. A further series of 40 samples yielded 13–6 clones at day 11 for 0.8 ml AF and for controls 16–5 clones at day 9. Thus, by using 4 petridishes per diagnosis repeated amniocentesis will not be necessary as long as more than 3.2 ml of AF is available. Since the number of clones obtained from 0.8 ml AF is comparable to the number obtained in protocols for early amniocentesis1, it might be worthwhile to investigate the feasibility to also perform early diagnosis with smaller volumes of AF. 1Kennerknecht I, Baur-Auberle S, Grab D and Terinde R (1992) First trimester amniocentesis between the seventh and 13th weeks; evaluation of the earliest possible genetic diagnosis; Prenat Diagn12; 595- 601

P0620. The Belgian experience of prenatal diagnosis in Marfan syndrome (MFS).

B. Loeys[1], *L. Nuytinck*[1], *M. Bonduelle*[2], *K. Sermon*[2], *B. Hamel*[3], *A. Sanchez*[4], *L. Messiaen*[1], ***A. De Paepe***[1]
[1]*University Hospital Ghent; Gent, Belgium;* [2]*Free University of Brussels; Brussels, Belgium;* [3]*University Medical Center; Nijmegen, The Netherlands;* [4]*Hospital Clinic; Barcelona, Spain*
bart.loeys@rug.ac.be

Marfan syndrome (MFS) is an autosomal dominant disorder with an incidence of 1 in 5000 live births. Symptoms are variable from skeletal overgrowth, cutaneous striae to ectopia lentis and aortic dilatation leading to dissection. Since the disease causing mutations are dispersed throughout the fibrillin-1 gene (FBN1), prenatal diagnosis was until recently usually performed by linkage analysis in familial cases. However, mutation detection has become feasible with thorough screening methods. The phenotypic variability of the MFS makes reproductive options difficult, as molecular diagnosis cannot predict clinical severity of the disease. We present data on 14 prenatal and/or pre-implantation diagnoses (PGD) in eight families, originating from Belgium, the Netherlands, Spain and France. In four families prenatal diagnosis was carried out using linkage analysis, whereas in four other families the causative FBN1 mutation was characterised. Four PGD cycles in two couples lead to one ongoing pregnancy, and in addition two amniocenteses and eight chorion villus samplings were performed. In four pregnancies an affected foetus was diagnosed. In one of them, the couple chose to continue the pregnancy and an affected child was born, whereas the 3 other couples decided to terminate the pregnancy. We expect that the availability of molecular tests will increase requests for prenatal diagnosis. PGD represents an acceptable alternative in couples facing ethical reproductive dilemmas.

P0621. Late gestation chorionic villus sampling; genetic and economic implications for rapid prenatal diagnosis of high-risk pregnancies in the second and third trimesters.

L. P. Shulman[1], *O. P. Phillips*[2], *J. Matheson*[1], *E. Powell*[1], *S. Elias*[1]
[1]*University of Illinois at Chicago; Chicago, IL United States;* [2]*University of Tennessee, Memphis; Memphis, TN United States*
shulman@uic.edu

Cytogenetic assessment of high-risk pregnancies during the second and third trimesters has primarily been achieved by amniocentesis, despite the reported efficacy and safety of chorionic villus sampling (CVS) for second and third trimester cytogenetic analyses. Indeed, the concomitant use of fluorescent in situ hybridization (FISH) to obtain rapid cytogenetic information from amniotic fluid samples is gaining popularity in the United States and Europe. However, FISH analysis of amniotic fluid cells is frought with diagnostic limitations and additional expense. We seek to compare the use of five-color FISH analysis to CVS for the assessment of late-gestation (>14.0 weeks gestation) pregnancies at increased risk for fetal chromosome abnormalities. Materials/Methods; Late gestation CVS was performed on 97 women at increased risk for fetal chromosome abnormalities from January 1995 through November 2000; seventy-nine women for positive maternal serum screen outcomes (and nonelevated AFP) and 18 for fetal structural abnormalities (without evidence of neural tube defects). Results; Cytogenetic results were obtained from all samples. Five of the 79 samples obtained from pregnancies identified by maternal serum screening were characterized by chromosome abnormalities (3 cases of trisomy 21 and single cases of 45,X and trisomy 18). Nine of the 17 samples obtained from women carrying fetuses with structural defects were characterized by karyotypic abnormalities (3 cases of trisomy 18 and single cases of trisomy 21, trisomy 13, 45,X, 47,XX,+mar, partial trisomy 8 and der17). Of the 14 cases of karyotypic abnormalities, 3 (21.4%) would not have been detected by FISH analyses for chromosomes X, Y, 13, 18 and 21. In addition, FISH analyses added $75 to $240 to the cytogenetic cost of each case. Conclusion; Late gestation CVS provides the opportunity to obtain rapid and comprehensive cytogenetic information in a time frame similar to that required for FISH analyses. FISH also adds additional cost while providing less cytogenetic information than that obtainable by CVS. CVS should thus be considered when a prengnacy at high risk for fetal chromosome abnormalities (without a concomitant increased risk for AFP-related abnormalities) is detected after 14 weeks gestation.

P0622. A retrospective cytognetics study about 185 cases of twin-gestations.

M. A. J. M. JAMAR[1], *C. Lemarchal*[2], *V. Lemaire*[2], *C. Herens*[2]
[1]*CHU Sart Tilman; LIEGE, Belgium;* [2]*CHU Sart Tilman; LIEGE, Belgium*
Mauricette.Jamar@chu;ulg.ac.be

From January 1st 1990 until December 31st 1998, we collected 13899 prenatal diagnosis (on amniocentesis and chorius villus sampling). Three hundred thirty-nine samples concerned 185 cases of twin-gestations ; 154 cases for which we received samples for both fetuses, 31 cases with only one sample. The indications were clearly given in 122 pregnancies (65.9%); the most frequent were maternal age (49/185 =26.5%), positive ultrasound (21/185 =11.4%), medically assisted procreation (15/185 =8.1%). Q-banding karyotyping was performed following classical treatment. In 116 (62.7%) twin gestations, both fetuses were of the same sex (51 female; 65 male); 58 cases (31.4%) concerned twin couples; in 11 cases (5.9%), sex could not be determined for both fetuses, because most often of in vitro growth failure. Chromosome abnormalities were found in seven twin-pregnancies (3.8%) ; four with only one fetus affected [47,XX,+18; 45,XX,t(13;14); 47,XYY; 47,XXX], two with both fetuses affected, showing the same chromosomal abnormality [inv(11)(q21q25); 47,XX,+18] and one with only one fetus tested [47,XX, +18]. In total, we found seven autosomal abnormalities, three balanced and four unbalanced (all Trisomy 18), and two sex chromosome abnormalities, which were both observed in IVF pregnancies. One pregnancy exhibited a male fetus 47,XYY and a normal male fetus 46,XY. The other showed a female fetus triplo-X and a normal 46,XY fetus. Results will be discussed in the poster.

P0623. Facial photoanthropometric findings in a group of healthy newborns

I. Chomczyk, *E. Hubert, R. Posmyk, E. Burak, A. T. Midro*
Department of Clinical Genetics and Clinic of Maxillofacial Surgery; Bialystok, Poland
midro@amb.ac.bialystok.pl

As the knowledge on dysmorphic traits distinctive for children with genetic syndromes is essential in the diagnostic process, a support of appropriate photographic documentation is indispensable. Moreover, the clinical trait evaluation may be ensured by certain measurements made on those photographs. However, data elicited from a normal population are needed for concluding on the findings of children being diagnosed. They are needed in particular for newborns due to the principal concern of early diagnosing. The aim of our study was to determine the index values of selected anthropologically defined facial traits in a group of healthy newborns. 85 randomly selected healthy newborns (51 females and 34 males) aged from 1 to 14 days, born at term spontaneously or by Caesarean section, were studied. The photoanthropometric analysis was made according to Stengel-Rutkowski. A range between the 3rd and 97th percentiles was delineated for discrimination of the values considered as dysmorphic. No significant differences related to age, sex, or delivery passage were found. We suggest that the obtained database may be useful as an approach of the normal population for comparing with the respective data elicited from newborns with genetic syndromes.

P0624. Prenatal ONTD and Down syndrome Screen Positives Rate Reduction Using Dried Blood Samples.

P. D. Buchanan[1], *D. A. Krantz*[2], *T. W. Hallahan*[2], *J. W. Larsen*[3], *J. N. Macri*[1]
[1]*GeneCare Medical Genetics Center; Chapel Hill, NC United States;* [2]*NTD Laboratories; Huntington Station, NY United States;* [3]*The George Washington University Medical Center; Washington, DC United States*
genecare@earthlink.net

In 1996, we reported the use of dried maternal blood samples for ONTD, Down syndrome, Trisomy-18 screening (Macri, et.al.) We present an expanded comparison of our second trimester screen positive rates. Dried blood samples provide significant advantages over liquid samples by reducing biohazard and eliminating broken tubes, centrifugation, and sample degradation. The biggest advantage is the smaller standard deviations of analyte levels observed with dried blood compared to liquid serum. In both second trimester and first trimester screening, we observed smaller than previously reported SD.

	Macri et al 1996	Current Experience
Total Screened	7,497	102,487
Initial Positive Rate;		
ONTD	4.4%	3.4%
Down syndrome	3.6%	3.4%
Trisomy 18	NR	0.6%
False Positive Rate after revision;		
ONTD*	2.7%	2.0%
Down syndrome	2.8%	2.4%
Trisomy 18	NR	0.5%

NR = Not reported. *This number based on results after second specimen collected. Cut-off Risks; ONTD 1/400; Down s 1/380; t18 1/500.

Recent studies indicate first trimester screening using nuchal translucency, free B-hCG and PAPP-A is effective. We have prospectively evaluated over 25,000 first trimester patients exclusively with dried blood specimens. In summary, the data indicate the screen positive rate in our expanded dried blood screening is similar to the initial report showing significantly decreased screen positive rates.

P0625. Preliminary Report On Technical Issues Concerning The In-vitro Growth Of Extracoelomic Cells For Chromosomal Studies Using the Pregnant Baboon Model.

J. Santolaya-Forgas[1], *T. Hewitt*[2], *Z. Shen*[3], *M. McCorquodale*[3]
[1]*Dept Ob/Gyn. Texas Tech Health Sciences Center at Amarillo; Amarillo, TX United States;* [2]*University of Illinois at Chicago; Chicago, IL United States;* [3]*Michael Reese Hospital; Chicago, IL United States*
jsf@cortex.ama.ttuhsc.edu

In selected patients, prenatal diagnosis of genetic abnormalities could be performed between 7 to 9 weeks of pregnancy via the coelocentesis procedure (Jurkovic 1993, Pandya 1995). PCR and FISH based genetic studies have been performed in extra celomic fluid (ECF) obtained from women prior to a termination of pregnancy. However, only one author has been able to obtain a conventional karyotype in ECF (Gruger 1996). Our objective was to gain further insight into the previously described technical variables that may determine a successful culture on a small ECF sample. We performed 7 ultrasound guided coelocentesis (2-4 mL) in 7 time pregnant baboons at 36-40 days post-conception. The initial 0.5mL was discarded. ECF samples were ramdomly assigned to 2 groups in which the only differences were the container and the type of medium. TABLE shows; Animal#; ECF mL; Type of Container; Type of Medium; Culture-Days; Karyotype results and Pregnancy outcome. Medium at equal volume to ECF; (1)=Amniomax; (2)= 1+used Amniomax from fibroblast culture (1;1). Container (A) Small Petri dish keeping medium+ ECF on the small cover slip; (B) Plastic flaskettes which allows the medium+ECF to spread on the larger cover slip. CONCLUSIONS; We confirm that standard cytogenetic studies can be done in very small samples of ECF. This preliminary data also suggests that it is keeping the cells together what may determine a succesful ECF culture.

	Macri et al 1996	Current Experience
Total Screened	7,497	102,487
Initial Positive Rate;		
ONTD	4.4%	3.4%
Down syndrome	3.6%	3.4%
Trisomy 18	NR	0.6%
False Positive Rate after revision;		
ONTD*	2.7%	2.0%
Down syndrome	2.8%	2.4%
Trisomy 18	NR	0.5%

P0626. Real-time PCR for foetal RhD status determination in maternal serum.

E. Gautier, *P. Ernault, L. Lohmann, J. Costa*
H pital Am ricain de Paris; Neuilly, France
jean-marc.costa@ahp-paris.com

Prenatal determination of foetal Rhesus D (RhD) status has great implications in RhD-negative woman at risk for foetal alloimmunization. No further investigations are necessary if the fetus is RhD-negative, while anti-D prophylaxis and clinical management of sensitized pregnant women are required. Foetal RhD status can actually be easily performed on amniotic fluid or chorionic villus sampling by detection of a specific sequence of the RhD gene. However, these sampling procedures are invasive and at risk for foetal loss. A more advisable approach has been recently proposed to determine fetal RhD status by cell-free foetal DNA analysis in maternal plasma (Lo et al, NEJM, 1998). A new real-time PCR based on the LightCycler technology using hybridization probes was therefore developed to detect specific foetal RhD sequences in maternal serum. Ninety one sera from pregnant women were analyzed using this new technique. Maternal blood was obtained during the first trimester [mean gestational age=13.2 weeks of gestation (8-14)] in 48 cases and during the second trimester [mean gestational age=16.4 weeks of gestation (15-29)] in 43 cases. All sera were blind tested and the results were compared with those obtained later in pregnancy on amniotic fluid cells.

P0627. Epidemiological Study of Some Sentinel Anomalies and Down s syndrome

O. Tymchenko, *O. Nikula, O. Turos, T. Pokanevich, L. Piotrovich, O. Makaruk*
Laboratory of Genetic Monitoring, Institute of Hygiene and Medical Ecology; Kiev, Ukraine
otimch@usch.kiev.ua

It is known that the sentinel anomalies (SA) and Down s syndrome (DS) make a considerable contribution into infant morbidity and mortality. These defects are diagnosed well and can provide important clues in the detection of teratogenic agents.An anencephalia, spina bifida, limb reduction, oesophageal atresia and traheo-oesophageal fistula, ano-rectal atresia, cleft of a labium with / or without cleft of a palate, multiple congenitals anomalies and Down syndrome were taken into account.

Objective. To estimate the relative risk (RR) of the live-borns with SA and DS in different Ukrainian regions.

Methods. Data on live-borns only were received from the annual reports of seven medical genetic centres of Ukraine (Southern, Western, North, Northern-Eastern, Central, South-Eastern, South-Western). Period of the observation - from 1993 to 1999. The precise data about still-borns with birth defects are unknown in Ukraine. RR was calculated according to Epi

Info program for each selected defects.
Results. It was established that RR evenly disseminated among the newborns of the country. RR for sentinel anomalies is demonstrated in the Table. RR was lower in the Northern and the South-Eastern regions (0,86 at the 0.81-0.90 confidence interval; 0.88; CI 0.84-0.93). RR was the highest in the Southern (1.10; CI 1.02-1.19) and the Northern-Eastern regions (1.19; CI 1.14-1.25)(Table).

Region	RR	95% CI
Northern	0.86	0.81-0.90
Southern	1.10	1.02-1.19
Central	1.00	0.96-1.05
Western	1.00	0.97-1.04
Northern-Eastern	1.19	1.14-1.25
South-Eastern	0.88	0.84-0.93
South-Western	1.03	0.98-1.08

Conclusion. The distinctions in the rates of observed defects can reflect not only the difference of incidence in population, but mainly the difference in the levels and the quality of medical care and other socio-economic factors.

P0628. A Pilot Study On QF-PCR Analysis For Prenatal Diagnostics Of Down Syndrome In Bulgaria

S. Andonova[1], *V. Vladimirova*[1], *V. Dimitrova*[2], *V. Mazneikova*[2], *T. Chernev*[2], *E. Simeonov*[3], *D. Toncheva*[4], *I. Kremensky*[1]
[1]*Laboratory of Molecular Pathology; Sofia, Bulgaria;* [2]*Department of Fetal Medicine; Sofia, Bulgaria;* [3]*Section of Clinical Genetics, Department of Pediatrics; Sofia, Bulgaria;* [4]*Department of Medical Genetics; Sofia, Bulgaria*
sandonova@netscape.net
Down syndrome (trisomy 21) is the most frequent human chromosomal disorder, occurring in approximately 1 in 700 newborns. Prenatal diagnosis is offered because of advansed maternal age, abnormal maternal serum screening or ultrasound examination suggesting fetal abnormalities. It is usually performed by cytogenetic analysis of fetal cells, but this procedure is long lasting, expensive, requires high technical experience and exists some risk of culture or cytogenetic analysis failure. Here we report the results from our initial experience of introducing a quantitative fluorescent PCR based analysis for rapid determination of trisomy 21 status. A total of 38 fetus DNA samples were extracted from amniotic cells, chorionic villi and cultivated fibroblasts; all samples were analysed in blind. Fluorescent labeled primers were used to amplify 2 STRs on chromosome 21 and PCR products were analyzed on an automatic DNA sequencer to identify the presence of two or three copies of chromosome 21. In this study D21S11 microsatellite gave an informative results by QF-PCR for a group of 32 samples, while marker D21S1270 - for another 32 samples. All samples were informative for at least one marker; 35 fetuses were identified as having two copies of chromosome 21, and 3 fetuses - as having trisomy 21. Our results indicate that QF-PCR could be used as an alternative rapid and accurate method for prenatal diagnosis of Down syndrome, espessially when ultrasound or biochemical analyses already suggest fetal chromosome disorders. Rapid results will allow a termination of pregnancy at an early stage of gestation.

P0629. Deletion Screening , RFLPs and CA Repeats Analysis of Duchenne and Becker Muscular Dystrophy for Prenatal Diagnosis and Carrier Detection in Iranian Population

F. Pourfarzad[1,2], *A. R. Nadji*[1], *R. Karimi-Nejad*[1], *M. Azad*[1], *S. Jalil-Nejad*[1], *S. Kheradmand Kia*[2], *M. Karimi-Nejad*[1], *Y. Shafeghati*[2], *H. Najmabadi*[2,1]
[1]*Karimi-Nejad Pathology and Genetics Center; Tehran, Islamic Republic of Iran;* [2]*University of Welfare and Rehabilitation; Tehran, Islamic Republic of Iran*
maryamneishabury@yahoo.co.uk
Duchenne and Becker Muscular dystrophies (DMD/BMD) are X-linked disorders affecting 1;3500 and 1 in 18000 live male birth, respectively. Both DMD and BMD result from heterogeneous mutations in the dystrophin gene and in about 65% of the cases the gene has one or more deleted exons and the remaining cases are revealed as point mutation. One third of cases arise from new mutation and two thirds are familial. In order to determine the range of the deletion in Iranian patients and prenatal diagnosis, a deletion screening and linkage analysis were preformed in a group of 30 unrelated DMD/BMD patients. Three sets of multiplex PCR were used to screen for twenty of the most frequent deletions of dystrophin gene. Three intragenic RFLPs (pERT87-15/Bam HI, pERT 87-8/Taq I, pERT87-15/XmnI) and Two CA repeats (3 -Dys and 5 -Dys MSA), which have the most hetrozygostiy frequency were used to carrier detection and linkage analysis. Deletions were detected in 53% of the patients and most of the deletions were in exons 46(13.5%),47-50 (11.8% each) and the number of deleted exons varied from one to nine. So far 8 prenatal diagnosis have been performed.

P0630. No association between severe preeclampsia and a mutation in plasma platelet-activating factor acetylhydrolase (Val279Phe) in a Japanese population

S. Sohda, *N. Yamada, H. Watanabe, H. Hamada, K. Yamakawa-Kobayashi, T. Arinami*
University of Tsukuba; Tsukuba, Japan
sato@sohda.com
Platelet-activating factor (PAF), a phospholipid with a multiple actions that include thrombosis and inflammation, is immediately inactivated by a plasma enzyme, PAF acetylhydrolase (PAF-AH). Deficiency of this enzyme in plasma is caused by a missense mutation in the gene (Val279Phe). Surprisingly, 4% of the Japanese population lacks the extra cellular isoform of this enzyme, plasma PAF-AH, due to this mutation. However, the role of a deficiency of this enzyme caused by this mutation in the etiology of sever preeclampsia has not been determined. In this study, we investigated a possible association of this mutation with the risk of sever preeclampsia in the Japanese population. The allele frequency of Val279Phe mutation was 18.9% in 111 patients with sever preeclampsia and 21.0% in 188 healthy pregnant controls women (P = 0.60). The frequency of the homozygotes for the 279Phe allele was not significantly different between the patients and the control pregnant women (P = 0.75). These findings indicate that plasma PAF-AH deficiency due to Val279Phe mutation has no major effect on the etiology of sever preeclampsia in the Japanese population.

P0631. Molecular Basis of Cystic Fibrosis in Yugoslavia

D. Radivojevic[1], *M. Guc-Scekic*[1], *E. Kanavakis*[2], *E. Kanavakis*[2], *M. Tzetis*[2], *M. Tzetis*[2], *T. Antoniadi*[2], *T. Antoniadi*[2], *M. Djurisic*[1], *T. Lalic*[1], *J. Savic*[1], *P. Minic*[1]
[1]*Institute of Mother and Child Health Care Dr Vukan Cupic ; Belgrade, Yugoslavia;* [2]*University of Athens, 1st Department of Pediatric and Choremio Research Lab Unit of Molecular Medicine, St.Sophia s Children s Hospital; Athens, Greece*
pdarko@beotel.yu
Since the cloning of the cystic fibrosis (CF) gene, a great deal of information has been contributed by various centres worldwide. More then 800 mutations, together with their distributions and frequencies, have been identified in various populations. In order to determine the heterogeneity of mutations in Yugoslavian (YU) CF patients, we analyzed 244 CF chromosomes by PAGE, DGGE and direct sequencing of PCR-amplified genomic DNA. The major mutation deltaF508 accounts for 68,44% of CF chromosomes (167 CF alleles), whereas another 18 mutations account for 13,12% (32 CF alleles). Finally, 18,44% of YU CF chromosomes (45 CF alleles) remain uncharacterized. At present, we have identified 15 mutations that have frequency of less then 1%. That indicates the high degree of mutational heterogeneity in Yugoslavia. In this work, authors will discuss their results, comparing them with datas from other European populations.

P0632. Molecular analysis of 88 Iranian patients with Duchnne/Becker Muscular Dystrophies by Multiplex - PCR And RFLPs

S. Keradmand-Kia[1], *S. Zeinaly*[1], *H. Najmabadi*[2,3], *F. Pourfarzad*[2], *A. Mowjodi*[1], *D. Farhud*[4]
[1]*Biotech. Dept. Pasture Institute; Tehran, Islamic Republic of Iran;* [2]*University of Welfare and Rehabilitation; Tehran, Islamic Republic of Iran;* [3]*Karimi-Nejad Pathology and Genetics Center; Tehran, Islamic Republic of Iran;* [4]*School Of Public Healt, Tehran Medical University; Tehran, Islamic Republic of Iran*
maryamneishabury@yahoo.co.uk
Duchenne Muscular Dystrophy (DMD) and the milder allelic Becker Muscular Dystrophy (BMD) are X-linked disorders affecting 1 out of 3500 & 1 out of 18000 live male birth, respectively. Both DMD & BMD result from heterogeneous mutation in the dystrophin gene and in about 65% of the cases one or more exons of the gene are deleted or duplicated. One third of cases arise from new mutations & two third are familial. To analyze the prevalence of deletion in Iranian patients, a deletion screening was performed on a group of 88 unrelated DMD/BMD patients. Three set of multiplex PCRs were used to screen 18 exons of dystrophin gene. Deletions were detected in 56.8% of patients. Seventy four percent of deleted exons were located in the major hot spot region, whereas 26% were in the minor hot spot region. The most frequently deleted exons were exons 50, 48 & 47 (16.2%, 16.2% & 12% respectively). No deletion was detected in exon 43). The intragenic RFLPs analysis (pERT87.15/ BamHI & pERT87.8/ TaqI) were carried out on DNA samples obtained from 22 Iranian unrelat-

ed families (196 males & females) showing DMD & BMD clinical symptoms, that 45% of them had informative patterns. The percentage of heterozygosity was 22.75% for BamHI intragenic RFLP, and 22.75% for TaqI intragenic RFLPs.

P0633. Molecular Diangosis of Spinal Muscular Atrophy in Iran

S. Teimourian[1,2], *Y. Shafeghaty*[2], *G. H. Baba Mohammady*[3], *N. Almadany*[3], *M. Azad*[3], *S. Jalilnejad*[3], *R. Karimi-Nejad*[3], *H. Najmabadi*[2,3]
[1]*Institute of Biophysics and Biochemistry, Tehran University; Tehran, Islamic Republic of Iran;* [2]*University of Welfare and Rehabilitation; Tehran, Islamic Republic of Iran;* [3]*Karimi-Nejad Pathology and Genetics Center; Tehran, Islamic Republic of Iran*
maryamneishabury@yahoo.co.uk

All three types of autosomal recessive spinal muscular atrophy map to chromosome 5q12 and are associated with deletions or mutations of the SMNt gene. The availability of a test to distinguish between the SMNt gene and its nearly identical centromeric copy SMNc allows molecular diagnosis. We have analysed patients from 25 Iranian families for the presence or absence of a deletion in the SMNt gene. To compare the telomeric with centromeric portion, we have enployed method by (Chang et al). Our results show that in 23 family, (92%)of SMA patients exon 7 of SMNt has been deleted and in about 1 family (4%) of SMA patients exon 8 of SMNt has been deleted. For the remained patients the probability of mutations in SMNt gene exists. Prenatal diagnosis for 6 family was done and 2 affected 4 carrier 4 carrier and 2 normal were identified.

P0634. Deletion screening of dystrophin gene in Yugoslav DMD/BMD patients

T. N. Lalic[1], *M. Guc Scekic*[1], *M. Djurisic*[1], *D. Radivojevic*[1], *D. Zamurovic*[1], *S. Todorovic*[2]
[1]*Mother and child health institute dr Vukan Cupic ; Belgrade, Yugoslavia;* [2]*Institute for neurology and psychiatry for children and youth; Belgrade, Yugoslavia*
majkaidete@ptt.yu

To initiate the complete charcterization of mutations in the dystrophin gene in Yugoslav Duchenne/Becker muscular dystrophy patients, we screened 140 patients for the presence of deletions in the dystrophin gene, using multiplex PCR reaction for the simultaneous amplification of 17 deletion-prone exons and muscular promoter. Intragenic deletions were found in 75 of 121 patient with DMD (62%) and in 7 of 19 patients with BMD (36,8%). The majority of deletions were clustered in the 3 region of the gene (76,8%) while deletions in the 5 region were less abundant (18,3%). Loss of muscular promoter region was found in one patient and large deletions(more than 25 exons) were detected in 3 probands. This results will be further discussed and compared with the results from other populations.

P0635. Rare and New Beta thalassemia mutations in Iran

H. Najmabadi[1,2], *A. A. Poorfathollah*[3], *N. Amirizadeh*[1], *M. Neishabury*[1], *A. Hatamy*[1], *K. Javan*[1], *N. Amirizadeh*[1], *C. Oberkanins*[4], *W. Krugluger*[5]
[1]*University of Welfare and Rehabilitation; Tehran, Islamic Republic of Iran;* [2]*Karimi-Nejad Pathology and Genetics Center; Tehran, Islamic Republic of Iran;* [3]*Iran Blood Transfusion Services; Tehran, Islamic Republic of Iran;* [4]*Vienna Lab, Labrodiagnostika GmbH, A-1110; Vienna, Austria;* [5]*Institute of Clinical Chemistry, Rudolfstiftung Hospital; Vienna, Austria*
maryamneishabury@yahoo.co.uk

Ten years of widespread testing for beta-thalassemia mutations in Iran using amplification refractory mutation system (ARMS) and Reverse Hybridization, focused on 25 beta-thalassemia mutations, which have been assigned to cover the majority of mutations in the Iranian population. However, depending on the geographical location, beta-thalassemia mutations of 15% to 30% of the patients remained unidentified, indicating the presence of additional beta globin mutations in Iran. The aim of this study was the characterization of the molecular defect in a group of patients with unknown mutations. Over a 3 years period, a total of 70 DNA samples from unidentified beta-thalassemia cases, representing different geographical areas and ethnical groups, were collected from various prenatal diagnosis laboratories in Iran. DNA was subjected to sequence analysis and so far sequencing of 30 of these samples has been performed. Sequence analysis has reveald 2 new mutations not previously reported. These include a mutation in cd 67 T-G leading to a hemoglobin variant with a Val-Gly substitution and a possible beta-thalassemia mutation (T-C substitution at IVS2.840). Furthermore, 8 known mutations (cd 42/43 +T, cd 24/25 -GGT del, cd 82/83 -G del, -88 C-A, -87 C-G, cd 15-T, cd 39 +G and IVS-1/130 G-C) were identified, which can be added to the list of rare b-thalassemia mutations in Iran. These results further characterize the heterogenous spectrum of b-thalassemia mutations in Iran and will improve the abilities in prenatal diagnosis and carrier detection for b-thalassemia in order to prevent this prevalent disease in this country.

P0636. A Clinical Experience On Prenatal Diagnosis Of Down s Syndrome by FISH Using Alu-PCR Amplified YAC Clones In Uncultured Amniocytes

S. Mohaddes Ardebili
Tabriz University of Medical Sciences; Tabriz, Islamic Republic of Iran
Mohaddes@tbzmed.ac.ir

In Fluorescence In Situ Hybridisation (FISH) experiments, the hybridisation efficiency is generally less than satisfactory when total yeast genomic DNA isolated from pulsed-field gels is used as probes. to overcome these problems Alu-PCR products of a chromosome 21-specific YAC, 831B9 was used to detectd the number of chromosome 21 copies on uncultured lymphocytes and amniocytes by FISH. In all of the experiments carried out using the 831B9 YAC clone, strong signals were produced. signal intensities were comparable to those observed using chromosome-specific alphoid DNA probes. Ninety percent or more of the randomly elevated nuclei from uncultured blood cells and 86% or more of the uncultured amniocytes showed two distinct signals, levels which compare favourably with similar studies in this field. Our results obtained from application of the technique on 1000 peipheral blood samples and more than 550 amniotic fluid samples suggests that this approach can be reliably used for prenatal and postnatal detection of chromosome 21 aneuploidy.

P0637. CGG Repeats Analysis of the FMR1 Gene in Mental Retarded Individuals with Clinical Symptoms of the FragileX syndrome in Iranian Population

F. Taghizadeh[1], *S. S. Hosseini Amini*[1], *S. Teimourian*[1], *R. Karimi-Nejad*[1], *Y. Shafeghati*[2], *M. H. Karimi-Nejad*[1], *H. Najmabadi*[2,1]
[1]*Karimi-Nejad Pathology and Genetics Center; Tehran, Islamic Republic of Iran;* [2]*University of Welfare and Rehabilitation; Tehran, Islamic Republic of Iran*
maryamneishabury@yahoo.co.uk

FragileX syndrome is considered as the most common form of inherited mental retardation, that is dominantly inherited in males as an x-linked dominant trait. This syndrome is known to be the result of a dynamic mutation at the 5 UTR (Untranslated Region) of the FMR1(FragileX Mental Retardation 1) gene. The number of CGG repeats in this area is amplified to more than 200 repeats resulting methylation of CpG islands and decrease or absent of FMR1 expression in affected individuals. The result of this methylation is the absense of FMRP (FragileX Mental Retardation Protein) that plays an important role in development of brain neurons. The clinical phenotypes in males consist of mental retardation, special somatic features and macroorchidism. For the first time in Iran we used PCR (Polymerase Chain Reaction) and southern blot analysis for detection of the fragileX mutation. DIG-labeled probes were used for the detection of bands in the southern blot analysis(No radioactive materials were used in this study). 180 individuals from 127 families with at least one mentally retarded child were examined, 79 cases had a full mutation(FM), 10 with a premutation(PM) and 91 were normal(N). In prenetal diagnosis that was performed for 8 fetuses from these families 2 normal males, 1 normal females, 3 fullmutation males and 2 fullmutation females were detected

P0638. Mutation Detection and Prenatal Diagnosis in Iranian §-Thalassemia Traits

D. D. Farhud[1,2], *P. Derakhshandeh*[1,2], *H. Najmabadi*[3], *M. Zakernia*[4], *M. Haghshenas*[5], *S. Kheradmand*[2], *A. Attariani*[2]
[1]*Human Genetics, School of Public Health, Tehran Univ. of Medical Sciences; Tehran, Islamic Republic of Iran;* [2]*Genetic Clinic, Valie-Assr Sq. 16; Tehran, Islamic Republic of Iran;* [3]*Genetic Research Center, Welfare and Rehabilation University Tehran; Tehran, Islamic Republic of Iran;* [4]*MRI Center; Shiraz, Islamic Republic of Iran;* [5]*Dept. of Hematology, Shiraz Univ. of Medical Sciences; Shiraz, Islamic Republic of Iran*
pupak-peykar@yahoo.com

§-Thalassemia is the most frequent single gene defect in the world (1). Nearly 25000 indivdual with b-Thalassemia major living in Iran. Over 150 mutations have been reported sofar.

Mutation detection in b-globin gene: The mutation spectrum of §-Thalassemia in Iran is very heterogenously, because of different ecological, ethnical and geographical feature of the population, therfore the mutation analysis was carried out in five main provinces, north, south, west, east and central.

During the last nine years, 720 individuals with b-Thalassemia have been diagnosed in the Genetic Clinic in Tehran.

85 % of the patients have been screened for 21 common §-globin gene mutations in Iran, by directed PCR-ARMS methods (2, 3) and 12 % of

other patients by nine RFLP systems (4). For 3 % of the patients , it was not possible to determine the mutations of both alleles.
43.3 % of the total marriages have been first or second cousin , out of these consanguineous marriages, 74.35% had the same mutations.
Prenatal diagnosis: Out of 305 CVS and amniotic fluid from 610 patients (84.7%) used for prenatal diagnosis (5), 26.1 % were normal for §-globin gene , 48,9 % trait (Heterozygote) and 25 % major §-Thalassemia.
A comparision between frequency of § globin gene mutations: Results have shown, that IVSII-1 mutation is the most frequent type in Iran (49%), 52.6 % of which is distributed in northern provinces, specially on the Caspian Sea (4), as the first most frequent mutation in §-globin gene. The lowest in centeral provinces (14.0 %) and again higher in the southern provinces (28 %).
IVSI-1 and C39 are most frequent mutations in southern provinces specially on the persian golf region.
Other frequent mutation in Iran are as follows ;
IVSI-5 (9 %), Fr 8-9 (6 %), C30 (5 %), IVSI-25 (5 %), IVSI-110 (5 %)., C44(4 %), IVSI-1(4 %), C39 (4 %), IVSI-745 (3%) , FR 36-37 (3 %), C8 (2 %), IVSI-6 (1 %), Fr41-42, Fr16, -88, C15, IVSI-130 (1 %).
b-Thalassemia gene flow: The mutation spectrum of the b-Thalassemia in Iran should be compared with the mutations of the neighbouring countries. Therefore it was necessary to study in a north-south,- west-east- §-globin gene flow in Iran. It shows the following results;
The frequencis of IVSII-1 is decreased from north to south ,while the frequencies of Fr 36-37, C39 and IVSI-1 Show an opposite direction.
The frequency of C44 and C30 decreased from west to east and the frequency of the IVSI-1 increase from west to east.
IVSI-1 as a splice junction mutation (C >T) is a prevalent mutation of Asian,Indian and Chinese (South -east countries) (7).
The frequency of the Fr 36-37 from the west to east of Iran is the same.

P0639. Carrier Analysis and prenatal diagnosis in haemophilia A families of north India by RFLP and STR markers.

G. S. Pandey[1], *S. R. Phadke*[2], *B. Mittal*[2]
[1]*Sanjay Gandhi Post Graduate Institute of Meducal Sciences; Lucknow, India;* [2]*Sanjay Gandhi Post Graduate Institute of Medical Sciences; Lucknow, India*
gspandey@sgpgi.ac.in

Haemophilia A is an X-linked recessive genetic disorder affecting 1/5,000-10,000 males, resulting from mutations in factor VIII gene. Mutational heterogeneity causes difficulties in identification of mutations. Carrier analysis and prenatal diagnosis is possible by DNA linkage analysis using intragenic factor VIII gene linked DNA markers. In order to find out informativeness of STR markers (intron 13 and 22) in Indian population, DNA samples from 50 normal females were amplified by PCR followed by separation in denaturing sequencing gel. Allele sizing was carried out by comparing the bands with pUC sequencing ladder in the same gel. Heterozygosity of RFLP marker was studied in 60 normal females each for Hind III and Bcl I by PCR followed by restrction digestion analysis. Observed heterozygosity for intron 13 STR was 60% (29/50) and for intron 22 STR was 40% (21/50) respectively. Number of alleles seen for intron 13 and 22 were 5 and 4 respectively. Observed heterozygosity for RFLP markers for Hind III and Bcl I were 63% (38/60) and 60% (36/60) respectively. Both STR and RFLP markers were used in carrier analysis for 35 haemophilia A families and 3 prenatal diagnosis were performed. In order to cover 100 percent families, direct detection of intron 22 inversion mutation is under progress.

P0640. Thrombophilia in Neonatal Stroke

C. J. Curry[1], *J. W. Holmes*[1], *E. R. Roeder*[1], *A. L. Storm*[1], *J. Wall*[2]
[1]*Genetic Medicine,Valley Children s Hospital/UCSF; Madera, CA United States;* [2]*Molecular Diagnostics,University of California; San Francisco, CA United States*
ccurry@valleychildrens.org

Neonatal stroke (NS) accounts for about 25% of all childhood stroke and is an incompletely ascertained cause of childhood death, mental retardation, seizures and cerebral palsy. Factor V Leiden (FVL) and other thrombophilic factors in the child have been implicated in causation but maternal factors have not been systematically evaluated. Between 1998-2000 we evaluated 32 patients with NS looking at thrombophilic factors in the mother and child as well as multiple clinical characteristics.Patients were ascertained in clinical settings and ranged in age from newborns to 10 years. NS was defined on the basis of history and cranial imaging;CT(20), MRI(3), both(8), and autopsy(1). Global ischemic injury and watershed infarcts were excluded. Mutations studied included FVL, Prothrombin 20210 (PRO), methylenetetrahydrofolate reductase 677 (MTHFR) and, in most cases, Protein C, S, and Antithrombin III activities, anticardiolipin antibodies (ACA) and lupus anticoagulant(LAC). Testing was performed on 27 mother/child pairs, 4 child only and one mother only. Overall, 17/32 (53%) of NS were found to have one or more abnormalities in mother, child or both, with a total of 25 abnormalities detected. In 6/31 children (19%) we found 2 with FVL heterozygosity(H), 2 with MTHFR homozygosity(HZ), 3 with PRO(H) and one with ACA. In 13/28 mothers (46%) we found 3 with FVL(H), 4 with MTHFR(HZ), one with PRO(H), 6 with ACL, one with LAC and 2 with confirmed type III Protein S deficiency. Four mothers and one child had combination defects. Only one mother/child pair were concordant for the same defect. Our data suggest that maternal thrombophilia may be at least as important as these defects in the fetus. Mechanisms to explain the apparent role of maternal thrombophilia may include chronic fetal hypoxemia from placental dysfunction, for which we have some anecdotal evidence, functional breakdown of the placental barrier via thrombosis and infarction and other undoubtably complex maternal-fetal interactions. A complete evaluation for thrombophilia in both child and mother is indicated in NS. Identification of maternal factors is critically important for implementation of prevention strategies. Additional studies, including the availability of population-based controls and placental evaluations are needed to confirm and better understand our observations.

P0641. Beckwith Wiedemann; Further Prenatal Characterization of the Syndrome

O. Reish[1], *I. Lerer*[2], *A. Amiel*[3], *D. Abeliovich*[2]
[1]*Genetic Institute, Assaf Harofeh Medical Center; Zerifin, Israel;* [2]*Genetic Institute, Hadassah Medical Center; Jerusalem, Israel;* [3]*Genetic Institute, Meir Medical Center; Kefar Saba, Israel*
oreish@post.tau.ac.il

Purpose; To further characterize the prenatal findings in Beckwith Wiedemann Syndrome (BWS). Methods; Two patients had prenatal ultrasonic anomalies suggestive for BWS. Cytogenetic analysis and PCR amplification of polymorphic loci mapping to 11p15.5 region were used. Results; Initial amniotic karyotype was normal in both. Postnatal evaluation revealed partial trisomy of 11p15.5 due to paternal translocation in one and paternal uniparental disomy (UPD) in the second patient. Conclusions; The described ultrasonic findings should suggest the syndrome. Prenatal PCR analysis for 11p15.5 region in addition to conventional cytogenetic analysis is suggested to detect potential paternal UPD or allelic duplication.

P0642. Study of p53 tumor suppressor gene in spontaneous abortions

D. Toncheva[1], *B. Zaharieva*[1], *L. Georgieva*[1], *A. Savov*[1], *S. Atanassova*[1], *A. Markoff*[2], *I. Kremenski*[3]
[1]*Medical University Sofia; Sofia, Bulgaria;* [2]*Institute fur Medizinishche Biochemie, ZMBE, Westfalische Wilhelms-Universitat; Munster, Germany;* [3]*Laboratory of Molecular Pathology, University Hospital Maichin dom ; Sofia, Bulgaria*
draga@sofiaonline.com

We suggested that p53 is a good candidate gene to be associated with spontaneous abortions due to the fact that p53 inactivation could lead to accumulation of genome imbalances and miscarriages. For the first time mutation screening of exons 4 to 9 of p53 gene was carried out in 31 spontaneous and 30 voluntary abortions. SSCP shift was detected in exons 6 and 9 only in the spontaneous abortions analyzed (23.3%). The DNA sequencing in 1 out of the 7 samples with changed SSCP mobility revealed misscence mutation GAC to GGC in codon 260 leading to Asp->Gln substitution near to the so could hot-spot region (third domain), caracterized by high mutability rate. In two patients the sequencing of exon 6 revealed neutral polymorphism g13399 A>G, in two - polymorphism in intron 9 IVS9nt+12T>C, and in two - normal sequence. Our results of the p53 mutation screening are the first data supporting the hypothesis of possible role of p53 mutation in spontaneous abortions in human.

P0643. Maternal UPD 20 in an Infant from a Pregnancy with Mosaic Trisomy 20

C. Hoppe[1], *F. von Eggeling*[1], *U. Claussen*[1], *S. N. MacGregor*[2], *I. S. Salafsky*[3]
[1]*Institute of Human Genetics and Anthropology; Jena, Germany;* [2]*Division of Maternal and Fetal Medicine, Evanston Hospital, Northwestern University Medical School; Evanston, IL United States;* [3]*Division of Genetics, Evanston Hospital, Northwestern University Medical School; Evanston, IL United States*
fegg@mti-n.uni-jena.de

Uniparental disomy (UPD) has been reported for the majority of human chromosomes. In most cases this is thought to arise from trisomic or monosomic rescue of an aneuplodic cell line. Adverse phenotypic effects have been documented for chromosomes 6, 7, 11, 12, 14, 15 and 16. But there are only two reports in the literature of UPD 20 with abnormal phenotypic

effects. Here we present the first case of a maternal uniparental disomy (UPD) 20 with normal karyotype. It was found in a 35-month old girl, the product of a pregnancy complicated by a prenatal diagnosis of mosaic trisomy 20. Phenotypic abnormalities included pre- and postnatal growth failure, microcephaly, minor dysmorphic features and psychomotoric developmental delay. Chromosomal analysis on cord blood revealed only an apparently normal 46, XX karyotype. Maternal UPD was confirmed by microsatellite analysis of 27 chromosome 20 loci, whereas 11 markers were found to be informative. Maternal heterodisomy was detected in two and maternal isodisomy in three loci. In the remaining six loci a noninformative maternal UPD was displayed. The change from iso- to hetero- to iso- to heterodisomy in four segments can best be explained by two recombination events. Whether this UPD is due to a meiosis I or meiosis II error cannot be decided, because the recombination creates two partial isodisomies. A meiosis I error seems to be more likely as the microsatellites located at the centromere display a heterodisomy. This case suggests that a search for UPD in pregnancies complicated by a prenatal diagnosis of mosaic trisomy 20 would be desirable.

P0644. Allele Frequency of the Uncoupling Protein-1 Bcl I Polymorphism and the TRP64ARG Variant of the §3-Adrenergic Receptor in SIDS Victims

A. Fatemi[1,2], *C. Item*[2], *S. St ckler-Ipsiroglu*[2], *O. Ipsiroglu*[2], *W. Patsch*[3], *W. Sperl*[4], *W. Strobl*[1]
[1]*Dept. of Medical Chemistry; Vienna, Austria;* [2]*Dept. of Pediatrics; Vienna, Austria;* [3]*Dept. of Laboratory Medicine, LKA; Salzburg, Austria;* [4]*Dept. of Pediatrics, LKA; Salzburg, Austria*
ali.fatemi@akh-wien.ac.at

Disturbances of thermoregulation in brown adipose tissue (BAT) have been postulated as a cause of sudden infant death syndrome (SIDS). The human uncoupling protein-1 (UCP-1), expressed in BAT, dissipates the trans-mitochondrial proton gradient as heat and plays a central role in energy homeostasis and thermogenesis. The UCP-1 activity is regulated by the §3-adrenergic receptor. A Bcl I polymorphism at -3826 relative to the transcription start site of the UCP-1 gene and a variant of the §3-adrenergic receptor (Trp64Arg) are associated with alterations of energy homeostasis and with reduced UCP-1 mRNA. To determine, whether the UCP-1 Bcl I polymorphism is associated with the occurrence of SIDS, we extracted DNA from Guthrie cards of 53 Austrian SIDS victims and 54 controls. The clinical characteristic of the majority of the SIDS patients have been reported previously. A 350 bp fragment of the UCP-1 promoter was amplified by nested PCR and digested with Bcl I. We found that the allelic frequencies for the UCP-1 Bcl I polymorphism did not differ between the SIDS group (0.65/035) and the control group (0.72/0.28, chi 2; 1.26, p; 0.26). Our data do not support an association of the UCP-1 Bcl I polymorphism with SIDS. Considering the limited number of subjects studied and the heterogeneity of SIDS, however, a role of UCP-1 in the pathogenesis SIDS can not be ruled out. The role of the of the §3-adrenergic receptor, which is involved in UCP gene regulation, is under investigation.

P0645. The Fragile X Syndrome; Screening And Prenatal Diagnosis

W. T. Brown, *S. Nolin, C. Dobkin, E. Jenkins*
New York State Institute for Basic Research; Staten Island, NY United States
WTBIBR@AOL.COM

Fragile X syndrome is the most common inherited form of mental retardation. Its molecular basis is usually an expansion of a repetitive CGG triplet sequence located in the 5 untranslated region of the fragile X gene, FMR1. Because of its prevalence and medical importance, efficient means for accurate diagnostic screening and prenatal testing are needed. Polymerase chain reaction (PCR) is rapid and requires little DNA. We have carried out screening and prenatal diagnosis for the fragile X syndrome using a PCR protocol we have developed that accurately resolves normal alleles as well as detects premutations and most full mutations (>90%). Follow-up Southern blotting is carried out on prenatal samples and other selected samples. Testing of 2095 males with developmental delay of unknown etiology revealed 61 (3%) were positive for fragile X. Our prenatal experience now includes screening of 624 pregnant women, who had a family history of mental retardation of undetermined etiology. We have found 12 of the women to be carriers; 3 full mutations, 3 premutations and 6 with unstable borderline alleles. Two additional fragile X families were identified although the women were not carriers. Thus, 2.7% (14/624) of women were carriers or came from fragile X families or had unstable alleles. Additionally, 25 carriers (40%) with prior unknown carrier status were identified among 62 pregnant members of previously identified fragile X families. Prospective prenatal testing of 230 carrier women correctly detected 98 fetal samples with full mutations and 16 with premutations. Follow up information on all samples obtained so far, including approximately 30% of the terminated products of conception, indicates no false positive or negative results. Maternal cell contamination has presented problems in 3 cases. Thus, highly reliable screening of at-risk pregnant women for fragile X status and subsequent prenatal diagnosis is now possible.

P0646. Measure of X-inactivation using a Methyl-PCR method; Prenatal study in a case with Xq terminal deletion and diaphragmatic hernia

R. L. Touraine, *F. Prieur, V. Adouard, B. de Fr minville, B. Lauras*
CHU de Saint Etienne; Saint Etienne, France
renaud.touraine@univ-st-etienne.fr

Classical methods to measure X-inactivation require the use of methylation-sensitive restriction enzymes and either Southern blot or PCR analysis. Numerous technical drawbacks can limit the utilization of these methods. We developed an original method using methyl-PCR. We illustrate the use of this method with the case of a malformed female fetus diagnosed prenatally with a deletion of one chromosome X. A young woman was referred to us because of diaphragmatic hernia diagnosed in her fetus at 22 weeks of amenorrhea. This was the first child of unrelated parents originating from Algeria. Familial medical history was unremarkable. No abnormalities were diagnosed at the previous ultra sound examination done at 12 weeks. Fetal karyotype analysis done on amniotic cells showed a terminal deletion of the long arm of one chromosome X in a girl; 46,X,del(X)(q27). Karyotype analysis was normal for both parents. Considering the de novo nature of this chromosomal abnormality, we worried about its role in the malformation observed. We therefore studied the fetus X-inactivation status. X-inactivation was measured with our methyl-PCR method at 3 loci, namely, FMR1, FMR2 and the Androgen Receptor (AR) genes, corresponding respectively to Xq27.3, Xq28 and Xq11.2. First, we confirmed the fetus hemizygoty at the FMR1 and FMR2 genes loci, the fetus having 2 alleles at the AR gene locus. Second, we established that X-inactivation was almost completely skewed with the normal chromosome X always active. This was further supported by a normal Iduronate Sulfatase activity in cultured amniotic cells. We concluded rather unlikely any causative link between the chromosomal abnormality and the diaphragmatic hernia in that fetus. Ultra sound examination follow-up was favorable and pregnancy was continued. Soon after delivery, at 32 weeks, diaphragmatic hernia was surgically cured. No other malformation was observed in this baby weighting 1920g. Preferential inactivation of the deleted chromosome X was confirmed postnatally in different tissues. Methyl-PCR represents an easy and rapid method to measure X inactivation, requiring a limited amount of DNA and without the inconvenient use of methylation-sensitive restriction enzymes.

P0647. Rapid Detection Of Common Aneuploidies And Zygosity Determination By Fluorescent Multiplex Polymerase Chain Reaction

C. F. Rodrigues, *P. M. Jorge, M. R. Santos*
Instituto de Gen tica M dica Jacinto Magalh es; Porto, Portugal
carina.rodrigues@ijm.min-saude.pt

We report the results of a study using fluorescent polymerase chain reaction (F-PCR) of polymorphic tandem repeat markers, for rapid foetal sexing, detection of common aneuploidies and determination of zygosity, in a total of 203 pregnancies. Indications included X-linked disorders, anomalies detected by ultrasound scanning, advanced maternal age combined with other risk factors and twinning. Testing was carried out directly on 1.5 ml amniotic fluid (n=199). Three analyses were done on chorionic villi, and one on a failed amniocyte culture. Whenever possible samples from both parents were collected on filter paper. A previously described combination of markers for chromosomes 21, 13, 18 and X was used, in addition to others for chromosomes 18, 21, Y and the X-linked dystrophin gene.During the initial pilot study, cultured amniocytes from 10 Down syndrome cases were used as controls. Nineteen foetuses were correctly diagnosed with aneuploidies involving chromosomes 21 (n=12), 13 (n=2), 18 (n=2) and X (n=3). Markers were uninformative in 4 positive cases (as confirmed by cytogenetic analysis). Five, in a total of 16 sets of twins, were monozygotic. A case of Down syndrome was detected in one twin of a dizygotic pregnancy. In the total of samples studied, 98% were informative, thus allowing the exclusion of common aneuploidies in 175 foetuses within 24-48 hours. This test also allowed rapid foetal sexing whenever necessary and rapid determination of zygosity which is advantageous essentially in like-sex twins with uncertain chorionicity.

P0648. Prenatal Diagnosis of Beta-thalassemia in Iranian Population, Results of Ten Years Study

F. Sahebjam[1], *R. Karimi-Nejad*[1], *S. Teimourian*[1], *M. Karimi-Nejad*[1], *H. Najmabadi*[2]
[1]*Karimi-Nejad Pathology and Genetic center; Tehran, Islamic Republic of Iran;* [2]*GeneticsResearch Center, Welfare Sciences and Rehabilitation University; Tehran, Islamic Republic of Iran*
sahebjam@MAILANDNEWS.COM

Beta-thalassemia is the most common genetic disorder in Iran. It is estimated that at least 25,000 affected patients and 2 million carriers live in Iran. Therefore prenatal diagnosis is at present a primary goal for prevention. As a first center, 10 years ago we started mutation screening and prenatal diagnosis of §-thalassemia in Iranian families. During 10-year period we have made prenatal diagnosis for 314 cases (178 amnion samples and 136 CVS samples). We used a 23 primers-based panel for diagnosing the mutations of the parents and samples. Using this panel along with RFLF we were able to provide a reliable prenatal diagnosis for over 95% of pregnancies. Out of these 314 cases, 81 (26%) cases were normal, 147 (47%) cases were heterozygotes for a single Beta-thalassemia mutation, and 72 (24%) cases were either homozygotes or compound heterozygotes. In 9 samples we could not detect the mutations. Our data showed a very close Mendelian distribution as expected for a Mendelian type mode of inheritance. The frequency of detected mutations also will be discussed.

P0649. Molecular Analysis of Neurofibromatosis Type 1 in Turkish Families Using Polymorphic Markers

S. Oguzkan, *M. Cinbis, S. Ayter, B. Anlar, S. Aysun*
Hacettepe University, School of Medicine; Ankara, Turkey
soguzkan@yahoo.com

Neurofibromatosis type 1 (NF 1) is a common autosomal dominant disorder characterized by multiple neurofibromas, caf -au-lait spots, and Lisch nodules of iris. NF1 gene is located at chromosome 17q11.2 and encodes an mRNA containing 60 exons.The NF1 gene product neurofibromin is a large protein of 2818 amino acids. The protein acts as a negative regulator in the ras signal transduction pathway. Diagnosis of NF1 is based on established clinical criteria. The disease has a high mutation rate and wide range of expression most patients are expected to have different mutations, so molecular analysis and genetic counselling is limited to the identification of the specific mutation in each patient or family. This situtation means that genetic counselling has to rely mainly on indirect diagnosis using linked markers. We have analysed 10 Turkish families for a total of 27 affected individuals and 34 non-affected relatives with the use of polymorphic sequences, 4 intragenic and 5 flanking markers. We have started diagnosis by using extragenic RFLPs (pHHH 202, p11.3C4.2, pEW206, pEW207, p2F9.8). Then the use of intragenic microsatellit markers (EVI20-CA repeat, Alu-AAAT repeat, IVS38GT53.0-CA/GT repeat, IVS27AC28.4-AC/TG repeat) has increased the informativity in our series of NF1 families. As a result, we can provide the prenatal and the presymtomatic diagnosis to these families.

P0650. Prenatal testing for Huntington s disease - a description of tests accomplished from 1993 - 1998; a European collaborative study

S. A. Simpson[1], *M. Zoeteweij*[2], *G. Evers-Kiebooms*[3], *K. Nys*[3], *P. S. Harper*[4], *A. Durr*[5], *G. Jacopini*[6], *C. Yapijakis*[7]
[1]*Grampian University Hospitals Trust; Aberdeen, United Kingdom;* [2]*Clinical Genetics Centre; Leiden, The Netherlands;* [3]*Centre for Human Genetics; Leuven, Belgium;* [4]*Institute of Medical Genetics; Cardiff, United Kingdom;* [5]*INSERM-Unite 298; Paris, France;* [6]*Istituto di Psicologia; Rome, Italy;* [7]*Department of Neurology; Athens, Greece*
s.a.simpson@abdn.ac.uk

This poster will describe the experience of 6 countries participating in a multidisciplinary study of prenatal tests in pregnancies at risk of Huntington s disease. Additional data will also be presented from 7 centres within the 6 countries. A total of 305 tests were performed. The average age of the prospective parent at risk of Huntington s disease (HD) was 30.8 years, and 53.9% of this group were female. 57.4% of the prospective parents were known to have the gene for HD, whereas the others had not undergone predictive or diagnostic testing. 65.2% of the pregnancies were investigated using mutation analysis, the remainder by exclusion testing. Exclusion testing allows the at risk prospective parent to have children at negligible risk of HD, without defining accurately their own risk, as it shows whether the fetus has inherited the at risk chromosome 4 from the affected grandparent. If so, then the fetus shares the 50% risk of the prospective parent, if not, then the fetus is at negligible risk. 43% of the total tests gave an unfavourable result. 8 of these high risk pregnancies were continued. Data from the 7 participating centres will be given which describe in more detail 202 of the 305 tests. These data also describe 114 pregnancies in 41 couples who had 2 or more prenatal tests (range 2 - 5 tests). Although prenatal testing is used by the minority of those at risk of HD, it is clear from this study that both direct testing for the HD mutation and exclusion testing are valuable tools in preventing transmission of HD for individuals who belong to an HD family.

P0651. Bayesian risk of cystic fibrosis in fetuses with echogenic bowel reported from a eight-year experience of prenatal screening performed in Brittany (France), where the disease is frequent

V. Scotet[1], *M. De Braekeleer*[1], *M. P. Audr zet*[1], *I. Qu r* [1], *B. Mercier*[1], *I. Dugu p roux*[1], *J. Andrieux*[1], *M. Blayau*[2], *C. F rec*[1]
[1]*CHU, UBO; Brest, France;* [2]*CHU; Rennes, France*
virginie.scotet@univ-brest.fr

Echogenic bowel has been associated with an increased risk of cystic fibrosis (CF). The situation is particularly difficult when one CF mutation is detected in an echogenic bowel fetus. We determined the residual risk of CF in such fetuses, from the experience performed in Brittany, a region where the disease is frequent. A total of 113 pregnant women were referred for a prenatal diagnosis of CF based on an initial analysis of three exons of the gene (87% mutation detection rate) followed by an extended analysis when only one mutation was detected (98.5% detection rate). Among those 113 fetuses, 11 were CF and 10 heterozygous. The incidence of CF (9.7%) and of heterozygosity (8.8%) were significantly higher than expected ($p<0.001$). We used Bayesian calculations to estimate the residual risk of CF, which depends on CF incidence, on carrier rate and on mutation detection rate. Assuming a carrier rate of 8.8% (1/11), the residual risk was 1/501 when no mutation was detected by our protocol and 1/4.6 when one mutation was identified. The complementary analysis performed in this last case, which leads to a mutation detection rate of 98.5%, enables to reduce this risk to 1/32. Our results are of first importance for clinicians who order testing for CF following the ultrasound diagnosis of echogenic bowel. They also highlight the importance of efforts made to identify a second mutation in heterozygous fetuses, what enables to reduce the residual risk of CF.

P0652. Quantitation of fetal DNA in maternal serum in normal and aneuploid pregnancies

N. Miharu, *Y. Ohashi, O. Samura, H. Honda, K. Ohama*
Department of Obstetrics and Gynecology, Hiroshima University School of Medicine; HIroshima, Japan
miharu@mcai.med.hiroshima-u.ac.jp

We investigated whether the amount of circulating cell-free fetal DNA in maternal serum is influenced by fetal karyotype, using real-time quantitative polymerase chain reaction (PCR) assay. Serum samples were obtained from pregnant women at week 15 to 17 of gestation, prior to undergoing amniocentesis. We examined 70 samples in total, consisting of 55 pregnant women with 46,XY, 5 cases with 47,XY,+21, 3 cases with 47,XY,+18, a single case with 46,XY,dup(1) and 2 cases with twins of 46,XY, and 4 cases with 46,XX which were used as negative controls. We measured the concentration of the SRY sequence as a molecular marker for fetal DNA. The SRY sequence was detectable and measurable when the fetuses were male except for one case with 47,XY,+18. This case showed fetal growth retardation and bradycardia. No amplification signals of the SRY sequence were detected when the fetuses were female. The mean concentration of fetal DNA in maternal serum was 31.5 copies/ml in the pregnancies with 46,XY, 23.5 copies/ml in the pregnancies with 47,XY,+21 and 21.5 copies/ml in the pregnancies with 46,XY,+18. No significant difference in the concentration of fetal DNA was observed +between pregnancies with fetuses of normal karyotype and those with fetuses of abnormal karyotype.

P0653. Prenatal diagnosis using fetal cells and free fetal DNA in maternal blood

S. Hahn, *X. Zhong, L. Dudarewicz, C. Carrillo, W. Holzgreve*
University of Basel; Basel, Switzerland
shahn@uhbs.ch

Our group has previously shown that fetal erythroblasts can be enriched for from maternal blood using magnetic cell separation (MACS), by which means we were able to identify fetal aneuploidies before an invasive procedure. This aspect is currently being investigated in the large scale NIH funded NIFTY study, in which our group is participating. We have recently extended upon these early observations by examining the possibility of determining fetal genetic loci by single cell PCR in individual erythroblasts isolated by micromanipulation. This study, which for convenience, focused on the fetal rhesus D and SRY loci, indicated that it is possible to determine multiple fetal loci with a high degree of accuracy by these means. This

aspect has been confimred in a further collaborative study, in which we examined for fetal hemoglobinopathies. We have also intensely pursued the recent finding of free fetal DNA in maternal plasma. By using the same sensitive PCR assay that we have established for the analysis of single fetal cells, we have shown that multiple fetal loci can be reliably determined using free fetal DNA. In order to optimise this assay for a possible clinical setting, we have recently developed a multiplex realtime PCR assay, as this methodology is more amenable to automation. By the use of such real-time quantitative PCR assays, we have also made the interesting finding that free fetal DNA levels are elevated in pregnancies with trisomy 21 fetuses, but not in those with trisomy 18 fetuses. This result, which confirms and extends upon a report made by Lo and Bianchi, could form the basis of a new additional screening assay for the detection of fetal aneuploidies.

P0654. 5 Cases of low hCG in 2 nd Trimester Screening, which resulted in Chromosomal Abnormalities.

M. Simandlova[1], *M. Havlovicov* [1], *M. Mal kov* [1], *V. Krut lkov* [1], *S. Vejvalkov* [1], *A. Puchmajerov* [1], *M. Macek*[1], *E. Koc rek*[1], *D. Novotn* [1], *E. Seemanova*[1], *P. Goetz*[1], *E. Kulovan?*[2], *J. Marek*[3], *R. Kodet*[4], *V. Dudkov* [5]
[1]*Institute of Biology and Medical Genetics of 2nd Medical Faculty, Charles University Hospital; Prague, Czech Republic;* [2]*Dpt. of Obstetrics and Gynaecology, 2nd Medical Faculty, Charles university; Prague, Czech Republic;* [3]*Centre of Pediatric Cardiology and Cardiac Surgery, 2nd Medical faculty, Charles university; Prague, Czech Republic;* [4]*Dpt. of Pathology of 2nd Medical Faculty, Charles University; Prague, Czech Republic;* [5]*Dpt. of Radioisotopy, Homolka Hospital; Prague, Czech Republic*
martina.simandlova@lfmotol.cuni.cz

Low HCG in the triple test is ussually associated with low uE3 and together indicate higher risk for Edwards or Patau syndrome, triploidy or pregnancy with Turner syndrome without hydrops. hCG can be also lowered in some cases of Smith-Lemli-Opitz syndrome (estriol is low more often) and in pregnancy with bad course (i.e. missed abortion). We present five cases of low hCG associated with chromosomal abnormalities. In the fist there was low hCG (0,35 MoM) and high AFP (2,41 MoM), in the karyotype was 5,45 %mosaicism for trisomy 8. In the second case hCG was 0,31 MoM, uE3 0,42 MoM, risk for M. Edwards 1;19, karyotype 46,XY. Immediately after birth critical defect led to the defect of this newborn baby, FISH analysis confirmed 22 q 11.2 microdeletion. In the third case hCG was 0,35 MoM, there was evaluated duodenal obstruction by US investigation in the 32nd week of pregnancy, karyotype was 47,XXY. In the forth case hCG was 0,23 MoM, karyotype was 47, XYY. In the last case hCG was 0,23 MoM, echokardiogram showed cardiac failure, fetal ultrasound investigation described ren arcuatum, karyotype was 46,XX. By the method QFPCR we proved partial trisomy of the 18 th chromosome, TOP was provided. Conclusions; We recommend better control of pregnancies with low hCG, detailed ultrasound and echocardiography investigation of the fetus, karyotyping of amniocentesis eventually. There is about 8% pregnant woman with hCG bellow 0,5 MoM, therefore we stress the necessity of each evaluation individiually.

P0655. Amniotic fluid alpha fetoprotein difference between twin pairs; does zygosity or gender have an impact?

R. Sharony, *A. Amiel, Y. Grinshpun-Cohen, S. Markov, M. D. Fejgin*
Sapir Med Ctr, The Genetic Inst; Kfar Saba, Israel
sharonyr@netvision.net.il

Objectives;To examine the assumption that AFAFP levels are different in female and male fetuses. To look for a possible difference in the levels of AFAFP between monozygous and heterozygous twins. Since the production of AFAFP is similar in monozygous twins the levels of AFAFP should reflect these changes as well. Design;Comparison of the levels of AFAFP between pregnancies with female and male fetuses in gender-concordant and gender-disconcordant twin. In addition, AF levels of AFP in monozygotic and heterozygotic twins were compared. A t-test of P< 0.05 was considered significant. Material and Methods;Between 1995-1999 we performed 201 amniocenteses on twin pregnancies at Meir Hospital, Kfar Saba, Israel. One hundred and nine of them were concordant for gender (62 females and 47 males) while 92 had a different sex. Amniotic fluid AFP levels of each sac were measured using fluorescent immunoassay methods. Results;There was no significant difference in the levels of AFAFP in female compared to male twins. Average AFAFP was lower in female twins compared to their male counterpart (p=0.07). Nevertheless, there was no difference between AFAFP of male versus female fetuses in gender-discordant fetuses. AFAFP did not differ significantly in cases of identical twin from non-identical or unknown zygosity twins Conclusions;We could not detect a significant difference between AFAFP levels in monozygous versus heterozygous twin. However, the levels of AFAFP were higher in male twins concordant for gender in comparison to female. No such difference was found between female versus male fetuses of disconcordant twins for gender.

P0656. The correlation between first and second trimester markers for Down s syndrome screening

P. De Biasio[1], *F. Prefumo*[1], *P. Marchini*[1], *I. Bruzzone*[1], *G. Ginocchio*[1], *S. Ferrero*[1], *S. Canini*[2], *P. L. Venturini*[1]
[1]*Department of Obstetrics and Gynaecology, Institute; Genova, Italy;* [2]*Clinical Laboratory, Institute G. Gaslini ; Genova, Italy*
bnhdeb@tin.it

Recently, a new screening method for Down s syndrome based on the integration of sequential measurements of first and second-trimester markers into a single test was described. This integrated test is supposed to detect more cases of trisomy 21 with a lower false positive rate than currently available first trimester combined test (nuchal translucency, free beta-hCG, PAPP-A) and (hCG, unconjugated estriol, alpha-fetoprotein). The estimates of its performance are based on there being little or no correlation between the first and second trimester markers used. We verified this assumption in an unselected pregnant population. We studied 927 consecutive singleton. All the women had both first trimester combined test and second trimester triple test. All the maternal serum marker levels were assayed by immunoradiometric measurements. Nuchal translucency (NT) and biochemical marker measurements were transformed as gestation-specific multiples of the median (MoM). The correlation between each pair of markers was analysed by Pearson s r regression coefficient. No marker showed a significant correlation with any other one with the exception of free beta-hCG subunit versus total hCG (* p<0.001). Thus, the informations supplied by first and second trimester screening markers are different, with the obvious exception of free beta-hCG and total hCG. Then it is correct to integrate first and second trimester markers levels.

		Second trimester markers			First trimester markers	
		hCG	uE3	AFP	Free beta-hCG	PAPP-A
First trimester markers	NT	0.015	-0.05	0.003	-0.13	0.08
	PAPP-A	0.08	0.12	0.04	0.16	
	Free beta-hCG	0.48*	0.06	0.003		
Second trimester markers	AFP	0.24	0.22			
	uE3	-0.04				

P0657. Elevated alphafetoprotein levels in a rare case of aplasia cutis congenita.

B. S. Reddy, *S. S. Reddy*
National Medical Diagnostic Laboratory; Southfield, MI United States
balireddy@hotmail.com

A case of aplasia cutis congenita with elevated maternal serum and amniotic fluid alphafetoprotein and a positive acetylcholinesterase is described. The patient was 29-year-old Asian woman, gravida 3, para 2. The maternal serum drawn at 18 weeks revealed a high level of alphafetoprotein,392ng/ml(8.4 multiples of the median). Amniotic fluid alphafetoprotein was also elevated,at 49.6ug/ml(8.2 multiples of the median). Acetylcholinesterase was positive and amniotic fluid culture revealed a normal 46,XX karyotype. At 28 weeks, the patient presented with ruptured membranes, chorioamnionitis. Following cesarean delivery the neonate was found to have lacked >85% of its skin and died. Autopsy revealed aplasia cutis congenita involving >85% of the body with absent scalp hair and eyelashes. The other noted dysmorphic features were a flat facial profile, a down-turned mouth, an abnormally curled right ear and the left ear fused to the scalp. The plausible theories for the pathogenesis were discussed. Furthermore, it was discussed whether the acetylcholinesterase-to-pseudocholinesterase ratio is diagnostic tool in differentiating neural tube defects from other anomalies. It was advocated that aplasia cutis congenita must be considered in the differential diagnosis in a pregnancy evidenced with elevated levels of serum and amniotic fluid alphafetoprotein, positive acetylcholinesterase and normal ultrasonography outcome.

P0658. The First Trimester Screening is the best approach to prenatal diagnosis

G. Tsukerman[1,2], *N. Gusina*[2], *H. Zinkevich*[2], *I. Kirillova*[2]
[1]*Reproductive Genetics Institute; CHICAGO, IL United States;* [2]*Institute for Hereditary Diseases; Minsk, Belarus*
genescreen@flashcom.net

By the beginning of the first trimester screening (FTS), we have already

had experience in the screening of almost 100,000-second trimester pregnancies, including 6.7% of women of 35 and older. The detection rate of Down s syndrome was 66%, which is probably the highest possible result for double-markers screening. This figure could be a good background for future comparison with results of any new type of prenatal screening. The pilot study of the FTS started in 1996, and, by 1999, 35,942 pregnancies were screened by ultrasound. The age distribution in this group is similar to the one in the second trimester. The vast majority of pregnancies were screened between 10th and 13th weeks of gestation. During the pilot study, 8 types of ultrasound scanners were used, and only two of them proved to be suitable for the FTS. Besides that, 16,000 serum samples from unaffected pregnancies and 42 Down s syndrome cases were tested for AFP, Free b-hCG and partly for PAPP-A. The median level of AFP, Free b-hCG and PAPP-A in single unaffected pregnancies for each CRL was derived. Several versions of PAPP-A kits were tested and 30% difference in concentration between kits was discovered. The expected detection rate for different marker combinations was determined. A modeling exercise for screened population with maternal age, NT, AFP, PAPP-A and Free b-hCG predicts a 92% detection rate for Down s syndrome in FTS at 5% FPR. Based on the predictions collected from the pilot program, we started a prospective population FTS. The risk of 1;360 at the expected date of delivery was chosen as the cut-off for an invasive procedure. 4, 384 of unselected pregnant women were tested according to the protocol. 93% of pregnant women were screened at CRL of the fetus between 38 and 70 mm. 6 cases of trisomy 21 were detected with the expected number 5.03. Since the acceptance of invasive procedures was only 75%, we could not estimate a real detection rate for aneuploidies until all pregnancy outcomes were known. Currently we know all pregnancy outcomes for screened pregnancies; 1 case of trisomy 21 was registered because a woman with the risk of 1;32 refused to have an invasive procedure, and 1 case of trisomy 13 was also registered since the risk calculation for trisomy 13 had not been applied yet. Practically, no cases of trisomy 21 were missed.

P0659. Elevated first trimester free beta-human chorionic gonadotropin (hCG) is not associated with adverse pregnancy outcome

Y. Yaron, *Y. Sapir, Y. Ochshorn, A. Orr-Urtreger*
Prenatal Diagnosis Unit & Genetic Institute, Tel Aviv Sourasky Medical Center; Tel Aviv, Israel
yyaron@post.tau.ac.il

Human chorionic gonadotropin (hCG) is associated with Down syndrome (DS) and is therefore used in most second trimester maternal serum DS screening programs. Elevated hCG is also known to be associated with adverse pregnancy outcomes such as preeclampsia, miscarriage and fetal growth restriction (FGR). This has been attributed to placental abnormalities. Recently, first trimester DS screening has been introduced, using nuchal translucency (NT) and maternal serum biochemical markers (free beta-hCG and PAPP-A). The purpose of this study was to evaluate whether first trimester maternal serum free beta-hCG is also associated with adverse outcomes. The study included 1269 patients with singleton pregnancies who underwent first trimester DS screening. The rates of various pregnancy outcomes were evaluated according maternal serum free beta-hCG levels. A cut-off value of 3.0 multiples of the median (MoM) was employed. 92 patients (7%) had elevated free beta-hCG, however no significant increase was found in these patients for any of the outcomes evaluated;

Adverse Pregnancy Outcome	Free beta hCG >= 3.0 MOM (n=92)	Free beta hCG < 3.0 MOM (n=1177)
FGR	2.2 %	2.3 %
Oligohydramnios	1.1 %	0.2 %
Preeclampsia	2.2 %	2.8 %
Preterm delivery	3.3 %	3.3 %
Miscarriage	0 %	0.8 %
Chromosome aberration	1.1 %	1.5 %
Fetal anomaly	1.1 %	1.3 %
Abruptio placentae	0 %	0.1 %

No other statistically significant cut-off values for free beta-hCG were detected. We conclude that unlike second trimester beta-hCG, first trimester free beta-hCG is not a predictor of adverse pregnancy outcomes.

P0660. Serum markers in pregnancy after in vitro fertilisation

T. Kascheeva, *V. Vakharlovskiy, Y. Paikacheva, O. Arzhanova*
Institute of Obstetrics & Gynecology; St.Petersburg, Russian Federation
TIV@ti2629.spb.edu

A new group of high risk pregnancy in the Down screening program has been identified in our lab since 1994. Serum markers for Down s syndrome in women subjected to in vitro fertilisation were quite differed from these ones in common pregnancies. To examine the Down s syndrome (DS) screening positive rate among in vitro fertilisation (IVF) pregnancies, we measured second trimester serum markers in 60 singleton and in 32 twin IVF pregnancies. Total beta-human chorionic gonadotropin (hCG) and alpha-fetoprotein (AFP) were measured. Median AFP levels were of the same order and median hCG was 40% lower in IVF singleton pregnancies with threatened abortion compared to control values, meanwhile median AFP was 50 % higher (P=0,05) and median hCG was 55% higher (P= 0,001) in IVF pregnancies without threatened abortion. Women after IVF in the screening program could be attributed to high DS risk group because of their age (mean age was 32 (P=0.001)) and usually high hCG. Thus AFP and hCG values should be adjusted to avoid the high screen positive rate in programs for IVF pregnancies. Th first DS fetus was recovered after IVF in St.Petersburg. Its karyotyping on the 19 w.p. was provoked by previous biochemical (16 weeks) and US markers (18 weeks).

P0661. Evaluation of pregnancy-associated plasma protein A (PAPP-A) as an additional serum marker in risk assessment for fetal chromosomal disorders.

U. Sancken
University of Goettingen; Goettingen, Germany
usancke@gwdg.de

In order to evaluate serum-PAPP-A as a risk marker for fetal trisomies PAPP-A concentrations were assayed in 33 serum samples of pregnancies affected by fetal trisomy 18 (13 cases) and trisomy 21 (20 cases) and in 593 controls between 9th and 13th week of gestation. The median PAPP-A MoM values were 0.22 MoM for trisomy 18 and 0.38 MoM for trisomy 21, thus significantly lower than the median of the controls (1 MoM). A risk algorithm was modelled on the basis of the found PAPP-A distributions and additionally on hCG and AFP distributions of 98 pregnancies affected by fetal trisomy 21 and those of 588 matched controls of the second trimester. Compared with the Triple-test the new algorithm results both in a higher sensitivity and in a higher specificity as to risk assessment for trisomy 21. Subsequently 644 prospective examinations were carried out by combining the serum-markers PAPP-A (first trimester) and hCG/AFP (second trimester) and by applying the new risk algorithm. In this cohort two cases with fetal trisomy 21 were detected because of high risk (>1;380) and one case with fetal trisomy 18 was found solely by an extremely low PAPP-A concentration (0,2 MoM). The false-positive rate was 5,5% and there were no false-negative results.

P0662. Placental Growth Hormone in prenatal screening of fetal chromosomal abnormalities and congenital malformations

E. Papadopoulou[1], *S. Sifakis*[2], *Y. Koumantaki*[3], *E. Angelakis*[2], *S. Smbirakis*[1], *N. Karkavitsas*[4], *E. Koumantakis*[2]
[1]*Dept of Pediatrics, University of Crete; Heraklion, Greece;* [2]*Dept of Obstetrics-Gynecology, University of Crete; Heraklion, Greece;* [3]*Dept Hygiene-Epidemiology, University of Athens; Athens, Greece;* [4]*Dept of Nuclear Medicine, University of Crete; Heraklion, Greece*
sifakis@excite.com

Aim; to examine the predictive value of maternal serum Placental Growth Hormone (hPGH) in prenatal diagnosis of fetuses affected by congenital anomalies. Material-Methods; Serum samples were obtained at 16th-17th week from 122 high-risk pregnancies of an abnormal fetus. The increased risk was identified by advanced maternal age (#48), pathological biochemical markers (#31), ultrasound scanning (USS) (#19), positive family history (#10), increased nuchal translucency (#9) and diabetes mellitus (#5). Amniocentesis was performed in 76 pregnancies, and high-quality USS at 20th-22nd week in the remaining 46 cases. Maternal serum hPGH was measured by a solid phase immunoradiometric assay, using specific monoclonal antibodies for two different epitopes of hPGH. T-test and Post Hoc-test were used for statistical analysis. Results; Karyotype analysis revealed 7 fetuses with chromosomal aberration, and USS detected 19 fetuses with minor or major malformation. The mean value of hPGH serum concentration of the 26 pathological fetuses was 6.28 ng/ml (SD –4.32) that was significantly higher than the mean value (2.79 ng/ml –1.68 SD) in normal fetuses (p<0,05). The hPGH determination in the detection of abnormal fetuses had 42% sensitivity, 75% specificity, 41% positive and 80% negative predictive value. Conclusion; our results show that hPGH is

a specific marker for prenatal detection of fetuses with chromosomal abnormalities and congenital malformations. However, its moderate sensitivity and positive predictive value do not allow at present, to be used as an additional marker in prenatal screening tests. Further investigations, in larger obstetrical populations are needed to establish hPGH in prenatal diagnosis.

P0663. Choroid Plexus Cysts in the Mid-Trimester Fetus; Modification of risk including triple marker screening results

L. M. Bird[1,2,3], *B. Dixson*[3], *D. Masser-Frye*[3], *L. Mestre*[3], *L. Ribas*[3], *L. Mullen*[2], *K. Kalla*[4], *K. Carder*[3], *V. Catanzarite*[3], *M. Jones*[1,2,3]
[1]*Children s Hospital; San Diego, CA United States;* [2]*University of California; San Diego, CA United States;* [3]*Sharp & Children s Prenatal Diagnostic Center; San Diego, CA United States;* [4]*Kaiser Permanente; San Diego, CA United States*
Lbird@chsd.org

Most studies regarding the risk for Trisomy 18 when a choroid plexus cyst (CPC) is found on prenatal ultrasound employ an average risk of approximately 1/150. The major difficulty in interpreting most of the previous studies has been the failure to consider other variables that impact the risk of aneuploidy, such as maternal age or serum screening results. Gupta et al. (1997) analyzed prospective studies of unselected populations and suggested a relative risk of 9 when the CPC is isolated and a relative risk of 1800 when the CPC is associated with other ultrasound abnormalities. We present a study of more than 300 CPCs detected in the 2nd trimester after the advent of triple marker screening. The results are presented using both the average-risk method most commonly employed in the clinical literature, as well as using the likelihood ratios suggested by Gupta et al. to modify individual risk assessment. Two hundred eighty patients had isolated CPCs, and 43 patients had complicated CPCs. Of the 43 complicated CPCs, there were 9 karyotypic abnormalities (8 trisomy 18 and one inherited chromosome 9 inversion). Of the 280 isolated CPCs, there were 3 karyotypic abnormalities (one trisomy 18, one 45,X/46,X,iso(Xq), and one de novo chromosome 16 inversion). The incidence of finding a significant chromosomal anomaly in fetuses with isolated CPCs is thus 1/93 in our referral population if an average risk method is employed. If trisomy 18 risk had been modified according to the likelihood ratios established by Gupta et al., 97 women would have had a midtrimester risk greater than or equal to 1/100 and would have been offered amniocentesis. This level of risk (1/100) was chosen because it is at this risk for trisomy 18 that amniocentesis is funded by the state prenatal diagnosis program. All 9 cases of trisomy 18 would have been ascertained using this method, as well as the de novo chromosome 16 inversion and the familial chromosome 9 inversion. The only chromosomal abnormality that would not have been ascertained is the case with 45,X/46,X,iso(Xq). However, because of her age, this patient was eligible for amniocentesis irrespective of the trisomy 18 risk. With individualized risk assessment that included triple marker screening results, 65 amniocenteses would have been performed, instead of 218 that were performed using the average risk method of counseling. There would have been no reduction in sensitivity for trisomy 18, and specificity would have increased greatly. In addition to fiscal savings, the theoretical loss of one fetus due to a procedure-related loss would have been avoided. We conclude that the individualized risk assessment using the method advocating by Gupta et al. is preferable to an average risk in counseling patients with CPC about the risk for Trisomy 18.

P0664. First trimester risk screening for aneuploidy; Results from a prospective trial in a single center

P. Miny[1,2], *W. Holzgreve*[3], *C. Batukan*[3], *A. Gerber*[4], *C. Szente*[5], *F. Wenzel*[6], *S. Tercanli*[3]
[1]*Division of Medical Genetics, University Children s Hospital; Basel, Switzerland;* [2]*Aristogen GmbH; Ingelheim, Germany;* [3]*University Women s Hospital; Basel, Switzerland;* [4]*Institute Dr. Viollier; Basel, Switzerland;* [5]*Div. Medical Genetics, University Children s Hospital Basel; Aristogen GmbH, Ingelheim; Basel; Ingelheim, Switzerland;* [6]*Div. Medical Genetics, University Children s Hospital; Basel, Switzerland*
peter.miny@unibas.ch

First trimester risk screening was introduced in late 1997 using the sonographic measurement of the fetal nuchal translucency (NT) and participating in the quality control scheme of the Fetal Medicine Foundation, London. Later maternal serum markers (free § hCG, PAPP A) were included. Patients were ascertained at the University Women s Hospital, Basel, from the outpatient clinic and those refered for specialized ultrasound services. Risk screening was offered to all patients with a fetal crown rump length between 38 and 84 mm (i.e. gestational age 10+3 to 13+6 weeks) after extensive counseling. A total of 2339 consecutive pregnancies will have reached term by the end of April 2001 following risk screening. So far, a total of 38 unbalanced chromosomal anomalies (including 13 trisomies 21, 11 trisomies 18, 7 Turner syndromes) were diagnosed mainly by CVS or amniocentesis. A preliminary analysis based on NT measurements alone and a risk cut off of 1;400 revealed a detection rate of 36 out of 38 unbalanced chromosome anomalies (95 %) with a false-positive rate of 10 %. Risk screening in the first trimester appears to have clear advantages as compared to second trimester screening using maternal serum markers.

P0665. Prenatal diagnosis of 2q37 deletions resulting from three different mechanisms of chromosomal rearrangements.

S. W. Cheung, *A. A. Ybarra, L. Cooper*
DYNAgene; Houston, TX United States
swcheung@dynagene

Terminal 2q37 deletion is a recognizable syndrome with growth retardation, developmental delay, microcephaly, dysmorphic features, cardiac defects, genital anomalies, and syndactyly/clinodactyly. We report three prenatal cases with normal ultrasound findings and subtle terminal deletions of 2q37, each of which was derived from different mechanisms of chromosome rearrangements. Case 1, a paternal pericentric inversion of chromosome 2 [inv(2)(p25.2q37.2)] lead to an imbalance in the fetus. The result showed that the fetus carried a rec(2) with a partial duplication of 2p at band p25.1 and a monosomy for 2q at band q37. Case 2, a paternal translocation involving 2q37 and 11p13.3 was only detectable by cytogenetics after the FISH result was available. Initial results were 46,XY,add(2)(q27), which was subsequently found in the father. FISH analysis carried out with a probe specific for the 2q telomere (BAC 172113) revealed monosomy 2q in the amniotic fluid sample. FISH analysis using the same probe on the father identified a balanced translocation between 2q and 11p. These results verify paternal transmission of the unbalanced translocation resulting in a fetus with a partial duplication of 11p13.3 and monosomy for 2q at band q37. Case 3, a de novo deletion was detected by standard cytogenetics and verified by FISH using telomeric probes indicating a fetus affected with monosomy 2q at band q37. Detailed cytogenetic studies for chromosome rearrangements, FISH analysis and follow up studies will be presented.

P0666. Prenatal diagnosis of mosaic tetrasomy 8p.

M. J. Le Bris[1], *M. P. Audrezet*[1], *P. Marcorelles*[1], *P. Parent*[1], *H. Le Guern*[1], *M. Collet*[1], *P. Heren*[2], *C. F rec*[3], *M. De Braekeleer*[3]
[1]*CHU Morvan; Brest, France;* [2]*CH; Lannion, France;* [3]*CHU Morvan & Universit de Bretagne Occidentale; Brest, France*
marc.debraekeleer@univ-brest.fr

Tetrasomy 8p is a rare chromosomal disorder that has only been detected in a mosaic form. At the present time, 11 cases have been reported; their phenotype included agenesis of the corpus callosum, enlarged ventricles, minor facial dysmorphism, rib and vertebral anomalies, and mild to moderate developmental delay. To the best of our knowledge, tetrasomy 8p has never been prenatally diagnosed. This 43 years old woman was referred for amniocentesis at 20 weeks gestation because of advanced maternal age. Amniotic fluid cells were cultured according to standard techniques by the in situ method. A supernumerary chromosomal marker was detected in a single clone of amniotic cell cultures and interpreted by RHG banding as an isochromosome of the short arm of chromosome 8 (i(8p)). The ultrasound investigation at 27 weeks gestation revealed enlarged ventricles and agenesis of the corpus callosum which were confirmed at fetal autopsy after medical termination of the pregnancy. Chromosomal analyses, including RHG banding and FISH, of several tissues showed different levels of i(8p) mosaicism. Whereas no i(8p) was detected on cytotrophobast nor additional amniotic fluid cells, 97% and 30% of cells from long term cultures of placenta and lymphocytes respectively had the i(8p). Using DNA markers, the isochromosome 8p was interpreted as the result of a prezygotic event during maternal meiosis. Our findings suggest that the i(8p) is the subject of tissue selection. Tetrasomy 8p might be underdiagnosed during pregnancy; therefore, we recommend karyotyping on a fetal blood sample following detection of agenesis of the corpus callosum when no chromosomal abnormality has been found on the amniotic fluid cell cultures.

P0667. Application of Inter-Fluorecence in situ hybridization (FISH) of Chromosome 13/21 Satellite Probe in Amniotic cells for Prenatal Diagnosis Trisomy 21 Syndrome

W. Li[1], *Y. Wu*[2], *Z. Ye*[2]
[1]*Institute of Medical Genetics of Zhuhai; Zhuhai, China;* [2]*Institute of Medical Genetics of Zhuhai; Zuhai, China*
liwendian@21cn.com

Objective To investigate the prenatal diagnosis of trisomy 21 syndrome using chromosome 13/21?satellite probe Fluorecence in situ hybridization

(FISH) on uncultured interphase cells from amnic fluid.
Methods The interphase amniocytes of 10 fetus who were detected normal and 3 fetus who were detected trisomy by prenatal cytogenetic diagnosis were selected. We did FISH which used chromosome 13/21 ? satellite probe directly on the uncultured amniocytes of these 13 samples.
Results The total rate of the hybridization were 36.7% and 38.6% in controls group and observation group respectively, which showed no significantly difference. In controls group, there are 36.5% of the cells which have four signals in the nucleus and 4.0% of the cells which have five signals in the nucleus. In observation group, there are 3.9% of the cells which have four signals in the nucleus and 36.1% of the cells which have five signals in the nucleus. The controls group and observation group showed significant difference by the statistical X^2 values ($P<0.01$). Trisomy 21 syndrome was diagnosed when cells which have five signals in nucleus were more than 36.1%.
Conclusion Chromosome 13/21? satellite probe is a very reliable and valuable method for the prenatal diagnosis on trisomy 21 syndrome.

P0668. Prenatal Characterisation of 27 Autosomal Marker Chromosomes and Outcomes of Pregnancies

G. Hickmann[1], *M. Mazauric*[1], *S. Weik*[1], *O. Bartsch*[2], *P. Kozlowski*[1]
[1]*Center for Prenatal Diagnosis and Genetics; Duesseldorf, Germany;* [2]*Institut for Clinical Genetics; Medical Faculty, Technical University; Dresden, Germany*
institut@praenatal.de

We present 27 cases of prenatal marker chromosomes (mar) identified after amniocentesis, chorionic villus sampling or cordocentesis. Investigations included conventional cytogenetics (GTG-, CBG-, NOR-banding) and FISH using locus- and centromere-specific probes and whole chromosome paints. Eleven mar were present in mosaic status with normal cells and 16 were present in all cells. Twenty mar were found to have arisen de novo and 7 were found to be familial (3 mat, 4 pat). Markers originated from chromosomes 1, 8, 11;22, 12, 13 or 21 (4x), 15 (5x), 15;21 or 13;15, 16, 18 (2x), 19, 21, and 22 (8x). Outcomes; One woman had an abortion. Seven pregnancies were terminated, 6 of them after abnormal ultrasound findings indicated a high risk for a handicapped child. One woman with a moderately increased risk for a handicapped child chose to terminate the pregnancy without ultrasonografic abnormalities after being counselled . A total of 19 children were born of which seventeen are healthy (range 0-5 years, 6 females and 11 males) One male twin, karyotype 47,XY,+mar(11;22), with severe malformations (enterothorax, diaphragmatic hernia, hydrocephalus, atrioventricular septal defect, renal malformations) died one hour after birth; the other twin is healthy (balanced 11;22-translocation). One other male child demonstrates congenital abnormalities (cat-eye syndrome; preauricular pit, renal dysfunction and moderate growth and motor retardation). A subset of the healthy children had high-risk marker chromosomes. Aspects of genetic counselling will be discussed.

P0669. Deletion 22q11 and fetal cardiac pathology

D. E. McFadden[1], *W. P. Robinson*[2]
[1]*C&W Hospital and University of British Columbia; Vancouver, BC Canada;* [2]*University of British Columbia; Vancouver, BC Canada*
dmcfadden@cw.bc.ca

The incidence of microdeletion 22q11 in fetal populations is not known but is probably lower than in liveborns given the greater role that aneuploidy plays in fetuses. All cardiac anomalies diagnosed in the Fetal Pathology Laboratory at C&W Hospital in a 2 year period were collected. Method of ascertainment, presence of other anomalies, cytogenetic results, and results of del22q11 FISH testing were noted. Chromosomally normal cases in which del22q11FISH testing had not been performed were tested molecularly. Cardiac anomalies were present in 102 of 484 fetal autopsies. 32 cases were terminated after prenatal diagnosis of chromosome abnormality, 17 were spontaneously aborted, and the rest were terminated after prenatal diagnosis of fetal anomalies, including 14 cases diagnosed with cardiac anomaly. Most (90/102) cases had other anomalies (complex); only 12 had isolated cardiac lesions. 5 of the isolated cases and 64 of the complex group were chromosomally abnormal. Excluding those 32 cases terminated on the basis of chromosome abnormality (4 isolated, 28 complex), 1/8 (12.5%) isolated cardiac lesions and 37/62 (59.7%)complex cases were abnormal. The most common abnormality was VSD. Tetralogy of Fallot was present in 7 cases and there were 2 cases of truncus arteriosus and 3 of pulmonary atresia. Eight chromosomally normal cases tested by FISH showed no del22q11, including TOF (2) and PA (2). The remaining cytogenetically normal cases were tested by molecular markers and showed no evidence of deletions. These results suggest that del22q11 does not play a major role in fetal cardiac pathology and that aneuploidy is a more significant factor than del22q11 in this population, even when excluding cases ascertained through abnormal chromosomes.

P0670. Immunological abnormality at partial trisomy 3p syndrome

N. Nikitina[1], *V. Sorokina*[1], *E. Nikolaeva*[1], *G. Pavlov*[2], *M. Devaikina*[1]
[1]*Medico-Genetic Centre; Ekaterinburg, Russian Federation;* [2]*Ural Medical Academy; Ekaterinburg, United States*
gvp@online.ural.ru

The proband is a 1 y. o. female infant, the first child of an unrelated 31-y. o. mother and 33 y. o. father. Two previous pregnancies ended in regress. Chromosome studies showed that the father had a balanced reciprocal translocation t(3;14) (p24;q23). The family refused from the prenatal examination for this pregnancy. The proband was delivered by C-section. The baby s weight was 2700 g. and his length was 46 cm. On the 4th day after the birth the baby s status got poorer. The proband was transferred to the intensive care with encephalitis and ulceronecrosic gastritis diagnosis. The stomach s perforation was operated successfully. At 2 months the baby suffered from sepsis of mixed etiology (Klebsiella and Proteus), at 8 months -from broncopneumonia. And what s more - upper respiratory tract infection. At 2 and 9 months the proband was tested immunologically; decreased serum IgA, IgG, decreased E-rosette formation, decreased activity of phagocytes were revealed. At the age of 1 the child s height was 71,5 cm and weight - 9000 g. Dysmorphic features of the face were obviuos; a broad and flat nasal bridge, full cheeks, a short nose. Chromosome studies confirmed partial trisomy 3p paternal. In 6 cases previously published, children were diagnosed with frequent infections and in 3 cases the decrease of immunological indexes was established. So we can speak about immunological disfunction as one of the clinico-laboratory characteristics of the syndrome.

P0671. Prenatal Diagnosis of a de novo unbalanced rearrangement by CGH and FISH

S. Park[1], *Y. Kim*[1], *M. Kim*[2], *S. Hong*[3], *H. Ryu*[2]
[1]*Laboratory of Medical Genetics, Samsung Cheil Hospital; Seoul, Republic of Korea;* [2]*Department of Obstetrics and Gynecology, Samsung Cheil Hospital; Seoul, Republic of Korea;* [3]*Department of Pathology, Samsung Cheil Hospital; Seoul, Republic of Korea*
genelab@samsung.co.kr

Unbalanced translocation was identified in a fetus with cardiac anomaly at 21 weeks of gestation; 46,XX,der(9)t(7;9)(p21;p23). Parental karyotypes were normal, indicating a de novo origin of the unbalanced translocation in the fetus. By comparative genomic hybridization (CGH) and fluorescence in situ hybridization (FISH), we defined the composition of an unbalanced de novo translocation. CGH revealed the DNA amplification at distal 7p (7p21` pter) and the deletion at 9p (9p23` pter). FISH with chromosome 7 and 9 painting probes confirmed the partial trisomic status of the short arm in chromosome 7 and the partial monosomy of the short arm in chromosome 9. Resampling for special banding to confirm the precise breakpoint was declined and the parents opted for termination at 23 weeks of gestation. Therapeutic termination of the pregnancy was done. Autopsy showed cardiac anomalies of right ventricle hypoplasia, pulmonary atresia and left heart enlargement with prominent aorta, and urogenital anomalies of hypoplasia of internal genital organs with cystocele. This case demonstrates that FISH and CGH are useful for the identification of chromosomal material of unknown origin.

P0672. Prediction of prognosis in apparently balanced de novo rearrangements at prenatal diagnosis; Role of molecular cytogenetic study and fetal ultrasound

H. Ryu[1], *S. Park*[2], *Y. Kim*[2], *M. Kim*[1], *Y. Lee*[3]
[1]*Department of Obstetrics and Gynecology, Samsung Cheil Hospital; Seoul, Republic of Korea;* [2]*Laboratory of Medical genetics, Samsung Cheil Hospital; Seoul, Republic of Korea;* [3]*Department of Radiology, Samsung Cheil Hospital; Seoul, Republic of Korea*
genelab@samsung.co.kr

De novo balanced reciprocal translocations incidentally found at prenatal diagnosis raises serious prognostic dilemmas. In our series of 5,541 prenatal diagnoses during the past 5 years, there were 5 cases of de novo balanced reciprocal translocations. High resolution banding and molecular cytogenetic study (FISH and CGH) were performed as an adjunct to G-banding for characterization of the abnormal chromosomes. In two cases, there were fetal anomalies found using ultrasound; one had thickened fetal myocardium, another had a markedly enlarged right atrium and ventricle. The parents decided to terminate the pregnancy and the autopsy showed consistent results with the prenatal ultrasound. In the third case, a prenatal diagnosis indicated due to thickened nuchal folds at second trimester of

pregnancy. Pregnancy was continued and a 3500gm healthy baby was delivered with no anomalies except simian creases on both hands and cryptochidism. At present, he is a healthy at the age of 6. In the last two cases, there were no fetal anomalies found on the ultrasound. The couples were counseled that the risk of phenotypic abnormality from a de novo reciprocal translocation has been estimated at approximately 6.1% [Warburton, 1991]. Both couples decided to continue pregnancy and delivered healthy babies. The children showed normal development during follow up examinations up to the age of 5 and 2 years, respectively. In our series, detailed fetal ultrasound including fetal echocardiogram played an important role for predictions of fetal prognosis.

P0673. Diagnostic Of Mosaic Karyotype 46, Xx / 46, Xy In Amniotic Fluid

G. Radivojevic-Pilic[1], *S. Nikolic*[1], *S. Brankovic-Niksic*[1], *M. Stevanovic*[2]
[1]*Institute of Mental Health; Belgrade, Yugoslavia;* [2]*Institute of Genetic Engineering; Belgrade, Yugoslavia*
nbrankov@eunet.yu

The paper discusses the case of a patient who, after the ultrasound finding, was referred to prenatal cytogenetic diagnosis. The analysis of amniotic fluid cells indicated the presence of the following karyotype in the fetus; 46, xx / 46, xy. The conformation of this mosaic karyotype was made by classic cytogenetic analysis and FISH of the cells of peripheral blood and fibroblast of fetus s skin. Once again, this case has indicated that the problem of mosaic is one of the most difficult problems in prenatal diagnosis.

P0674. Rapid prenatal screening for chromosome X, Y, 21, 13 and 18 aneuploidies by quantitative fluorescent polymerase chain reaction (QF-PCR) on 551 uncultured amniotic fluids.

V. Cirigliano[1,2], *M. Ca adas*[1], *M. Ejarque*[1], *E. Lloveras*[3], *A. Plaja*[3], *C. Fuster*[2], *J. Egozcue*[2]
[1]*Dept. of Molecular Biology, General Lab; Barcelona, Spain;* [2]*Dept. Biologia Celular i Fisiologia, Universitat Autonoma de Barcelona; Barcelona, Spain;* [3]*Dept. of Cytogenetics, General Lab; Barcelona, Spain*
v_cirigliano@hotmail.com

Since 1993 it has repeatedly been shown that rapid and accurate diagnosis of selected chromosome aneuploidies can be achieved by Quantitative Fluorescent Polymerase Chain Reaction (QF-PCR) amplification of genomic repeated sequences known as Small Tandem Repeats (STR). In the last few years, this approach has been successfully used in large experimental and clinical trials on hundreds of amniotic fluids and CVS samples. Due to the early shortage of highly polymorphic STR markers on the X and Y chromosomes, the great majority of studies focused on the detection of aneuploidies involving chromosomes 21, 13, 18 and the fetal sex. Recently it has been shown that the newly identified pentanucleotide repeat X22, which maps in the pseudoautosomal region (PAR 2) of the X and Y chromosomes, together with a modified sequence of the amelogenin region, allows accurate detection of sex chromosome copy number. We developed a multiplex QF-PCR assay including these sequences together with highly informative autosomal STRs for the rapid prenatal detection of aneuploidies involving chromosomes X, Y, 13, 18 and 21. The test was used in clinical trial on 551 uncultured amniotic fluids allowing the assessment of sex chromosome copy number in 100% of samples, including one case of Klinefelter and two of Turner syndrome. In the course of this trial 17 autosomal trisomies were also correctly diagnosed. The assay proved to be efficient and reliable allowing the detection of all numerical variations of the examined chromosomes within 24 hours from collection of the sample.

P0675. Prenatal diagnosis of trisomy 2 mosaicism

S. Sifakis[1], *V. Velissariou*[2], *E. Papadopoulou*[1], *E. Angelakis*[1], *S. Christopoulou*[2], *M. B. Petersen*[2], *E. Koumantakis*[1]
[1]*Department of Obstetrics Gynecology, University of Crete; Heraklion, Greece;* [2]*Department of Genetics and Molecular Biology, MITERA Maternity and Surgical Center; Athens, Greece*
sifakis@excite.com

Trisomy 2 mosaicism is very rare in prenatal diagnosis. Published data from eleven cases showed a risk >60% for an abnormal outcome. We present a case of mosaic trisomy 2 detected at amniocentesis in a 35 year-old G3P2 woman. Amniocentesis was performed at the 16th gestational week because of advanced maternal age. There was no family history of congenital abnormalities or genetic diseases. The culture of amniocytes and the use of GTG banding revealed a 47,XY,+2(4)/46,XY(21) karyotype in two different cultures. At the time of amniocentesis there were no pathological ultrasonographic findings and the fetus weight was normal for gestational age. The use of serial high-resolution ultrasound examination of the fetus for detecting major abnormalities was offered as an alternative option to the couple during genetic counseling. They decided termination of pregnancy. Fetal autopsy did not reveal malformations. There was no cytogenetic examination for confirmation of the trisomy neither in fetal nor in placental tissues. Trisomy 2 mosaicism is related with no specific type of abnormalities, IUGR, and fetal demise or stillbirth. The only reported case with normal outcome had only 4% trisomy 2 cells in amniocytes. Cytogenetic studies have shown confirmation rates about 81-88% in cases with abnormal outcome. Confined placental mosaicism may also be considered in differential diagnosis, while some of the fetuses with trisomy 2 placental mosaicism may have uniparental disomy. In conclusion, the rarity of trisomy 2 mosaicism in prenatal diagnosis makes the diagnostic approach, the management and the genetic counseling difficult.

P0676. Features of Cornelia de Lange and Cri-du-Chat Syndromes in a Foetus with a Derivative Chromosome from a Maternal 3;5 Translocation

N. O. Teles[1], *M. Pinto*[1], *O. Pinho*[2], *R. Nogueira*[2]
[1]*Instituto de Gen tica M dica J.M.; Porto, Portugal;* [2]*Centro Hospitalar V. N. Gaia; Vila Nova de Gaia, Portugal*
natalia@igm.min-saude.pt

Cornelia de Lange and Cri-du-Chat syndromes are well known entities described for the first time in 1933 and 1963, respectively. The main features of Cornelia de Lange are; bushy eyebrows, hirsutism, synophrys, thin downturning upper lip, micromelia and mental retardation; in some cases it is associated with 3q26.1 duplication. On the other hand, Cri-du-Chat features are much less obvious and include microcephaly, downward slant of the palpebral fissures and mental retardation, and it is caused by a deletion of 5p15. Chromosome studies were performed on amniotic fluid cells of a 21 years old woman referred for ultrasound abnormalities and revealed an abnormal karyotype with a derivative of a 3;5 translocation inherited from the mother.The authors describe the ultrasound, cytogenetic and post-mortem findings in the foetus terminated at 20 weeks with characteristics suggesting both Cornelia de Lange and Cri-du-Chat syndromes. As far as we know this is the first case where both syndromes have been found in the same foetus.

P0677. Prenatal Diagnosis of the Fetus with Mosaic del(12)(q21.32q22); First Prenatal Case

N. Basaran[1], *T. Sener*[2], *B. Durak*[1], *H. Berkil*[1], *Y. H. Ozon*[1]
[1]*Osmangazi University, Medical Faculty, Department of Medical Genetics; Eskisehir, Turkey;* [2]*Osmangazi University, Medical Faculty, Department of Obstetrics and Gynecology; Eskisehir, Turkey*
nbasaran@ogu.edu.tr

Proximal or interstitial 12q deletions are very rare, and only six patients were described previously (Funderbunk et al., 1984; Meinecke and Meinecke, 1987; Watson et al., 1989; Tonoki et al., 1998; Rauen et al., 2000; Zollino et al., 2000). All of the reported cases were diagnosed postnataly. The couple were referred to our department because of the advanced maternal age and the amniocentesis was applied in 18th weeks of gestation. The fetal karyotype was revealed as 46,XY/46,XY,del(12)(21.32q22)(87/13). This mosaic karyotype was also confirmed by the cordocentesis (86/14) and the pregnancy was terminated at 24th weeks of gestation. The postmortem physical examination of the fetus showed minor facial dysmorphism. This case is especially important because of the chromosomal abnormality (interstitial 12q deletion) is the first prenataly diagnosed.

P0678. The Usage Of Comparative Genomic Hybridization Technique In Prenatal Diagnosis

Y. H. Ozon[1], *T. Sener*[2], *S. Artan*[1], *M. H. Muslumanoglu*[1], *N. Basaran*[1]
[1]*Osmangazi University, Medical Faculty, Department of Medical Genetics; Eskisehir, Turkey;* [2]*Osmangazi University, Medical Faculty, Department of Obstetrics and Gynecology; Eskisehir, Turkey*
hozon@ogu.edu.tr

As it is known, the majority of chromosome abnormalities in newborn related aneuploidies of chromosomes 13, 18, 21, X and Y, are nearly 95 percent of all the chromosome abnormalities diagnosed prenatally. Full karyotype analysis from amniocentesis and/or CVS have been performed to the pregnancies at risk for chromosomal syndromes. However it is time consuming and labour-instensive. Comparative Genomic Hybridization (CGH) employed to uncultured cells has a new to investigate chromosome aneuploidies. In this study, we report the results of the first study evaluated the DNA of these samples for CGH analysis of uncultured amniocytes/CVS. The results of 40 amniotic fluid samples/CVS were analysed by CGH and the results were compared with the ones from standart cytogenetics. CGH allowed accurate sex and chromosome enumeration in 38 uncultured amniocytes/ trophoblasts (95%), consistent with the result

obtained by traditional cytogenetic analysis. Neither false positive nor false negative results were obtained. Morever, the signals/nucleus for each probe was also evaluated. This experience demonstrates that CGH can provide a rapid and accurate clinical method for prenatal idendification of chromosome aneuploidies and sexes.

P0679. The high recurrence probability of early died child with unbalanced partial monosomy 7q with trisomy 13q in large pedigree of t(7;13)(q34;q13) carriers.

A. T. Midro, *B. Panasiuk, A. Sawicka, B. Stasiewicz-Jarocka*
Department of Clinical Genetics; Bialystok, Poland
midro@amb.ac.bialystok.pl

Holoprosencephaly and cyclopia is very often result of unbalanced karyotype and influences at early death. We observed partial monosomy 7q with trisomy 13q as result of familial translocation t(7;13)(q34;q13). From literature data we know that both involved chromosome 7 and 13 are responsible for holoprosencephaly. Pedigree analysis show sixty members with 5 carriers and ten early died children with similar malformations. It was enough to estimate probability of recurence of unbalanced progeny. Genetic risk was estimated according to the method of Stene and Stengel-Rutkowski. Figure 8/29 i.e. 27.6% +/- 8.3% was obtained after ascertainment corretion. It could be useful for genetic counselling.

P0680. Trisomy 18 and 21 Associated with a Partial Hydatidiform Mole

U. Tantravahi[1,2], *H. Pinar*[3], *M. Steinhoff*[3], *D. Singer*[3]
[1]*Brown University; Providence, RI United States;* [2]*Women and Infants Hospital; Providence, RI United States;* [3]*Women and Infants Hospital, Brown University; Providence, RI United States*
utantrav@wihri.org

Partial hydatidiform moles have triploid chromosome constitution except for few cases where they are diploid. We describe a case of partial hydatidiform mole with unusual chromosomal findings. A 32-year-old woman whose pregnancy history was remarkable for two previous early fetal losses, presented at 14 weeks gestational age with intrauterine fetal demise. Products of conception after a dilatation and curettage procedure consisted of two populations of villi; one was small and sclerotic and the other large hydropic with trophoblastic proliferation. Some of these hydropic villi had scalloping borders and trophoblastic inclusions. Occasional villi had blood vessels with nucleated red blood cells. Some sections revealed severely macerated embryonic fragments. These findings were consistent with a partial hydatidiform mole. Although almost all partial hydatidiform moles are triploid, a DNA flow cytometric analysis revealed a diploid peak. Chromosomal analysis of the villi as well as the fetal tissue using Fluorescent Suppression In situ Hybridization (FISH) studies using chromosome enumeration DNA probes revealed trisomy for chromosomes 18 and 21. Products of conception with morphological changes consistent with partial hydatidiform mole but diploid DNA flow analysis, should probably be evaluated with FISH to rule out unexpected chromosomal abnormalities. Although hydropic villi have been associated with aneuploid pregnancies, chromosomal abnormalities as identified in this case showing typical partial hydatidiform mole morphology have not been previously described.

P0681. Routine use of QF-PCR for prenatal diagnosis of the most common aneuploidies

C. Bili[1], *A. Divane*[2], *A. Apessos*[3], *A. Maravelaki*[4], *L. Florentin*[1]
[1]*Molecular Biology and Cytogenetics Center, Alfalab Medical Institute of Research and Diagnosis; Athens, Greece;* [2]*Molecular Biology and Cytogenetics Center,Alfalab Medical Institute of Research and Diagnosis; Athens, Greece;* [3]*Molecular Biology and Cytogentics Center,Alfalab, Medical Institute of Resesarch and Diagnosis; Athens, Greece;* [4]*Molecular Biology and Cytogenetics Center, Alfalab Medical Institute of Resesarch and Diagnosis; Athens, Greece*
lfloren@hellasnet.gr

We have routinely performed prenatal diagnosis of the most common aneuploidies of chromosomes 13, 18, 21, X and Y using multiplex quantitative fluorescence PCR on more than 700 amniotic fluid samples. Our results were all confirmed by cytogenetics analysis. In most cases results were based in two informative markers for each chromosome, in some cases we had to use at least three or four markers to obtain a result and occasionally due to lack of heterozygosity results were based on a single marker for a specific chromosome. We have found that the markers we have used were informative in the Greek population also with a heterozygosity frequency ranging within 0.75-0.90, similar to the frequencies reported in the literature. There was accordance between the quantitative fluorescence PCR and cytogenetic results in all but 2 cases. We had one false negative result of a trisomy 18 and an ambiguous result for which no result was reported for chromosome 18 (cytogenetic analysis showed a trisomy 18). We detected 21 abnormal cases (trisomy 21, 18, 13, 69XXX and a mosaic (46,XX(15)/45,X(15)). Additionally, more then 30 blood stained amniotic fluids were studied. Only 4 gave ambiguous results (triallelic pattern for most markers or abnormal ratios). The results of this study clearly support that quantitative fluorescence PCR for common aneuploidies is an accurate and rapid adjunct to conventional cytogenetic prenatal diagnosis and can be used as part of the diagnostic routine in a prenatal unit.

P0682. Rapid aneuploidy screening using QF-PCR; data from a UK diagnostic laboratory

K. L. Mann, *S. Fox, Z. Docherty, C. Mackie Ogilvie*
Guy s Hospital; London, United Kingdom
kmann@hgmp.mrc.ac.uk

Prenatal diagnosis of chromosome aneuploidies currently involves the culture and karyotype analysis of chorionic villus or amniotic fluid samples. The average UK reporting time using this approach is 13.8 days, and there is increasing pressure to develop techniques that provide a more rapid result. Quantitative-fluorescence PCR uses dosage analysis at polymorphic loci and is suited to high throughput. We have set-up and assessed QF-PCR as a diagnostic test for trisomy 13, 18 and 21. Trisomic samples were characterised by 3 allele peaks or 1;2 or 2;1 dosage differences between 2 allele peaks. One multiplex PCR was designed to include 10 tetranucleotide repeat markers (3, 4 and 3 for chromosomes 13, 18 and 21 respectively). The use of three fluorescent labels enabled all chromosomes to be analysed in a single injection on a capillary-based fluorescence analyser. DNA was prepared from uncultured amniotic fluid or chorionic villi using a rapid, resin-based approach. The strategy was evaluated with blind and pilot studies and QF-PCR has now been successfully integrated into our cytogenetic service. All amniotic fluid and chorionic villus samples are tested and results issued within 3 working days (mean reporting time 1.58 working days, median reporting time 1 working day). Abnormal results are confirmed by rapid-FISH and all samples have follow-up karyotype analysis. To date 631 diagnoses have been made with no false positive or negative results. Our experience of QF-PCR as a clinical service over a 12 month period (estimated to include 1,200 samples) will be presented.

P0683. Preimplantation Diagnosis For Beta-globin Gene Mutations

A. Kuliev[1], *S. Rechitsky*[1], *O. Verlinsky*[1], *G. Kalakoutis*[2], *M. Angastiniotis*[2], *Y. Verlinsky*[1]
[1]*Reproductive Genetics Institute; Chicago, IL United States;* [2]*Makarios Hospital; Nicosia, Cyprus*
Anver_Kuliev@immc.org

Prevention of beta-globin gene mutations is presently based on prospective carrier screening, prenatal diagnosis and termination of affected pregnancies. We introduced preimplantation genetic diagnosis (PGD) to preselect unaffected embryo and establish healthy pregnancies avoiding pregnancy termination. Using a standard IVF setup, the oocytes or embryos were biopsied and tested for the presence of beta-globin gene mutations by multiplex nested PCR, involving simultaneous mutation and linked marker analysis in single blasromeres for paternal, or first and second polar bodies for maternal mutations. Overall, 40 PGD cycles were performed from 26 couples at risk for having children with sickle cell disease (7 cycles) or different thalassemia mutations, including IVS1-110, IVSII -745, IVSI-1, IVSI-6, codon 39, 619 bp deletion and IVSI-5 (33 cycles). Of the 265 resulting embryos with DNA results, 101 unaffected embryos were pre-selected for transfer back to patients in 40 clinical cycles, resulting in 14 clinical pregnancies and 7 healthy children born by the present time. The remaining embryos were avoided from transfer either because they were predicted to contain an abnormal gene, or had not available confirmatory marker analysis. Of 116 embryos from this group available for follow up confirmation analysis the genotype was correctly predicted in 114 (98.2%), demonstrating a high accuracy of PGD for beta-globin gene mutations using multiplex nested PCR analysis in single blastomeres or polar bodies.

P0684. Clinical Outcome Of Preimplantation Aneuploidy Testing In Ivf Patients Of Advanced Maternal Age

Y. Verlinsky, *J. Cieslak, S. Evsikov, A. Kuliev*
Reproductive Genetics Institute; Chicago, IL United States
Anver_Kuliev@immc.org

Selection of embryos for transfer in IVF is currently based on morphological parameters, which do not correlate with chromosomal make up, leading to incidental transfer of aneuploid embryos. We introduced preimplantation aneuploidy testing of oocytes to improve the clinical outcome in the IVF patients of advanced maternal age. Using standard IVF procedure, the

first and second polar bodies were removed following maturation and fertilization of oocytes in 87 clinical cycles from patients of 39 years and older. Polar bodies were tested for aneuploidies by five-color FISH probes specific for chromosomes 13, 16, 18, 21 & 22 (Vysis). Overall, 56% of oocytes were aneuploid, resulting from errors in the first (40.8%) and second (42.1%) meiotic divisions, and were not transferred. The embryos resulting from aneuploidy free oocytes were transferred back to uterus and the pregnancy and implantation rates yielded in this group were compared to that in the control group, involving 77 clinical cycles from routine IVF patients of the same age. On the average, 1.8 aneuploidy-free embryos per cycle were pre-selected and transferred in the test group, compared to 2.8 in the control. Despite this difference, 8% implantation and 14.5% pregnancy rates were observed in the test group, compared to 3.4 and 7.9%, respectively, in the control, demonstrating a two-fold improvement of the clinical outcome of IVF following pre-selection of aneuploidy-free embryos.

P0685. Preimplantation Diagnosis for Single Gene Disorders Combined with HLA Testing

S. Rechitsky[1], *O. Verlinsky*[1], *W. Schoolcraft*[2], *C. Strom*[1], *A. Kuliev*[1], *Y. Verlinsky*[1]
[1]*Reproductive Genetics Institute; Chicago, IL United States;* [2]*Colorado Center for Reproductive Medicine; Denver, CO United States*
Anver_Kuliev@immc.org

Preimplantation diagnosis provides an option for avoiding an affected pregnancy together with pre-selection of an HLA compatible donor for an affected sibling. We performed preimplantation HLA testing for leukemia, Fanconi anemia (IVS 4+4 A-T mutation) and thalassemia (619bp deletion), the latter two combined with mutation analysis. Haplotype analysis for family members (including father, mother and affected child) was performed to identify different polymorphic STR alleles corresponding to specific markers in HLA genes. Linked STRs scattered through HLA genes were applied to increase accuracy of analysis and detect potential crossover between HLA-A, HLA-B, HLA-C, HLA-E and HLA-DQB genes. Single blastomeres were biopsied in a standard IVF setting and tested by multiplex nested PCR analysis to analyze simultaneously for mutations, linked markers, and HLA genes. Genotyping for Fanconi anemia (four cycles) and thalassemia (one cycle) mutations was possible in 33 and 8 embryos, respectively, 6 and 3 of which were affected, and 24 and 5 unaffected. HLA testing of these unaffected embryos, and 28 embryos obtained in clinical cycles for leukemia, revealed 13 HLA matched embryos overall, including 5 for Fanconi anemia, 3 for thalassemia and 5 for leukemia. These embryos were transferred back to patients, resulting in a clinical pregnancy and birth of an unaffected child in case of Fanconi anemia, whose cord blood stem cells have been transplanted to the affected sibling.

P0686. Testing Times; Creating a protocol for Preimplantation Genetic Diagnosis for Huntington s Disease

A. Lashwood[1], *F. A. -. Flinter*[2], *P. R. -. Braude*[3]
[1]*Centre For Preimplantation Genetic Diagnosis; London, United Kingdom;* [2]*Centre for Preimplantation Diagnosis, Guy s & St. Thomas Hospital; London, United Kingdom;* [3]*Centre for Preimplantation Genetic Diagnosis, Guy s & St. Thomas Hospital; London, United Kingdom*
alison.lashwood@gstt.sthames.nhs.uk

Preimplantation genetic diagnosis (PGD) combines in vitro fertilisation technology with molecular and cytogenetic analysis. PGD is an alternative reproductive option for couples at risk of having a child with a genetic disorder. Huntington s disease (HD) is an autosomal dominant neurodegenerative adult onset disorder. Individuals at risk can have a gene test prior to the onset of symptoms. Prenatal diagnosis is available, but the uptake is low. PGD for Huntington s disease (HD) has been considered by The Guy s & St. Thomas Centre For Preimplantation Diagnosis. Couples where one partner has received an HD gene positive test result will be considered for PGD treatment. In accordance with the 1990 Human Fertilisation & Embryology Act, a licence to practise PGD is required from the Human Fertilisation & Embryology Authority. Unlike other single gene disorders for which we offer PGD treatment, there are several issues relating to PGD for HD which require special attention. Æ To ensure accuracy in a PGD cycle, HD allele sizes are needed from the non gene carrying partner. Æ If confirmatory prenatal testing is not undertaken following a successful PGD cycle, no confirmation of diagnosis can be obtained at birth. Æ The welfare of children born into a family where one parent will become symptomatic of HD. In order to address these issues it has been necessary to create a detailed working protocol for the management of PGD for HD. We believe that this is the first such document and the details will be discussed in this paper.

P0687. Meiotic outcomes in female translocation carriers ascertained in three-day human embryos.

C. Mackie Ogilvie, *T. L. Kirby, P. R. Braude, P. N. Scriven*
Guy s & St. Thomas Centre for PGD; London, United Kingdom
caroline.ogilvie@kcl.ac.uk

Chromosomes involved in reciprocal translocations form quadrivalents at meiosis. These complexes segregate by alternate, adjacent-1, adjacent-2, 3;1 or 4;0 modes, to give gametes with different balanced or unbalanced chromosome complements. The lack of direct access to female gametes has meant that until recently data concerning frequency of segregation mode in female carriers has not been easily available. With the development of preimplantation genetic diagnosis (PGD) for chromosome rearrangements, these data are now emerging. We have carried out PGD cycles for 15 couples with translocations; of these, data were obtained for six female carriers of reciprocal translocations and three female carriers of Robertsonian translocations. Following PGD and transfer of embryos with a normal/balanced PGD result, the remaining embryos from each cycle were spread and tested for confirmation of diagnosis and segregation mode. Not all embryos gave informative results, as arrested embryos gave nuclear fragments and multinucleated cells made interpretation difficult. Of those which gave an informative result, 66% (range 60% to 75%) of embryos (n=48) from female reciprocal translocation carriers were consistent with alternate segregation, compared with 77% (range 60% to 100%) (n=27) for Robertsonian translocations. Interestingly, in the reciprocal translocation cycles, only 16% of embryos were consistent with adjacent-1 segregation, the mode that gives rise to the most common unbalanced products seen at prenatal diagnosis. Six of the couples established a successful pregnancy following PGD. These results, together with further data currently being collected, will be presented and discussed.

P0688. Establishing a one-step multiplex fluorescent PCR for the diagnosis of Huntington«s disease on single cells

C. Gro§[1], *L. A. Kunz-Schughart*[2], *U. Hehr*[1], *D. Seifert*[1], *F. Hofstaedter*[2], *B. Seifert*[1], *A. Hehr*[1]
[1]*Center for Gynecological Endocrinology, Reproductive Medicine and Human Genetics; Regensburg, Germany;* [2]*Institute of Pathology, University of Regensburg; Regensburg, Germany*
claudia.gross@humangenetik-regensburg.de

Huntington«s disease is an autosomal dominant neurodegenerative disorder caused by a CAG-trinucleotide repeat expansion in the 5« coding region of the HD gene. Here we report a one-step multiplex fluorescent PCR protocol to detect simultaneously the disease causing mutation as well as the fragment sizes of closely linked highly polymorphic markers at the single cell level. The repeat expansion itself and five neighbouring microsatellite markers were amplified using fluorescently labelled primers and analysed with the Genescan software on an ABI 310 genetic analyser. The multiplex PCR was optimised with human genomic DNA of normal controls and subsequently on nucleated single cells, separated from whole blood after enrichment with Histopaque (Sigma) by fluorescence-activated cell sorting. In the next step, this protocol was applied for the analysis of single cells from a known Huntington«s disease carrier with 19 and 44 repeats, respectively. Although the normal Huntington allele was preferentially amplified, a reproducible amplification of both repeat alleles could be demonstrated. In order to reduce the risk of misdiagnosis due to allelic drop out or contamination and to increase the reliability of the test, the repeat was coamplified with 2 additional closely linked informative markers. The here presented PCR protocol on single cells without preamplification and the simultaneous detection of the coamplified fragments can be completed in less than 6 hours. Potential applications of this protocol include the analysis of polar bodies obtained during assisted reproduction of female Huntington«s disease carriers in order to transfer fertilized oocytes with the normal allele.

P0689. Successful IVF treatment of couples with chromosomal aberrations

Y. Wettergren[1], *L. Nilsson*[2], *L. Hamberger*[2], *J. Wahlstr m*[3], *C. Hanson*[2]
[1]*Department of Surgery, Sahlgrenska University Hospital/ stra; G teborg, Sweden;* [2]*Department of Obstetrics and Gynecology, Sahlgrenska University Hospital; G teborg, Sweden;* [3]*Department of Clinical Genetics, Sahlgrenska University Hospital; G teborg, Sweden*
yvonne.wettergren@dep-surg.gu.se

Carriers of translocated or inverted chromosomes usually have reduced fertility caused by chromosome imbalances formed during gametogenesis. Generally, these carriers are subjected to a higher frequency of miscarriage as well as an increased risk of having children affected by chromosome imbalances. The actual risk depends on the chromosomes involved in the aberration and the position of the breakpoints. Couples affected by

reduced fertility caused by translocations or inversions often turn to the IVF clinic for treatment. After thorough penetration of the possible genetic risks we decided to make a pilot study comparing 10 subfertile couples with chromosomal aberrations. These couples did undergo up to three stimulated IVF cycles using intracytoplasmic sperm injection (ICSI) when indicated. All couples were strongly recommended to have a prenatal genetic diagnosis in case of pregnancy. Here, we present the outcome of the IVF treatment of the 10 subfertile couples (9 translocation carriers and one inversion carrier). Thirty-two treatment cycles resulted in 8 pregnancies;

2 spontaneous abortions
5 deliveries
1 ongoing pregnancy

In total, 7 healthy children were born. Although preimplantation genetic diagnosis (PGD) may be an attractive alternative for couples with chromosomal aberrations, traditional IVF treatment in combination with prenatal diagnosis seems to be an option in cases where PGD is not acceptable or possible.

P0690. Sex determination and detection of delta F508 mutation in human blastomeres by F-PCR

Z. Ban, *B. Nagy, P. Fancsovits, J. Urbancsek, Z. Papp*
Semmelweis University; Budapest, Hungary
banz@noi1.sote.hu

OBJECTIVE; Preimplantation genetic diagnosis requests highly sensitive methods, that are very sensitive for contamination to detect genetic alterations in single blastomeres. Fluorescence polymerase reaction (F-PCR) is a reliable method for single cell PCR. This study summarizes our experience in the detection X and Y chromosomes for sex determination and in the detection of delta F508 mutation in single blastomeres. METHODS; We analysed 108 blastomeres. 46 blastomeres (23 preembryos) for ˇF508 mutation and 62 blastomeres (31 preembryios) for X and Y chromosomes. The amplification of the delta F508 region was performed with CF1a and CF2 primers, for the identification of X and Y chromosomes we used the AmelA and AmelB primers. RESULTS; 54 preebryos were biopsied successfully (2 intact blastomeres). Amplification of the delta F508 region was successful in 42 blastomeres, we accepted results only in cases, when the two blastomeres from the same preembryo showed the same results. This was the case in 40 preembryos (87%). Detection of X/Y chromosomes was successful in 58 blastomeres (29 preembryos) We could accept the results in 28 preembryos (90%). CONCLUSION; In our study F-PCR appears to be a reliable method for the preimplantation genetic diagnosis of the delta F508 mutation and gender determination in X-linked recessive disorders.

P0691. Aneuploidy screen of blastomeres in at least 2 and occasionally 3 sequential fluorescence in situ hybridizations using probes from 7 chromosomes (13, 16, 18, 21, 22, X and Y).

K. S. Reddy[1], *V. Sulcova*[1], *K. Lundin*[2], *U. Selleskog*[3], *S. Ziebe*[4], *L. Helmgaard*[5], *J. C. Arce*[5]
[1]*Quest Diagnostics Inc.; San Juan Capistrano, CA United States;* [2]*Enheten for Reproduktionsmedicin, Su/Sahlgrenska; Gothenburg, Sweden;* [3]*Fertility Center, Carlanderska; Gothenburg, Sweden;* [4]*Fertilitetsklinikken, Rigshospitalet; Copenhagen, Denmark;* [5]*Novo Nordisk A/S; Bagsvaerd, Denmark*
reddyk@questdiagnostics.com

To assess the chromosome status of individual blastomeres from human embryos sequential FISH screen was performed using PB probe (Vysis MultiVysion PB 5-color mix of DNA probes for chromosomes 13,16,18,21 and 22), followed by X/Y probe (CEP X /CEP Y (alpha satellite) (Vysis Inc.). For both hybridization, results were obtained on 100% of the nuclei [42/42] tested. Six nuclei were subject to a third hybridization. In 3 cases, the third hybridization was to backtrack and complete the screen using PB probe when a nuclei was detected only on the second sequential X/Y hybridization. In 3 cases, a third hybridization confirmed one large signal consisted of two overlapping signals by using single copy probes DGS for 22, CBFB for 16 and KAL for X chromosome. The technical aspects of this study that require caution in interpretation are; in the PB hybridization the aqua and blue fluorescence signals bleed through and hence interpreting the probes labeled with these fluorophores requires cross checking to see if they are overlapped or are in different location. Secondly, short (4-hour) sequential hybridization also resulted in the bleed through of the PB green and red signals from the first hybridization, but the carry over of the signals were eliminated in a longer sequential hybridization (overnight). Conclusion; Two sequential hybridizations worked on a consistent basis. In those nuclei that were subject to the third hybridization, 100% gave results. This study shows that our FISH protocol to screen blastomeres is robust and that there is a potential for expanding this FISH test to screen ploidy status of more chromosomes in sequential hybridizations by populating the screen with more probes per hybridization. reddyk@questdiagnostics.com

P0692. The Ethical and Psychological Aspects of Prenatal Diagnostics (PD)in Tbilisi

M. Chipashvili[1], *J. Kristesashvili*[2]
[1]*E.Pipia Tbilisi Medical Institute; Georgia, Georgia;* [2]*Zhordania Institute of Human Reproduction; Tbilisi, Georgia*
maka@kheta.ge

The aim of this research was to study women s attitude towards PD, the possibilities of abortion due to medico-genetic reasons, the birth of defective child. Investigation carried out by annonymous questionnaire in 4 Maternal Houses of Tbilisi among 922 women with different age and educational level. 102 Women refuse to complete a questionnaire. The most of interviewed women (90,2%)prefer the early methods of PD in first trimester of pregnancy and in the case of defective fetus they checked an abortion as a right conclusion. Approximately the half of inquired women(48%)considered an abortion as non-ethical in the late stages of pregnancy even of abnormal fetus. There is a relatively large number of women (37%) who suppose doctor s preferance toward their defective child s future(Children s home, Family). A great majority of all inquired women would like to avoid the problems concerning to defective child s future, they prefer stillborn defective child rather then living one(40%). Correlation between age, educational level, pregnancies and ethical and psychological attitudes were find out. Conclusion; 1. 90,2% of all women prefer the early methods of Pd; 2.Women before the age 25 suppose to care for defective child in Children s Home, the most women aged 25-35 would-n t wish to look at their children in Children s Home; 3. 47,5% women with high education and 10% with secondary education considered that defective child must grow up in the family; 4. In all groups; as early appears Maternal feeling in women, they prefer to care for defective child in the family.

P0693. Diagnostic dilemma in prenatal diagnosis of sex chromosomal anomalies

A. T th[1], *E. P.Tardy*[1], *K. Hajdu*[1], *J. B torfi*[1], *S. Gombos*[2]
[1]*Semmelweis University; Budapest, Hungary;* [2]*R kus County Hospital; Budapest, Hungary*
dr.atoth@netposta.net

Couples are facing with a very difficult decision when a fetal sex chromsome abnormality is identified. The decision is more complicated when the result of prenatal investigation presents mosaic karyotypes. Postnatal studies show that even in cases of apparently pure 45,X result there is a high possibility of tissue specific discrepancies. Since Turner syndrome is basically a lethal abnormality, it has been proposed that liveborn patients should have a mosaic chromosome complement. However, it is extremely problematic to predict by prenatal investigations that the foetus will have gonadal dysgenesis, mixed gonadal dysgenesis with ambiguous genitalia or normal male with or without infertility. We report eight cases with prenatally diagnosed sex chromosomal anomalies. Studies were carried out both by conventional cytogenetic methods and FISH from chorionic villi and amniotic fluid. Based on the literature and our results we aim to present a strategy to establish correct diagnosis counselling and management.

P0694. Technical and ethical considerations raised by prenatal diagnosis in non lethal and clinically heterogeneous disorders evidence the necessity of a multidisciplinary approach ; experience based on Charcot-Marie-Tooth (CMT) disease

R. Bernard[1], *P. Negre*[1], *A. Vandenberghe*[2], *J. Pouget*[3], *N. Philip*[1,4], *N. L vy*[1,4]
[1]*Departement de Genetique Medicale; Marseille, France;* [2]*Unite de Genetique Moleculaire; Lyon, France;* [3]*Service de neurologie et des maladies neuromusculaires; Marseille, France;* [4]*Inserm U491 Medical Genetics and Development ; Marseille, France*
rafaelleb@free.fr

CMT disease is a typical example of a dominant non-lethal disease with a wide spectrum of severity ranging from asymptomatism to severe motor and sensory disability. Since molecular diagnosis is available, prenatal diagnosis is required by many at risk families. Our laboratory has to face frequent requests for CMT prenatal diagnosis. Here, we present our experience in developping molecular procedures as well as genetic counselling and psychological issues for at risk parents. Oppositely from adult testing, results delay, severity s prediction, and the elements of the final decision regarding a possible pregnancy termination are crucial. We thus developped a multidisciplinary approach including clinical and molecular geneticists, obstetricians, psychiatrists, neurologists and patients associations. In order to optimize the safety as well as the delay of the results, we designed

technical molecular procedures potentially allowing a very short result s delay. The ability to use these approaches will be compared to classical methods. We insist on the heavyness of the process engaged for the parents when considering the very high risk pregnancy, compared to the questions and doubts regarding the phenotype s severity in case of positive diagnosis. Paradoxically, the unpredictable degree of severity is also the reason why prenatal diagnosis is required and must be ethically adressed. Ethical questions raised by prenatal diagnosis in CMT evidence that molecular diagnosis in non-lethal genetic diseases can not be standardized, and should be actively and multidisciplinary considered. Eventually, the accessibility of preimplantation embryo diagnosis that our approach should make feasible will also be discussed.

P0695. Prenatal diagnosis - do males have a clue?

***U. Dresler**, J. Kunze, J. Pelz*
Institut fuer Humangenetik; Berlin, Germany
science2000uk@yahoo.co.uk

About 65% of women with an age of 35+ years decide to make use of invasive prenatal diagnosis during pregnancy in Berlin (Germany). Several factors have an influence on this decision. Subject of this study was the impact of their partners and an evaluation of their knowledge about the diagnostic procedure. Personal interviews with 200 women (35+ years old) who just gave birth to a healthy child and with 117 of their partners are reported. Interviews were conducted on maternity wards of 11 hospitals in Berlin. Males were less well informed about prenatal diagnosis (PD) than their female counterparts. The male s level of knowledge depends on that of their partners. It differs significantly regarding the men s intention to have a child, the size of the family, whether or not the woman took advantage of the invasive PD and which diagnostic procedure she chose (amniocentesis or chorion villi sampling). Males knowledge about PD is depending neither on their religion nor on their age. The male s influence on the female decision whether or not to undergo invasive PD is small. It does not depend on the male s knowledge and the magnitude of its impact is estimated differently by both partners in 25%

P0696. PCR-bassed Analysis of Infectious Teratogen in Amnion Fluid

***A. R. Nadji**[1], S. Kashani[1], S. Jalil-Nejad[1], A. Karimi-Nejad[1], H. Najmabadi[2,1]*
[1]Karimi-Nejad Pathology and Genetics Center; Tehran, Islamic Republic of Iran; [2]University Of Welfare and Rehabilitation; Tehran, Islamic Republic of Iran
maryamneishabury@yahoo.co.uk

Molecular biology approaches has led to new orientation in the fields of Pathobiology Sciences.The consequences of this changes are getting the result sooner, increasing the specifity and sensitivity of the assay and more reliability on the test result. Generally, maternal teratogenic infections are detected by the seroconversion of mother and fetus against the microorganism, Invivo and Invitro culture of microbe from body secretions of fetus, amnion fluid and autopsy samples. For this purpose, the sensitivity of serological assays, isolation and culture of pathogen may be insufficient and are time consuming. PCR, a very sensitive specific and rapid assay, helps us to detect the teratogen genome in the amnion fluid and plays an important role in prenatal diagnosis of teratogenic infections. In order to extend our Amnion synthesis pannel in this center, we have decided to include the infectious teratogen detection (CMV, HSV) by PCR method.So far, we have analysed 40 samples. From these , eight HSV positive and two CMV positve cases were detected. This study is now in progress on more number of samples.

P0697. NXF5, a novel member of the nuclear RNA export factor family, involved in a syndromic form of mental retardation

***G. F. V. Froyen**, J. Lin, P. Marynen*
VIB; Leuven, Belgium
guy.froyen@med.kuleuven.ac.be

Mental retardation provides a basis for the identification of genes involved in the development of cognitive functions. Here, we report on the identification of a gene that is interrupted by an inv(X)(p21.1;q22) in a male patient with a syndromic form of MR. The Xp-breakpoint is positioned in the gene contig Xpter-CYBB-TCTE1L-breakpoint-SRPX-RPGR-OTC-Xcen . However, a male with normal mental development, with a microdeletion on Xp21 extending from CYBB to RPGR was reported. Molecular analysis of the Xq-breakpoint region identified a novel gene called Nuclear eXport Factor 5 (NXF5) that is split by the breakpoint leading to its functional nullisomy. The predicted protein likely is a novel member of the nuclear RNA eXport Factor family since it shows 78% similarity with TAP/NXF1. Three other genes highly similar to NXF5 were found within a region of 1 Mb on Xq22. The genomic organization of the NXF gene cluster is Xcen-GLA-NXF5-NXF2-NXF4-NXF3-PLP-Xqter. We identified five different isoforms of NXF5 and the one that produces the longest ORF (NXF5a) was shown to bind to RNA as well as to p15, which points to a role in mRNA nuclear export. NXF5 is expressed at very low levels and expression profiling of the two mouse nxf-homologs (nxf-a and nxf-b) which also map on X, demonstrated the highest mRNA levels of both genes in the brain. The expression of nxf-a is predominantly detected in the cerebrum while for nxf-b, the expression in the cerebellum was more pronounced. A mutation detection analysis of NXF5 in 117 mentally retarded patients revealed single-base pair polymorphisms but no disease-associated changes were identified in this population of patients. Our results point to an important role of the potential nuclear RNA export factor NXF5 in the development of cognitive functions.

P0698. RhD/d status determination by gene dosage using a quantitative real-time duplex PCR.

***P. Ernault**, E. Gautier, L. Lohmann, M. Girard, J. Costa*
H pital Am ricain de Paris; Neuilly, France
jean-marc.costa@ahp-paris.com

Rhesus D (RhD) antigen is encoded by an autosomal gene (1p34.3). It has been demonstrated that the RhD-negative phenotype is mainly caused in Caucasian population by a complete deletion of this gene. A method for RhD/d status determination could have application in couples where there is an RhD-negative woman at risk for fetal allo-immunization. If the father is homozygous (D/D), all his children will be RhD-positive; invasive procedure to determine fetal RhD genotype becomes unnecessary. Serological methods do not indicate easily wether one or two copies of the RhD gene are present in RhD-positive individuals. Therefore, a quantitative duplex PCR using real-time technology was developed to determine the number of RhD gene copies. Both the RhD gene and a second autosomal reference gene were co-amplified by PCR. Each of the PCR products were simultaneously and specifically detected using hybridization probes, respectively labelled either with LCRed640 (for the RhD gene) or LCRed705 (for the reference gene). Serial dilutions of a control DNA was used to establish a standard curve for both the two genes. A relative quantitation expressed as a RhD gene/reference gene ratio was calculated for each sample. One hundred and one blood samples were tested using this assay. Results showed that two clear non overlapping populations are identified (ratio ;1.30 +/-0.09 vs ratio ; 0.65+/-0.04) suggesting that discrimination of RhD homozygotes from RhD heterozygotes can be easily achieved by gene dosage. Results were compared to those obtained from phenotypic studies.

P0699. Nuchal Translucency amd Chromosome Anomalies

***S. V. Boulytcheva**, O. K. Rusanova, I. V. Popova, O. V. Buntova*
Laboratory of Prenatal Diagnostics, Astrakhan Medico-Genetic Consulting Centre; Astrakhan, Russian Federation
coolworld@astranet.ru

A three- level ultrasonic screening of pregnant women is held in the Medico- Genetic Consulting Centre in Astrakhan. Analysing results of screening of 10 - 14 week pregnancy discovered that > 3 mm nuchal translucency of a fetus makes it possible to find out 9% of its chromosome anomalies. The growing level of the nuchal translucency increases the frequency of chromosome anomalies in the following proportion; when the nuchal translucency is >3mm, chromosome anomalies make 9,4 %, when the nuchal translucency is >4mm, chromosome anomalies make 27 %, the nuchal translucency >4 mm corresponds to 100 % chromosome anomalies, as well as the nuchal translucency of 10 mm. A pregnant woman of 29, somatically was observed in our centre. It was her first pregnancy. Her genetic background has no aggravations. The spouse s karyotype has no peculiarities. The ultrasonic examination at 11 - 12 week pregnancy discovered monochorial twins, the nuchal translucency being found 6 mm in both fetuses. The prenatal karyotyping by the method of transabdominal chorioincentesis was held at 11 -12 week pregnancy. The samples were prepared by the direct method, the common colouring of the sample was used. Both fetuses karyotype is 92, xxxx (tetraploid). The pregnancy was not interrupted, as the woman desired to keep it. At the term of 20 - 21 week the ultrasonic examination showed numerous congenital defects in both fetuses. The pregnancy was interrupted on the 21-st week. Results of the pathoanatomical examination are as follows; The first fetus Weight is 400 gr., height is 26 cm. Peculiarities of the phenotype are; docichocephalia, skewed forehead and occiput, hypertelorism, wide sunken nose bridge, the nose being short and snubbed, the filter is long and well formed, the lips are narrow with their rim inside, hypoplasia of the lower jaw. The auricles are low placed, ear lobes are attached, the neck is short. The cross palm wrinkles are seen on both sides of the hand, klinodactylia

of the second to fifth fingers. All the toes are of the same length, ectonia of the first toes is observed. There is obvious edema of the neck s back surface going down to the body and the neck hygroma. The chest is cylindrical. The division of lungs into lobes is correct. The heart s size is normal, there is one common ventricle, the ventricular septum is absent. The artery trunk goes out of the common ventricle, then two pulmonary arteries are ramified from it, the diameter is 1 - 2 mm. The oval window is open. The kidney is normally structured and placed, the ureters are a bit widened, wound. The adrenals are of normal size and position. The uterus and adnexa are normally sized and placed. There is observed hypoplasia of thick intestine. The turn of the intestine is incomplete. The outside reproductive organs are developed according to the female type. There is hypoplasia of labia majora, the clitoris is enlarged. Proximal sections of the limbs are shortened. The second fetus; The weight is 400 gr., the height is 26 cm. Peculiarities of the phenotype are as follows ; dolihocephalia, auricles are low placed, hypertelorism, short snub nose, long filter, thin lips, short neck, there are wing-like skin folds on the neck, the lower jaw is hypoplastic. There is arachnodactylia, klinodactylia of the second, third, fourth finger, cross palm wrinkles on both hands. All the toes have the same length. Shortness of proximal limb sections is observed. The chest has a wide aperture. Lungs division into lobes is correct. In the membranous part of the ventricular septum there is a defect, its diameter is 0,2 cm. The length of the plumonary artery curcumference is 1 cm, of the aortha is 0,9 cm. The pulmonar artery goes out of the right ventricle, the aortha goes out of the left one. The size of kidney is 2,2x1x1 for the right one and 2x1x1 for the left. There is distension of renal pelvis and calyces. The ureters are widened, wound, their diameter is 0,2 cm. There is stenosis in the vesicular-urethral segments. The turn of the intestine is incomplete. The uterus and adnexa are normally structured and placed. Outside genitals are developed according to the female type, there is hypoplasia of labia majora, the cliterus is enlarged. The cerebrum is obviously edemic, division into hemispheres is shown, side ventricles are widened.

P0700. Nuchal Translucency as a Marker of Hereditary and Congenital Pathology

***S. V. Boulytcheva**, O. K. Rusanova*
Laboratory of Prenatal Diagnostics, Astrakhan Medico-Genetic Consulting Centre; Astrakhan, Russian Federation
coolworld@astranet.ru

The laboratory of the prenatal diagnostics of the Astrakhan Medico-Genetic Consulting Centre holds prenatal karyotyping of pregnant women of the risk group. The groups are formed when the women turn to the Centre to be genetically examined. Ultrasonic screening is held at the term of 10-14 and 20-24 week pregnancy. Special attention is paid to early diagnosing of chromosome anomalies at the term of 10-14 weeks. The principal ultrasonic marker of chromosome anomalies is found to be nuchal translucency of the fetus > 3 mm.

The prenatal karyotyping is held at the term of 10-14 week pregnancy by the method of transabdominal biopsy of the koryon in the following risk groups; the presence of nuchal translucency- 22 cases (24,7 %), the age of the pregnant woman above 36 years- 3 cases (3,4 %), nuchal translucency in combination with the mentioned age -5 cases (5,6 %), one of the spouses having chromosome reconstruction - 6 cases (6,7 %), birth of a child with congenital or hereditary diseases - 2 cases (2,3 %), other cases- 51 (57,3 %). The total of 89 pregnant women have been examined. There were found 8 cases (9 %) of digital chromosome anomalies; trisomy 18 - 2 cases, trisomy 21 - 2 cases, monosomy according to sexual chromosomes- 2 cases, monosomy according to autosomes- 1 case, tetraploidia - 1 case. The majority of chromosome anomalies was found to belong to the first risk group.

The results of the investigation held made it possible to come to the following conclusions; it is reasonable to hold the first ultrasonic prenatal screening of pregnant women at the term of 10 - 14 week pregnancy; forming groups of women with a high degree of the risk of giving birth to children with congenital and hereditary pathology it is necessary to consider not only conventional medico- genetic recommendations but the discovery in the fetus of ultrasonic markers of hereditary and congenital diseases, such as nuchal translucency.

P0701. Multicolor FISH for Detection of Common Aneuploidies in Fetuses from Recurrent Spontaneous Abortion

***V. Jobanputra**, K. Kucheria*
Department of Anatomy, All India Institute of Medical Sciences; New Delhi, India
vjobanputra@hotmail.com

Aneuploidy is a leading cause of reproductive wastage. About 50% of spontaneous abortions have abnormal chromosomal complement. Chromosomal analysis of the aborted conceptus provides valuable information regarding recurrence risk and possible therapies for the couple. Conventional karyotyping depends on tissue culture and analysis of metaphase preparations which is time consuming with high rate of culture failure and false results due to maternal cell contamination. Interphase FISH is a powerful molecular cytogenetic technique which has been used for several applications in cytogenetic diagnosis. The objective of our study was to compare the results of multicolor interphase FISH with conventional chromosomal analysis for he detection of the most common aneuploidies in spontaneous abortions and to test whether interphase FISH can eliminate the need for culture in these samples. For this we have used the chromosome specific probes for 13, 15, 16, 18, 21, 22, X and Y for interphase FISH analysis on the products of conception from 52 women with recurrent spontaneous abortions. Aneuploidies were detected in 26 samples using these probes. Conventional karyotyping in all these samples confirmed the results of FISH analysis except in 2 cases where the probe for 15 was cohybridizing with polymorphic region on chromosome 14 (thereby showing false trisomy 15 on the interphase cells) and 2 cases where aneuploidy of chromosome 7 and 17 (for which the probes were not used) were detected. The results suggest that multicolor interphase FISH overcomes the limitations of conventional karyotyping and readily identify common aneuploidies associated with spontaneous abortions eliminating the need for tissue culture in a substantial number of samples.

P0703. 49,XXXXY; Case Report in Prenatal Diagnosis

***G. Di Cola**[1], C. Perfumo[1], C. Caporale[1], P. Caggiati[1], S. Rossi[1], M. Savi[2]*
[1]Bio-Tech Laboratories; Parma, Italy; [2]Istituto Genetica Medica, Universit di Parma; Parma, Italy
bbiotech@tin.it

The pentasomy 49,XXXXY is one of the rarest sex chromosome defects, occurring with an estimated incidence of 1 in 85 000 male births. This condition is associated with pre- and postnatal growth deficiency, severe mental retardation, hypogenitalism, and other skeletal, facial and cardio-vascular anomalies. We report on the prenatal diagnosis, genetic analysis of a 49,XXXXY fetus.

A 33-year-old, primigravida woman was referred for amniocenthesis and quantitative fluorescent polymerase chain reaction (QF-PCR) with small tandem repeat (STR) markers specific for chromosome 13, 18, 21, X and Y at 16 weeks gestation for positive second trimester triple test (1;16) and increased nuchal translucency.

Quantitative fluorescent polymerase chain reaction (QF-PCR) with small tandem repeat (STR) markers specific for chromosome X and a pentanucleotide marker X22 for the Xq/Yq pseudoautosomal region PAR2 rapidly detected the X-chromosome polysomy from amniotic fluid cells. Cytogenetic analysis revealed a karyotype of 49,XXXXY.

Our case also shows that QF-PCR assays with sex chromosome specific STR markers provide rapid prenatal diagnosis of numerical sex chromosome aneuploidies.

P0704. Identification Of Fetal Dna In Systemic Lupus Erythematosus

***M. P. S. Alvarenga**[1], E. C. Pavarino Bertelli[2], M. Abbud F...[2], R. A. Toledo[2], I. M. M. Fernandes[2], F. A. Malara[2], E. H. Tajara[3], E. M. Goloni Bertollo[2]*
[1]Famerp - Faculdade de Medicina de S o Jos do Rio Preto; S o Jos do Rio Preto - SP, Brazil; [2]Faculdade de Medicina de S o Jos do Rio Preto; S o Jos do Rio Preto -SP, Brazil; [3]Instituto de Bioci ncias, Letras e Ci ncias Exatas de S o Jos do Rio Preto - UNESP; S o Jos do Rio Preto - SP, Brazil
eny.goloni@famerp.br

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by diverse clinical manifestations and the presence of multiple autoantibodies. SLE affects approximately 1 in 2000 individuals and is more prevalent in women, particularly their reproductive years.There are multiple etiological factors involved in this pathogenesis. Recent studies indicate that fetal cells can survive in the maternal circulation for many years post partum. This finding suggests that fetal microchimerism could be involved in the autoimmune diseases, including rheumatoid arthritis, Sj grens syndrome and scleroderma. We used the polymerase chain reaction (PCR) to identify Y-chromosome sequences in DNA extracted from peripheral-blood cells and renal biopsy from women with SLE and health women both had had male children. We describe the presence, of the y-chromosome-specific sequence in DNA extracted from the peripheral-blood and biopsy of women with SLE. These observations provide support for the hypothesis that a fetal antimaternal graft-versus-host reaction may be an immunopathogenic mechanism in the development of SLE in some women.

Financial support; FAPESP - Funda o de Amparo Pesquisa do Estado

de S o Paulo,
FAMERP - Faculdade de Medicina de S o Jos do Rio Preto

P0705. Microsatellite instability in the FRAXA-FRAXF region; implications for the mechanisms of trinucleotide repeat expansion.

V. V. Strelnikov, *O. Drozd, M. Nemtsova, D. Zaletayev*
Research Centre for Medical Genetics; Moscow, Russian Federation
vstrel@iname.com

Reported associations between fragile X (FX) mutations and adjacent polymorphic markers may reflect founder effects as well as microsatellite instability (MI). To better understand the bases of mutational processes in the FX populations, we analyzed allele frequencies and mode of inheritance of DXS584, FRAXAC1, FRAXAC2, DXS1691, DXS1123 and DXS1113 microsatellites in normal and FX individuals in Russia. The study of inheritance mode for DXS584, FRAXAC1, FRAXAC2, DXS1691, DXS1123 and DXS1113 revealed 3 DXS548, 4 FRAXAC1 and 2 DXS1123 unstable FX maternally derived meioses among 54 tested, thus showing that not only an FMR1 (CGG)n repeat is unstable on FX chromosomes, but other microsatellites as well. The proximal bounder of the region of FX-associated microsatellite instability is no closer than 150 kb to FRAXA (DXS548 being unstable) and the distal bounder is no farther than 1.8 Mb (DXS1123 being unstable and DXS1113 being stable). The boundaries of this region coincide with the boundaries of delayed replication zone detected on X(q27.3-q28) in the cell lines from the FX patients. Spreading of late replication zone changes the replication timing leading to the inability of mismatch repare system to correct the replication mistakes naturally occurring in repetitive sequences and in trinucleotide repeats particularly. It means that FX mutations arise not on a specific haplotype background, but are themselves results of specific occurrences of MI, thus depending not solely on the internal properties of FMR1 (CGG)n repeat, but on the chromosomal background as well, representing an example of position effect on human X-chromosomes.

P0706. A Glu to Ala Polymorphism Leads to Loss of Function of the Human P2X7 Receptor

W. Zhang, *B. Gu, R. Worthington, R. Sluyter, P. Dao-Ung, J. Barden, J. Wiley*
University of Sydney; Sydney, Australia
Weiyi.Zhang@swsahs.nsw.gov.au

The P2X7 receptor is a ligand-gated cation selective channel which mediates ATP-induced apoptosis of cells of the immune system. We and others have shown that P2X7 is non-functional both in lymphocytes and monocytes from some subjects. To study a possible genetic basis, we sequenced genomic DNA coding for the carboxy terminal tail of P2X7. In 9 of 45 normal subjects, a heterozygous nucleotide substitution (A1513C) was found while 1 subject carried the homozygous substitution which codes for glutamic acid to alanine at amino acid position 496. Surface expression of P2X7 on lymphocytes was not affected by this A1513C polymorphism demonstrated both by confocal microscopy and immunofluorescent staining. Monocytes and lymphocytes from the A1513C homozygous subject expressed non-functional receptor while heterozygotes showed P2X7 function which was half that of germline P2X7. Results of transfection experiments showed the mutant P2X7 receptor was non-functional when expressed at low receptor density but regained function at a high receptor density. This density-dependence mutant P2X7 function was also seen on differentiation of fresh monocytes to macrophages with interferon-gamma which upregulated mutant P2X7 and partially restored its function. P2X7-mediated apoptosis of lymphocytes was impaired in homozygous mutant P2X7 compared with germline (8.6% versus 35.2%). The data suggest that the glutamic acid at position 496 is required for optimal assembly of the P2X7 receptor.

P0707. Genomic organization of the human respiratory chain complex I 13-kDa subunit gene NDUFA5.

K. Tamboom[1], *I. Pata*[2], *I. Wittig*[3], *M. Wehnert*[3], *A. Metspalu*[2]
[1]*Institute of Experimental and Clinical Medicine; Tallinn, Estonia;* [2]*Institute of Molecular and Cell Biology, Tartu University, Estonian Biocentre; Tartu, Estonia;* [3]*Institute of Human Genetics, Ernst-Moritz-Arndt University Greifswald; Greifswald, Germany*
kristiin@ekmi.ee

NADH; ubiquinone oxidoreductase (complex I) is the first multisubunit enzyme complex in the respiratory electron transport chain of mitochondria. It catalyzes the transfer of electrons from NADH to ubiquinone coupled to proton translocation across the membrane. Complex I contain at least 42 subunits, seven of them are encoded by mitochondria and remainders are the products of nuclear genes and are imported into the organelle. Here we report the identification of one complex I gene; NDUFA5. The NDUFA5 spans approximately 14 Kb of genomic DNA and is composed of five exons (Gene Bank ac. No. AF044415-AF044418). The promoter region was studied for putative transcription factor binding sites and it reveals that the gene is controlled by housekeeping transcriptional machinery. As usual for housekeeping genes, the 5 end of NDUFA5 is embedded in a CpG-island. In the human genome there are several processed pseudogenes of NDUFA5. The transcriptional start site of human NDUFA5 was mapped by primer extension on a single T residue 109 bp upstream the translation start codon. The functional gene was localized by FISH to 7q31. The position was future refined using two NDUFA5 specific STSs on radiation hybrid panel. NDUFA5 maps 3 cR distal to the genetic marker D7S648 that is positioned within YAC883_a_2. By heteroduplex analysis there was found one variable nucleotide at the position —318 (T/C). The frequencies of this variation were analyzed in Complex I deficiency patients and age matched control group, no significant difference was found. The variant seems not to be related to any disease phenotype.

P0708. Sequencing of the LDLR gene and of the coding exons of LRP1 on individual DNA samples reveals novel mutations in both genes.

F. Van Leuven
Experimental Genetics Group; Leuven, Belgium
fredvl@med.kuleuven.ac.be

Methods were developed using long-range amplification to sequence all the 89 exons of the LRP1 gene and the entire LDLR gene (exons 2-18 and introns) on individual DNA samples. Analysis of 3 test-groups of 22 healthy individuals, 29 Alzheimer patients and 18 individuals with different clinical and molecularly uncharacterized lipid metabolism problems, revealed 5 coding polymorphisms in de LRP1 gene, i.e. A217V, A775P, D2080N, D2632E and G4379S. No genetic defect was evident in the LRP1 gene of any of the AD patients, further excluding LRP1 as a major genetic problem in AD. LRP A217V (exon 6) was present in the 3 groups as a normal polymorphism, while D2632E was observed only once, in a healthy volunteer. On the other hand, LRP1 alleles A775P, D2080N, and G4379 were encountered only in patients with FH or with undefined problems of lipid metabolism. This finding forced us to analyze their LDLR gene entirely from exons 2 to 18. This yielded a sequence contig of 33,567 nucleotides, and thereby - finally - an exact physical map of the LDLR gene. This corrects the published and web-posted maps in many positions, and include not only the exact size but also their content of about 250 Alu-related repeat sequences distributed all over the gene. In addition, 4 novel LDLR mutations that cause FH were defined, i.e. del e7-10, exon 9 mutation N407T, a 20 bp insertion in exon 4, and a double mutation C292W/K290R in exon 6. No evidence for pathology connected to the LRP1 mutations was obtained by subsequent screening for the 5 LRP1 variants in larger groups of 110 FH patients and 118 patients with molecularly undefined, clinical problems of cholesterol and/or lipid metabolism. In three individuals with a mutant LDLR gene also a variant LRP1 allele was present, but without direct, obvious clinical compound effects, indicating that the variant LRP1 alleles must, at this moment, be considered polymorphisms.

P0709. Abnormal mRNA splicing resulting from four different mutations in the CFTR gene

M. Tzetis[1], *A. Efthymiadou*[2], *S. Doudounakis*[3], *E. Kanavakis*[2]
[1]*Athens University; Athens, Greece;* [2]*Athens University, Medical Genetics; Athens, Greece;* [3]*St Sophia s Chil dren s Hospital; Athens, Greece*
mtzeti@cc.uoa.gr

Four different putative splicing mutations in the CFTR gene have been studied by analysing mRNA extracted from nasal epithelial cells harvested from patients with cystic fibrosis and one heterozygote with reccurent episodes of pneumonia. Four patients carried the 621+3A>G mutation (2 with F508del, 1 with 1898+1G>T and 1 with W1282X), 1 carried the 2751+2T>A (with F508del), another the 296+1G>C (with 3130+1G>A) and 1 heterozygote 1717-9T>C (with D565G and R668C in cis). For each case two non-CF control subjects were also studied for the appropriate cDNA fragment. The 621+3A>G mutation resulted in activation of an alternative splice site within exon 4 (28.3–5.9% of total cDNA) and skipping of exon 4 (6.75–1.35% of total cDNA). The 2751+2T>A mutation resulted in skipping of exon 14a (52.6% of total cDNA), the 296+1G>C mutation resulted in skipping of exon 2 (56% of total cDNA). The patients carrying the above mutations presented with PI and all except the patient carrying the 296+1G>C mutation were diagnosed between birth and 8mos. Mutation 1717-9T>C resulted in skipping of exon 11 as a minor product and skipping of exons 9 and 11 as a major product. The patient was diagnosed with reccurent episodes of pneumonia and has normal sweat test. Open reading frame is maintained in transcpirts produced by mutations

621+3A>G, 2751+2T>A, and 296+1G>C (except for the change in the last aa of exon 1 from Ser to Arg), but deletion of exon 11 as a consequence of mutation 1719-9T>C results in a stop codon within exon 12.

P0710. Genomic Structure And Functional Analysis Of The Human Ssadh Gene

P. Malaspina[1], *P. Blasi*[1], *M. Corvaro*[1], *F. Amaldi*[1], *A. Novelletto*[2], *F. Loreni*[1]
[1]*University Tor Vergata ; Rome, Italy;* [2]*University of Calabria; Rende, Italy*
patrizia.malaspina@uniroma2.it

Mitochondrial NAD+ dependent succinic semialdehyde dehydrogenase (SSADH, OMIM 271980) deficiency is a defect in the GABA degradative pathway that results in 4-hydroxybutyric aciduria, a rare inborn error of metabolism inherited in an autosomal recessive manner [1]. We had already identified and physically mapped the SSADH gene in 6p22 [2]. Analysis of cDNA clones encompassing the entire coding sequence allowed to predict a 535 residues amino acid sequence (accession n. Y11192). We used this information to detect the first two mutations that affect SSADH expression by causing exon skipping during mRNA maturation [3]. Northern blot analysis revealed the presence of two SSADH mRNAs (about 4 and 2 Kb), whose relative abundance seems to vary in different tissues. These data suggest that, although having a central role in the cellular metabolism, a tissue-specific expression of the SSADH gene could exist. The different length of the two trascripts could be partially due to the alternative usage of two polyadenilation signals that we identified and localized 78 bp and 2 Kb downstream the ORF. In order to investigate the qualitative and quantitative difference between the two mRNAs we first decided to identify putative promoter region(s) through transfection of reporter gene constructs cloning different portions of 5 Kb region upstream the ATG start codon. The results show that a 180 bp region immediately upstream the ATG is able to drive considerable activity of the luciferase reporter gene as contrasted with more distal DNA portions. Acknowledgment; This work was supported by Telethon Onlus Italia grant. E.818 (P.M.) References [1] Chambliss K.L., et al. J. Biol. Chem. 270;461-467, 1995. [2] Malaspina P., et al. Genomics 36;399-407, 1996. [3] Chambliss K.L., et al. Am. J. Hum. Genet. 63;399-408, 1998.

P0711. Alternative first exons of PTCH1 are differentially regulated in vivo and may confer different functions to the PTCH1 protein

P. Kogerman[1], *D. Krause*[2], *F. Rahnama*[2], *L. Kogerman*[1], *A. Und n*[2], *P. Zaphiropoulos*[2], *R. Toftg rd*[2]
[1]*National Institute of Chemical Physics and Biophysics; Tallinn, Estonia;* [2]*Karolinska Institutet; Huddinge, Sweden*
priitk@kbfi.ee

PTCH1 gene is a human tumor suppressor frequently gene mutated in basal cell carcinoma (BCC) and several other tumor types. It encodes a receptor for soluble factors of the hedgehog family. Binding of hedgehog to the receptor relieves its inhibitory action on the transmembrane co-receptor SMOH. In this study we described alternative first exons of the PTCH1 tumor suppressor gene and shown that they are differentially regulated in normal tissues, exon 1B being expressed at very low levels and the major mRNA species containing exon 1 or 1A. Exon 1B transcripts were found to be specifically upregulated in nodular BCCs. All PTCH1 alternatively spliced isoforms encode proteins that colocalize and interact with SMOH in doubly transfected cells. Furthermore, functional assays demonstrated that whereas all PTCH1 isoforms can inhibit the activity of SHH, only the PTCH1B isoform is capable of fully inhibiting SMOH activity. These results indicate that in tumor cells the PTCH1B promoter is specifically activated and importantly, that the N-terminal part of PTCH1 including exon 1B is required for full inhibition of SMOH signaling but not for physical interaction with SMOH.

P0712. Germ cell-Sertoli cell interaction; Functional analysis of THEG gene

A. U. Mannan, *G. Nica, P. Burfeind, W. Engel*
Institute of Human Genetics; G ttingen, Germany
amannan@gwdg.de

Stage-specific interactions between Sertoli cells and germ cells are central in the regulation of spermatogenesis, but their molecular mechanisms are still poorly understood. In the present study, a differentially expressed gene, named THEG, which is specifically expressed in spermatids, is characterised. Its expression is up regulated by some unknown factor/s from Sertoli cells. The gene is approximately 10 kb pairs in size and contains 8 exons. The ORF of 376 amino acids encodes for a 42.7 kDa putative nuclear protein. To elucidate the potential role of the THEG, we deleted the gene in mice through homologous recombination. Both male and female mice heterozygous for THEG mutation appeared normal and fertile. Homozygous male and female mice also exhibits normal phenotype, however THEG -/- male mice are infertile. Further molecular, histological and physiological analyses are in progress, which will give us insights into the critical role of THEG protein in male germ cell development. The cellular type and subcellular localization of the THEG protein in the testicular sections of adult mice were determined by immunostaining with a polyclonal antibody against THEG protein. A predominant signal was detected in the nucleus of round spermatids, whereas no specific staining was observed in other germ cell stages and in somatic cells. A human homologue of THEG (hTHEG) was cloned. The complete genomic structure was characterised from sequence analysis of a genomic hTHEG PAC clone and from the release of the working draft sequence of the human genome in the GenBank.

P0713. Relationship between transcription of carnitine palmitoyltransferase (CPT) in rat liver and in human peripheral blood mononuclear cells and carnitine levels

H. Karlic[1], *S. Lohninger*[2], *C. Laschan*[3], *A. Lohninger*[2]
[1]*Ludwig Boltzmann Institute for Leukemia Research and Hematology; Vienna, Austria;* [2]*Institute of Medical Chemistry, University of Vienna; Vienna, Austria;* [3]*3rd Medical Department, Hanusch Hospital; Vienna, Austria*
molzell@adis.at

This study focused on the influence of dietary L-carnitine supplementation on the transcription rate of carnitine palmitoyltransferases (CPT-1A and CPT-1B) which are key enzymes of fatty acid metabolism. The expression of these enzymes is related to the metabolic availability of L-carnitine which is decreased in aging subjects and in metabolic disorders such as hyperlipidemia. mRNA of all samples was determined by the LightCycler SYBR green technique (RT-LC-PCR). In animal experiments 100 mg/kg/bw/d L-carnitine was administered orally to old (21months) male Spraque Dawley rats for a period of 3 months. Old (21 months) and adult (7 months) control animals received tap water exclusively. Livers from carnitine treated old rats showed an 8-12 fold higher transcription rate regarding ratios both of CPT/§-actin and CPT/G6PDH. Further, PBMC (peripheral blood mononuclear cells) from 10 healthy humans and 5 patients with hyperlipidaemia type IV were analyzed. In cases with hyperlipidaemia type IV we observed an up to 100-fold increase in CPT transcripts relative to §-actin or G6PDH (glucose-6-phosphate-dehydrogenase) after at least 4 weeks of L-carnitine supplementation (1g/d), but no further rise when in a comparable range to controls. To our knowledge this is the first report to date providing direct evidence for a stimulatory effect of dietary L-carnitine on the transcription rate of CPT in PBMC. This appears to reflect a metabolic situation comparable to that in liver. Thus, this study provides a non-invasive approach for monitoring the effect of L-carnitine deficiency and L-carnitine supplementation on oxidative metabolism.

P0714. Quantitative Analysis of Immune-Mediated Stimulation of Tumor Necrosis Factor in Macrophages at the mRNA-level using RT-PCR and LightCycler SYBR Green¤ Technology and at the Protein Level using ELISA

M. Weghofer[1], *H. Karlic*[1], *A. Haslberger*[2]
[1]*Ludwig Boltzmann Institute for Leukemia Research and Hematology; Vienna, Austria;* [2]*Institute of Microbiology and Genetics, University of Vienna; Vienna, Austria*
molzell@adis.at

Expression of cytokines such as TNF (Tumor Necrosis Factor) as induced by lipopolysaccharides (LPS) from the outer membrane of gram negative bacteria has been associated with septic shock. As macrophages and dendritic cells have been considered as the most important target cells, the focus of this study was to investigate quantitative changes in mRNA synthesis rates of TNF. The stress-sensitve murine macrophage cell line RAW 264.7 was chosen an an in vitro model. RAW 264.7 cells were stimulated for 2, 4, 6 and 18 hours with 100 ng/ml LPS. Using RT-PCR and LightCycler SYBR Green¤ Technology we could show a 3-10 fold increase in the LPS-induced transcription rate of TNF which decreased after 18 hours down to the 2-fold rate of untreated controls. Analysis of TNF-synthesis at the protein level using ELISA indicated a 40-70 fold rise in the TNF synthesis rate after 2 hours which was still elevated 10 - 30 fold after 18 hours compared to untreated controls. These data indicate that expression of TNF is regulated at the posttranscriptional level.

P0715. The isolation of new of human minisatellite UPS29 and search for its homologs in animals.

I. Ogneva, *I. Suchkova*
Institute for Experimental Medicine; Saint-Petersberg, Russian Federation
molgen@molgen.iem.ras.spb.ru

The molecular mechanism of the micro- and minisatellite DNA instability

remains mainly unclear, though the role of GC-content is known. The main aims of this work were an isolation of new GC-rich human minisatellite and the search for its homologs in animals. The computer analysis of the cosmid with a 34175 bp clone of human DNA, performed by Dr G. Vergnaud, revealed the presence of gene containing a minisatellite sequence in one of its introns and named UPS29. We determined the repeated element of this sequence to be 46 bp long with the total length of 745 bp. Our computer analysis showed that the remarkable feature of repeat is the presence of one direct and two inverted short repeats (5 bp long) in the middle of repeat. These short repeats are analogous to the elements, found in sopC-locus of F-plasmid in E. coli. UPS29 contains a large number of sites, that are homologous to hot spots of recombination of various organisms and it also contains four sites for topoisomerase I. Further analysis revealed a sequence in Mus musculus, that is expressed in hypothalamus and codes a 322 bp mRNA. Some exons of this sequence, including the once flanking UPS29 element, are highly homologuos (86%) to human one. Described computer analysis gave us an opportunity to isolate human UPS29 with the help of our modification of Southern analysis and its mouse homolog using PCR.

P0716. The structure, expression, and evolution of the human nucleoporin 155 (NUP155) gene

X. Zhang[1], *H. Yang*[2], *J. Yu*[3], *N. Gregersen*[4], *E. Niebuhr*[5], *L. Bolund*[1]
[1]*Aarhus University; Aarhus, Denmark;* [2]*Chinese Academy of Sciences; Beijing, China;* [3]*University of Washington; Seattle, WA United States;* [4]*Aarhus University Hospital; Aarhus, Denmark;* [5]*Copenhagen University; Copenhagen, Denmark*
xqzhang@humgen.au.dk

Nucleoporins (NUPs) are the main components of nuclear pore complex (NPC) which are involved in the regulation of bi-directional trafficking of molecules, especially mRNAs and proteins, through the nucleopores between nucleus and cytoplasm. We have obtained the complete sequence and genomic organization of the gene for human NUP155, one of the most abundant nucleoporins in the NPC, by sequencing and annotating a 165.6 kb human BAC clone. The human NUP155 gene is estimated to be 90 kb in length and contains 36 exons. It is expressed in all the tissues tested with two major alternatively-spliced transcripts resulted from the alternative usage of a 5 cryptic splice donor in Intron 1 and two polyadenylation signals. We have also cloned a full-length cDNA of the mouse Nup155 gene, as well as the Nup153, a Nup155 orthologue, from the pufferfish (Fugu) genome, which is only 9 kb in length with similar genomic organization but much smaller introns. Comparative analyses of the NUP155 orthologues in human, mouse, rat, Fugu, Arabidopsis, Drosophila, and yeast, reveal a loss of one of the two paralogues, increased numbers of introns in higher organisms and highly conserved amino acid sequences in eukaryote evolution. The regions harboring the NUP155 orthologues in the human and Fugu genomes have different gene orders, advocating cautious interpretation of synteny in comparative genomics analysis even in the vertebrate lineage.

P0717. Exclusion of Gemin3 and Profilin II as modifying genes for spinal muscular atrophy (SMA) using denaturing high performance liquid chromatography (DHPLC)

C. Helmken, *B. Wirth*
Institute of Human Genetics; Bonn, Germany
C.Helmken@gmx.de

Spinal muscular atrophy (SMA) is caused by homozygous mutations of the survival motor neuron gene 1 (SMN1). SMN, located on chromosome 5q13, is part of an 800 kDa protein complex with a crucial role in snRNP biogenesis, pre-mRNA splicing and a presumable function in neural transport. In rare cases sibs with identical 5q13-homologs and identical SMN1 mutations can show variable phenotypes, suggesting that SMA is modified by other, yet unknown factors. Recently, we excluded SIP1 and Htra2-beta1, two SMN-interacting proteins, as putative SMA modifying genes. Here we report the analysis of Gemin3 and Profilin II, two further SMN-interacting proteins, as putative SMN modifiers and searched for mutations/polymorphisms using DHPLC. We studied the complete coding region in 36 sibs belonging to 15 SMA families displaying identical 5q13 haplotypes and SMN1 mutations but variable phenotypes. Two different polymorphisms were detected in Gemin3 exon 11 but with no significant association to the phenotype. To look for quantitative differences between transcription isoforms and the total amount of RNA, we performed quantitative analysis of RT-PCR products from 26 sibs of 11 families with identical genotypes without identifying any significant difference between phenotypically discordant sibs. Based on these data, we suggest that the intrafamilial phenotypic variability in SMA families is not caused by polymorphic variants or transcription differences within Gemin3 or Profilin II.

P0718. Expression and alternative splicing of the candidate tumor suppressor gene DICE1

I. Wieland, *A. R pke, P. F. Wieacker*
Institute for Human Genetics, Otto-von-Guericke-University; Magdeburg, Germany
ilse.wieland@medizin.uni-magdeburg.de

The candidate tumor suppressor gene DICE1 is located within a previously reported LOH critical region telomeric to the RB1 gene in chromosomal region 13q14.3. Intron/exon mapping of the DICE1 gene revealed 18 exons that are preceded by a GC-rich promoter. The DICE1 mRNA is expressed in a variety of normal tissues and it is downregulated in tumor cells suggesting tumor-specific inactivation of DICE1 by gene silencing. In adult brain an alternatively spliced DICE1 transcript (ASP1) has been identified that appears to be differentially expressed. In the variant DICE1 transcript ASP1 exon 3 is spliced out which results in a DICE1 protein lacking a potential protein kinase C phosphorylation site. In order to determine expression of the DICE1 mRNAs in different brain regions RT-PCR based expression profiling of a regional brain panel was performed. Expression of DICE1 was observed in frontal and temporal lobe, cerebellum, substantia nigra, caudate nucleus, thalamus, hypothalamus, pons, medulla and spinal cord but not in hippocampus and amygdala. The ASP1 transcript appears to be expressed predominantly in substantia nigra. From these results we conclude that in the brain alternative splicing and expression of the DICE1 mRNA is regulated in a region-specific manner.

P0719. Analysis of expression and function of the mammalian pelota gene

M. Sallam, *H. Lee, R. Shamsadin, W. Engel, I. Adham*
Institut f r Humangenetik; G ttingen, Germany
mahmoud2001@yahoo.com

During spermatogenesis, spermatogonia undergo extensive mitotic divisions forming primary spermatocytes, which then remain in an extended premeiotic G2 phase bevor entry into two meiotic divisions and produce haploid spermatids. Several genes that regulate the meiotic division in Drosophila have recently been identified. In the male homozygous for a mutation in pelota gene, the germline mitotic divisions are normal but the cell cycle arrests prior to the first meiotic division. The resulting 4N germ cells in testis of pelota mutants develop to spermatids with head and tail. The continued differentiation of 4N spermatids after meiotic failure suggested that the pelota gene is required only for transition of the germ cells from premeiotic G2 phase to the meiotic phase. To understand the function of pelota gene in mammals, we have isolated the murine and human gene and determined its chromosomal localization. The predicted murine and human proteins share a 65 % sequence identity with the Drosophila pelota protein. Southern blot and fluorescence in situ hybridization (FISH) analysis reveal that the pelota gene is present as a single copy gene in murine and human genome and is localized on murine chromosome 13 and on human chromosome 5q11.2. Northern blot analysis indicates that the human gene is expressed in two alternative spliced mRNAs (1.6 and 2.1 kb). The 1.6 kb mRNA is found in all tissuse studied, while the 2.1 kb transcript is detected predominantly in testis. To determine the function of the pelota gene in mammalian spermatogenesis, we have generated the Pelota mutant mice and analysed the phenotype of homozygous mutant. In contrast to normal development of the Drosophila pelota mutant, the pelota deficient mice die in theearly embryonic development.

P0720. Functional characterisation of an atypical LINE1 element (LIPMA2) contained in intron 11 of the human dystrophin gene.

A. Ferlini[1], *F. Gualandi*[1], *P. Rimessi*[1], *E. Calzolari*[1], *B. Cardazzo*[2], *T. Patarnello*[2], *M. Dunckley*[3], *F. Muntoni*[3]
[1]*Sezione di Genetica Medica, University of Ferrara; Ferrara, Italy;* [2]*Dipartimento di Biologia, University of Padova; Padova, Italy;* [3]*Department of Paediatrics, HH, Imperial College School of Medicine; London, United Kingdom*
fla@dns.unife.it

Several recent observations suggest that LINE1 mobile elements may not only represent junk DNA. The finding that they can co-mobilise 3 tails, which may be relevant both for exon shuffling and other regulatory functions raises the question of whether and how these elements may function in the genome. We carried on functional as well as phylogenetic studies on an atypical LINE1 element, LIPMA2, which contains an unusual 3720 bp tail at its 5 end. A truncated (but preserving the 3 end 800 bp of the tail) LIPMA2 element is normally present in the dystrophin gene intron 11 and is involved in a deletion causing a dystrophinopathy. We have defined this rearrangement finding out that it brought the LIPMA2 tail close to a cryptic splice site and caused a splicing pathology. This observation suggests that

the LIPMA2 5 tail might play a role in the splicing process. In order to functionally and phylogenetically investigate this novel element of L1 class, we firstly tested by Zoo-blot and PCR/sequencing the presence of the 3 end 800 bp of the LIPMA2 5 tail in genomic DNA of several species. Results showed that this sequence is a primate specific repeat element that appeared firstly in lemur, at least 25-45 million years ago, when the lineage of the common ancestor of old world monkeys and hominoids emerged. Comparative analysis also showed that The LIPMA2 5 tail contains several regulatory motifs, particularly in its 3 end (last 800 bp), that are adjacent to the LINE1 consensus, and at least 7 tail regions are well conserved among primates as well as human chromosomes. In order to functionally test this tail, we used a reporter construct containing the beta-globin exon 2 , intron 2 splicing borders, and beta-globin exon 3, under the T7 promoter control for in vitro transcription. We inserted in this construct the entire 3 end 800 bp of the tail. We also made a control construct similar in length and containing the full beta-globin intron 2. From these two constructs, together the control-construct, DNA templates have been obtained by PCR and have been in vitro transcribed. A splicing assay was than carried out using HeLa cell extracts as splicing substrate. The experiments showed that the construct containing the LIPAM2 tail is affecting the beta-globin splicing. The presence of several regulatory motifs, including at least one purine-rich SR binding site and other transcription controllers in the conserved regions, together with the splicing capacity of this tail, suggests that it may represent a conserved non-coding sequence (CNS). This observation might have several implications in the general role(s) retroposon-related sequences may have in genome plasticity. Acknowledgement This work has been supported by the EU grant FINGER , number QLG2-CT-1999-00920 (to AF)

P0721. Ceruloplasmin Receptor Involved In Copper Metabolism Is a Member Of Transmembrane Ceruloplasmin-like Multicopper Oxidase Family.

N. Platonova[1], *A. Vasin*[2], *B. Mitshenko*[2], *N. Tsymbalenko*[1], *L. Puchkova*[1]
[1]*Institute for Experimental Medicine; St. Petersburg, Russian Federation;* [2]*State Technical University; St. Petersburg, Russian Federation*
chemist@ctinet.ru

Electrophoretically pure ceruloplasmin receptor (CpR), which is the protein of metabolic copper system (MCS) in mammalian, was isolated from human erythrocyte membranes using the affinity chromatography. The clone containing the insert of CpR cDNA was isolated from the expression cDNA library from human placenta. The comparative BLAST analysis of the two sequencing clone fragments in the GenBank database shows these sequences are homologous to human Cp cDNA. The 638 b.p. sequence (NAF211154) contains fragments that are homologous to Cp exons 4-7 (81% identity). The deduced amino acid sequence has 83% of identity to E216 — E427 segment of Cp. One of the two glycosylated sites of the compared Cp region remains unchanged in CpR. All ligands for mononuclear copper type 1 site of Cp are conservative in CpR. The 257 b.p. sequence (NAF211153) has 76% sequence identity with Cp cDNA exon 16. This deduced amino acid has no homologies to any a.a. sequences in the Genbank database. According to the analysis with the PC/GENE system it contains a putative transmembrane domain. Thus we can relate CpR to the family of transmembrane CP-like proteins. The computer analysis of 20 proteins participating in MCS of various organisms using CLUSTAL W and PHYLIP programmes found out CpR sequence NAF211154 is the most similar to Cp, Heph and Fet3. CpR sequence NAF211153 is the most similar to Aso and Ccc2. This data make us think that Cp, CpR and Heph have a common ancestral gene with Fet3 from S. cerevisiae.

P0722. Mutations in Latency Associated Protein (LAP) Cause Instability of the TGF-beta1 Complex and Camurati-Engelmann Disease (CED)

A. Kinoshita[1], *T. Saito*[2], *H. Sugawara*[1], *K. Yamasaki*[1], *Y. Makita*[3], *K. Yoshiura*[1], *N. Taniguchi*[2], *N. Niikawa*[1]
[1]*Dept. Human Genet., Nagasaki Univ. Sch. Med.; Nagasaki, Japan;* [2]*Dept. Biochemistry, Osaka Univ.; Osaka, Japan;* [3]*Dept. Public Health, Asahikawa Med. Coll.; Asahikawa, Japan*
d399006a@stcc.nagasaki-u.ac.jp

CED (MIM *131300) or progressive diaphyseal dysplasia (DPD1) is an autosomal dominant disorder characterized by hyperosteosis and sclerosis of the diaphyses of the long bones. We identified, in 10 CED families (8 Japanese and 2 Caucasians), four different missense mutations (R218H, R218C, C223R and C225R) in the TGFB1 gene at a domain corresponding to the C-terminus of LAP of latent TGF-beta1. TGF-beta1 is translated as a precursor protein with 390 amino acids, and dimerized by a disulfide bond at the cysteine residues. The TGF-beta1 precursor protein consists of signal peptide, LAP and mature TGF-beta1. LAP associates with mature TGF-beta1 by noncovalent binding and represses the activation as latent form. To know how these mutations cause CED, we performed functional analysis of mutated TGF-beta1. Pulse-chase analysis and ELISA revealed that all mutations cause instability of the TGF-beta1 and LAP complex and the increase of free TGF-beta1. This instability is inhibited by in vitro administration of dexamethasone. The results gave an insight into not only the pathogenesis of CED but also the function of TGF-beta1 in bone remodeling.

P0723. Intracellular localization of the y+LAT-1 amino acid transporter; green fluorescent protein (GFP) fusion studies

M. Heinonen
University of Turku; Turku, Finland
minna.heinonen@utu.fi

Lysinuric protein intolerance (LPI) is an autosomal recessive disease in which the absorption of cationic amino acids is defective in small intestine and renal tubules. This leads to excretion of cationic amino acids in urine. The symptoms associated with LPI are caused by defective amino acid uptake leading to protein malnutrition, which causes growth failure and osteoporosis. The absorption defect also causes vomiting and sometimes hyperammonemia after protein feeding, and children with LPI often develop protein aversion. LPI belongs to the Finnish disease heritage, but appears globally. All together, there are more than 100 LPI cases worldwide of which half of the cases are reported in Finland. LPI is caused by mutations in SLC7A7 (solute carrier family 7 member 7) gene encoding y+LAT-1 amino acid transporter polypeptide. The SLC7A7 gene comprises of 11 exons and 10 introns spanning about 48 kb of genomic DNA. All Finnish patients have the same founder mutation 1181-2 A T or LPIFin, a splice site mutation leading to a 10 base-pair deletion in the cDNA level, which is absent elsewhere. The patients outside Finland have a variety of different mutations. y+LAT-1 (y+L amino acid transporter-1) has 12 hydrophobic transmembrane domains, 6 extracellular and 5 intracellular loops. Both the carboxy and amino termini are cytoplasmic. The y+LAT-1 protein forms a heterodimeric structure with the 4F2 cell surface antigen receptor heavy chain (4F2hc). The heterodimer is responsible of the cationic amino acid transport activity on the basolateral membrane of the polar epithelium cells in the small intestine and renal tubules. In this study, the cellular localization of the wild type and mutant y+LAT-1 is studied by using GFP-reporter protein fusions in different cultured cells. Also, the heterodimeric interactions of y+LAT-1 with the 4F2 heavy chain are studied by using fluorescent microscopy and fluorescent fusion proteins of both of the subunits of the active transport system.

P0724. Cloning of the human DDAH1 gene; a new player in the pathogenesis of renal cell carcinoma?

D. Steinemann[1], *B. Hinzmann*[2], *S. Gesk*[1], *H. J. Heidebrecht*[3], *G. Beckmann*[2], *L. Harder*[1], *W. Grote*[1], *A. Rosenthal*[2], *B. Schlegelberger*[1], *R. Siebert*[1]
[1]*Institute of Human Genetics; Kiel, Germany;* [2]*metaGen Gesellschaft f r Genomforschung; Berlin, Germany;* [3]*Institute of Hematopathology; Kiel, Germany*
steinemann@medgen.uni-kiel.de

Nitric oxide (NO) is an important regulator of many cellular processes, including inflammation, necrotic and apoptotic cell death. NO is produced by the enzyme nitric oxide synthase, which is competitively inhibited by endogenously produced asymmetrically methylated arginine residues. The enzyme dimethylarginine dimethylaminohydrolase (DDAH) specifically hydrolyzes these methylated arginines to citrulline and methylamines and thereby regulates NOS activity and thus the NO level in vivo. During the identification and characterization of a new apoptosis inducing gene, BCL10, mapping to the human chromosomal region 1p22, we identified the human DDAH1 gene. This gene is highly conserved during evolution. It is almost identical to the rat DDAH gene and shows considerable homology to genes encoding arginine deiminases from B. burgdorferi, P. aeruginosa, S. pyogenes and C. elegans. The gene structure of the human DDAH1 implies 6 exons of 627bp, 102bp, 73bp, 119bp, 143bp and 572bp (including 5 and 3 UTR) interspaced by five introns of 31kb, 7169bp, 971bp, 8491bp 25.5kb und 3171bp. Electronic expression analysis of DDAH1 revealed wide expression of the DDAH1 gene. Remarkably in renal tumor tissue DDAH1 was significantly downregulated as compared to normal renal tissue implying a role of DDAH1 in the pathogenesis of renal cell carcinomas. To identify mechanisms leading to downregulation of the DDAH1gene in renal tumors we screened the six exons of DDAH1 for mutations of the coding region by PCR-SSCP and partial sequencing. In 20 renal cell carcinomas analysed no inactivating mutations were observed. Subsequently, monoclonal antibodies were raised against the

recombinant DDAH1 protein. Current immunhistochemical experiments with these antibodies address for other mechanisms like mutations in regulatory elements which could result in decreased expression or mRNA instability. This study was supported by the Hensel-Stiftung (Kiel, Germany)

P0725. Identification of Eight Novel 5 -Exons in Cerebral Capillary Malformation Gene-1 (CCM1) Encoding KRIT1

***I. Eerola**, B. A. S. McIntyre, M. Vikkula*
Christian de Duve Institute of Cellular Pathology; Brussels, Belgium
iiro.eerola@bchm.ucl.ac.be

Cerebral capillary malformations (CCM), also known as cerebral cavernomas, are vascular malformations of the brain. These malformations are usually multiple and consist of dilated capillary-venous channels, which tend to grow in size. The average age of clinical presentation is 33 years and the most common symptoms include headache, seizures and cerebral haemorrhage. CCMs often show familial aggregation and genetic linkage has been established to three chromosomal loci, 7q21-22 (CCM1), 7p13-15 (CCM2) and 3q25.2-27 (CCM3). Identification of mutations in the CCM1 gene encoding KRIT1 (Krev-1 Interaction Trapped 1) has provided the first clue to the molecular mechanisms causing CCM. In addition, we showed that vascular defects caused by CCM1 mutations are not restricted to cerebral vasculature, but can also cause cutaneous lesions defined as hyperkeratotic capillary-venous malformations. However, CCM1 mutations have not been identified in all the families linked to CCM1. Here we demonstrate that the CCM1 gene contains eight additional 5 -exons which may thus encompass the missing mutations.

P0726. Four novel testis specific ADAM family genes- studies on their expression and function.

***T. Rzymski**, E. Bolcun, P. Grzmil, W. Engel*
Institut f r Humangenetik; G ttingen, Germany
trzymski@yahoo.com

The ADAM (a disintegrin and a metalloprotease)protein family consists of at least 31 members, wich are all characterized by the presence of metalloprotease- and disintegrin- like domains. The latter has been suggested to mediate cell- cell and cell- matrix interactions by binding to appropriate receptors of the integrin type. In this poster we present data concerning four ADAMs genes that are only expressed in testis; testase 1, testase2, testase 3 and ADAM 27. Testase 1, 2 and 3 show high homology to each other and to ADAM 20- human orthologous gene to the mouse fertilin a, The three testases probably function as active metalloproteases. ADAM 27 is more related to cyritestine, a putative adhesion protein, which was found to be necessery for mouse fertility. All of these proteins are precisely regulated during postnatal testis development. Testase 1 expression was found to start at day 5, testase 2 at day 20, testase 3 at day 5, ADAM 27 at day day 15 of the postnatal testis development. Two of these genes, testase2 and ADAM 27 are expressed exclusively in male germ cells. RT-PCR analyse revealed that testase 2 was haploid and the other three ADAMs were diploid expressed. In order to investigate the function of these genes, knock- out mice are generated. The results of these functional analyses are reported.

P0727. Cell-specific expression of genes associated with renal injury in a knockout mouse model for kidney stone disease

***A. Sahota**, E. Tzortzaki, D. Glass, M. Yang, L. Deng, J. Tischfield*
Rutgers University; Piscataway, United States
sahota@nel-exchange.rutgers.edu

A prominent feature of adenine phosphoribosyltransferase (APRT) deficiency is 2,8-dihydroxyadenine (DHA) nephrolithiasis. In a knockout mouse model for DHA lithiasis, we recently demonstrated changes in renal mRNA expression for a subset of genes associated with inflammation, chemotaxis, fibrosis, and calcification. As a first step in understanding the role of these genes in DHA-induced renal injury, we have used in situ hybridization (ISH) or RT-PCR ISH to identify the cell types expressing these genes at different stages of disease progression. Digoxigenin-labeled oligonucleotide probes were hybridized to paraffin-embedded kidney sections, and adjacent sections were stained with hematoxylin and eosin for histological evaluation. Messages of low (APRT), medium (1,25-dihydroxyvitamin D3-24 hydroxylase, 24-H; and matrix gla protein, MGP), or high (kidney androgen-regulated protein, KAP) abundance could be readily detected by ISH, but RT-PCR ISH was required for the detection of messages of extremely low abundance (imprinted multi-membrane spanning polyspecific transporter-like gene 1, IMPT-1). APRT was expressed in all cell types in wild-type mice, but no expression was observed in the knockout mice, demonstrating the specificity of the hybridization reaction. The expression of 24-H, the enzyme that degrades vitamin D3, is tightly regulated by this vitamin. 24-H was expressed in proximal and distal tubules in wild-type mice, but expression was not detected in APRT-deficient mice, suggesting diminished circulating levels of vitamin D3 in these mice. MGP is an inhibitor of soft tissue calcification and its expression in glomeruli, cortex, and medulla was up-regulated in APRT deficiency, possibly to limit further harmful calcification. KAP is the most abundant mRNA in the mouse kidney and its expression is under sex-hormone control. KAP expression in S3 proximal tubules was decreased in one-month-old APRT-deficient mice, and expression was not detected in wild-type or mutant mice at three or six months. This decrease was not related to renal tubular loss in diseased animals, but probably reflects the effects of age-related hormonal changes. IMPT-1, which is expressed preferentially from the maternal allele, is involved in ion transport, and its expression in proximal tubules was decreased in APRT-deficiency, possibly due to loss of cells expressing this gene, or to epigenetic inactivation. These observations suggest that hormonal imbalances, tissue calcification, and impaired ion transport adversely affect renal function in APRT-deficient mice. The identification of genes expressed at different stages of disease progression, and the cell types expressing these genes, may lead to a better understanding of the molecular and cellular pathogenesis of nephrolithiasis. Supported by NIH grant DK38185.

P0728. Conditions for DNA phase transition induced by ligand binding.

***V. B. Teif**[1], D. Y. Lando[2]*
[1]Institute of Atomic and Molecular Physics; Minsk, Belarus; [2]Institute of Bioorganic Chemistry; Minsk, Belarus
vlad_teif@yahoo.com

Cooperative binding of ligands to DNA lattice may arise in result of two types of interaction between ligands bound to DNA. Firstly, there are direct contact interactions or any other kind of short-range interactions between ligands that occupy adjacent base pairs. An example of contact interactions is glue ends of subunits of lac-repressor that govern their assembling on DNA. Secondly, there are long-range interactions that are occurred due to alteration of DNA structure or/and DNA charge density around bound ligands or of the whole DNA molecule. Various antibiotics and drugs, metal ions and many other compounds give rise to such interactions. In our recent work (1) we showed that some types of long-range (but not short-range) interactions may give rise to ligand binding to DNA with the character of phase transition. Such sharp transition may be caused by changes in DNA topology or by DNA condensation. Here we study influence of energetics and stoicheometry of binding on the character of phase transition. Physical meaning of these transitions is discussed. 1. Lando D. Y., Teif V. B. Long-range interactions between ligands bound to a DNA molecule give rise to adsorption with the character of phase transition of the first kind. // J. Biomol. Struct. & Dynam., V. 18, N 5., P. 903-911 (2000).

P0729. Role of mutated BIGH3 in corneal dystrophies with amyloid and non-amyloid deposits

***S. Morand**[1], C. Bonny[1], F. L. Munier[2], D. F. Schorderet[1]*
[1]Division of Medical Genetics; Lausanne, Switzerland; [2]Jules Gonin Eye Hospital; Lausanne, Switzerland
Sabine.Morand@chuv.hospvd.ch

Mutations in the BIGH3 gene are responsible for autosomal dominant corneal dystrophies (CD) linked to 5q. CD lattice type I is associated with amyloid deposits and an R124C mutation while the Groenouw type I produces non-amyloid deposits and is due to an R555W sequence variation. We investigated the mechanisms leading to deposits in 5q31-linked corneal dystrophies. Although these 2 mutations represent hot spots, other sequence variations have been reported in lattice type I-like phenotypes. Methods ; The full length BIGH3 sequence without stop codon was amplified from plasmids pGEM-BigWT, pGEM-BigR124C and pGEM-R555W and subcloned in pEGFP-N2 to give pEGFP-BigWT, pEGFP-BigR124C and pEGFP-BigR555W. The non classical mutations N622K, G623D, H626R and V631D were introduced using oligonucleotides directed mutagenesis to give pEGFP-BigN622K, pEGFP-BigG623D, pEGFP-BigH626R and pEGFP-BigV631D. Apoptosis was measured in transiently transfected HeLa and HCE, a human corneal epithelial cell line, by Hoechst /PI nuclear staining after 24 or 48 hrs of culture. Results ; The following rates of apoptosis were observed in HeLa (and in HCE) ; pEGFP ; 3% (4.1%); pEGFP-BigWT ; 2.5% (2.8%); pEGFP-BigR124C ; 12% (16%); pEGFP-BigR555W; 14.7% (9%); pEGFP-BigG623D ; 8% (8%); pEGFP-BigH626R ; 6% (7.6%); pEGFP-BigV631D ; 13.5% (8.7%). Measurement of LDH activity showed similar differences between the constructs. Conclusion ; Transient overexpression of mutated BIGH3 in HeLa and HCE cells induces apopto-

sis. These results indicate that apoptosis plays an essential role in cornea dystrophies and amyloid formation. Thus, because of the accessibility of corneas, CD represent good models in which the formation and solubilization of amyloid can be investigated.

P0730. The importance of regulation ceruloplasmin gene expression in mammary grand cells for copper homeostasis in newborns

N. Gyulikhandanova[1], *R. Povalihin*[2]
[1]*St. Peterburg State Technical University; St. Petersburg, Russian Federation;* [2]*Institute for Experimental Medicine; St. Petersburg, Russian Federation*
marsa@newmail.ru

Ceruloplasmin (Cp) is soluble copper contained glycoprotein which functions as an universal transporter of the copper (Cu) ions. We were shown that specific molecular form Cp is synthesised by mammary gland cells and its is secreted to milk. 7-fold decrease of Cp concentration in samples of breast milk during 20 days of lactation period was demonstrated, and Cp molecule contains 5-7 Cu atoms. So, the level of Cu (10 (g/kg body weight) is constant at the breastfeeding. Therefore, in the newborns Cu balance is controlled at the transcriptional level in mammary grand cells. By computer analysis of the 5 -flanking region of rat CP gene we found a potential sites for nuclear hormone receptor RORalpha1, for estrogen and progesteron; and specific cis-elements liver expression genes (HNF-1) and genes expressed in mammary grand (WAP). This region of CP gene (1700 nucleotides) was amplificated. Gel-shift analysis shown that PCR-product contain the specific sites for 9-cis-retinoic acids and thyroid hormone receptors. Also, PCR-product bound TF from liver and mammary gland were different. A fragment (2.3 kb) chromosome Cp gene was cloned in pTZ19. The fragment hybridized with Cp-cDNA containing from 520 to 2620 nucleotides of the Cp-mRNA. Antibodies to TF YY1 are detected YY1 protein in the 2,3 kb DNA\TF complex. The data were approved by gel-shift analysis. The YY1 protein was identified by western-blot hybridization manly in TF fraction from liver of adult rats. It is possible this data indicate existence of the tissue-specific regulation of CP gene expression on the transcriptional level.

P0731. Chromosomal mapping of the human Protein Kinase C Gene Module

K. Kofler*, M. Erdel, G. Utermann, G. Baier*
University of Innsbruck; Innsbruck, Austria
kurt.kofler@uibk.ac.at

Members of the protein kinase C (PKC) family of serine/threonine kinases play critical roles in the regulation of cellular differentiation and proliferation of diverse cell types (1). Molecular cloning and biochemical studies identified PKC enzymes as members of a distinct family that constitutes, at the gene level, nine mammalian members, i.e. alpha, beta, gamma, delta, epsilon, zeta, eta, theta & iota. In this study we have performed fine chromosomal mapping by fluorescence in situ hybridization, employing cosmid, BAC & PAC probes of all 9 PKC isoforms. As result the human PKC genes are not clustered and found at single loci. Additionally, employing HUGO and bioinformatic tools, genomic organization analysis of the PKC genes, as well as the search of neighboring polymorphic markers (useful for genetic linkage analysis) has been initiated. Comparison of chromosomal location of these fine-mapped PKC gene loci with human disease gene loci (and known orthologous regions within the mouse map) is currently performed. Along with tissue-specific and restricted spatial pattern PKC isoenzyme expression, information from biochemical signal transduction work and the phenotype of the established mouse PKC KO-lines (loss-of-function) will permit genetic studies in defined groups of patients in search for PKC associated genetic defects/abnormalities.

(1.) Altman A, Isakov N and Baier G, Protein kinase C theta; a new essential superstar on the T cell stage Immunology Today, Nov 1;21(11);567-573.

P0732. Two missense-mutations in C2C-domain of OTOF-long-splice-form cause non-syndromic autosomal recessive inherited hearing loss (DFNB9)

N. Blin[1], *H. Esmer*[2], *M. Pfister*[3], *H. Zenner*[3], *F. Mirghomizadeh*[2]
[1]*Molecular Genetics, Uni T bingen; T bingen, Germany;* [2]*IZKF, Univ. T bingen; T bingen, Germany;* [3]*HNO, Univ.-Klinikum; T bingen, Germany*
blin@uni-tuebingen.de

DFNB, the non-syndromic hearing loss with autosomal recessive mode of inheritance, constitutes the vast majority of genetically based severe to profound childhood forms of deafness, which normally lead to inability of speech acquisition. We analyzed a consanguineous family originating from eastern Turkey in which four siblings suffered from severe to profound hearing loss linked to the chromosomal region 2p23 (DFNB9). By SSCP analysis as well as by sequencing of the recently identified DFNB9-gene, OTOFERLIN, we excluded the nonsense mutation in exon 18 of OTOF-short splice-form as reported for four Lebanese families. Also no further DFNB9-relevant mutations in the additional 29 exons of this transcript were found. Moreover, we detected a 92 bp-intervening sequence in the 5«-UTR, at the position -121 to -29 of the OTOF-short splice form in all analyzed individuals (controls and members of this family) that was absent in the published 5«-UTR sequence of this cDNA. This could be indicative for an additional OTOF-splice form. In fact, we detected two missense mutations in exon 15 of OTOF-long splice form. Interestingly, exon 15 encodes the first of the four Ca2+ binding C2C-domains of this gene. Based on computer simulations our first mutation effects the secondary structure of C2C-domain through creation of a new alpha-helix structure. The second mutation results in substitution of the predicted C2C-domain specific §-sheet no. 7 through an alpha-helix structure and diminishes the likelihood of forming of §-sheet no. 1 under the significant limit of 0.7. These predicted changes in secondary structure of the C2-domain may cause an alteration the conformation of §-sheet dependent-Ca2+ binding loops in this protein. This would in turn result in altering Ca2+ binding capacity of OTOF, which is essential in signal transduction pathways or in Ca2+ triggered vesicle membrane fusion by changing the electrostatic potential of membrane in exocytosis processes.

P0733. Localisation and organisation of the gamma-glutamyl transferase (GGT) genes on human chromosome 22q

D. J. Morris-Rosendahl[1], E. Burgert[1], G. Uyanik[1], A. Jauch[2], K. Do[3], M. Cu nod[3], A. Mainberger[1]
[1]Institute for Human Genetics, University of Freiburg; Freiburg, Germany*;* [2]Institute for Human Genetics, University of Heidelberg; Heidelberg, Germany*;* [3]Centre for Research in Psychiatric Neuroscience, University of Lausanne; Lausanne, Switzerland
morrisro@humangenetik.ukl.uni-freiburg.de

Members of the gamma-glutamyl transferase (GGT) gene family are on chromosome 22q11, a region prone to rearrangements and deletions that cause human congenital anomaly disorders including Cat Eye (CES), Velo-Cardio-Facial/Di-George (VCFS/DGS), and derivative 22 (der(22)) syndromes. Low-copy repeats (LCR) in 22q11, containing sets of repeated genes, of which GGT is one, seem to mediate deletions or duplications. GGT is a major enzyme of glutathione (GSH) homeostasis, and the induction of GGT in response to oxidative stress is an essential element of the cellular response to oxidative injury. In order to determine whether any of the GGT genes may be involved in disorders assigned to 22q11, it was essential to clarify their exact chromosomal localisation and organisation. We have identified GGT gene-specific DNA sequences by sequencing YACs and PACs containing different GGT genes. Using these sequences, we have localised six GGT genes to a 6 Mb region of 22q11 via Blast searches in human chromosome 22 DNA sequence. We have named the most proximal gene GGT 13a, to distinguish it from a nearly identical gene which lies more distal, GGT 13b. Previously named GGT genes 3 and 11 occur on different YACs which map to the identical chromosome 22q sequence location, so are most likely allelic. Genes 1 and 2 consist only of exons 8 to 12, which represent the functional domain of the protein. Gene 6 is the most distal gene and is adjacent to gene 2. Sequence comparisons of the different genes indicate two groups of nearly identical genes within each group; GGT 13-like, comprising genes 3/11, 13a and 13b; and GGT 6-like, comprising genes 1, 2 and 6. The exact localisation of these genes on chromosome 22q has allowed us to highlight polymorphic markers which can be used in genetic studies to test the possible involvement of these genes in the above-mentioned disorders.

P0734. Structure and regulation of the murine homologue of the gene for human arylamine N-acetyltransferase 1, an enzyme involved in folate metabolism

S. Boukouvala[1], *N. Price*[1], *K. Plant*[2], *E. Sim*[1]
[1]*Department of Pharmacology, University of Oxford; Oxford OX1 3QT, United Kingdom;* [2]*Sir William Dunn School of Pathology, University of Oxford; Oxford OX1 3RE, United Kingdom*
sotiria.boukouvala@pharm.ox.ac.uk

Arylamine N-acetyltransferases (NATs) acetylate arylamine and hydrazine drugs and carcinogens. In humans, there are two isoenzymes encoded by genes located on chromosomal region 8p22. The NAT1 isoenzyme can also N-acetylate p-aminobenzoylglutamate. The product of this reaction is a major folate catabolite in vivo, which suggests that NAT1 could be involved in the metabolism of folate, a compound of key importance during early embryogenesis. In mice, the genes encoding for three NAT isoenzymes have been localised on chromosome 8, in a region syntenic with

human 8p22. We describe the structure and expression of the gene for murine NAT2, the functional equivalent of human NAT1. Murine Nat2 is the only Nat gene expressed at the blastocyst stage, as demonstrated by RT-PCR. We show that the Nat2 gene has an unusual structure, with an intronless coding region separated from a short non-coding exon by a 6kb intron. The transcription initiation site was accurately mapped by RNase protection assay and a promoter was identified upstream of the non-coding exon by reporter gene assay. The promoter has the typical GC-rich sequence of housekeeping genes lacking a TATA-box. The intron is unlikely to contain an alternative promoter, but may contain other regulatory elements, as indicated by reporter gene assays. The unusual splicing pattern of the Nat2 transcript was detected in embryonic stem cells, embryonic liver cells and several adult tissues of the mouse. Investigation of the factors regulating expression of murine Nat2 will aid in understanding its possible role in development. [Supported by the Wellcome Trust and Action Research (SPARKS)].

P0735. Genomic structure of GJB6 encoding Cx30 in a connexin cluster on chromosome 13q11

J. Lamartine[1], *E. Callouet*[2], *G. Waksman*[1]
[1]*Universit d Evry/CEA; Evry, France;* [2]*Genethon; EVRY, France*
lamartin@dsvidf.cea.fr

The connexin (Cx) gene family codes for protein subunits of gap junction channels, which permit the rapid exchange of ions and metabolites between neighbouring cells. Mutations in genes encoding connexins have been identified in various human inherited diseases, especially skin diseases and hearing loss. We have recently shown that mutations in the GJB6 gene encoding Cx30 are responsible for Hidrotic Ectoderma Dysplasia (Clouston syndrome), a rare genodermatosis. Another mutation of GJB6 was also identified in non syndromic autosomal dominant deafness. The control of GJB6 expression in various human tissues appears as a key point to understand the molecular basis of these two diseases. In order to localise the promoter region of GJB6, we reconstructed the genomic structure of the gene. A complete GJB6 cDNA was cloned by 5 RACE from a human brain cDNA library. The sequence of this full length cDNA was compared with genomic sequences obtained from BAC clones previously mapped in this region. We found that GJB6 is composed of 4 exons spanning more than 10 kb of genomic sequence. The coding sequence is entirely contained in exon 4. The identification of the transcription initiation site allowed us to localise the promoter and start to study it. By analysing the genomic sequence around GJB6, we were able to position two other genes encoding connexins ; GJB2 encoding Cx26 and GJA3 encoding Cx46. These 3 connexin genes are clustered in a region of 60 kb. This connexin region on chromosome 13q11 could be the result of duplication events of an ancestral common gene.

P0736. Hfb1 brainspecific sequence as a part of the extended 3 -untranslated region of the human complexin 2 gene

I. Vladychenskaya[1], *N. Raevskaya*[1], *L. Dergunova*[1], *G. Sulimova*[2], *E. Klimov*[2], *S. Limborska*[1]
[1]*Institute of Molecular Genetics RAS; Moscow, Russian Federation;* [2]*Institute of General Genetics RAS; Moscow, Russian Federation*
irinavl@img.ras.ru

Hfb1 brain-specific sequence (Acc. No. Y15167) was obtained from the human cDNA forebrain cortex library.The genomic clone from the human chromosome 5 cosmid library hybridizing with Hfb1 was investigated. The nucleotide sequence more than 4 kb in length (Ghfb, Acc. No. AF318943) was determined for the part of this genomic clone.100% identity between the 5 -end of Ghfb sequence and 3 -untranslated region of the human complexin 2 mRNA was revealed by the comparison of Ghfb with the sequences from the GenBank database.Long transcripts with their 5 -ends coinciding with the human complexin 2 sequence and 3 -ends coinciding with Ghfb sequence were revealed by RT-PCR. These data make us possible to suppose that Hfb1sequence is the part of yet not-described human complexin 2 gene exon which is coding for one of the possible ends of the corresponding mRNA. PCR screening of the GeneBridge 4 RH-panel using Ghfb-specific primers allowed to map Ghfb sequence within the 5q35 chromosomal region. The localization site for Ghfb appeared to be near the same with that determined for human complexin 2 gene thus confirming the close vicinity of these two sequences.

P0737. KCNK10; a new model for 5 -alternative splicing in potassium channels

W. Gu[1], *G. Schlichth rl*[2], *C. Derst*[2], *J. Daut*[2], *O. Steinlein*[1]
[1]*Inst. f r Humangenetik; Bonn, Germany;* [2]*Inst. f r Normale und Pathologische Physiologie; Marburg, Germany*
eeg@mailer.meb.uni-bonn.de

Alternative splicing provides for maximal variability of coding products and regulation patterns from limited numbers of genes. In the K+ channel superfamily the 5 -alternative splicing affecting the first exons is quite common. The alternative usage of the first exons does not always change the N-terminal sequence at the amino acid level. The functional significance of the 5 -splicing of various K+ channel subunits is not yet known. We have cloned a novel human potassium channel subunit KCNK10 which belongs to the tandem pore domain K+ channel gene family and has 85 % amino acid sequence identity to KCNK2. So far, we have identified three different 5 -splice variants of KCNK10. These variants provide an opportunity for studying the functional implications of N-terminal alternative splicing of potassium channels. Two of the three different KCNK10 splice forms are already expressed in HEK293 cells. Both showed outward rectification using 5 mM K+ bath solution. The channels have mean open times in the order of 100 s and measured single channel conductances were between 119 and 187 pS. With symmetrical K+ in the pipette solution, the single channel current voltage relation was linear. RT-PCR experiments demonstrated a tissue-specific expression pattern of the KCNK10 splice variants. Further studies will show if their variable N-terminals are associated with different biophysical and pharmacological properties.

P0738. Proliferative effects of TSC1 are independent of TSC2; first evidence for separable functions of tuberous sclerosis genes

M. Hengstschl ger, *A. Miloloza, M. Kubista, M. Rosner*
Prenatal Diagnosis and Therapy; Vienna, Austria
Markus.Hengstschlaeger@akh-wien.ac.at

The autosomal dominant disease tuberous sclerosis is caused by mutations in either TSC1 on chromosome 9q34, encoding hamartin, or TSC2 on chromosome 16p13.3, encoding tuberin. The biochemical functions of these two proteins have not been clarified so far. The observations that the phenotypes associated with mutations in these genes are similar (although not identical) and that these two proteins can interact in vivo suggested that hamartin and tuberin function in the same complex. Earlier, we have shown that these two molecules affect the control of mammalian cell proliferation (J Biol Chem 272; 29031; Oncogene 16;2197; PNAS 95; 15653; Hum Mol Genet 9;1721).We now found that hamartin can affect proliferation independently of the presence of functional tuberin. Different molecular approaches demonstrated that the two TSC proteins can affect cellular proliferation by independent mechanisms. This is the first evidence that these two genes can have separable functions and can, beside creating a complex, also act independently. These data have clear impact for understanding the phenotypical differences of the TSC1- and the TSC2-associated disease and for understanding of the functions of hamartin and tuberin.

P0739. Isolation and characterisation of a novel human chromosome 21q22.1 gene encoding an RNA associated protein

F. Chapot-Skovsgaard
Faculty of Medecine, Geneva; 1211 Geneve 4, Switzerland
chapot6@etu.unige.ch

To contribute to the development of the transcript map of human chromosome 21 (HC21) and thus identify candidate genes for Down syndrome and phenotypes that map to HC21, we have used RT PCR, RACE and cDNA library screening to isolate a novel cDNA, named C21orf66. This new gene spans more than 40kb of genomic sequence on 21q22.1 (between SYNJ1 and C21ORF49 genes) and is split in 20 exons. C21orf66 is expressed in all 20 adult and foetal tissues tested, except colon and stomach. Alternative splicing permits the synthesis of 4 mRNAs encoding proteins of 469, 511, 835 and 917 amino acids that contain several Nuclear Localisation Sequences, an S1 RNA binding domain, and a PfamB 3456 domain of unknown function. The occurrence of these domains suggests that this protein is localised in the nucleus and binds RNA. C21orf66 protein is strongly homologous to a putative C.elegans peptide (F43G9.12, 27% identity over 758 residues). Double stranded RNA interference of F43G9.12 leads to 90% or more embryonic lethality as well as low post-embryonic growth (Nature, vol 408, 2000). Almost the full length mouse homologue cDNA has been cloned and showed 90 % amino acid identity with the human protein. C21orf66 overexpression might consequently disturb pre-mRNA splicing, general cellular translation, and lead to some phenotype, causing C21orf66 to emerge as a good candidate for some of the Down syndrome phenotypes.

P0740. Molecular basis of CFTR exon 9 alternative splicing

F. E. Baralle[1], *E. Buratti*[1], *T. Doerk*[2], *E. Zuccato*[1], *C. Stuani*[1], *F. Pagani*[1]
[1]*International Centre for Genetic Engineering and Biotechnology; Trieste, Italy;* [2]*Medizinische Hochschul; Hannover, Germany*
pagani@icgeb.trieste.it

We have analyzed the cis- and trans-acting factors involved in the regulation of CFTR exon 9 alternative splicing, which have been associated to monosymptomatic forms of Cystic Fibrosis. We have identified in intron 8, in the exon itself and in the intron 9 5 proximal region critical cis-acting elements regulating exon 9 splicing. At the 3 splice site of intron 8,there are two elements composed by polymorphic (TG)m(T)n repeats. We report the identification of TDP-43, a nuclear protein not previously described to bind RNA, as the factor binding specifically to the (TG)m repeats. Antisense inhibition of endogenous TDP43 expression increased inclusion of exon 9. Paradoxically, SR proteins inhibit exon 9 splicing, through the interaction with sequences located both in the coding region and in intron 9 where we have mapped an Intronic Splicing Silencer. Interestingly, some missense mutations, such as G424S, I444S and A455E, previously considered to cause CF only by altering protein function, showed an aberrant splicing pattern in transient transfection assay, indicating that the nucleotide changes affect exonic splicing enhancers. Therefore, mutations in the exons and the composition of the (TG)m(T)n polymorphic repeats affect alternative splicing and can lead to phenotypes of variable severity according to the individual concentration of splicing factors. In addition antisense inhibition of TDP43 may provide a new therapeutic target to correct exon 9 skipping.

P0741. Three new exons of the ATM gene activated by aberrant splicing due to intronic mutations

A. Sobeck[1], *D. Schindler*[1], *R. Bendix*[2], *T. D rk*[2]
[1]*Biozentrum, University; W rzburg, Germany;* [2]*Medical School; Hannover, Germany*
doerk.thilo@mh-hannover.de

The ATM gene that is mutated in ataxia-telangiectasia (A-T) encodes a 350 kDa protein kinase which orchestrates the radiation-induced DNA damage response and cell cycle regulation. The mutational spectrum of the ATM gene is still undergoing further expansion although several extensive studies have investigated the molecular basis of A-T in large numbers of patients (e.g., Sandoval et al. 1999). We have explored the presence of aberrantly spliced ATM mRNA isoforms in lymphoblastoid cell lines from a subset of A-T patients. Three new exons of 166, 58 and 65 bp in size were identified by detection of their sequence being inserted into the reading frame of the ATM transcript. The underlying molecular events appear to be the consequence of two point mutations and one four-bp-deletion, respectively, that were decovered deep in the introns 15, 19 and 20 of the ATM gene in a total of four A-T patients. One of these mutations generates a canonical splice donor signal. The second mutation produced only a subtle change of the consensus splice donor site score but nevertheless proved functional as a new splice site. Finally, the third mutation disrupted a sequence that appears to act as a splicing silencer element in its normal context. We conclude that even subtle changes in introns can activate cryptic exons and are sufficient to cause the classical course of A-T. Mutations deep in the introns may account for a number of failures to detect ATM mutations by genomic mutation scanning.

P0742. SALL1, which is mutated in the Townes-Brocks syndrome, is a transcriptional repressor and interacts with UBC9 and the small ubiquitin like modifier 1 (SUMO-1).

C. Netzer, *L. Rieger, J. Kohlhase, S. K. Bohlander*
Institute of Human Genetics, University of G ttingen; G ttingen, Germany
cnetzer@web.de

The SALL1 gene encodes a putative zinc finger transcription factor (1324 aa) which is mutated in Townes Brocks syndrome. This syndrome is inherited in an autosomal dominant fashion and is characterized by imperforate anus, preaxial polydactyly, and dysplastic ears. Little is known about the cellular function of SALL1. To investigate its possible role as a transcription factor we performed reporter gene assays expressing portions of SALL1 fused to the GAL4 DNA binding domain transiently in mammalian cells. These experiments revealed that the central 1000 aa of SALL1 strongly repress the expression of a luciferase reporter gene controlled by the thymidine kinase promoter and GAL4 DNA binding sites. Trichostatin A, an inhibitor of histone deacetylases, was not able to relieve this repression suggesting that SALL1-mediated repression does not lead to the recruitment of mSin3A, N-CoR or histone deacetylases. We also employed the yeast two-hybrid system to identify protein interaction partners of SALL1. One of these interacting proteins is UBC9 which is known to mediate the covalent modification of other proteins by SUMO-1. The SALL1/UBC9 interaction was confirmed in vitro by a GST pulldown experiment. We could also demonstrate interaction between SALL1 and SUMO-1 in the yeast system indicating that SALL1 is modified by sumoylation. Sumoylation is known to target proteins to certain subnuclear compartments. Expression of a GFP-SALL1 fusion protein in NIH3T3 cells showed indeed localization of GFP-SALL1 in distinct nuclear speckles.

P0743. A combined analysis of the cystic fibrosis transmembrane conductance regulator; implications for structure and disease models

J. Chen[1], *C. Cutler*[2], *C. Jacques*[3], *G. Boeuf*[4], *E. Denamur*[5], *G. Lecointre*[6], *B. Mercier*[3], *G. Cramb*[2], *C. Ferec*[3]
[1]*Etablissement Fran ais du Sang-Bretagne; Brest, France;* [2]*University of Saint Andrews; Fife, United Kingdom;* [3]*Universite de Bretagne Occidentale and Centre Hospitalier Universitaire; Brest, France;* [4]*Laboratoire ARAGO; Banyuls-sur-Mer, France;* [5]*INSERM U458; Paris, France;* [6]*Museum National d Histoire Naturelle; Paris, France*
Jian-Min.Chen@univ-brest.fr

Over the past decade, more than 900 mutations have been identified in the cystic fibrosis transmembrane conductance regulator (CFTR) gene; and an enormous wealth of information concerning the structure and function of the protein has also been accumulated. This data, evaluated together in a sequence comparison of all currently available CFTR homologues, has recently enabled us to define a functional R domain of the CFTR. Here, such a combined analysis has been augmented by the determination of two full-length cDNA sequences (sCFTR-I and sCFTR-II) from the Atlantic salmon (Salmo salar), an alternatively spliced exon 12- transcript of the sCFTR-I, and an additional partial coding sequence from the kangaroo (Macropus giganteus) and has been extended to refine the boundaries of the two nucleotide binding domains and the COOH-terminal tail. This approach also provides further insights into the differential roles of the two halves of CFTR, and highlights several well conserved motifs that may be involved in inter- or intra-molecular interactions. Moreover, the serious possibility that a certain fraction of missense mutations identified in the CFTR gene may not have functional consequences, is discussed. Finally, phylogenetic analysis of all the full-length CFTR amino acid sequences, and an extended set of exon 13 coding nucleotide sequences, reinforces the idea that the rabbit rather than the mouse may represent the optimal CF model and strengthens the assertion that a long branch attraction artifact separates the murine rodents from the rabbit and guinea pig, the other Glires.

P0744. Towards a complete transcription map of the Williams-Beuren syndrome deletion region

L. F. Magano, *M. Bay s, R. Flores, L. A. P rez Jurado*
Genetics Unit, Dept. of Experimental Sciences, Universitat Pompeu Fabra; Barcelona, Spain
luis.magano@cexs.upf.es

A common 7q11.23 heterozygous deletion of ~1.6 Mb that arises as a consequence of unequal crossing over between highly homologous repeat blocks has been defined in the great majority of Williams-Beuren syndrome (WBS) patients. Up to 17-18 genes located in the deleted interval have been reported to date. A map of the ortologous region showed that the order of the intradeletion genes is fully conserved in mouse. Based on the physical maps and high-throughput genome sequences from both species released by the Genome Projects, we have identified seven novel genes located between WBSCR14 and ELN, by comparative analysis using the integrated NIX service (http;//www.hgmp.mrc.ac.uk). On Northern blots, the novel genes (provisionally named WBSCR16-22) are expressed as single transcripts with sizes ranging from 1.2 to 4.5 kb, and with variable intensities in the different tissues. We have characterised the complete cDNA and genomic structure of 5/7 genes, while two genes are still incomplete at the 5 end. Homology searches and prediction of putative protein domains suggest these new genes code for; 1- a DNAJ family member, 2- an alpha/beta hydrolase, 3- a new claudin family member, 4- two different methyltransferases, and 5- two novel proteins with no predicted domains. Idenfication of all genes in the WBS critical region is required to define those whose haploinsufficiency may contribute to the clinical phenotype and to establish precise clinical-molecular correlations in patients with partial deletions.

P0745. Physiological role of the ClC-2 chloride channel

C. A. Huebner, *V. Stein, M. R. Boesl, A. A. Zdebik, T. J. Jentsch*
Zentrum f r Molekulare Neurobiologie Hamburg; Hamburg, Germany
chuebner@zmnh.uni-hamburg.de

ClC-2 is a member of the large family of voltage-gated chloride channels which includes at least nine different members in mammals. Expression of ClC-2 mRNA correlates with the presence of a hyperpolarization-activated

chloride current with similar voltage dependence and kinetics as ClC-2. ClC-2 is almost ubiquitously expressed. Though ClC-2 is largely closed under resting conditions, it can be slowly activated by strong hyperpolarization. In the Xenopus oocyte expression system ClC-2 can be activated by osmotic cell swelling, suggesting a role in the regulation of cell volume, and by acidic extracellular pH, which was postulated to be important for gastric acid secretion. It has been proposed that expression of ClC-2 in certain neurons ensures an inhibitory response to GABA by preventing intracellular chloride from rising above its electrochemical equilibrium. Immunocytochemistry has revealed that ClC-2 is expressed in apical membranes of respiratory epithelial cells, the same site where CFTR is normally expressed. To investigate the physiological role of ClC-2 we generated ClC-2 null (ClC-2-/-) mice. ClC-2-/- mice developed at the expected Mendelian ratio and appeared normal at gross morphological evaluation. Life expectancy was not altered. Lung development was normal and respiratory related symptoms were absent. We did not observe spontaneous epileptic seizures nor did we find an altered response to the seizure inducing agent flurothyl. Secretion of gastric acid was not reduced in Clc-2-/- mice. Though female Clc-2-/- mice were fertile, we observed a progressive degeneration of the testes in Clc-2-/- mice, leading to male infertility.

P0746. E2Tag Epitope Tagging System for Monitoring and Purification of Proteins

***R. Kurg**, A. Ilves, N. Kaldalu, M. Ustav*
Institute of Molecular and Cell Biology, Tartu University and Estonian Biocentre; Tartu, Estonia
rkurg@ebc.ee

As functional genomics becomes proteomics, researches will look for easier, less time intensive ways in which to express, isolate, and detect proteins. Epitope tagging is a recombinant DNA technique by which a protein is made immunoreactive to a pre-existing antibody. A short amino acid sequence (epitope tag) is incorporated into the primary sequence of a recombinantly expressed protein, the tagged protein is expressed and can be detected and purified with antibodies directed against the epitope tag. Epitope tags are small and they have minimal, if any effects on the biological function of the tagged protein. Epitope tagging eliminates the time consuming work of producing a new antibody every time a different protein is studied. So, epitope tagging is a technology, which simplifies the detection, characterization and purification of proteins. Here we describe the use of the bovine papillomavirus type 1 E2 protein sequence, SSTSSDFRDR as an epitope tag. The new tag, E2Tag, can be fused to the N- or C-terminus or in the middle of the protein and the activities of the tagged proteins have been tested in different assays, including Western blot assay, immunofluorecsence staining of cells, immunoprecipitation as well as the DNA binding and DNaseI footprinting assays. The anti-E2Tag antibody interaction with its respective epitope tolerates high salt concentrations up to 2 M NaCl, this permits immunoprecipitation and immunopurification of the tagged proteins in high-salt buffers and reduction of the nonspecific binding of the contaminating proteins. We suggest that the E2Tag is a useful tool for detection and purification of proteins.

P0747. Functional and molecular characterization of the cardiac transient outward current Ito and its putative implication in cardiac arrhythmias

***B. Wollnik**[1], N. Decher[2], O. Uyguner[1], B. Karaman[1], C. R. Scherer[2], A. Ghanbari[1], M. Yuksel-Apak[1], K. Steinmeyer[2]*
[1]Center of Molecular Cardiac Arrhythmia, Division of Medical Genetics, Child Health Institute; Istanbul, Turkey; [2]Aventis Pharma Deutschland GmbH, DG Cardiovascular Diseases; Frankfurt, Germany
wollnik@superonline.com

Alterations of the cardiac action potential due to dysfunction of heart specific ion channels are known to cause cardiac arrhythmias. Recently, mutations in different ion channel genes have been identified in families with long QT syndrome, a cardiac repolarization abnormality leading to ventricular tachycardias and sudden cardiac death. However, nothing is known yet about the molecular basis of various other common rhythm abnormalities of the heart. The KCND3 gene encodes the Kv4.3 A-type potassium channel, a molecular correlate of the cardiac transient outward current Ito. In the present study, we cloned the Kv channel-interacting protein 2 (KChIP2) cDNA from human heart and identified two functional splice variants of the gene. The human KChIP2 cDNA encodes a 252 amino acid protein and is expressed in heart and brain. Using functional expression of the Kv4.3 and KChIP2 proteins in Xenopus oocytes, we show that KChIP2 interacts with Kv4.3 and modifies its biophysical properties. The resulting current resembles the native cardiac transient outward current, suggesting that KChIP2 contributes to the heterogeneity of Ito in human heart. The Ito current is important for the initial fast repolarisation of the cardiac action potential and dysfunction of this current may lead to an abnormal action potential duration and thereby may cause cardiac arrhythmias. Therefore, we further analyzed the genomic organization of the KChIP2 gene, which consists of 9 exons, and determined its chromosomal localization using FISH analysis. Subsequently, a mutation-screening test was established for both, the KCND3 and the KChIP2 gene, which will allow targeted screening for mutations in patients with various forms of arrhythmias.

P0748. Characterization, tissue expression, and chromosomal assignment of the human RAB22A gene belonging to Rab small GTPases

***S. Ku§mann**, S. Worch, A. Hehr, I. Hansmann, D. Schlote*
Institut f r Humangenetik und Medizinische Biologie; Halle, Germany
dietmar.schlote@medizin.uni-halle.de

The mouse chromosome 2 segment (MMU2) corresponding to human chromosome 20 (HSA20) is known to be involved in both, maternal as well as paternal noncomplementation (genomic imprinting). Uniparental disomies for distinct regions of MMU2 result in different neonatal lethalities with opposite anomalous phenotypes, strongly suggesting the presence of imprinted genes in this region. These chromosomal regions show a conserved synteny of gene loci to human 20q13 segment, predicting the presence of imprinted genes in this syntenic human chromosomal region. Two mouse models have been generated carrying reciprocal translocations which served to define chromosomal segments of the parental source effect. We have identified a new gene in this region of interest which is located on a BAC RPCIB753L051096 proximal to GNAS1 on HSA 20q13. Cloning and sequencing the full-length cDNA revealed a novel isoform of the human RAB22 subfamily of small GTP-binding proteins playing an important role in the regulation of vesicular trafficking. Based on the EST WI-12997 this new isoform was isolated containing 2242 nucleotides and is designated RAB22A. Structurally, the RAB22A encodes a polypeptide of 194 amino acids which has 97% identity to the canine rab22a. Northern blot analysis revealed ubiquitous expression slightly increased in heart. The genomic structure was solved by database analysis and sequencing of the isolated BAC clone. The gene consists of 7 exons spanning about 50 kb of genomic sequence. Supported by a grant of the DHGP.

P0749. Regulation Of The Human Hfe Gene Expression ; Cis-elements Of The Promoter And Trans-acting Factors

***C. Mura**, G. Le Gac, C. Ferec*
INSERM, CHU, UBO; Brest, France
Catherine.Mura@univ-brest.fr

Hereditary hemochromatosis (HC) is a common autosomal recessive genetic disorder of iron homeostasis. The human HFE gene is clearly involved in HC. Two missense mutations (C282Y and H63D) account for approximately 90% of HC chromosomes and other rare or private mutations have been described. However, the role of HFE remains unclear and the mechanism of the regulation of HFE expression is unknown. Here, we examined the functional organization of the HFE gene promoter and defined the cis-elements and trans-acting factors. The analysis of HFE-driven luciferase reporter gene activity performed by transient transfection into human cells indicated that the -1485 to -8 sequence of the 5 -flanking region relative to the first coding nucleotide contains several elements that regulate the transcriptional activity of the HFE gene. We determined that the level of HFE expression was cell-line dependent, the promoter activity was 2.8-fold higher in HepG2 cells and 1.7-fold higher in HT29-19A cells compared with HeLa cells. We identified a promoter region where activity was maximal, which extended from the part of the untranslated first exon to position -558, and additional positive and negative elements extending to -1057 and -1485, respectively. Cotransfection with vectors expressing the ubiquitous Sp1 and the tissue-specific GATA-1 transcription factors showed a trans-activation with a maximum increase in HFE-directed transcription of 7.1-fold and 8.7-fold, respectively. The data revealed that GATA-1 transcription factor, implicated in the up-regulation of a number of genes involved in iron homeostasis, allowed a coordinated expression of HFE with this group of genes.

P0750. Functional characterization of a new human gene family

***M. Tuson**[1], L. Hjelmqvist[1], E. Herrero[2], G. Marfany[1], S. Balcells[1], R. Gonz lez-Duarte[1]*
[1]Department of Genetics, University of Barcelona; Barcelona, Spain; [2]Department of Basic Medical Sciences, University of Lleida; Lleida, Spain
mtuson@bio.ub.es

With the completion of the human genome project, a huge amount of sequence data is available for genomic analysis and the challenge is to

understand the roles of gene products. Although functional clues are obtained by database comparisons, a significant number of predicted genes remains unclassified. We here describe one such a case, a new gene family with at least three members in human and orthologous genes in mouse, yeast, Arabidopsis and Drosophila. The first human member was found while searching candidate genes for Retinitis Pigmentosa, and additional members were identified on the basis of sequence homology. To approach function, Northern analysis and GFP-fusion proteins are being performed for the human members. Additionally, yeast knockouts have been constructed and their phenotypes are being characterised.

P0751. Genetic analysis of a 22 bp deletion of the Hfe gene.

S. A. M. Taylor[1,2], *S. M. Kinkley*[3], *B. Brown*[4], *A. T. Lyng*[5], *G. N. Schep*[6], *D. P. Lillicrap*[3]
[1]*Pathology, Queen s University; Kingston, ON Canada;* [2]*Pathology, Kingston General Hospital; Kingston, Ontario, Canada;* [3]*Queen s University; Kingston, ON Canada;* [4]*Queen s Unversity; Kingston, ON Canada;* [5]*Pathology, KIngston General Hospital; Kingston, ON Canada;* [6]*South Lake Regional Health Centre; Newmarket, ON Canada*
taylor@cliff.path.queensu.ca
The mutations of the Hfe gene which are most commonly associated with hemochromatosis are H63D and C282Y. They are easily tested for by PCR amplification of exons 2 and 4 of the Hfe gene followed by diagnostic restriction enzyme digestion. To date our laboratory has performed testing on more than 3500 individuals. In one individual we observed altered PCR fragments which suggested the presence of a deletion of exon 2 of the Hfe gene. The affected individual is a 32 year old female of Celtic descent. The patient, who is described as being very thin, presented with malabsorption, nonspecific abdominal pain, and swollen arthritic fingers. Increased ferritin levels were present while a liver biopsy did not reveal evidence of abnormal iron stores. She has had one normal pregnancy. Treatment by phlebotomy resulted in reduction of ferritin levels to below normal after the removal of six units of blood. Further investigation of this individual revealed that she is a compound heterozygote for the C282Y mutation as well as a deletion of exon 2. A 22bp deletion of the Hfe gene beginning at residue 369 (Accession # NM_00410) was confirmed by sequence analysis. The deletion results in the addition of 30 nonspecific amino acids beyond the alanine residue at codon 48. This to our knowledge is the first report of a truncating mutation in the coding region of the Hfe gene that is associated in humans with iron overload.

P0752. TFNR - a subunit of TFIIIB - is a candidate gene for atypical forms of SMA with cerebral atrophy

A. Kelter*, J. Herchenbach, B. Wirth*
Institute of human genetics; Bonn, Germany
akelter@gmx.de
The transcription factor-like nuclear regulator (TFNR) is a novel human gene that maps on 5q13, distally to the duplicated region which includes SMN1, the spinal muscular atrophy (SMA) determining gene and allowed us to design an evolutionary model of the SMA region. TFNR shows homology to yeast B«« protein which is required for transcription of both TATA-less and snRNA-type RNA polymerase III promotors and thus is an essential factor of the basal RNA polymerase III transcription machinery. Northern blot and transcription start point analysis allowed us to determine a transcript length of ~ 10 kb. The TFNR transcript is highly expressed in cerebellum and weakly in all tissues tested. TFNR encodes a 2254 amino acids (aa) protein. The protein contains a SANT domain, and ssDNA- and dsDNA-protein interactions were shown. Immunocytological studies showed that the TFNR protein is highly expressed in the granule cell layer of the cerebellar cortex. Based on its proximity to SMN1 and its expression in the cerebellum, we investigated several SMA patients with large deletions in the SMA region. Quantitativ analysis of an SMA patient with cerebral atrophy, showed a heterozygous deletion of TFNR and no mutation in the remaining copy. Therefore, we speculate that the cerebral atrophy is due to a hemizygous effect of TFNR. Human and mouse antibodies from the N- and C- terminus were established and will allow ontogenetic and functional analysis of TFNR. The construction of a TFNR knock-out mouse is in progress.

P0753. Murine Gtf2ird2- another member of the TFII-I family of transcription factors with unique HLH domains.

M. Tassabehji[1], *C. Wilmot*[2], *A. Read*[1]
[1]*University of Manchester; Manchester, United Kingdom;* [2]*University of Leeds; Leeds, United Kingdom*
m.tassabehji@man.ac.uk
We have isolated and characterised a novel murine gene, Gtf2ird2, which appears to be a member of the TFII-I transcription factor family of genes containing a unique helix-loop-helix domain (HLH). These genes are clustered together in a region of conserved mouse-human synteny on chromosome 5G. The two members of this family, TFII-I (Gtf2i) and BEN (Gtf2ird1), contain large multiple HLH domains unlike other basic HLH proteins, suggesting more complex multiple roles. In fact TFII-I has been shown to be a multifunctional phosphoprotein with roles in transcription and signal transduction and BEN is thought to be involved in transcriptional regulation. The primary sequence of Gtf2ird2 contains two HLH domains with homology to the HLH domains present in six copies in both TFII-I and BEN. Although the role of Gtf2ird2 is unknown this motif homology suggests that it belongs to this unique family of HLH transcription factors. Gtf2ird2 is expressed as a 3.5kb transcript in most adult tissues tested but a smaller transcript (~3kb) is found in testis. The ORF encodes a protein of 936 amino acids with a molecular weight of 104578Da that is predicted to be slightly acidic. Structurally Gtf2ird2 comprises 16 exons and spans a genomic region of 33.940kb. We have also isolated the human orthologue (GTF2IRD2) located on 7q11.23 within the duplicated region flanking the Williams syndrome (WS) critical region. WS is a developmental disorder with characteristic physical, cognitive, and behavioural traits caused by a microdeletion of ~1.5Mb. Of all the deleted genes, only haploinsufficiency for elastin has been associated with a WS phenotype (the heart abnormality, SVAS). Functional and mouse studies will be required to determine if hemizygosity for GTF2IRD2 contributes to the aetiology of the syndrome.

P0754. Analysis of transcriptional startpoints and methylation patterns of the PMP22 promotors B and C in tumor cell lines containing a PMP22 amplification.

K. Huehne*, B. Rautenstrauss*
Institute of Human Genetics; Erlangen, Germany
khuehne@humgenet.uni-erlangen.de
Recent data prove that the peripheral myelin protein 22 (PMP22) is alternatively transcribed from promotors A, B and C. Promotor A is active in myelinating Schwann cells and contains elements important for peripheral-nerve specific expression. Promoter B is predominantly activated in non-neuronal tissues and transcript C appears in relatively high amounts in tumor cell lines carrying a 7 to 11 fold PMP22 amplification. The tumor cell lines used were SF763 (glioblastoma) and RH30 (osteosarcoma).Transcript C has also been identified in human fetal lung and, albeit at lower levels, in sciatic nerve tissue.With the gene racer method gaining full-length cDNA clones we discovered that transcript A always starts from a distinct nucleotide. However, transcript B and C revealed multiple starting points in several distinct regions and furthermore from neighbouring nucleotides. Additionally, we studied the effect of CpG island methylation in the PMP22 promotors B and C by methylation specific PCR performed with DNA from sciatic nerve, leucocytes and tumor cell lines. In leucocytes and sciatic nerve the two promotors did not contain methylated CpGs. However, hypermethylation of promotor B was discovered in SF763, which indicates a silencing effect. In RH30 most copies of promotors B and C were methylated but the few remaining hypomethylated copies were obviously sufficient for strong expression of PMP22. The biological sense of the transcript C in tumor cell lines remains to be determined.

P0755. Identification and characterization of the novel human metalloproteinase genes; MMP-21, MMP-26 and MMP-28.

G. Marchenko
The Burnham Institute; La Jolla, CA United States
marchenko@burnham.org
Matrix metalloproteinases (MMPs) are strongly associated with cancer cell invasion and metastasis, tumor neovascularization and other pathologies such as inflammation, arthritis, cardiovascular and neurodegenerative diseases. Increasing understanding of the role for MMPs in complex regulatory and extracellular matrix remodeling processes has stimulated the search for novel genes encoding proteinases with unique functions, regulation and expression patterns. To date about 20 human MMP genes have been cloned and partially characterized. In our strategy for identification of novel genes, we combined subtraction of the known MMP genes from gene libraries and enrichment of the pool of unknown MMP genes using the magnetic bead technology followed by PCR with degenerate primers and RACE. This facilitated identification and cloning of three novel genes MMP-21, MMP-26 and MMP-28 with relatively low representation in human cDNA libraries. Here we report the molecular cloning, nucleotide sequences, the chromosomal localization (11p15.3, 17q21.2, and 10q21 for MMP26, MMP28 and MMP21 respectively) and genomic organization with a unique intron/exon distribution, as well as expression of novel genes in human normal and tumor tissues. Catalytic domains of the novel proteins were expressed in E. coli and partially characterized in terms of substrate specificity and inhibition by natural and synthetic inhibitors of MMPs.

The unique amino acid sequence and transcription regulation of these genes by transcription factors such as AP1, AP2, AP4, Sp1, RORA suggest a critical function of these enzymes in malignancy.

P0756. Human genes for K+-dependent Na/Ca-exchangers, NCKX1, NCKX2 and NCKX3; genomic structure, comparative analysis of promoter regions and expression patterns.

A. Reigo[1,2], *A. Metspalu*[1,2]
[1]*Tartu University Institute of Molecular and Cell Biology; Tartu, Estonia;* [2]*Estonian Biocentre; Tartu, Estonia*
3pusa2susa@hot.ee

Cytoplasmic Ca2+ level is an evolutionarily old signal used throughout the life cycle of an organism in an universal, yet versatile manner, providing a regulatory crosstalk between different signaling pathways. Restoring the resting state and avoiding lethally high Ca2+ concentrations, expelling Ca2+ through ATP-driven pumps and Na/Ca-exchangers is essential for cell survival and function. The exchangers, characterized as having low affinity, yet high capacity for intracellular calcium, fall into two classes (NCX and NCKX) according to their K+-dependence and stoichiometry of action. All the NCKX genes analyzed here perform 4Na+ exchange for 1Ca2+ plus 1K+. We have determined full genomic structures for three distinct human genes encoding K+-dependent Na/Ca-exchangers, NCKX1 (stretches >40kb in 15q22, has 4 alternative transcripts of 10, 9, 8 and 6 exons), NCKX2 (encompasses 272kb in 9p22, has 2 alternative transcripts of 10 and 9 exons) and NCKX3 (covers 150kb in 20p12, encodes 2 alternative transcripts of 17 and 16 exons). Analysis of their promoter regions and cis-regulatory elements, as well as functional domains, reveals highly conserved motifs important for transcriptional regulation and function. Complete mRNA sequences have been refined, based on 5 - and 3 -RT-PCR and underlying genomic clone sequencing results (additional sequence tracks are added to those available in public databases). We also present comparative expression patterns of the alternative transcripts of the three genes. Additional data from this study, specific expression of NCKX1 and NCKX2 in retinal photoreceptors and their involvement in light adaptation brings all the three genes forth as good candidate disease genes for inherited retinopathias.

P0757. A member of the MAGE-family cloned from the pericentromeric region of chromosome X

P. Wieacker[1], *K. Langn se*[2], *B. Seidel*[2], *M. Wehnert*[3]
[1]*Institute of Human Genetics, Otto-von-Guericke-University; 39120 Magdeburg, Germany;* [2]*Institute of Medical Neurobiology, Otto-von-Guericke-University; Magdeburg, Germany;* [3]*Institute of Human Genetics, Ernst-Moritz-Arndt-University; Greifswald, Germany*
peter.wieacker@medizin.uni-magdeburg.de

In a search for candidate genes for X-linked mental retardation (XLMR) we have cloned from Xp11 the human gene 11B6 (meanwhile known as MAGE-D) containing 13 exons and covering 8.3 kb of genomic DNA. The open reading frame for 606 amino acids contains several nuclear localization signals. The deduced protein sequence shows about 30% similarity to the MAGE proteins and necdin with highest similarity in the central part (aa286-480) of the protein. The 2.2kb mRNA is expressed in brain, skeletal muscle, heart, placenta and pancreas and in lower amounts in kidney and lung. Northern analysis in various rat tissues revealed highest expression in a brain subfraction (brain stem, striatum, thalamus and hypothalamus) and in skeletal muscle. In situ hybridization studies in rat brain revealed prominent labelling in the hippocampal formation, nucleus supraopticus, cerebellar purkinje cells and plexus choroideus. In situ hybridization in rat testis revealed high expression in Leydig cells, in contrast to the expression data of other MAGE genes mainly expressed in spermatogenic (mouse Mage-a, Mage-b) or Sertoli cells (rat MAGE-D1/SNERG-1). By sequencing the exons as well as the exon/intron boundaries in 6 independent XLMR patients with linkage in this critical region no causative mutation could be detected. Five single nucleotide polymorphisms (SNPs) encoding synonymous amino acids have been identified in the protein coding sequence. The allele frequencies of these SNPs have been determined in different ethnic populations.

P0758. Characterization of transgenic mice overexpressing human frataxin

C. Miranda[1,2], *K. Ohshima*[1], *M. Santos*[1,2], *M. Pandolfo*[3], *M. Pandolfo*[3], *M. Pandolfo*[1]
[1]*H pital Notre-Dame, CHUM; Montr al, PQ Canada;* [2]*UnIGENe, IBMC, Univ. Porto; Porto, Portugal;* [3]*H pital Notre-Dame, CHUM; Montr al, Portugal*
cjtmiranda@hotmail.com

Friedreich ataxia is the most common autosomal recessive ataxia. Almost all cases are caused by a homozygous (GAA)n expansion in intron 1 resulting in decreased levels of frataxin, a highly conserved mitochondrial protein that is thought to prevent toxic iron accumulation in mitochondria. With the aim to better understand the function of frataxin we have generated transgenic mice expressing different levels of human frataxin. The full length human frataxin cDNA was inserted into a pCI-neo vector, under the control of the CMV promoter. Three independent lines were established, with copy number ranging from 1 to 6. Overexpression was confirmed at RNA and protein levels, and high levels were found in several tissues, but particularly in heart and pancreas. However, because of the inactivation of the CMV promoter during early embryonic development, these transgenics could not rescue the early embryonic lethality of the homozygous frda knock-out. Transgenic mice, up to one year of age, did not show any obvious abnormality. Detailed studies of iron metabolism, mitochondrial function and resistance to oxidative stress are in progress. Preleminary results show that plasma iron and total serum iron binding capacity are in the normal range and resistance to the cardiotoxicity of adriamicin, which is thought to be mediated by an oxidative stress mechanism, is not increased.

P0759. Molecular analysis of the complexity of the DAZ gene family in AZFc reveals partial DAZ deletions in men with severe oligozoospermia as novel mutation event

S. Fernandes[1], *J. Goncalves*[2], *H. Ditton*[1], *K. Huellen*[1], *M. Sousa*[3], *A. Barros*[4], *P. H. Vogt*[1]
[1]*Institute of Human Genetics; Heidelberg, Germany;* [2]*INS Dr. Ricardo Jorge; Lisboa, Portugal;* [3]*Instituto de Ciencias Biomedicas de Abel Salazar; Porto, Portugal;* [4]*Centro de Genetica da Reproducao; Porto, Portugal*
susana_fernandes@med.uni-heidelberg.de

One of the strongest candidates for the azoospermia factor (AZF) is the DAZ (Deleted in Azoospermia) gene family, exclusively expressed in male germ cells and mapped to the AZFc male fertility locus located in distal Yq11 (Vogt et al., 1996). AZFc deletions are a major cause for the occurrence of male infertility as they were found in men with idiopathic azoospermia or severe oligozoospermia with a frequency between 4-20 % (Vogt, 1998). Recent analysis of the DAZ gene cluster by restriction mapping, Fiber-FISH and sequence analysis in different men identified a variable number of DAZ gene copies in AZFc (3 DAZ genes; Yen, 1997; 4 DAZ genes; Saxena et al. 2000; 7 DAZ genes, Gl ser et al., 1998) suggesting the presence of DAZ pseudogenes. We, therefore set out to identify those DAZ gene copies which are essential for human male fertility by DAZ copy specific transcription and translation analyses and by a molecular screen for partial DAZ deletions in men with severe oligozoospermia. For the identification of partial DAZ deletions the structure of each DAZ gene copy was marked by a set of single nucleotide variants (DAZ-SNVs) and DAZ copy specific restriction maps. In this way a series of copy specific DAZ haplotypes were established and screened for their presence in the AZFc locus of patients with severe oligozoospermia. Up to now 3 individuals with partial DAZ deletions were identified and confirmed by Southern blotting experiments (Fernandes S. et al. in prep). Our results identify DAZ1 as the first DAZ gene copy essential for human male fertility. References; Gl ser B, Yen PH, Schempp W (1998) Fibre-fluorescence in situ hybridisation unravels apparently seven DAZ genes or pseudogenes clustered within a Y-chromosome region frequently deleted in azoospermic males. Chromosome Res. 6; 481-486. Saxena R, de Vries, JWA, Repping S, et al. (2000) Four DAZ genes in two clusters found in the AZFc region of the human Y chromosome. Genomics 67; 256-267. Vogt PH, Edelmann A, Kirsch S, et al. (1996) Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. Hum. Mol. Genet. 5; 933-943. Vogt PH (1998) Human Y chromosome deletions in Yq11, AZF candidate genes and male infertility; history and update. Mol. Hum. Reprod. 4; 739-744. Yen PH, Chai, NN, Salido EC. (1998) The human DAZ genes, a putative male infertility factor on the Y chromosome, are highly polymorphic in the DAZ repeat regions. Mam. Genome 8; 756-759.

P0760. Cloning And Characterisation Of The Gene Encoding The Human Npl4, A Component Of The Nuclear Pore Complex Which Interacts With The Ubiqutin Fusion Degradation Protein- 1(ufd1l)

A. Botta[1], *C. Tandoi*[2], *G. Fini*[3], *G. Calabrese*[4], *B. Dallapiccola*[2], *G. Novelli*[1]
[1]*Tor Vergata University of Rome; Rome, Italy;* [2]*Chair of Medical Genetics, La Sapienza University of Rome and CSS-Mendel Institute; Rome, Italy;* [3]*Tor Vergata University of Rome and CSS-Mendel Institute; Rome, Italy;* [4]*Institute of Biology and Genetics, University of Chieti; Chieti, Italy*
botta@genetica.med.uniroma2.it

UFD1L gene encodes for the human homologue of the yeast ubiquitin

fusion-degradation 1 protein, an essential component of the ubiquitin-dependent proteolytic turnover and mRNA processing. Although UFD1L gene has been mapped in the region commonly deleted in patients with DiGeorge syndrome (DGS), it is not clear the link between its haploinsufficiency and some specific features of the DGS phenotype. Since a Ufd1l/Npl4 protein complex has been recently described in rat, we have cloned and characterised the human counterpart of the Npl4 gene. Human Npl4 gene encodes for a protein showing 96%, 44%, 34% homology with Npl4 from rat, C. elegans, S. cerevisiae respectively and, similarly to the C. elegans and rat orthologs, contains a single C-terminal RanBP2 zinc finger motif. Fluorescent in situ hybridisation (FISH) experiments on human metaphases localised the Npl4 gene on the most telomeric region of chromosome 17q. Northern blots analysis on fetal and adult human tissues revealed a ~ 4.5 Kb transcript most abundant in heart, brain, skeletal muscle and kidney. Given the involvement of the yeast Npl4p in the nuclear targeted protein transport, the identification of the Ufd1l/Npl4 complex in mammals reveals a potential relationship between nuclear transport defects and the DGS phenotype. Npl4 gene could therefore be considered a good candidate to perform mutations screening in patients with DGS and no detectable 22q11 deletion. Work supported by Telethon grant 364/bi and E723.

P0761. Mutational analysis of the SH2D1A gene in two Danish families

K. Gr¿nskov, *S. Timshel, A. Sand, K. Br¿ndum-Nielsen*
John F. Kennedy Institute; Glostrup, Denmark
kag@kennedy.dk

X-linked lymphoproliferative syndrome (XLP or Duncan disease), is characterized by an extreme sensitivity to Ebstein-Barr virus (EBV), leading to severe and often fatal infectious mononucleosis. The disease is caused by mutations in the SH2D1A gene (Xq25), containing four exons. The mRNA of 2.5 kb encodes a protein of 128 amino acids, consisting of a 5 aa N terminal sequence, a Src homology 2 (SH2) domain and 25 aa C-terminal tail. SH2D1A have been found to bind a phosphotyrosine of the signalling lymphocyte activating molecule (SLAM) protein thereby inhibiting recruitment of a tyrosine phosphatase, SHP2. SLAM is a transmembrane receptor with two immunoglobulin-like domains expressed in T and B cells. We have performed mutational analysis of the SH2D1A gene in two Danish families. In a boy with symptoms of XLP we found a R55X mutation. This mutation has been found in several XLP patients and are known to be disease causing. In another family, a 16 year old boy died from mononucleosis. Mutational analysis was performed on DNA extracted from tissue embedded in paraffin. Exon 2 was not amplified and we did not find any mutations in the remaining exons, however, in DNA from the mother and sister we found a missense mutation in exon 3 in the C-terminal tail, Q112H. The interpretations of our findings are; a) Q112H is a rare polymorphism unrelated to disease, b) Q112H is disease causing and the results in the boy was due to artifact and poor quality of DNA

P0762. Role of histone acetylation in genomic imprinting of human 15q11-q13 and mouse 7C.

M. Takahashi[1], *K. Takano*[1], *T. Wada*[1], *T. Ohta*[2], *R. D. Nicholls*[2], *S. Saitoh*[1]
[1]*Hokkaido University; Sapporo, Japan;* [2]*Center for Neurobiology and Behavior, Department of Psychiatry, University of Pennsylvania; Philadelphia, PA United States*
makmak@med.hokudai.ac.jp

To understand the role of histone acetylation in genomic imprinting, we have investigated the status of histone acetylation of imprinted loci in human 15q11-q13, which is associated with Prader-Willi syndrome (PWS) and Angelman syndrome (AS), and the syntenic region in mouse 7C. We performed Chromatin immunoprecipitation (ChIP) assays using anti-acetylated histone H3 and H4 antibodies to assess histone acetylation. Lymphoblastoid cells from PWS and AS patients, and controls, as well as fibroblasts from PWS and AS model mice carrying a 4 Mb deletion, and control mice were used. We have previously identified parent-of-origin specific histone acetylation confined to the CpG island of SNURF-SNRPN. In this study, we extended the study to 6 human loci including 5 paternally-expressed loci (MKRN3, MAGEL2, NDN, SNURF-SNRPN, and IPW), and 1 maternally-expressed (UBE3A), as well as 4 mouse paternally-expressed loci (Mkrn3, Magel2, Ndn, and Snurf-Snrpn). Although the CpG island of SNURF-SNRPN demonstrated a clear difference of histone acetylation, other human loci did not demonstrate such parent-of-origin specific differences even in the CpG island of those genes where parent-of-origin specific DNA methylation has been demonstrated. In contrast, in mouse fibroblasts, the maternally derived inactive allele is less acetylated than the paternally derived active allele at all tested loci, with the most striking difference detected at the CpG island of Snurf-Snrpn. These findings suggest that 1) histone acetylation may not strictly correlate with the DNA methylation status at each imprinted locus, and 2) chromatin structure of the imprinted domain may vary between mouse fibroblasts and human lymphoblasts.

P0763. Analysis of Microdeletions in the Y Chromosome of Idiopatic Azoospermia and Severe Oligospermia with Infertil Men by using Multiplex PCR

M. Muslumanoglu[1], *M. Turgut*[2], *O. Cilingir*[1], *C. Can*[2], *Y. Ozyurek*[2], *N. Basaran*[1]
[1]*Osmangazi University, Medical Faculty, Department of Medical Genetics; Eskisehir, Turkey;* [2]*Osmangazi Univ, Medical Faculty, Department of Urology; Eskisehir, Turkey*
mhamza@ogu.edu.tr

It has been known that some infertile men with azoospermia and oligospermia have microdeletions in the Y chromosome. However, the frequency of such microdeletions among the infertile men is unknown. We have studied 14 men with azoospermia and oligospermia. Our study consist of 18 primer pairs that are homologous to previously identified and mapped sequence tagged sites. These primers amplify nonpolymorphic short DNA segments from Sthe Y chromosome when used in polymerase chain reactions. Primers have been combined into four sets for using in multiplex PCR. Molecular deletion analyses of azoospermic and oligospermic males have suggested existence of Azoospermia Factor(AZF) residing in deletion intervals 5 and 6 of the human Y chromosome and coinciding with three functional regions associated with spermatogenic failure. Additionaly, it was investigated fourth, AZF region called AZFd which is located between AZFb and AZFc. Of 14 patients, we found microdeletions lying in different regions of in three (21.4%) cases.

P0764. An additional alternative exon of the Neurofibromatosis Type 1 (NF1) gene called 10a-2 codes for a possible transmembrane segment

B. Bartelt, *P. Kruse, W. Vogel, O. Kenner, R. M ller, D. Kaufmann*
University of Ulm; Ulm, Germany
britta.bartelt@medizin.uni-ulm.de

An additional splice product of the Neurofibromatosis Type 1 (NF1) gene was found. It contains an insert of 45 bp of intron 10a. This insert, termed exon 10a-2, qualifies as an exon as it is surrounded by splice sites with sufficiently high splice site scores, contains no in-frame stop codon and does not alter the reading frame of the following in-frame exon 10b. This additional splice product was expressed in HeLa cells at a level of 10% of the wildtype allele. It was found to be expressed at comparable levels in all human primary and tumor cell cultures examined. Of human tissues, a higher expression was seen in parts of the brain. Exon 10a-2 was also found to be expressed in cells derived from primates and cat, but not in cells from cow, rat or mouse. Sequence comparison between exon 10a-2 and the NF1 sequences of fugu rubripes and drosophila melanogaster showed no high homology. Investigation of the putative 10a-2 protein sequence suggests that its predisposes to transmembrane helices. To test this, fusion proteins of EGFP and NF1 exon 9 to 10b were expressed in HeLa and brown melanoma cells. Fusion protein without exon 10a-2 showed only a cytoplasmic localization. In contrast, fusion protein containing exon 10a-2 exhibited predominantly perinuclear granular signals.

P0765. XOS9, a non-coding transcript antisense to SOX9

U. Dohrmann[1], *D. Pfeifer*[1], *F. Poulat*[2], *G. Scherer*[1]
[1]*Institute of Human Genetics and Anthropology; Freiburg, Germany;* [2]*Institut de G n tique Humaine; Montpellier, France*
udohrmann@mm31.ukl.uni-freiburg.de

SOX9, the gene mutated in the human skeletal malformation syndrome campomelic dysplasia with XY sex reversal, is an essential regulator of chondrocyte and testis differentiation and is expressed in a variety of embryonic and adult tissues. Genomic sequence analyses showed that SOX9 is the only protein-coding gene within a region of 2.5 Mb on distal 17q. Database searches for ESTs revealed the presence of a non-coding, polyadenylated RNA transcribed from the SOX9 antisense strand. We named the corresponding gene XOS9. It extends over 67.5 kb and consists of 14 exons with a total length of at least 1250 bp, with each exon flanked by canonical splice donor and acceptor sites. The 5.4 kb SOX9 gene is embedded within the 17 kb first intron of XOS9, and XOS9 exon 2 overlaps with part of the SOX9 promoter. The processing of the XOS9 transcripts appears to be complex, as at least 8 different alternatively spliced XOS9 variants are found in various tissues of different developmental stages and in cell lines. Reporter gene constructs containing the XOS9 promoter showed enhanced reporter gene activity in some eukaryotic cell lines but not in others, revealing specific regulation of the XOS9 promoter.

Interestingly, cells that activate the XOS9 promoter are derived from tissues known to express SOX9. Work is in progress to establish a role for XOS9 in the complex regulation of the SOX9 gene.

P0766. Suppression analysis of the human beta-globin nonsense mutation CD15 (TGG->TGA)

F. Almeida, *A. Miranda, J. Lavinha, L. Romao*
Instituto Nacional de Saude Dr. Ricardo Jorge ; Lisbon, Portugal
fatima.almeida@insa.min-saude.pt

Nonsense mutations may cause the synthesis of truncated proteins from a mutant gene. Because the protein is incomplete and deleterious, its mRNA is often rapidly degraded by a mechanism termed nonsense-mediated decay (NMD).A CD15 nonsense mutation was found in portuguese beta-thalassemia carriers (Ribeiro et al., Brit. J. Hematol. 1992) and cloned. Surprisingly, the corresponding mRNA was expressed at levels similar to wild-type in vivo as well as in vitro (Romao et al., Blood 2000). Since the CD15 mRNA is not subject of NMD, there is a therapeutic interest in studying if this mutation may be suppressed. It is known that some aminoglycoside antibiotics may suppress nonsense mutations during translation by competing with protein factors that promote the release of the polypeptide from the ribosome.In order to test this hypothesis, murine erythroleukemic (MEL) cells were cotransfected with the CD15 nonsense mutated gene or, as a positive control, with the wild-type gene, and the puromycin-resistance gene (selection marker). The pools of transfected cells were induced to erythroid differentiation, i.e. to transgene expression, and the aminoglycoside G418 was added in varying concentrations. Cytoplasmic extracts of MEL cell pools expressing either transgene in the presence or absence of G418 were prepared and protein expression analysis was performed by immunoblotting.Preliminary results indicate that the amount of beta-globin resulting from suppression of CD15 is low. For a quantification, transfected MEL cell lines are currently being subcloned from the pool.

P0767. Use of the GFP as reporter in an adhesion test system to determine in vivo effects of Myelin Protein Zero (P0) mutations

A. Ekici[1], *E. Nelis*[2], *C. Van Broeckhoven*[2], *B. W. Rautenstrauss*[1]
[1]*Institute of Human Genetics; Erlangen, Germany;* [2]*Laboratory of Neurogenetics, Born Bunge Foundation, University of Antwerp; Antwerp, Belgium*
aekici@humgenet.uni-erlangen.de

P0 is a well characterized cell adhesion molecule playing a crucial role during myelination in peripheral nervous system. GFP is a helpful tool to observe cellular events in vivo and is multiple used for this purpose in various cells or even organisms. P0 mutations cause Charcot-Marie-Tooth disease type 1B (CMT1B), Dejerine-Sottas-Syndrome (DSS) and congenital hypomyelination (CH), diseases of different severity, even if the same position is substituted by different amino acids. We used two insect cell lines, S2 and HighFive, in order to develop an adhesion test system to determine effects of P0 mutations. We analyzed 3 pathogenic mutations for their effect on adhesion capability in a S2 cell system and found a direct correlation of adhesion capability and severity of the disease phenotype. We constructed fusion proteins with GFP for P0wt and one mutation to visualize the effect in vivo both on the intracellular localization and on the changes in the adhesion capability. The GFP-P0wt fusion revealed the expected adhesion capability and moreover the membrane insertion of the fusion protein was clearly visible in a fluorescent microscope. The GFP-P0 mutation showed expression, but no membrane insertion and may explaine the lack of its adhesion capability. Hence, this system is suitable to predict the severity of the phenotype based on expression of in vitro mutated P0 and visualize the effect of mutations in vivo.

P0768. Aberrant Splicing in Several Human Tumors in the Tumor Suppressor Genes NF1, NF2 and TSC2

O. Kenner[1], *D. Kaufmann*[1], *W. Leistner*[2], *P. Kruse*[1], *S. Hoffmeyer*[3], *C. Hein*[4], *W. Vogel*[1], *L. Messiaen*[5], *B. Bartelt*[1]
[1]*Department of Human Genetics; Ulm - Donau, Germany;* [2]*Max-Delbr ck-Center for Molecular Medicine; Berlin, Germany;* [3]*Epidauros; Bernried, Germany;* [4]*Department of Neurosurgery; Ulm - Donau, Germany;* [5]*Department of Medical Genetics; Ghent, Belgium*
oliver.kenner@medizin.uni-ulm.de

Mutations at splice sites or surrounding sequences have been reported to cause aberrant splicing. Splicing errors, however, can also occur without sequence alterations. We investigated three tumor suppressor genes for aberrant splicing in tumors. At a low frequency per exon it was found for 5 of 7 investigated in-frame exons of the neurofibromatosis type 1 (NF1) gene, for 2 of 3 exons of the neurofibromatosis type 2 (NF2) gene and for 1 of 3 exons of the tuberous sclerosis 2 (TSC2) gene. It was detectable in all human tumor tissues tested (NF1 neurofibroma, sporadic intramedullar neurinoma, sporadic meningiomas, NF2 schwannoma, NF2 meningioma, basalioma and naevus) as well as in cultured tumor cell lines and cultured primary cells. Hence our data show that aberrant splicing is a very common process. According to simulations of the secondary structures of the pre-mRNA we suggest that it is due to the rare occurrence of alternative structures at the splice donor site not recognised by the splice machinery. In HeLa cells aberrant splicing is increased at elevated temperature and low pH in vitro, conditions often found in tumor tissues. We suggest that increased aberrant splicing due to environmental factors represents an additional mechanism for reduction of the amount of tumor suppressor mRNA in the absence of relevant mutations in the tumor.

P0769. Towards understanding the molecular pathology of vitelliform macular dystrophy (Best disease)

F. Kr mer[1], *V. Milenkovic*[1], *H. St hr*[1], *R. S. Molday*[2], *B. H. F. Weber*[1]
[1]*Human Genetics; Wuerzburg, Germany;* [2]*Department of Biochemistry; Vancouver, BC Canada*
fkraemer@biozentrum.uni-wuerzburg.de

The autosomal dominant vitelliform macular dystrophy (Best disease, VMD2) is characterized by a typical bilateral accumulation of lipofuscin-like material within and beneath the retinal pigment epithelium (RPE). The egg yolk-like macular lesion and the absence of the light-rise in the electro-oculogram (EOG) are key features associated with this disease. Recently, the gene underlying Best disease has been identified and more than 75 distinct mutations have been reported. Of these, almost all are found to be clustered in four regions of the N-terminus implying functional significance for these regions. To gain insight into the pathogenic mechanisms of mutant VMD2 protein, we developed a heterologous cell expression system. Monoclonal (mAb_H6) and polyclonal (pAb_333/334) antibodies were generated against different epitopes of the VMD2 protein and their specificity was verified by Western blotting. Both antibodies label a specific band in the detergent phase of human RPE extractions but not other tissues tested. COS-7 and ARPE-19 were chosen as suitable cell lines for the transfection experiments, as no endogenous VMD2 expression is detectable in these cell systems. A number of different mutations found nearby and within the predicted transmembrane domains of VMD2 were selected for this study. Using site-directed mutagenesis, A10T, R92S, Y227N, A243V and DI295 were introduced into the full length cDNA of VMD2. The mutant constructs and the intact VMD2 were transiently transfected into COS-7 and ARPE-19 cell lines. The current study will present the subcellular localisation of mutant and intact VMD2 protein as a first step in the functional characterization of its biological role in the RPE.

P0770. Is the IVS2 Region of the Human Agamma Globin Gene an Important Regulatory Region?

L. Osorio-Almeida, *I. Gon alves, A. Raimundo, P. Baptista*
FCT/UNL; Caparica, Portugal
mlo@mail.fct.unl.pt

Our work performed in beta thalassemia and HbLepore heterozygotes with variable levels of fetal hemoglobin (HbF) has shown that one important contribution to the observed HbF elevation comes from cis acting sequences located in the betaglobin gene cluster. The genetic markers consist of two polymorphic microsatelites lying in the Agamma globin IVS2 gene [(TG)n] and 5 to beta globin gene [(AT)xTy]. We observed a statistically significant correlation between the presence of allele (TG)13 in Agamma; IVS2, in linkage disequilibrium with the C to T transition at -158 of Ggamma gene, and HbF elevation. The AgammaIVS2 has been previously identified as a region required for the inducible expression of gamma globin gene by hemin. To further characterize this region, we have footprinted two introns with different (TG)n alleles and have analysed regions of interest by electrophoretic mobility shift assay. Through these studies we identified two binding sites for the erythroid regulatory factor GATA-1 and five sites bound by general transcription factors (Sp1, NF1, YY1, BP1 and Oct 1). Additionally, we have found in the two alleles studied [(TG)13 and (TG)9(CG)5(TG)8] that the microsatelite region interacts with different proteins. These proteins, not yet identified, are present in adult erythroid nuclear extracts. All these data suggests that AgammaIVS2 contains DNA elements functionally relevant for gamma to beta globin gene regulation. We started to test the functional importance of the Oct 1 binding site by a PCR-based method of site directed mutagenesis in the (TG)13 allele. The normal and mutated introns were inserted into a plasmid p2g§301 (a gift from Dr. Swee Lay Thein), able to express the gamma and the beta genes under the control of HS2 site. We are currently performing stable transfection assays with the normal and the mutated constructs into MEL cells to investigate if the Oct 1 site is functionally relevant for the gamma globin gene expression.

P0771. Aggregation and toxicity in mammalian cells of proteins containing homopolymeric (Gln)- or (Leu)- repeats.

J. C. Dorsman, *B. Pepers, D. Langenberg, J. T. Den Dunnen, G. B. Van Ommen*
LUMC; Leiden, The Netherlands
J.C.Dorsman@LUMC.NL

Several neurological disorders are caused by the expansion of a trinucleotide repeat. The largest group of these diseases, including Huntington s disease (HD) and several spinocerebellar ataxia s (SCAs), are associated with an expansion of a (CAG)n repeat in the coding region of the gene. Since the instability of the (CAG)n repeat lies at the DNA level there is no a priory reason why the sequence (CAG)n or its complement (CTG)n would not occasionally be translated as (Gln)n, (Leu)n, (Ser)n, (Ala)n or (Cys)n, depending on reading frame and translated strand. Yet, in the neurodegenerative disorders only Gln-repeat expansions are found, while the occurrence of repeats encoding (very) long stretches of other amino acids has not been documented. To understand this observation and to determine the potential effect of other repeats we transformed mammalian cells with constructs expressing different single amino acid stretches. To overcome the inherent instability of long homogeneous trinucleotide repeats we employed the redundancy of the genetic code to express homogeneous single amino acid stretches from mixed sequence DNA oligomers. These sequences replicate stably in pro- and eukaryotes and facilitated the expression of proteins containing up to 300 glutamines or leucines. Transfections of cultured mammalian cells show that proteins containing (Gln)300- and (Leu)300-repeats are toxic and display a high propensity for aggregation with Leu-repeats producing a significantly increased toxicity compared to Gln-repeats. In addition, various cellular transcription factors co-aggregate in these aggregates, including the transcriptional co-activators CBP and p300. We are currently studying the effects of the expression of proteins containing single amino acid repeats in mammalian cells using DNA chip and micro-array technology. These studies should provide insight in the cellular changes and the toxic effects of these aggregation-prone proteins.

P0772. The autosomal dominant hypophosphatemic rickets (ADHR) gene is a secreted fibroblast growth factor (FGF23)

B. Lorenz-Depiereux[1], *A. Benet-Pages*[1], *K. E. White*[2], *S. Hauck*[1], *M. Grabowski*[1], *G. Carn*[2], *M. Ueffing*[1], *M. J. Econs*[2], *T. Meitinger*[1], *T. M. Strom*[1]
[1]*Technische Universit t M nchen und GSF Forschungszentrum f r Umwelt und Gesundheit; M nchen, Germany;* [2]*Indiana University School of Medicine; Indianapolis, IN United States*
bettina@pedgen.med.uni-muenchen.de

Autosomal dominant hypophosphatemic rickets (ADHR) is a phosphate wasting disorder, characterised by low serum phosphorus concentrations, rickets, osteomalacia, lower extremities deformities, short stature, bone pain, and dental abscesses. Recently, we identified missense mutations in a novel fibroblast growth factor (FGF23) as the cause for ADHR. Patients with ADHR display many clinical and laboratory characteristics that are observed in patients with oncogenic hypophosphatemic osteomalacia (OHO). OHO is thought to arise by the secretion of a phosphate wasting factor. We therefore investigated whether FGF23 is a secreted factor. An affinity purified polyclonal antibody was produced against a peptide corresponding to residues 229-243 of human FGF23. After stable transfection of HEK293 cells with a plasmid (pcDNA3.1) encoding full-length human FGF23 with an C-terminal 6xHis tag, two protein species of approximately 35 and 15 kDa were detected in the conditioned medium but not in the cell lysate by Western blot analysis. Detection by an anti-His antibody gave the same results, indicating that the affinity-purified anti-FGF23 antibody recognised recombinant human FGF23 protein. FGF23 without the signal peptide of 24 amino acids is predicted to have a molecular mass of 25.3 kDa. If the molecular mass is correctly estimated by SDS-PAGE, our findings thus imply that FGF23 undergoes post-translational modifications. To investigate whether FGF23 is a substrate of the putative endopeptidase PHEX, we prepared constructs for the coexpression of wildtype FGF23 and mutant FGF23 with PHEX. In summary, the FGF23 protein is a secreted factor and may have an important role in human phosphate homeostasis.

P0773. Development of an insect cell expression system for in vivo analysis of TIGR-GFP fusion proteins

S. Oezbey, *A. B. Ekici, E. Kierysch, K. Michels-Rautenstrauss, B. Rautenstrauss*
Institute of Human Genetics; Erlangen, Germany
soezbey@humgenet.uni-erlangen.de

Primary open angle glaucoma (POAG) is the most common form of glaucoma and between 1 and 2 % of the population over age 40 is affected by this disease. Recently, mutations in the trabecular meshwork-inducible glucocorticoid response (TIGR) gene, also known as myocilin (MYOC), were identified for juvenile open angle glaucoma (JOAG). TIGR/MYOC is located on human chromosome 1q23-24 and his function remains unknown. As prerequisite for TIGR expression analysis three different green fluorescent proteins have been transfected into S2-insect cells. First the GFP-coding region was cleaved from pQBI25 and subcloned into the expression/selection plasmid. The assay uses the expression/selection vector pIB/V5-His. The highest concentrations of blasticidin (10g/ml) resulted in good and contamination free growth of the cell culture. In this analysis the GFP derived from the pQBI25 vector revealed the highest transfection efficiency accompanied by bright fluorescence. Analysis of BFP proteins, however, was hampered by the blue fluorescence of S2 cells themselves. In analogy to experiments performed with Myelin Protein Zero-GFP Fusions in this system we perform currently expression analysis of TIGR-GFP fusion proteins. This will allow to determine the correct ATG codon as well as intracellular localization and a postulated secretion of the protein.

P0774. Interspecies comparison of SMN genes and screen for cis-elements regulating transcription.

L. R. SIMARD, *A. Semionov, C. Rochette*
Centre de Recherche H pital Sainte-Justine; Montr al, PQ Canada
simardlo@medclin.umontreal.ca

The spinal muscular atrophy (SMA) region on chromosome 5q13 contains an inverted 500-kb duplication and deleterious mutations in one of the survival motor neuron (SMN) genes, namely SMN1, cause this common lethal childhood neuropathy. The major difference between SMN1 and SMN2 is a single nucleotide that, in SMN2, favors the exclusion of exon 7. SMA severity parallels the amount of available full-length SMN. We have demonstrated that SMN gene duplication occurred >5 Mya in a common ancestor of humans and chimpanzees; however, SMN2 first appeared in Homo sapiens indicating that SMN1 is the ancestral gene. RNA and protein data suggest both temporal and spatial regulation of SMN production. We have used two approaches to identify potential cis-elements involved in SMN gene regulation. First, we performed an interspecies sequence comparison to generate a percent identity plot. PipMaker detected 3 major clusters of highly conserved (60-70%) DNA stretches between the mouse and human SMN genes. These segments also harbored a number of putative binding sites for known transcription factors. In addition, the region surrounding exon 7 shared 77% identity. Second, functional assays of enhancer/silencer activity of the entire 14-kb mouse Smn gene revealed two DNA segments that affected the promoter activity of a CAT reporter gene. One of these mapped to a region of 70% interspecies sequence identity. This high degree of sequence conservation was lacking in the second element. In conclusion, we have identified two elements potentially involved in transcriptional regulation of SMN gene expression. This work could provide some clues towards manipulating SMN2 gene expression to potentially treat SMA. Funded by HSCF and MRC.

P0775. Subcellular localization of the polycystic kidney disease-1 and -2 gene products.

D. J. M. Peters, *M. H. Scheffers, H. Le, P. van der Bent, E. de Heer, M. H. Breuning*
Leiden University Medical Center; Leiden, The Netherlands
d.j.m.peters@lumc.nl

Autosomal dominant polycystic kidney disease (ADPKD) is a common hereditary disease characterized by the progressive development of fluid-filled cysts in renal tubuli, leading to chronic renal failure. To date two proteins have been identified to be involved in ADPKD, polycystin-1 and polycystin-2. These proteins are thought to function in signal transduction, cytoskeletal rearrangement and cell adhesion and probably act in a common cellular pathway as well as independently of eachother. Antibodies generated against both proteins have been used to study expression in tissues and in cultured epithelial cells. We also analyzed MDCK-cells, a renal epithelial cell line, transfected with a full-length Polycystin-2-enhanced green fluorescent protein (Pol2-EGFP) fusion protein construct. Pol2-EGFP overexpressing cells showed mainly diffuse cytoplasmic expression, consistent with endoplasmic reticulum/pre-middle Golgi localization reported by Cai et al. (JBC Oct 1999). Expression of Pol2-EGFP in the plasma membrane was detected only in a small subset of cells. Staining with polyclonal antibodies raised against polycystin-2 overlapped with Pol2-EGFP expression, which indicates that these antibodies recognize polycystin-2. Staining with antibodies raised against polycystin-1, partially overlapped in the cytoplasm which generally paralleled intensity of Pol2-EGFP expression. In the plasma membrane polycystin-1 co-localizes with desmosomes and possible colocalization with polycystin-2 was observed in the subset of

cells in which Pol2-EGFP was expressed at the plasma membrane. These results suggest cotransport of these proteins from the cytoplasm to the plasma membrane and that Polycystin-1 and -2 may interact and stabilize eachother in a cell cycle or transcription/translation dependent manner.

P0776. Human TSPY can be transcribed and processed in Mouse GC-1 cells

B. Skawran, *F. Dechend, S. Schubert, J. Schmidtke*
Institut f r Humangenetik, Medizinische Hochschule; Hannover, Germany
bskawran@yahoo.de

The human TSPY (testis specific protein, Y-encoded) is organized as a repetitive gene family of 30 to 60 copies with ist bulk mapping to yp.TSPY codes for a testis specific expressed m-RNA transkripts and could also be detected on Western blots as a 33/38kD protein, depending on its degree of phosphorylation. By immunohistostaining TSPY was detected in spermatogonia and to a lesser extent in primary spermatocytes, suggesting a role in spermatogenesis. Nevertheless the precise function of TSPY remains unknown. In contrast to human TSPY, mouse tspy appears to be a single copy pseudogene. To better understanding of TSPY regulation, we determined whether human TSPY can be correctly transcribed and spliced in a mouse spermatogonia cell line (GC-1).After transfection of these cells with a construct including the entire gene and the putative promoter of human TSPY, we could show that the hTSPY cDNA is spliced according to the major pattern known from in-vivo studies. This cellular model enabled us to investigate the human TSPY promoter region more precisely. We have cloned different reduced promoter fragments into a luciferase-vector (pGL3) (Promega) and tested their transcriptional activities in the GC-1 spermatogonia cell line. The distinct promoter regions showed different transcriptional activities, enabling us to determine length and functional domains of the TSPY-promoter.

P0777. Homologues To The First Gene For Autosomal Dominant Polycystic Kidney Disease (ADPKD) Are Pseudogenes

N. Bogdanova[1], *A. Markoff*[2], *M. McCluskey*[3], *J. Horst*[1], *B. Dworniczak*[1]
[1]*Institut f r Humangenetik, Univ. M nster; M nster, Germany;* [2]*Institut f r Medizinische Biochemie, Univ. M nster; M nster, Germany;* [3]*Centre for Human Genetics, Edith Cowan University; Jundaloop, Australia*
bogdano@uni-muenster.de

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common monogenic inherited disorders with an incidence of approximately 1 in 1000. The disease is characterized by development of fluid-filled cysts in both kidneys which, in most affected individuals, results in kidney failure. In affected individuals of European descent, mutations in the PKD1 gene, located on 16p13.3 are the most common cause for the disease and account for up to 85% of the cases. The PKD1 gene extends over ~52 kb of genomic DNA and contains 46 exons encoded by a 14 kb transcript. A large part of the gene, extending from exon 1 to the first 87 bp of exon 33, is duplicated in at least three homologous genes (HG), which are located on chromosome 16p13.1 and share approximately 95-97% homology with the PKD1 gene, thus heavily obstructing the mutation analysis of PKD1. Two of these genes have been recently covered in a large sequencing work on chromosome 16. It has been however not known for years if homologous to PKD1 genes code for functional polypeptides. We have identified four more homologues to PKD1 which are different from the previously published sequence by employing PCR screening of a bacterial artificial chromosome (BAC) DNA library and BLAST homology searches in publically available data bases. Deciphering the structural differences between HG and PKD1 allows for differential analysis of those genes. Assaying their transcripts in the model cell line T98G shows that HG are not translated. Taken together with the sequence information about the genes, these data show that homologues to PKD1 are pseudogenes. This is the first work on PKD1 homologous genes to demonstrate that HG belong to a PKD1 pseudogene family, the members of which emerged at about the same time in the course of molecular evolution. The precise structural characterization of HG allows in addition to create better reagents for conventional mutation analysis of the duplicated part of the PKD1 gene.

P0778. A new screening method applied to the identification of proteins interacting with the neurofibromin mRNA

A. Striebel, *J. Haeussler, W. Vogel, G. Assum*
Department of Human Genetics University of Ulm; Ulm, Germany
andrea.striebel@medizin.uni-ulm.de

Neurofibromatosis type 1 (NF1) is one of the most common genetic disorders in human with a prevalence of about 1 in 3500 individuals. The 8.5 kb coding sequence of the NF1 gene is followed by an unusually long 3«-untranslated region (3.5 kb) which is highly conserved between mouse and human. 3«UTRs are known to influence the fate of mRNAs in several ways, including intracellular localisation, control of its stability and regulation of translation efficiency. These functions are often mediated by proteins, binding to regulatory sequences within the 3«UTRs. Our group searched for protein binding sites within the NF1 3«UTR and defines the interacting proteins. Up to now we were able to identify five protein binding regions termed PBR1-5 in the NF1 3«UTR. Two of them were studied in more detail. PBR1 binds three different proteins with estimated molecular weights of 40 kDa, 68 kDa and 96 kDa. Cross-link reactions demonstrated that the protein interacting with NF1-PBR5 has an approximate molecular weight of 48 kDa. In order to identify the respective proteins we established a new screening method using high density protein expression filters from the RZPD (Resource Center German Human Genome Project). The filters contain 37200 clones expressing high levels of the protein-repertoire of human fetal brain. After incubation of the filters with radiolabelled PBR1 or 5 RNA we obtained 8 positive clones interacting specifically with one of the two PBRs. The identification and verification of the putative RNA binding proteins are on the way.

P0779. TSPY variants in six loci on the human Y-chromosome

S. Schubert[1], *F. Dechend*[1], *G. Williams*[2], *B. Skawran*[1], *M. Krawczak*[3], *C. Tyler-Smith*[2], *J. Schmidtke*[4]
[1]*Institut f r Humangenetik, Medizinische Hochschule; Hannover, Germany;* [2]*CRC, University of Oxford; Oxford, United Kingdom;* [3]*Institute of Medical Genetics, University of Wales; Cardiff, United Kingdom;* [4]*Institut f r Humangenetik, Medizinische Hochschule; Hannover, United Kingdom*
schubert.stephanie@mh-hannover.de

TSPY (testis-specific protein, Y-encoded) is a Y-specific, heterogeneous gene family. It was first discovered in humans but has subsequently been detected in many other mammalian species. The majority of human TSPY copies map to interval 3C on Yp11.2 (TSPYA) where each copy is embedded in a single unit of the DYZ5 tandem repeat array. TSPY thus forms the only known regular tandemly-repeated protein-coding gene family in the human genome. A minor locus (TSPYB) exists in an interval proximal to 3D, close to the centomere. FISH analysis revealed an additional minor locus on Yq11.23. Recently, a new member of the TSPY gene family (TSPYq1) was isolated from subinterval 6E, part of the AZFc region. Thus in humans, as in many other species, TSPY is organized as a repetitive gene family, with inter-individual variation in copy number ranging from about 30 to 60. The present study was performed in order to obtain more detailed insight into the structure, organization and genomic evolution of the human TSPY gene family by mapping three sequence variants identified through RT-PCR analysis onto genomic clones derived from two different YAC-contigs. TSPY gene family members occur in at least six locations on the human Y chromosome, and each cluster comprises a unique combination of variants. Our data further suggest that an 18 bp tandem duplication found in TSPY exon 1 originated from an unequal sister chromatid exchange between two tandemly arranged TSPY clusters.

P0780. Cloning and characterization of hOAT1, a novel organic anion transporter in kidney

D. Prawitt[1], *A. Bahn*[2], *T. Enklaar*[1], *H. Hauser*[1], *F. Quondamatteo*[2], *A. Hillemann*[2], *R. Herken*[2], *G. Burckhardt*[2], *B. U. Zabel*[1]
[1]*Universitaetskinderklinik; Mainz, Germany;* [2]*Zentrum fuer Physiologie und Pathophysiologie; G ttingen, Germany*
prawitt@wserv.kinder.klinik.uni-mainz.de

The human organic anion transporter 1 (hOAT1) plays a key role in the secretion of many clinically important drugs (e.g.antiviral drugs) in the proximal tubule cells of the kidney. In a PCR based approach we isolated a genomic hOAT1 PAC clone and determined the exon-intron structure of the gene. hOAT1 consists of 10 exons ranging from 104 to 369 bp. RT-PCR analysis on human kidney samples led to the detection of two new splice variants (hOAT1-3 and hOAT1-4), showing a 132 bp inframe deletion of the ORF as part of exon 9. Using Fluorescence in-situ-hybridization and radiation hybrid panels we mapped the hOAT1 gene to chromosome 11q13.1-q13.2. In order to further elucidate its function and involvement in regulatory processes expression experiments were performed with the human and the rabbit orthologue (rbOAT1). Transcription of the gene was not only observed in kidney and liver, but also in non secreting tissues like muscle and eye. In situ hybridization of the rabbit retina with the renal rbOAT1 as a probe revealed positive signals in the endothelium of inner blood vessels, the ganglion cells and several neurons in the bipolar cell layer. Since this is the first description of an organic anion transporter in neuron cells, it is tempting to speculate about hOAT1 playing a role in transport processes of neurotransmitter metabolites in the visual system.

P0781. Not like the others; Prm3 with unexpected properties.

D. Boinska, *G. Schl ter, W. Engel*
Institut f r Humangenetik; G ttingen, Germany
dagusia_b@yahoo.com

The protamine 3 gene (Prm3) is a member of the protamine gene cluster on a mouse chromoseme16. The transcriptional and translational pattern of Prm3 seems to be similar to these of the other genes from this locus (protamine 1, 2 and transition protein 2). Expression starts from the day 20 (postnatal) of mouse development. Prm3 protein can be found after a delay of 7 days. To our surprise, immunohistochemical studies show that unlike other protamines or transition proteins, Prm3 protein is not present in the nucleus, but in the cytoplasma of elongating spermatids and residual bodies of mature sperms. Also, unlike the other basic protamines and transition proteins, the Prm3 cDNA sequence seems to code for an acidic protein of 104 amino acids with a central domain of 24 consecutive acidic aminoacids (glutamic/aspartic). Correspondingly, on IEF westernblots we can detect a band at an IEP of about 4,5 pH. In contrast to many other testicular mRNAs, Prm3 mRNA seems to be translated relatively efficiently; about 60% of mRNA associated to polysomes, polysome spacing about 100nt. To determine the function of the Prm3 gene, knock-out and transgene studies with inducible aberrant Prm3 expression are underway.

P0782. Interaction Of Polycystin 1, The Major Protein Affected In Autosomal Dominant Polycystic Kidney Disease, With Annexin V

*A. Markoff[1], **N. Bogdanova**[2], U. Rescher[1], F. Qian[3], G. Germino[3], J. Horst[2], B. Dworniczak[2], V. Gerke[1]*
[1]Institut f r Medizinische Biochemie, ZMBE, Univ. M nster; M nster, Germany; [2]Institut f r Humangenetik, Univ. M nster; M nster, Germany; [3]Department of Medicine, Division of Nephrology, Johns Hopkins University School of Medicine; Baltimore, MD United States
markoff@uni-muenster.de

Polycystin 1 is the gene product of PKD1, the first gene identified to be causative for the condition of autosomal dominant polycystic kidney disease (ADPKD). Mutations in PKD1 are thought to be responsible for 85% of the ADPKD Europeans and 14% are attributed to the second gene, PKD2 . Polycystin 1 is proposed to be a transmembrane protein with multiple ligand binding domains. Modeling data indicate two complete leucine-rich repeats at the very N-terminus which could provide a site for protein-protein interaction. In order to characterise potential members of the PKD1-PKD2 pathway, we sought to identify the binding partner(s) of polycystin. We demonstrate that annexin V, a calcium and phospholipid binding protein, specifically interacts with the polycystin 1 LRR in vitro and in vivo. Our results from in vitro binding experiments show complex formation with the LRR of polycystin 1, specific for annexin V and its N-terminal portion. Display of LRR on the extracellular side allows for binding of annexin V to the cell surface in a model system. Binding of annexin V to polycystin 1 could possibly sequester annexin V molecules and prevent their clustering on endothelial cell membranes thereby not alowing for anticoagulatory activity.

P0783. Identification and localization of a novel human myotubularin related protein gene, MTMR8, on chromosome 8p22-p23

S. Appel[1], *K. Reichwald[2], A. Reis[3], A. Rosenthal[2], H. C. Hennies[1]*
[1]Max-Delbr ck-Centrum; Berlin, Germany; [2]Institute of Molecular Biotechnology; Jena, Germany; [3]Friedrich-Alexander-Universit t; Erlangen, Germany
siappel@mdc-berlin.de

Myotubularin and myotubularin related proteins belong to the dual specificity phosphatases that are able to dephosphorylate protein phosphotyrosine as well as phosphothreonine or phosphoserine. Several myotubularin related proteins have been identified so far. Mutations in the human myotubularin gene (MTM1), which is located on the X chromosome, lead to X-linked myotubular myopathy. We have isolated and localized a novel putative myotubularin related protein gene, MTMR8, on chromosome 8p22-p23 between the markers at D8S550 and D8S1759 by exon trapping experiments and RT-PCR. The gene consists of ten exons and spans about 43 kb genomic sequence. The corresponding cDNA is 7036 bp in length. The open reading frame, which starts in exon 2, predicts a protein of 464 amino acids and a calculated molecular mass of 54 kD. The myotubularin protein family is highly conserved from humans down to yeast. Like myotubularin related protein 5 (MTMR5 or Sbf1), MTMR8 shows significant similarities to other myotubularin related proteins but has no dual specificity phosphatase domain. It contains a double helical motif similar to the SET interaction domain, the function of which is still unknown but is thought to play a role in the control of cell proliferation. The chromosomal region 8p22-p23 is of special interest since it is associated with frequent loss of heterozygosity (LOH) and probably involved in different types of human cancer. Moreover, the gene causing keratolytic winter erythema (KWE), a skin disease characterized by hyperkeratosis and peeling of the palmar and plantar skin, has been localized to this chromosomal region.

P0784. Analysis of brain specific expressed genes frequently deleted in Neurofibromatosis type 1 patients

E. Moschgath, *W. Vogel, G. Assum*
Department of Human Genetics University of Ulm; Ulm, Germany
elke.moschgath@medizin.uni-ulm.de

The Neurofibromatosis type 1 (NF1) gene is located on chromosome 17q11.2, in a 1.5 Mb region which is flanked by two copies of a long range repeated sequences. Approximately 10% of NF1 patients carry a deletion of the entire region between the repeats. Most of these patients are more severely affected and suffer from learning disabilities as well as mental retardation besides other symptoms, the hallmarks of the NF1 phenotype. In order to identify the genes responsible for this specific phenotype we started a search for genes located in the deleted region which are expressed in brain. RT-PCR experiments showed that nine of twelve ESTs located in the critical region are expressed in human brain. We characterized the gene flanking the NF1 gene in 3 direction in more detail, termed FHN3 (flanking the human NF1 gene in 3 direction). The FHN3 gene spans 105 kb and comprises 18 exons. Alternative splicing and usage of multiple polyA sites were found by analysing a panel of fetal tissue specific cDNAs by RT-PCR. Northern Blot hybridisation experiments with different human adult tissues revealed the occurrence of a brain specific 9.5 kb mRNA in addition to the 3.5 kb transcript, which was also found in other tissues. The deduced amino acid sequence of the FHN3 gene shows no homology to other known proteins or functional domains. The brain specific isoform results from the usage of a downstream alternative polyA site while the protein coding part of the transcript is not altered.

P0785. Molecular identification of genes and pathways involved in skeletogenesis - potential candidates for human disorders

A. Winterpacht[1], *G. Mohrmann[1], S. Schlaubitz[2], C. Stelzer[2], P. Hermanns[2], C. M hlbauer[3], B. Lee[4], T. Hankeln[3], B. Zabel[2], E. R. Schmidt[3]*
[1]Institute of Human Genetics, University of Hamburg; Hamburg, Germany; [2]Children«s Hospital, University of Mainz; Mainz, Germany; [3]GENterprise GmbH, Mainz; Mainz, Germany; [4]Baylor College of Medicine; Houston, TX United States
winterpacht@uke.uni-hamburg.de

We initiated a project which is aimed at the systematic identification and characterization of novel genes and regulatory pathways involved in the complex processes of cartilage/bone formation, growth, differentiation and homeostasis. The goal is to generate a number of positional and functional candidate genes for inherited monogenic (skeletal dysplasias) as well as multigenic disorders (osteoarthritis/osteoarthrosis and osteoporosis).
Starting point of the project is a unique human fetal cartilage cDNA library. Sequencing of 400 randomly selected clones revealed that 62% of the clones showed sequence similarity to known genes (including collagen type II and osteonectin), 13% matched to known genomic sequences and 15% to other ESTs in the databases. The remaining 4,5% of clones showed no match to known sequences and were considered as putative novel cDNAs, most likely representing cartilage-specific transcripts. The generation of 10.000 human ESTs is now in progress. So far, 2000 ESTs have been generated and fifteen cDNAs that do not show any significant similarities to known genes are currently analysed in more detail. These cDNAs represent genes encoding transcription factors (one with a KRAB domain), putative transmembrane proteins as well as a protein with PHD domains. For novel or very rare transcripts the chromosomal localisation as well as the expression pattern will be determined and full length cDNA will be sequenced for a subset of transcripts (80-100). All sequences will be subject to comprehensive bioinformatic analysis, stored in a special database and will be used to generate DNA-microarrays for expression profiling with a focus on human disorders.

P0786. Wolf-Hirschhorn syndrome (WHS) - molecular dissection of a complex phenotype by comparative sequencing and functional studies

S. Endele[1], *N. Pfarr[1,2], S. Schlickum[1], C. Steglich[1], M. Oswald[2], R. Heller[1], B. Zabel[2], A. Winterpacht[1]*
[1]Institute of Human Genetics, University of Hamburg; Hamburg, Germany; [2]Children«s Hospital, University of Mainz; Mainz, Germany
endele@uke.uni-hamburg.de

Wolf-Hirschhorn syndrome (WHS) is a complex and variable malformation

syndrome resulting from the absence of the distal segment of one chromosome 4 short arm (4p16.3). Clinical and cytogenetic data indicate that WHS is a contiguous gene syndrome with an undefined number of genes contributing to the phenotype. The WHS critical region (WHSCR) has been confined to a 165 kb gene rich region on chromosomal subband 4p16.3. Our studies aim at the identification of all genes and regulatory regions in the WHSCR (including flanking regions) as well as the functional characterization of the gene products in order to identify those genes that are involved in the phenotype.
We and others have previously identified novel genes in the WHSCR and flanking region using exon trapping and computer based analysis of this region. Here we report the reinvestigation of the WHSCR using comparative sequencing of the corresponding chromosomal region in the mouse genome (chromosome 5) and interspecies sequence comparison to identify evolutionary conserved and therefore functionally important sequences. Besides the known genes *LETM1*, *WHSC1*, *WHSC2*, *POL4P* we identified additional putative transcribed sequences which may represent alternatively spliced exons or additional genes. One of these transcripts shows specific expression in several adult and fetal organs affected in WHS patients. Further activities will concentrate on the functional analysis of the transcribed sequences and on conserved non-coding regions. On the basis of these data we present a novel, complete transcript map of these region which is complemented by functional studies on some of the putative gene products.

P0787. Molecular characterization of human plexin B3 and demonstration of expression of its gene (PLXNB3) in brain

S. Krejcova[1], *A. Veske*[2], *P. Michelson*[2], *M. Schachner*[3], *U. Finckh*[2]
[1]*Institute of Human Genetics, University Hamburg and Institute of Biology and Medical Genetics, Charles University; Prague, Czech Republic;* [2]*Institute of Human Genetics, University Hospital Eppendorf, University Hamburg; Hamburg, Germany;* [3]*Center for Molecular Neurobiology, University Hospital Eppendorf, University Hamburg; Hamburg, Germany*
finckh@uke.uni-hamburg.de

Neurodevelopmental studies on semaphorin-mediated axon guidance showed that plexins are semaphorin receptors mediating cell repulsion and attraction in the developing nervous system. *PLXNB3*, the gene encoding plexin B3 maps to Xq28 and may be considered as candidate gene for X-linked neurological disorders including mental retardation. We isolated and characterized full-length (6142 bp) *PLXNB3* cDNA by screening fetal brain cDNA library and performing 5 RACE-PCR. Computer analysis predicted an ORF for a 1910 amino acid transmembrane protein containing all domains characteristic for plexins, with closest homology to plexin B1/SEP. The transcribed region spans more then 15 kb and contains a 5 -noncoding and 35 coding exons. Multiple tissue northern blot analysis revealed 6.7 and 8.6 kb transcripts and suggested a predominant expression in human brain. *In situ* hybridization showed complex and changing expression patterns during mouse embryonic development. In adult human CNS neuronal expression was observed in cortex, mesencephalon, pons, medulla oblongata, and spinal cord. Plexin B3 was also highly expressed in adult mouse ovary and testis. Two different specific rabbit-anti human plexin B3 antisera were obtained by immunization of animals with oligopeptides derived from extracellular plexin B3 domains. By using these antisera and a phosphotyrosine specific antibody, western blot analysis of various protein fractions of COS7 cells transfected with human cDNA containing the complete ORF showed that plexin B3 is a tyrosine phosphorylated transmembrane monomeric ~230 kDa glycoprotein. Western blotting also revealed a plexin B3 immunoreactive aberrant band in human glioma, suggesting tumor associated expression of *PLXNB3* with reduced glycosylation of its protein. Supported by DFG; SFB444, C3. Correspondence; veske@uke.uni-hamburg.de

P0788. Detection of messenger RNA in situ; P53 inducible transcripts accumulate at the site of transcription in a time dependent manner

H. Weninger, *S. Bartl, F. Watzinger, H. Kovar, T. Lion*
CCRI; Vienna, Austria
weninger@ccri.univie.ac.at

Detection of cytoplasmic mRNAs by in situ hybridization has been difficult in a number of instances on single cell level, particularly because of the diffuse distribution of transcripts, and thus, the low density of signals. We have therefore examined the possibility of detecting RNA transcripts in the nucleus where a higher concentration of target molecules, and thus, higher signal intensity might be expected. In search of a model system providing optimum controls, we have selected the Ewing tumor cell line SK-N-MC in which transcription of a p53 inducible gene is controlled by a recombinant p53 protein. This system offers a very good control for the specificity of hybridization reactions because the same cell line can be used under identical experimental conditions as positive and negative control, depending upon the presence or absence of p53 induction. Northern blot analysis was used to confirm the presence of the target RNA in nuclear samples derived from induced cells. For mRNA fluorescence in situ hybridization (RNA-FISH), PCR generated digoxigenin labeled probes were used. This method permitted detection of the expected RNA transcripts in the nucleus, as distinct spots and track-like structures depending on the time of induction, which were absent in the negative controls. Accumulation of the transcripts at site of transcription was visualized by combined DNA and RNA FISH. Specific detection of RNA transcripts in the nucleus may therefore provide a possible alternative to cytoplasmic hybridization in some instances. We are currently testing the possibility of the detection of other expressed genes.

P0789. Genetic Characteristics of the Homopolymeric Tract Length Heteroplasmy in the Control (D-loop) Region of the Mitochondrial DNA

H. Sudoyo[1,2], *S. Malik*[1], *P. Pramoonjago*[1], *H. Suryadi*[1], *E. Sahiratmadja*[1], *D. Safari*[1], *S. Marzuki*[1]
[1]*Eijkman Institute for Molecular Biology; Jakarta, Indonesia;* [2]*Departement of Medical Biology, University of Indonesia; Jakarta, Indonesia*
herawati@eijkman.go.id

Mutation in the mitochondrial DNA occurs at a high rate, and mtDNA mutations underlie a wide range of human diseases. Sequence polymorphisms in the mtDNA have been also suggested to be associated with either the generation or the expression of other mtDNA or nDNA mutations. Notable in this context are those in the D-loop control region, in particular the T to C sequence variant (T16189C) between nt16184 and 16193, which have been implicated as a predisposing factor for several diseases including diabetes mellitus. Elucidation of factors responsible for errors in mtDNA replication is important in our understanding of the generation of mtDNA mutations (pathological and normal variants). The mtDNA genome contains several homopolymeric tracts, ranging from five to twelve cytosines. Above the length of six the tracts started to show unstability as reflected by the presence of length heteroplasmy, indicating slip replication. The mtDNA T16189C variant is associated with the formation of poly-C of various lengths, ranging from five to 12, and thus providing an excellent system to study the generation of the length heteroplasmy and its determining factor(s). We have thus studied the genetic characteristics of this mtDNA region, taking advantage of our extensive collection of cell lines and various tissue samples obtained at autopsies. In this communication we present evidence to show that; 1. The pattern of length heteroplasmy is generated de novo during mitochondrial biogenesis, as shown (a) after severe mtDNA copy number reduction by exposing cultured fibroblast cells to ethidium bromide, and repopulation of the cells following its removal; (b) in single cell pick-up experiment regardless of random segregation of other mtDNA mutation marker; and (c) by the fact that the pattern is maintained in different tissues of an individual obtained at autopsy. 2. The pattern of length heteroplasmy is determined by both mitochondrial and nuclear genetic factors, in that (a) for homopolymeric tracts of more than nine cytosines the pattern is maternally inherited; and (b) for homopolymeric tract of eight cytosines or less nuclear influence is dominant as demonstrated in experiments involving the generation of cybrids.

P0790. The Molecular Basis of Intron 2 Mutation at Splicing Donor Site from GT to AT on the CYP21 Gene Occurred in Patient with CYP21-Deficiency

H. Lee[1], *S. Chang*[2]
[1]*Kingcar Food Industrial Co., Ltd. Yuan-Shan Research Institute, No. 86, Chen-Hsiang Rd. Yuan San; I-Lan, Taiwan Republic of China;* [2]*Graduate Institute of Cell and Molecular Biology, Taipei Medical University, No. 250, Wu-Hsing Street,; Taipei, Taiwan Republic of China*
hhlee@kingcar.com.tw

Maturation at the primary RNA transcript of eukaryotic gene often involves the removal of unwanted internal segments and rejoining the remaining segments. The fidelity of RNA splicing is dependent on the identity of the nucleotide sequences at exon/intron boundaries. It has been known that dinucleotides of GT started and AG ended in an intron have a considerable degree of conservation and to be functionally important in the splicing event. Substitution of GT to any other nucleotide eventually leads to a malfunction in RNA splicing processing. In this report, we used transient transfection of cloned CYP21 genome which contained the intron 2 mutation at the splicing site from GT to AT into COS-1 cells and analyzed the RNA transcript by RT-PCR. The results showed that skip at exon 3 occurred during RNA processing when the splicing donor site changed from GT to AT. The prominent splicing product was the transcript with whole exon 3 region

skipped. Other minor RNA processing products appeared to contain some additional intron 2 sequences. Similar results were obtained when the splicing donor site sequences were changed from GT to AT in human beta-globin gene, further emphasizing general consequence of the splicing donor site mutation for RNA processing.

P0791. Mapping and expression analysis of the human UBE4A gene

E. Pontieri[1,2], *A. Novelli*[1], *F. Amati*[1], *A. Botta*[1], *G. Carruba*[2], *G. Novelli*[1]
[1]*Universit Tor Vergata ; Roma, Italy;* [2]*Universit degli Studi; L Aquila, Italy*
eugenio.pontieri@dnaserver.med.uniroma2.it

UBE4A is a novel ubiquitination factor actively involved in the multiubiquitination chain assembly of target proteins in the proteasomal degradation process. E4 binds to the ubiquitin moieties of these conjugates and, together with E1, E2 and E3 enzymes drives the multiubiquitin chain assembly, yielding long chains. E4A is the human homologue of the yeast UFD2, also involved in the stress tolerance. Recently a chimeric protein Ufd2/D4Cole1e was identified in mouse and found associated to slow Wallerian degeneration (Wlds), a disease characterised by axon death at peripheral and central level. We characterised a full-length cDNA of 6060 bps of the human UFD2/UBE4A corresponding to the KIAA0126 cDNA deposited in GenBank. UFD2/UBE4A was mapped by FISH experiments on the human chromosome region 11q23.3. Northern blots analysis using adult tissues showed that this gene is expressed at high levels as a single band of 7.5 Kb in the heart, skeletal muscle, kidney, and to discrete levels in the brain, liver, and placenta. A faint band was evident in colon, thymus, spleen, small intestine, lung and peripheral blood leukocytes. A Northern blot containing fetal tissues confirmed the adult results. Because of the importance in ubiquitin pathway and its involvement in neuronal degeneration of this protein, we suggest that UBE4A can be considered a candidate for neurodegenerative diseases mapping on chromosome 11q.

P0792. Nucleotide Change At Hypersensitive Site-3 Of Lcr In Beta Thalassemia Major And Intermedia Patients

O. Guzeloglu Kayisli, *G. Bagci, G. Luleci*
Akdeniz University, Faculty of Medicine, Department of Medical Biology and Genetics; Antalya, Turkey
ozlem@hipokrat.med.akdeniz.edu.tr

Beta globin locus control region (LCR) is the first identified examples of a dominant control region located at a distance of many kilobases from the structural genes. It has very strong enhancer activity extending over the entire locus. b-LCR consists of four strong erythroid-specific DNase hypersensitive sites (5 HS-1, 2, 3, and 4). Most of the LCR activity is associated with 5 HS-2, 3 and 4. Hypersensitive site-3 (HS-3) of the b-LCR has been implicated as an important regulator of the b-like globin genes. To investigate the polymorphisms at HS-3 of the human b-LCR, both b-thalassemia patients and control group were initially amplified by PCR and then amplified products were screened for changes at HS-3 using direct DNA sequencing technique. According to our results, guanine deletion of 7415 position at the HS-3 was observed in a b thalassemia major patient. This segment including deletion of 7415 position is the core region of HS-3 which consists of binding sites for ubiquitous and erythroid specific regulatory factors such as GATA-1, YY1, and CSBP-2 transcription factors. These transcription factors play an important role in b-globin gene switching. To investigate whether there is any transcription factors bound this region we will aim to determine the interaction between these trans-acting factors and DNA using bandshift assay in the future studies.

P0793. A PI-3 kinase/Akt/mTOR pathway Mediates and PTEN Antogonizes TNF-induced Insulin Resistance

O. N. Ozes[1], *H. Akca*[1], *T. Maehama*[2], *J. E. Dixon*[2], *D. B. Donner*[1]
[1]*Department of Microbiology&Immunology, Indiana University School of Medicine and Walther Oncology Center; Indianapolis, IN United States;* [2]*Department of Medical Chemistry, University of Michigan Medical Center and Walther Oncology Center; Ann Arbor, MI United States*
ozes@hipokrat.med.akdeniz.edu.tr

TNF produced by fat cells in obese animals induces resistance to insulin through mechanisms that have not been completely identified. In NIH 3T3 cells, TNF inhibits insulin-promoted phosphorylation of IRS-1, an adaptor protein that links the insulin receptor to signaling pathways that promote insulin action, and activates the Akt serine-threonine kinase through the intermediacy of phosphatidylinositol 3-kinase (PI 3-kinase). Exposure to TNF was not required to induce insulin resitance in NIH 3T3 cells stably expressing constitutively-active Akt. In insulin-sensitive myotubes, TNF activated Akt and inhibited insulin-promoted phosphorylation of IRS-1. This effect, and the capacity of TNF to impair insulin induced glucose uptake into myotubes, was reversed by rapamycin, an inhibitor of the mammalian target of rapamycin (mTOR), a downstream target of Akt, which immunoprecipitated with, and phosphorylated, IRS-1. TNF also inhibited insulin-induced glucose transport in myotubes and rapamycin reversed this effect. Treatment of embryonic kidney 293 cells with TNF also impaired insulin-promoted tyrosine phosphorylation of IRS-1 and this was blocked by transitent expression of kinase that Akt. Transient expression of PTEN, a tumour suppressor which can dephosphorylated phosphatidylinositol (3,4,5)-triphosphate, and activator of PI-3-kinase, also blocked the ability of TNF to inhibit insulin-induced phosphation of IRS-1 in 293 cells. Thus TNF can promote insulin resistance by activation of a PI 3-kinase/Akt/mTOR pathway and this capacity is antagonized by PTEN.

P0794. Functional analysis of UFD1L (Ubiquitin Fusion Degradation 1 Like) gene product in eukaryotic cells

F. Amati[1,2], *E. Conti*[1,2], *I. Cond* [3], *F. Sangiuolo*[1], *B. Dallapiccola*[2,4], *R. Testi*[3], *G. Novelli*[1]
[1]*Dept. of Biopathology and Diagnostics Imaging, Tor Vergata University; Roma, Italy;* [2]*CSS-Mendel Institute; Roma, Italy;* [3]*Dept. of Experimental Medicine and Biochemical Sciences, Tor Vergata University; Roma, Italy;* [4]*Dept. of Experimental Medicine and Pathology, La Sapienza University; Roma, Italy*
amati@med.uniroma2.it

Ubiquitin-specific processes are essential in regulation of some apoptotic pathways. Spontaneous apoptosis, observed in T cells from DiGeorge patients, may be, at least in part, responsible for T cell deficiency in DiGeorge syndrome. UFD1L, a gene mapping in the typically deleted region for DiGeorge and velocardiofacial syndromes, encodes for the human homologue of yeast Ufd1 protein, an ubiquitin-dependent enzyme that takes part in a degradation pathway of ubiquitin fused substrates. Although the biochemical role of UFD1L in mammals is still unknown, it is presumable an active role in degradation processes mediated by ubiquitin also in human cells. We have analysed the in vivo cellular localization of UFD1L by means its cloning in an eukaryotic GFP-expression vector and then we used this chimaeric vector to analize the possible role of UFD1L protein in the regulation of programmed cell death triggered from Fas receptor (CD95/APO-1). NIH3T3 and Cos-1 cell lines were transfected with the UFD1L-GFP fusion construct and with the GFP-vector alone as a control. Both cell lines expressed UFD1L in the cytoplasm with a particular concentration around the nuclear envelope. UFD1L overexpression was performed in human T lymphoma HuT78 cells. In this condition, no anti-apoptotic effect was observed upon Fas and ceramide stimulation, thus excluding a role in the specific pathway of this death receptor. Our results indicate that UFD1L is a cytoplasmatic protein, particularly abundant around the nucleus, and that it is not involved in the Fas and ceramide specific apoptosis pathways. Nevertheless, further overexpression analysis involving other apoptotic stimuli and accumulation/degradation of key signaling proteins may provide elucidation of the possible pathways regulated by UFD1L. Work supported by Italian Ministry of Health and Telethon grants (N_i. E.723)

P0795. Visible transient expression of green fluorescent protein requires intranuclear microinjection of large copy numbers of pEGFP plasmid.

A. Laner, *D. Schindelhauer*
Ludwig Maximilians University; Munich, Germany
a.laner@gmx.de

In order to assess the copy number of a simple viral promoter/GFP-cDNA expression cassette required for visible transient expression, we microinjected pEGFP plasmid into the nucleus of the human immortalized epithelial cell line 16HBE, the tumor cell line HT1080, and primary human fibroblasts. pEGFP plasmid was diluted down from approximately 100 000 copies to 1 copy per injection volume (1 pl) in a DNA solution containing constant amounts of non expressing plasmid DNA. Independently of the cell line used, at least 100-1000 copies were required to detect green cells under the microscope. Between 100 and 10 000 copies the intensity was substantially increased. Above 10 000 copies no additional increase was detected. With higher copy numbers only the intensity was increased and not the number of expressing cells, since all dilutions from 100 to 100 000 copies led to approximately 60-80% surviving and expressing cells. Within a copy number group, most cells were found to show relatively equal intensity, however a 5-10 % fraction of cells showed extremely high intensity independent of the injected copy number, indicating the existence of occasional circumstances activating expression in the order of 1000-fold in those cells. We conclude that single copies of simple gene markers might not be suitable to readily detect the presence of transfected DNA. Data will be presented narrowing down the circumstances leading to the dramatic expression increase.

P0796. Measurement of cellular fluorescence parameters using a microfluidic, chip-based system

E. Broomfield[1], *A. Brecht*[2], *K. Hahnenberger*[3], *T. Preckel*[4]
[1]*Agilent Technologies; Bracknell, United Kingdom;* [2]*Agilent Technologies; Waldbronn, Germany;* [3]*Agilent Technologies; Palo Alto, CA United States;* [4]*Caliper Technologies; Palo Alto, CA United States*
elaine_broomfield@agilent.com

In 1999 Agilent introduced the 2100 Bioanalyzer as a first broadly available product for the life science laboratory using Caliper LabChipæ technology. So far separation based applications have been introduced, addressing DNA, RNA, and proteins. The compact, benchtop system is capable of two-color fluorescence detection and runs disposable microfluidic glass chips. The novel applications presented here are based on the controlled movement of cells by pressure-driven flow inside networks of microchannels. Cells are hydrodynamically focussed before passing the fluorescence detector in single file. Each chip accommodates several samples. Data acquisition is automated while analysis allows for user-specific settings. Specific advantages of the instrument are the low number of cells required for analysis and the ease-of-use. So far the technology has been used in cell transfection, and apoptosis detection. Transfection data were obtained from cells expressing green fluorescent protein and from antibody-stained cells. Counterstaining cells by a DNA-specific dye facilitated the accurate determination of transfection efficiency. For an apoptosis related parameter exposition of phosphatidylserine (PS) to the outer cell membrane leaflet was studied. Biotinylated AnnexinV was bound to externalized PS in camptothecin treated cells and labeled by Cy5-avidin. Calcein-staining was used to differentiate live and apoptotic cells from dead cells. Results obtained with the microfluidic chips showed good correlation with data obtained using a standard flow cytometer. The simple use and the low cell consumption of the microfluidic approach make the technique an ideal add-on for all cell based procedures. Further applications of this simple cell-analysis approach are under development.

P0797. Genetic counseling in Leber Hereditary Optic Neuropathy (LHON)

K. Huoponen, *A. Puomila, V. Juvonen, E. Nikoskelainen, M. Savontaus*
University of Turku; Turku, Finland
kirsi.huoponen@utu.fi

LHON is a maternally inherited disorder resulting in subacute visual loss. It is associated with mutations in mitochondrial DNA (mtDNA). About 15% of the families are heteroplasmic. Molecular diagnosis of LHON is usually made by means of mtDNA analysis in blood DNA, since the actual target tissues (optic nerve, retina) are not available for examination. According to genealogical data the general risk for optic atrophy in homoplasmic individuals is about 30-50% in males and 5-15% in females. However, genetic counseling in LHON is extremely difficult. We cannot detect the persons at risk for optic atrophy even in homoplasmic families, since all individuals with homoplasmic mutations do not express the disease. In heteroplasmic families the situation is even more complex. The offspring of a heteroplasmic mother can have dramatically different levels of heteroplasmy. Furthermore, the amount of mutant mtDNA may considerably vary between the tissues of the same individual, and therefore blood is not necessarily indicative of the mutation load in other tissues. We describe a family where the mother has less than 1% of mutant mtDNA, and yet she has transmitted a considerable amount of mutant mtDNA to her son. In his tissues, the proportion of mutant mtDNA varies from 50% in blood up to 86% in other tissues. Affected heteroplasmic individuals usually have more than 70% of mutant mtDNA in blood. However, the son with only 50% of mutant mtDNA in blood is severely affected. His sister has 0% of mutant mtDNA in her blood and no risk for optic atrophy. However, we cannot exclude a theoretical risk that she may transmit the disease to her offspring.

P0798. Genetic counseling in Romania - an acute issue

O. Gore[1], *A. Bobulescu*[2], *C. Gore*[3], *M. Isvoranu*[1]
[1] *Carol Davila University of Medicine and Pharmacy, Department of Human Genetics; Bucharest, Romania;* [2]*Central Military Hospital; Bucharest, Romania;* [3] *N.Gh.Lupu Clinical Hospital; Bucharest, Romania*
odette@home.ro

Having no effective treatment as yet, Duchenne Muscular Dystrophy (DMD) can be fought only by means of prevention. The aim of the present study was to assess the impact of genetic counseling in Romanian families affected by DMD. A total of 37 families having 47 offsprings diagnosed with DMD between 1996 and 1999 were interviewed and asked to fill out a questionnaire consisting of multiple choice and open ended questions. The data we recorded was structured into several categories ; general understanding of the disease and of its hereditary nature; awareness of risk in family planning; genetic counseling history; family pedigree. Some results were then compared with each patient s hospital records. Twenty-two families (59%) perceived their offspring s malady as an accident, with little risk of reoccurrence. Only two (5.4%) admitted a family history of DMD, suggesting either an unusually high frequency of de novo mutations, or, more likely, a poor knowlege of the disease. After the diagnosis was settled, 16 families (43%) planned to (and nine did) conceive another baby. Although some acknowledged it, the risk was seriously underestimated, and generally not linked to the mother s serum creatine kinase levels. Our data suggests that the beliefs, projects and decisions of the families affected by DMD suffered little scientifically-based influence. We consider the low level of medical education in the general population, together with the lack of a specific genetic counseling program, as being the leading causes for this situation, as well as the main issues that have to be corrected.

P0799. Attitudes towards prenatal diagnosis and termination of pregnancy among health professionals in Lebanon

L. F. Zahed[1], *M. Nabulsi*[2]
[1]*Department of Pathology and Laboratory Medicine, American University of Beirut Medical Center; Beirut, Lebanon;* [2]*Department of Pediatrics, American University of Beirut; Beirut, Lebanon*
lailaz@aub.edu.lb

Despite advances in the field of genetics, avoidance of many conditions still relies on screening, prenatal diagnosis and termination of pregnancy in case of an affected fetus. Attitudes towards such prevention techniques are influenced by many variables, including, at least to a certain extent, the attitudes of health professionals. Most of the studies have been conducted in the Western world. Therefore, to assess attitudes in a Middle-Eastern society, we have sent questionnaires concerning different genetic conditions, to geneticists, family doctors, pediatricians and obstetricians affiliated to one of the major medical centers in Lebanon. The questionnaires were based on similar published questionnaires and responses measured according to a similar scoring system. A total of 43 questionnaires were sent so far and responses received from 31 (72%). Generally-speaking, obstetricians seemed to be most favorable towards prenatal diagnosis and termination of pregnancy, both for medical and social conditions. They were followed by geneticists then family physicians, while the least favorable were pediatricians. A wide range of responses were noticed within each group and there was noted variability for particular questions. There was no clear correlation between response and sex, age or religious affiliation.

P0800. Suspicion on triple - X - syndrome in rapid prenatal FISH diagnosis on uncultivated amniotic fluid cells; Problems and pitfalls for genetic counselling

R. Hauschild, *V. Beensen, A. Heller, H. Starke, T. Liehr*
Institute of Human Genetics and Anthropology; Jena, Germany
rhau@mti-n.mti.uni-jena.de

A 27 year old pregnant woman with suspicion on triple - X - syndrome after prenatal rapid (FISH-) diagnosis (using a centromere -X- specific probe) was referred to genetic counselling. Prenatal diagnosis has been performed because of foetal nuchal translucency detected by ultrasound. The pregnant woman has been informed in detail about the result of the rapid test, the preliminary (!) nature of the finding, the possible existence of triple - X - syndrome, and the probable rare occurrence of important clinical findings. Additionally, we stressed the necessity of further cytogenetic investigations. However, the given information contributed to a considerable confusion of the pregnant woman. She presumed a very problematic disease of the expected child. The confusion was based mainly on discrepancies between the rapid prenatal FISH results and the cytogenetic investigations on cultured amniotic fluid cells. The latter revealed a strong evidence for a normal female karyotype of 46,XX, resulting of 3 metaphases analysed at first. Numerous additional conversations were needed to explain the details and the cytogenetic findings completed thereafter before the given situation has been understood and accepted. The further cytogenetic investigations on cultivated amniotic fluid cells revealed a very small marker chromosome (SMC) - nearly 0,5 m in diameter - and a mosaic karyotype of 47,XX,+mar(15)/46,XX(8) (GTG banding). Further FISH analysis defined this SMC as a der(X)(p11.1?q11), with no evidence for additional euchromatic material. The SMC was not found in both parents, but in cells of the placenta and umbilical cord blood of the phenotypic inconspicuous child at birth. Conclusion; Preliminary results obtained in rapid prenatal diagnosis on uncultivated amniotic fluid cells should be carefully handled in genetic counselling. Even in combination with unspecific abnormal sonographic findings their preliminary character should be explained in detail and one has to consider that 3 signals for a centromeric region of a chromosome does not always and automatically indicate to a trisomy of that chromosome. The interpretation of suspicion on a trisomy in rapid prena-

tal FISH diagnosis may be different in cases with trisomy specific sonographic findings.

P0801. Genetic Counseling in Indian Milieu

S. R. Phadke[1], *A. Pandey*[2], *S. S. Agarwal*[1]
[1]*Sanjay Gandhi PGIMS; Lucknow, India;* [2]*Queen Mary s Hospital,King George s Medical College; Lucknow, India*
shubha@sgpgi.ac.in

We have studied various psychosocial aspects of genetic counseling in Indian milieu by analyzing questionaires filled by consultands and parents of patients with genetic disorders; one on the first visit to genetic clinic and another after genetic counseling session. Multiple questions were used to judge the expectations, anxiety level, change of decision and satisfaction after genetic counseling. The analysis showed that most of the patients and parents came with the expectation of diagnosis and treatment. Only 30 out of 83 came primarily for diagnosis and information about risk of recurrence and other aspects of the disease. There was no change in the reproductive decision in 74 of 83 consultands after genetic counseling while it changed in 5 and 4 remained undecided even after genetic counseling. Presence or absence of normal live children and availability of prenatal diagnosis were the major factors affecting reproductive decision. 91% of those who decided against having more children had previous one or more normal children, while 67% of those who decided to plan for next child did not have any live normal child. Perceived severity of risk of recurrence did not appear to affect the reproductive decision. Though most of the consultands were satisfied with the genetic counseling, there was decrease in the anxiety in 35 of 83 persons while anxiety increased in 22 of 83. The genetic counseling was nondirective in all cases, but all of the parents though that the counsellor should have advised them about the decision to be taken.

P0802. Genetic testing, language and genetic counselling

J. Hartog
Universitaet Hamburg; Hamburg, Germany
hartog880@aol.com

Now that the precise genetic basis of some diseases has been determined, new diagnostic possibilities (tests) have emerged. While the psychosocial problems related to genetic testing are being explored, little attention has been paid to date to the underlying linguistic aspects of these psychosocial issues. Indeed, information is given in language and the processing of information occurs in language. The analysis of this linguistic level is fundamental to the understanding of the psychosocial problems arising from testing. In a qualitative pilot-study 10 counselling sessions (with 4 different counsellors) on genetic testing considerations in breast cancer patients were audiotaped, transcribed to the standards of discourse analysis and subsequently analysed linguistically. Testing for breast-ovarian cancer susceptibility was chosen as an example of problematic testing concerning its benefits. Results; In the problem-presentation at the beginning of the counselling sessions, counsellees tended to have clear-cut expectations about clear-cut benefits of testing. The linguistic analysis reveals how new chunks of knowledge and their assessment by professionals are gradually processed and integrated (or not) in the counsellees knowledge structures. By the end of counselling, in most cases, the benefits of testing were seen by the counsellees as more problematic than at the beginning of counselling, thus making decision-taking extremely difficult. The nature of the dilemma concerning BRCA1 and BRCA2 testing are revealed and differentiated by the linguistic analysis. Conclusions for the practice of genetic counselling are drawn.

P0803. Proposing a Genetic Counseling System in Japan; Experience in the Division of Clinical and Molecular Genetics, Shinshu University Hospital

Y. Fukushima*, K. Yoshida, M. Fujimori, H. Kawame, H. Kusabuka, A. Sakurai, M. Kanai, M. Tamai, K. Wakui, S. Ikeda*
Division of Clinical and Molecular Genetics, Shinshu University Hospital; Matsumoto, Japan
yfukush@sch.md.shinshu-u.ac.jp

Although genetic counseling is fundamental before and after the genetic testing, there is no official system for genetic counseling in Japan. We propose the team approach to genetic counseling. Shinshu University Hospital established a division of clinical and molecular genetics as one of its central service departments in 1996. It was officially approved by Ministry of Education in 2000. Our division is composed of several MDs from the departments of neurology, endocrinology, pediatrics, oncology, laboratory medicine and medical genetics, a clinical psychologist, and a genetic nurse. Persons who seek genetic counseling or genetic testing visit our clinic, usually twice or more. At the first visit, a staff member collects complete information including family history, correct diagnosis and examination results, and he clarifies the client s request. We have a staff meeting once a week to discuss each case for providing the next suitable counseling, and we discuss the ethical, legal and social issues (ELSI). At the second visit, genetic counseling is provided by an MD specialist and a clinical psychologist or a genetic nurse. Information of genetics or genetic testing is explained by an MD specialist, and psychological support is offered by a clinical psychologist or genetic nurse. Three hundred and eleven clients visited the clinic from May 1996 to March 2000; 170 for birth defects, 37 for obstetric problems, 47 for familial cancers, 29 for neurological disorders and 28 for other reasons. Regarding familial cancer, we performed genetic testing in 44 cases, including familial adenomatous polyposis, multiple endocrine neoplasia type 1 and 2, familial breast cancer, von Hippel-Lindau disease and Li-Fraumeni syndrome. We hope our approach will become familiar to other hospitals, and genetic services in Japan will consequently be improved.

P0804. Presymptomatic genetic testing in Myotonic dystrophy; counselling patterns and general lessons.

S. Fokstuen[1,2], *J. Myring*[1], *C. Evans*[1], *P. Harper*[1]
[1]*Institute of Medical Genetics, University of Wales College; Cardiff, United Kingdom;* [2]*Current address; Division of Medical Genetics, University Hospital; Geneva, Switzerland*
SivFokstuen@gmx.ch

Genetic counselling and presymptomatic genetic testing in myotonic dystrophy have to take into consideration the extreme variability in range of severity and age at onset due to the instability of the mutation. So far there is no specific model for presymptomatic testing in Myotonic dystrophy as for Huntington s disease or familial cancers. In this survey we retrospectively analysed the practice at the Institute of Medical Genetics, Cardiff. Since the availability of direct mutation analysis for myotonic dystrophy in 1992, clinical geneticists in Wales have undertaken 72 presymptomatic investigations. Six different approaches were found. The most common one (n=46) was to take the blood sample at the first counselling session. A two step approach as proposed for Huntington s disease was practiced in only 7 cases; it was offered but rejected by the applicants in two further cases. Factors which influenced on the choice of the counselling approach were mainly age, clinical assessment at the time of testing, familiarity with the disorder, family dynamic, the proband s perception of symptoms and time pressure. This survey illustrated by specific cases, should provide the starting point for further more systematic study as well as giving a general framework that may be useful for those involved with presymptomatic testing for this important and exceptionally variable condition.

P0805. Counselling before and after predictive genetic diagnosis for Huntington disease; A psychotherapeutic experience with 54 persons at risk

G. Wolff
Institute of Human Genetics and Anthropology; Freiburg, Germany
gwolff@uni-freiburg.de

Introduction; Huntington disease (HD) is a severe, autosomal dominantly inherited, late-manifesting, neuropsychiatric disease characterized by a progressive movement disorder, cognitive decline, dementia, affective disturbances, psychotic disorders, personality changes, and physical decay. Predictive testing by a direct molecular test of the CAG repeat expansion is possible since 1993. In Germany, guidelines for predictive testing for HD require psychotherapeutic counselling before and after the test procedure. Methods; Patients have been counselled in a psychotherapeutic setting before and after the molecular genetic test. Counselling aimed at 1. the clarification of possible psychological and social problems in the decision process, 2. a fully informed, autonomous decision in favour of or against the test, and 3. the elucidation of possible risks associated with a bad or good test result, and 4. stimulating the process of coping with the test result and 5. the prevention of psychosocial risks associated with the knowledge of the test result. In most of the cases, spouses or close friends of the person at risk were at least partly involved in the counselling process. Results; 54 persons at risk for HD (22 male, 32 female) with a mean age of 32.4 years most of them (72%) living in a stable partnership, and 40% having at least one child were counselled. In 5, a second degree relative was affected. 18 patient had symptoms, 5 had early signs of HD, 8 had symptoms indicative of HD, and 5 had psychosomatic symptoms. About half of the patients were firmly determined to have the test. There was a negative correlation between the decisiveness and the period of knowledge of the HD risk. The mean number of sessions before the test was 7.1, and after testing 6.5. Major themes in counselling before testing were the experience of HD in parents and family, intrafamilial relationships and conflicts, the personal experience of being at risk for HD, fear of early

symptoms, self-observation, hope in prevention and therapy, aspects of certainty and uncertainty, partner conflicts, and the private and professional situation. The treatment of the questions why testing now? , and what would be, if proved to be one of the most important measures in the counselling process, as well as the evaluation of the coping capacity. Nearly all patients dealt well with the test result up to now. Besides coping with the new situation and the relief due to certainty, major themes in the counselling after a bad test result were loss and gain of future, uncertainty about the age at onset and the possible course, coping with early signs of HD and future disability, the reaction of the partner, relatives and others, perspectives of family planning, and in general finding sense and a positive attitude. Besides the relief after a normal test result, major themes in those cases, in which problematic issues had been addressed, were loss of orientation in life planning, changes in intrafamilial relations, survivor guilt, partner conflict, and problems at work. Conclusions; A psychotherapeutic setting focussing on the basis and possible conflicts of the decision process before, and on foreseeable risks after testing, and integrating medical, genetic, and psychological aspects of the disease is an adequate and suitable measure for counselling persons at risk for HD. It helps patients to reach an informed decision regarding the test and fulfills the requirements demanded by the national and international guidelines for predictive molecular testing of this up to now unpreventable and untreatable disease. This setting seems to prevent at least short-term problematic reactions of those being tested irrespective of the test result.

P0806. Living with Marfan syndrome; the European experience.

I. Delvaux[1], *S. De Bie*[1], *R. Hennekam*[2], *B. Loeys*[1], *A. De Paepe*[1]
[1]*University Hospital Ghent; Gent, Belgium;* [2]*University of Amsterdam; Amsterdam, The Netherlands*
anne.depaepe@rug.ac.be

A specialised questionnaire, containing 88 multiple choice questions on medical and psychosocial aspects, was sent to patients with Marfan Syndrome (MFS) from 7 European countries. The results were statistically analysed with SPSS (9.0 for Windows). We present data from 857 individuals on the quality of life and the psychosocial well being as experienced by persons affected by MFS. A scoring system was established to assess the objective severity of the condition in each individual. The subjective severity that stands for the patients own perception of their condition was also questioned. We compared the results with an objective coping score based on questions relating to the psychosocial adjustment. The data show that MFS represents a significant burden on many aspects of daily life but that most individuals are coping effectively with the disorder. The level of coping and the quality of life is determined by the subjective rather than by the objective severity. This is illustrated by the fact that the subjective attitude towards the condition significantly influences the attitude towards professional activities, relationships and reproductive options. Many individuals have difficulties discussing problems associated with the MFS. It remains important to stimulate the support of family, friends and patient support groups since social isolation negatively correlates with depression and anxiety. Professional support must pay extra attention to the threat of social isolation. We conclude that creating a positive frame of reference is a very important element for their psychosocial well being.

P0807. Genetic counseling and follow up of livebirths with chromosomal abnormalities in Taiwan

S. Lin[1], *W. Tsai*[2], *M. Huang*[3], *M. Chen*[3]
[1]*National Cheng Kung University Hospital; Tainan, Taiwan Republic of China;* [2]*Chi Mei Hospital; Tainan, Taiwan Republic of China;* [3]*School of Nursing, Cheng Kung University; Tainan, Taiwan Republic of China*
sjlin@mail.ncku.edu.tw

To evaluate the genetic service in antenatal care and counseling provided in Taiwan, we conducted a nationwide study among women having children with chromosomal abnormalities. Interviewing with semi-structured questionnaire were conducted by trained counselors. Patient information was recruited from registrations of cytogenetic laboratories located in different regions of the island. In order to obtain the most recent data and provided counseling for most needed population, only live-births born from January 1, 1998 till October 31,1999 were included in this study. A among a total of 328 cases with chromosomal abnormalities obtained from 17 cytogenetic laboratories, 134 agreed to participate in the interview. 98% of mothers received antenatal care and 69% of them received maternal serum screening with triple markers and with results within low risk range. Although in Taiwan over 60% of mothers with advanced age received amniocentesis, there were 72% of them in this study group that did not receive prenatal diagnosis. In addition, we found that although maternal serum screening is performed popularly in antenatal care in Taiwan, genetic information is not provided adequately. These inadequacy in counseling raise confusion and unnecessary arguments when a child with chromosomal abnormalities was born. (This study is supported by a grant from Department of Health, Executive Yuan, Taiwan)

P0808. Individual approach, predictive genetic testing and help decision making system.

H. Baranova[1], *E. Albuisson*[2], *L. Chapy*[3]
[1]*Medical Faculty; Clermont-Ferrand, France;* [2]*Medical Faculty, University d Auvergne; Clermont-Ferrand, France;* [3]*IPPM; Clermont-Ferrand, France*
Baranova@nat.fr

Expert System (ES) is a highly personalised help decision making system for interpretation of different laboratory testing results (fatty acids spectrum and cardiovascular risk assessment, immune analyses with lymphocyte typing and protein profile; nutrition) and also predictive genetic testing. ES allows to perform more precise analysis and prognosis for each individual patient according to his peculiarities ; lifestyle factors, anamnestic and clinical data. Genetic program includes HLA typing and predictive genetic testing, where 54 main metabolic and predisposition genes are included up to date. Special goal of ES genetic program is education and presentation of genetically-based individual approach in modern medicine. Interpretation and all explanations in genetics are written in flexible and interactive manner with the other chapters of ES. Moreover, so called ¨ 3 dimentional principle ¨ is applied for all parts of ES, including genetics ; first brief result interpretation is followed by the second detailed explanation confirmed by the references (3d step). Other major goals of ES are ; 1) ES structure, which allows to add regularly new information and methodologies according to the latest achievements; 2) applied research and possibility to perform epidemiological studies based on the ES data bank. In conclusion, ES provides a physician with explanations, clinicobiological interpretations and advice, which helps to obtain a global vision of the patient s status. It also opens new opportunities for statistical analyses and mathematic modelling for investigation of possible new trends in different populations due to environment-gene interactions.

P0809. Mis-representation of genetic transmission during hereditary breast/ovarian and colon cancer genetic testing process

C. M. Julian-Reynier[1], *H. Sobol*[2,3], *F. Chabal*[1], *F. Eisinger*[1,4,3]
[1]*INSERMU379; Marseille, France;* [2]*INSERM E9939; Marseille, France;* [3]*Regional Cancer Center (Paoli-Calmettes Institute); Marseille, France;* [4]*INSERME9939; Marseille, France*
julian@marseille.inserm.fr

In Hereditary Breast/Ovarian and Colon cancer families, the probability to pass on mutated genes to offspring is a relevant knowledge issue to help patients to understand the reason for family disclosure of genetic risk. This factor could be considered as one indicator of informed choice. The objective of this study was to investigate the baseline level of knowledge, according to the type of cancer and the gender of the gene carrier. An ongoing prospective cohort survey (2000-2001) includes all patients attending at one cancer genetic clinics in France following the analysis of a first DNA sample (BRCA1/2; MLH1/MSH2). Face to face interviews were carried out before a 2nd genetic consultation aiming to confirm both the decision to be tested and the results on a 2nd DNA sample. Preliminary results were analysed in December 2000 (N=67; mean age 47, SD=12; 94% women, 72% cancer affected). Knowledge of the transmission of a breast/ovarian cancer gene to children when the mother is gene carrier, was correct in 34% and when the father is carrier in only 10% ($p<0.001$). In contrast, in the case of a colon cancer gene, the answers were correct in 25% and did not vary whether mother or father were considered as carriers (NS). These results suggest that for sex-related diseases inherited as an autosomal dominant trait, such as breast/ovarian cancer, understanding of risk transmission is particularly confusing. In this respect geneticists should be aware of the possible impact of this mis-representation in the screening of male and their offspring.

P0810. Genetic counseling and molecular testing for the most frequent mutant genes of the coagulation factors V and II may increase five-fold the prevention of venous thromboembolic incidents in individuals with family history of idiopathic thrombosis

C. N. Yapijakis[1,2], *Z. Serefoglou*[2], *N. Koufaliotis*[3]
[1]*Laboratory of Neurogenetics, Dept. of Neurology, University of Athens Medical School; Athens, Greece;* [2]*Dept. of Molecular Biology and Genetics, Bioerevna Diagnostic Center; Athens, Greece;* [3]*Dept. of Biopathology, Bioerevna Diagnostic Center; Athens, Greece*
cyapijakis_ua_gr@yahoo.com

Thrombophilia is a common inherited/multifactorial predisposition for venous thromboembolic incidents (estimated 10-15% in the Greek population), caused by inborn hypercoagulation of the blood.When a person s genetic predispodition is known, preventive anticoagulant therapy may prevent life threatening incidents.In the present study, we assessed whether genetic counseling combined with molecular testing for the two most common thrombophilia mutations may increase prevention in healthy index cases with a family history of idiopathic thrombosis.Two groups of unrelated Greek individuals were tested. Group A consisted of 48 individuals (26-55 years old), who asked for genetic counseling for various reasons, and they had at least one first degree and one second degree relative with thromboembolic incidents (in brain, heart, lungs, deep veins, placenta etc.).Group B consisted of 50 individuals (23-61 years old), who were tested in frame of routine prognostic check-up. Molecular investigation of blood cell DNA samples was performed with a combination of PCR and TaqI digestion analysis for thrombophilia mutations Leiden (G1691A) and G20210A in the genes of coagulation factors V and II(prothrombin), which display frequencies of 5% and 4.5% in Greek population, respectively.23 individuals of group A (48%), and only 5 individuals of group B (10%) were found to have at least one mutation.The observed frequencies correspond well to the expected respective risks of individuals with a parent with an autosomal dominant disorder (group A) and individuals of the general population(group B). We conclude that genetic counseling may increase five-fold the prevention of thrombotic incidents in individuals with a family history of thrombophilia.

P0811. The Utilization of Prenatal Diagnosis in the African American Population in Metropolitan Detroit

A. D. Gilbert[1], *B. Beyer*[1], *R. Aatre*[1], *S. Ramanadhan*[1], *A. Johnson*[1,2]
[1]*Hutzel Hospital; Detroit, MI United States;* [2]*Wayne State University; Detroit, MI United States*
gilbert@med.wayne.edu

As we expand our knowledge of genetic disease through the Human Genome Project and its potential benefit to prenatal diagnosis, it is imperative to explore the use of this information by all ethnic groups. Previous studies have shown that African American (AA) women are less likely than women of other ethnic groups to utilize prenatal diagnosis. We sought to explore whether or not there is a difference in the uptake of prenatal testing when access to genetic counseling services is equal. A 20-month retrospective study was performed to address these differences. During this time there were 486 AA and 977 non-AA women referred due to an increased risk of fetal aneuploidy based on maternal age (>= 35) or a positive maternal serum screen (MSS). Demographic comparisons noted a difference in Metropolitan Detroit residency (86% AA vs. 28% non-AA; p<0.0001) and private insurance policy holders (27% AA vs. 61% non-AA; p<0.0001). AA women were significantly less likely than non-AA women to chose prenatal testing following genetic counseling, (36% AA vs. 62% non-AA; p<0.0001). This was consistent in both maternal age patients (38% AA vs. 54% non-AA; p<0.001) and MSS patients (35% AA vs. 47% non-AA; p=0.001). Further analyses will determine if certain demographic or cultural variables influence the utilization of prenatal diagnostic procedures within our study population. Preliminary findings from our study demonstrate a need for additional investigations to uncover the inherent biases regarding prenatal diagnosis among specific ethnic groups.

P0812. Pregnancy and delivery in women with hereditary neuromuscular disorders

S. Rudnik-Sch neborn, *K. Zerres*
Institute for Human Genetics; Aachen, Germany
srudnik-schoeneborn@post-klinikum.rwth-aachen.de

Little advice can be given to women with rare hereditary neuromuscular disorders in child-bearing age who wish to have children. In a pro- and retrospective study on obstetric aspects, experiences of more than 100 mothers with various muscle diseases were documented. There were 15 patients with spinal muscular atrophy (SMA) types II-III, who mostly had uneventful pregnancies even in entirely immobile state. Caesarean section was undertaken in most cases with early onset SMA. Outcome was favourable in all infants. Despite some alarming reports, most pregnancies in 26 women with Charcot-Marie-Tooth disease were uneventful and completed by spontaneous deliveries. Similar experiences were made by 10 patients with facioscapulohumeral muscular dystrophy or 5 women with congenital myopathies. The perinatal complication rate was increased in 5 patients with limb girdle muscular dystrophy (secondary caesarean section rate), and persistent worsening of weakness related to gestation occurred in 3/5 women. Adverse effects of pregnancy and delivery were dramatically increased in 25 patients with myotonic dystrophy and included preterm birth, placenta praevia, fetal distress, and perinatal deaths. While most complications occurred in gestations with congenitally affected children, the obstetric risks in myotonic dystrophy seem to be independently increased due to the involvement of the genitourinary system. To conclude, women with slowly progressive disease courses that exclusively affect voluntary muscles, most likely will have uncomplicated pregnancies with a favourable outcome. Since most neuromuscular diseases are inherited, genetic counselling is recommended. It has also to be stressed that, even after complicated deliveries, most women are happy to have own children given that enough support can be provided by the individual social network.

P0813. Uncertainty management in genetic counselling for predictive testing

S. Sarangi, *L. Howell*
Health Communication Research Centre, Cardiff University; Cardiff, United Kingdom
sarangi@cardiff.ac.uk

Most counselling settings — in education, divorce mediation, dispute resolution etc — are characterised by a climate of uncertainty management. Both professionals and clients see these encounters as ‘information and ‘advice exchange systems as they draw upon the descriptive and evaluative functions of language. In the context of genetic counselling generally, and for predictive testing in particular, the issue of uncertainty assumes particular significance in relation to delivery of genetic (risk) information. Almost everything that is said by the clinician has the potential to be interpreted by the client as being ‘suggestive . In this paper, we approach the notion of uncertainty (as separate from ambiguity, vagueness and non-directiveness) from a qualitative, communication/discourse perspective. Based on detailed analysis of audio-recorded, transcribed data from Huntington s Disease and Breast Cancer clinics, we focus on the nature and role of various discourse strategies such as use of hedging and disclaimers, contrastive formulations, display of mutually shared knowledge etc. We argue that the interactional tensions are most evident when clients orient towards resolving uncertainty in personal and categorical terms, whereas the clinicians restrict themselves to their existing medical knowledge base and their professional experience of families with similar genetic disorders.

The paper is based on an on-going project titled ‘Communicative Frames in Counselling for Predictive Genetic Testing , funded by The Wellcome Trust.

P0814. Attitudes toward prenatal genetic testing and reproductive options among adults affected with achondroplasia and their first degree relatives

B. B. Biesecker[1], *H. Gooding*[1], *K. Boehm*[2], *D. Hadley*[1], *C. Francomano*[1]
[1]*NHGRI/NIH; Bethesda, MD United States;* [2]*NIDCR/NIH; Bethesda, MD United States*
barbarab@nhgri.nih.gov

Achondroplasia, the most common form of dwarfism, is caused by a mutation FGFR3. Since the discovery of the most common mutation in 1994, prenatal testing has become available. This study sought to collect empiric evidence for the awareness, interest in and use of prenatal genetic testing for achondroplasia by affected adults and their parents and siblings. Overall, there were 325 respondents, including 178 affecteds, who answered a self-report survey instrument. The groups differed in age, marital status and education level. The sex and ethnicity was similar, 71% females and 92% white. 70% of respondents were aware of prenatal testing; 62% of whom learned about it through the Little People of America. 13 affected individuals had used genetic testing compared to one relative. By regression analysis, affected individuals were 6.9X more likely than relatives to be interested in testing (p<0.001). Statistical modeling indicated that those in both groups who supported abortion were 3.4X more likely to express interest in genetic testing; 4.6X more likely to want to know a prenatal diagnosis of homozygous achondroplasia; 3.1X more likely to want to know a prenatal diagnosis of heterozygous achondroplasia; 2.9X more likely to want to know a prenatal diagnosis of an average statured fetus; and 10.0X more likely to express interest in terminating a fetus diagnosed with homozygous achondroplasia. Overall, both groups were supportive of population screening for achondroplasia and of the availability of testing for those at increased risk. There was a lack of interest in abortion of average statured fetuses out of preference for having a child affected similarly to themselves or their relative.

P0815. DNA goes to School! A pioneer educational project in Rio de Janeiro, Brazil

***M. Lachtermacher Rocha**, M. Rufier*
DNA goes to School; Rio de Janeiro, Brazil
lachtermacher@yahoo.com

As this new century begins, headlines about DNA catch our attention everyday and as the Human Genome Project approaches completion, it is critically important to bring science closer to society, to have educational projects that focus on DNA and to stimulate discussions of the issues inherent to the genetic era . Clearly, fundamental concepts as citizenship as well as important principles of ethics will be reshaped according to a new social order dictated by the introduction of this new technology in our society. In this sense, the young person s education should open horizons and prepare the young person for life, considering all of the transforming stages that our society is already facing. Because a free democratic society is not just a matter of having choices but of preparing the citizens to be able to understand these choices. For this reason, we have developed DNA goes to school, a pioneer educational project launched in 1999 in Rio de Janeiro, Brazil. The project was developed in order to provide an opportunity for high school students to have closer contact with DNA science. It combines hands-on DNA experiments with a variety of open discussions regarding different themes such as genetically modified food, cloning, The Human Genome Project, and other activities dealing with DNA and the genetic era . During the first year of establishment, more than 400 students have attended the course. The complete course is a 12 hours class divided into 4 different sections of 3 hours and is limited to a group of 16 students. We have evaluated the course and 98% of the students pointed out that the course is adequate for their level of knowledge, it has clarified important concepts and it has been a unique opportunity to have discussions about different issues concerning the new genetic era.

P0816. The Gap Between Services and Genetics Education of Allied and Counseling Health Professionals; HuGEM Survey Results

***C. Kozma**[1], E. Lapham[1], J. O. Weiss[2], J. L. Benkendorf[1], M. A. Wilson[2]*
[1]Georgetown University Medical Center; Washington, DC United States; [2]Genetic Alliance; Washington, DC United States
laphamv@georgetown.edu

A random sample survey of 3600 health professionals who are members of 6 national professional organizations in the US showed that the majority of allied and counseling health professionals are providing genetic services to at least some of their clients even though they have minimal education in genetics. The study was carried out as part of the Human Genome Education Model (HuGEM) Project of Georgetown University Medical Center and the Genetic Alliance and funded by the National Institutes of Health, National Human Genome Research Institute. The collaborating associations in the 1998 study included the American Dietetic Association, American Occupational Therapy Association, American Physical Therapy Association, American Psychological Association and National Association of Social Workers. At the time of the survey, the memberships of the six organizations totaled 570,000 with 330,909 members identified as direct service providers. Of the 1,958 (57%)responses received, 70% have discussed the genetic component of presenting problems with at least a few clients, 29% have provided counseling about genetic concerns while only 19% made referrals for genetic counseling, and 15% have referred for genetic testing. While the majority of respondents are providing genetic services, few have high confidence in what they are doing. Nearly two-thirds take family histories, however, only 1 in 4 is confident about eliciting genetic information as part of the history. Most social workers (87%) and psychologists (79%) in the study provide counseling, however only 26% and 29% respectively, have high confidence in counseling clients making decisions about genetic testing. Although two-thirds of the health professional s hold graduate degrees less than 21% have had one or more courses in genetics, 44% have had genetic content in course work and 33% have had no formal education in genetics. High confidence is related to amount of genetics education, independent of overall education. For example, 98% of psychologists have doctorates however their confidence in providing genetic services is correlated with amount of genetics education on eight measures ($p<.05$). Nearly two-thirds of respondents want continuing education in genetics. Priority topics identified were 1) Role of genetics in common disorders such as stroke, heart disease, and cancers, 2) Overview of human genetics, 3) Identifying genetic resources for clients, 4) Helping clients cope with new genetic diagnoses, 5) Genetic information and racial/ethnic concerns, 6) New treatments, 7) Privacy and confidentaility issues, and 8) Communicating genetic information to clients. The survey supports the need for incorporating genetics into the practice and teaching of all health professionals. It will take a coordinated effort among genetics professionals, professional associations, academic institutions and funding agencies to assure that health professionals receive the genetics education needed to meet the increasing needs of clients with genetic concerns. Questionnaires, survey results and model curricula will be available to participants.

P0817. Original Internet-System Myogene In The Process Of Teaching Medical Genetics

***L. Akhmadeyeva**, R. Magzhanov*
Bashkirian State Medical University; Ufa, Russian Federation
medic@agidel.ru

We have created a Web-site titled MYOGENE oriented to inherited neuromuscular disorders (INMD). It is the result of our own study in Bashkortostan (Russia) and our search of recent literature and the Internet. The main aims of this project are to help medical doctors and students to study INMD, to aid diagnosis, and to direct management and counselling. We have included spinal muscular atrophies (3 classical types and Kennedy disease), hereditary neuropathies, Myotonic Dystrophies (both types), myotonias (Thomsen s, Becker-type myotonia, Paramyotonia congenita and Potassium-aggravated myotonia), periodic paralysis, some congenital myopathies (Nemaline myopathy, Myotubular myopathy, Mini-core disease and Central core disease), several congenital muscular dystrophies (Classical congenital muscular dystrophy, Fukuyama congenital muscular dystrophy, Muscle-eye-brain disease and Walker-Warburg syndrome) and 9 progressive muscular dystrophies. MYOGENE offers to it s users several menus as a multiple choice questionnaire to choose presenting symptoms, family history, clinical signs and some basic data concerning electrophysiological and pathological investigations. Then it suggests a diagnosis or a list of the most relevant disorders. On the site there is a summary about each disorder and there are links to other Web-sites carrying more details, including OMIM. We continue to test and improving the system and recommend it for education in medical genetics. The preliminary results show that it is an effective tool both for counselling and for teaching. One can find a version of MYOGENE at http;//www.bashedu.ru/konkurs/akhmadeeva/ and e-mail us with recommendations to medic@agidel.ru. A project was supported by Open Society Institute (Soros Foundation).

P0818. Joining The Teaching Of Medical Genetics With Ethical Theory

***T. J. Meier**[1], R. C. Baumiller[2]*
[1]Xavier University; Cincinnati, OH United States; [2]Xavier University; Cincinnati, United States
soberdiver@excite.com

The teaching of human genetics at this dawn of the twenty-first century must include consideration of the ethical and moral implications of the information being presented. Advances in medical technology and understanding are rapidly communicated and assimilated, so there no longer exists the luxury of detached, dispassionate reflection upon what was once the incremental but slow progress of science. Geneticists and philosopher-ethicians need to work more closely to understand the impact of genetic knowledge on the society and culture into which it is incorporated. The revolution in the speed and trajectory of medical technology brought about by the advent of molecular biology techniques necessitates a revolution in the manner in which discoveries are taught in the classroom. Medical genetics must be presented in concert with the ethical and moral implications of that information, because all students are conditioned by the ethical and moral culture in which they live, and philosophical presuppositions must be brought to light so that the effects of the rapidly changing scientific knowledge base on people may be foreseen and rendered beneficial. We have developed a course for upper-level undergraduates, entitled Medical Genetics and Its Implications which presents a challenging curriculum of advanced scientific information along with a consideration of ethical theories by which to evaluate the impact of this knowledge on individuals, families, and societies. We believe that our course materials can be adapted easily and fruitfully for use in other undergraduate, graduate, and professional school courses.

P0819. Genetic education material for upper secondary schools

***H. Kaariainen**, M. Sipponen*
The Family Federation of Finland; Helsinki, Finland
helena.kaariainen@vaestoliitto.fi

The development of molecular genetics has created new possibilities for clinical applications, sometimes for whole populations. Simultaneously, human genetics has become a popular subject in the media which has increased public interest. In clinical practice it appears, however, that pub-

lic understanding of these issues has not increased and misconceptions are common. In Finland, after 9 years of basic education more than half of the students continue to upper secondary schools (gymnasiums). In the gymnasium curriculum there are two compulsory courses on biology and voluntary extra courses among which a course on genetics is very popular. However, the textbook used for this course is very theoretical and mainly concentrates on molecular genetics. To complement this book, we created material for a course of 12 lectures on clinical/practical aspects of human genetics. The team consisted of a clinical geneticist (HK), a genetic nurse (MS), three teachers of biology and two groups of students. The material consisted of texts and exercises for students, instructions for teachers and solutions for the excercises. Many of the exercises were problems on many levels; mendelian calculations, personal dilemmas, and ethical problems on the level of the community. We have also organized training for teachers based on this material. The response of both teachers and students has been enthusiastic but the real impact of such an approach to understanding of genetics will be difficult to assess.

P0820. Genetics Education; Adapting Global Strategies to Meet Local Needs

E. Balkite, *S. Ray*
Glaxo Wellcome Inc.; Research Triangle Park, NC United States
EAB52948@GLAXOWELLCOME.COM

Education in both the science of genetics and the issues in genetics is key to successful advancement of programs in the field and requires a combination of strategies. When Glaxo Wellcome established a Genetics Directorate in 1997 to incorporate genetics knowledge and technology into drug research and development, it made a commitment to education about its genetics initiatives to both internal and external stakeholders worldwide. After identifying the stakeholders and assessing their needs, global strategies were developed to raise awareness of the scope of Glaxo Wellcome genetics research and educational materials targeted to specific audiences were produced. Stakeholders and strategies are presented in Table 1. These strategies were adapted and modified to meet local genetics education needs depending on the country or regional needs. Evaluations were conducted and information shared to enhance future genetics education initiatives.

Table 1

STAKEHOLDERS	STRATEGIES
Employees	Symposia
Investigators	Courses
Ethics Committees	Workshops
Policymakers	Meetings
Regulatory Authorities	Newsletters
Patients	Websites
Public	Videotapes
Private Insurers	
University Faculty	

P0821. Affective, Behavioural And Cognitive Components Of University Students Regarding Genetics And Its Advance

M. I. Carb *, O. Pliego, M. B. Bravo-Luna, J. A. M. Cesolari, N. F. Peret*
Faculty of Medicine-UNR; Rosario, Argentina
mbravoluna@hotmail.com

Attitudes are determinant of educational process quality; hence, they must be included in its objectives. This proposal is a true change in Science-Didactics paradigm. The scientific, technological and social (STS) approach emphasizes the importance of the ethical aspects as well as the development of conscious opinion in the educational processes. Consequently, in a first stage we have investigated attitude components in a sample of students entering the medical career at the Faculty of Medicine, National University of Rosario (Argentina). In order to evaluate the affective and behavioural components we have prepared, validated and applied an instrument used in social psychology (Lickert s scale), analysing Genetics social image (GSI), teaching-learning process (TLP) and shared responsibility (SR). The results show a favourable attitude global trend, regarding Genetics and its advance [total mean; 3.73/5; GSI; 3.7/5; TLP; 3.7/5; SR; 3.8/5]. The cognitive component was assessed by means of an instrument demanding the solution of different activities offered to the students. Cognitive evaluation attained 34% of correctly resolved activities [x= 5.29/15.5]. See Figure displaying results according scores. Thus, it is clear that the knowledge of the students, in Genetics, is poor, this is a challenging goal to attain during the development of this first year of the career in which we are currently working.

P0822. The haze of Bayes - how to improve diagnostic inference and the understanding of risk by patients and by physicians

J. Pelz
Institut fuer Humangenetik; Berlin, Germany
joerg.pelz@charite.de

Genetic tests become increasingly accessible. Possible benefits of testing for susceptibility to chronic disease may be substantially outweighed by confusion raised by test results. The concepts of sensitivity, specificity and positive predictive value of a test are all complex and therefore not necessarily understandable to an uninitiated patient. On the other hand in order to make informed decisions on participation in genetic screening programs and in prenatal diagnosis patients should be told about these concepts. Moreover several studies have shown that even physicians have a poor understanding of probabilities and the predictive value of test results. Gigerenzer and coworkers hypothesised that due to human evolutionary development mental algorithms were not designed for probabilities and a Bayesian reasoning but for the understanding of natural frequencies. They showed that the use of this latter kind of representation can serve as an effective tool in inferring the predictive values of a test and to improve the communication of risks between physicians and patients as well. In order to test their suggestions for genetic counselling four problems were presented to more than 200 of our fellow students (representatives of an upper-middle class lay population) either as probabilities or as natural frequencies; (1) positive triple test and the risk of trisomy 21, (2) insulin dependent diabetes mellitus and DR3/DR4, (3) breast cancer and BRCA1/BRCA2, (4) inheritance of familial polyposis and symptom free ageing. Participants received in a randomised order all four problems, two presented as probabilities and two as natural frequencies. They generally ranked the natural frequency questions as less difficult and yielded a significant better understanding of the risk. The representation of complex concepts in natural frequencies rather than in probabilities can improve the understanding of patients and of physicians

P0823. Down syndrome - critical evaluation of information available in the Internet

A. Zahn, *J. Kunze, J. Pelz*
Institut fuer Humangenetik; Berlin, Germany
joerg.pelz@charite.de

Considering the increasing number of people using the internet as an source of knowledge about medical issues, we analysed the information of web-sites on Down Syndrome (DS). After a search for Down syndrome with the search engines Altavista and Yahoo (in German and in English), we examined medical and/or psycho-social information of web-sites within the first 100 hits yielded by each search engine. A predefined catalogue of health related data was used for a ranking of web-sites. Basic (required) and additional (facultative) information were evaluated separately, English and German web-sites were compared. The sample size was 324 hits (200 English, 124 German) of which only 77 (24%) were medical web-sites. Among these were 34 relevant German web-sites (27% of 124 hits) and 43 relevant English web-sites (22% of 200 hits). In both languages all levels of information (poor/excellent) were found. Only 24% of the German web-sites and 35% of the English web-sites gave more than 50% of the basic medical information. Looking at the additional information, there were 1 German and 5 English web-sites communicating more than 50% of the expected content. An impact of language and authorship (universities/personal homepage) on the substance could be established. English web-sites contained more material than German s. Since many homepages don t cover and are not intended to present most of the required facts, the usefulness of both the German and the English web-sites as a source of information on DS is limited. Careful work is essential to ensuring that the Internet takes shape in ways that support customers needs of health information. However, some examples show the potential of the internet, although the possibilities to educate people about DS are not completely realised.

P0824. Genetic disorders and congenital malformations in Iranian Population (Tehran)

R. Mokhtari[1], *A. Bagga*[2]
[1]*Self; Tehran, Islamic Republic of Iran;* [2]*University of Pune; Pune, India*
royakiana@yahoo.com

Over the last few decades there has been a gradual change in the pattern of diseases in many western countries and genetic disorders have been important causes of morbidity and mortality. In the present research, 800 patients coming to 3 genetic centers in Tehran (capital of Iran) were studied. The study focused on the prevalence of various kinds of congenital malformations and genetic diseases in Iranian Population. The effect of

consanguinity and the kind of congenital malformations and genetic disorders associated with consanguinity, the present position of genetic counseling facilities available in Iran and the extent of their utility, the role of education of the parents in prevention the spread of certain genetic disorders and their association with the consanguineous marriages was explored. Analysis of data revealed that major disorders were Down s syndrome, (22%), Primary Amenorrhoea (11%), {Psychomotor retardation (9.5%),Mental retardation(7.9%)and Turner Syndrome (5.1%). 43.6 % of the patients were born to the consanguineous parents out of which majority (37.8%) were from Parallel cousin ,28.9% were from Cross cousin marriages, and 33.2% were from the distantly related parents.Psychomotor retardation (20.8%), Primary Amenorrhoea (17.4%) and Mental retardation (10.5 %) topped the list of disorders encountered in the children born to consangineous parents. A Total of 45.4 % couples already having an affected child reported pregnancy wastage. In countries where the problems of malnutrition and infectious diseases have been overcome, society needs to turn its attention to the prevention and management of genetic diseases. This is what is strongly advocated for Iran because of its high rate of consanguinity.

P0825. Hemoglobinopathies Newborn Screening In Hospital De Base From S o Jos Do Rio Preto - Sp, Brazil.

F. A. M. Siqueira[1], *A. C. Fett-Conte*[2], *L. N. B. Borin*[3], *C. R. Bonini-Domingos*[4]
[1]*UNESP; Sao Jose do Rio Preto, Brazil;* [2]*Hospital de Base; 'Sao Jose do Rio Preto, Brazil;* [3]*Hospital de Base; Sao Jose do Rio Preto, Brazil;* [4]*IBILCE-UNESP; Sao Jose do Rio Preto, Brazil*
famendes@bio.ibilce.unesp.br

The hemoglobinopathies, mainly the sickle cell anaemia and the thalassemias, include a heterogeneous group of genetic alterations disseminated over the whole world. They consist of a problem of public health in several countries, including Brazil. The neonatal period is considerated the most effective for the screening of this alterations. This allows prophylaxis and prevention before the appearance of the first symptoms, allowing improvement on the patient s survival and guidance of the parents and heterozygote carrier. The present work aims the early detection of the abnormal hemoglobins to establishiment standard analysis and examine the viability of the prevent program. The samples were heel stick collected and through blood cord. Electrophoresis procedures, HPLC (Bio-Rad) and cytological and biochemical analysis were made for the abnormal hemoglobins characterization. From April, 98 to November, 99, 1478 neonatal blood samples were analyzed from Hospital de Base, S o Jos do Rio Preto, in which 216 (14,62%) hemoglobins alterations could be found, emphasizing 49 (3,32%) with Hb S, 9 (0,61%) with Hb C, 110 (7,44%) with suggestive alpha thalassemia, 23 (1,55%) with suggestive beta thalassemia, and 25 (1,70%) with interation alpha/beta thalassemia. The samples collected from the blood cord showed effectiveness in all analyses suitable while the blood samples hell stick collected, in filter paper, were applicable only to specific methodologies. The hemoglobinopathy suspected cases were reassessed after six months and they were led to genetic counseling and clinic attendance with their family members. The multiprofessional approach in programs of this kind is fundamental for its success.

P0826. Hsp70 polymorphism and HLA-DR diversity; Implications for tuberculosis susceptibility in the Cape Coloured population of South Africaility in the Cape Coloured population of South Africa

T. Boshoff[1], *A. E. Brune*[1], *E. G. Hoal van Helden*[2], ***L. Bornman***[1]
[1]*Biochemistry Division, Rand Afrikaans University; Auckland Park, South Africa;* [2]*Department of Medical Biochemistry, University of Stellenbosch; Tygerberg, South Africa*
LBO@na.rau.ac.za

Tuberculosis (TB) being one of the leading causes of death from infectious disease in the world, is also a major problem in the Cape Coloured population in the Western Cape of South Africa. Multiple genes influence susceptibility to TB. HLA class II polymorphisms have been associated with susceptibility to inflammatory diseases, but linkage disequilibrium of alleles in the major histocompatibility complex (MHC) complicates identification of disease-associated genes. Genes encoding members of the 70 kDa heat shock protein (HSP)family (Hsp70) are localised within the MHC. In the light of the protective role of Hsp70 in infection and immunity it is hypothesized that Hsp70 polymorhisms may contribute to disease susceptibility. Possible linkage disequilibrium of hsp70 alleles with HLA-DR alleles might lead to extended haplotypes which might act as additive TB susceptibility markers. Hsp70 gene polymorphism (hsp70-1, hsp70-2, hsp70-Hom and the polymorphic PstI site within the coding region of hsp70-2) and HLA class II DR polymorphisms were investigated in the Cape Coloured population inhabiting the Western Cape of South Africa. Polymorphic analysis of hsp70 and HLA-DR genes was performed on genomic DNA from patients suffering from TB (n=60) and matched control subjects (n=61) using PCR-RFLP and PCR-SSP respectively. Preliminary results showed no evidence for an independent role of hsp70 gene polymorphism in susceptibility to TB while DR3 (DRB1*0301-0302) and DR53 (DRB4*0101) were present at a higher frequency (p=0.068 and p=0.034 respectively) in TB patients, and DR12 (DRB1*1201-1203) in control subjects (p=0.028). An additional number of 300-400 cases and controls are currently analysed to meet statistical standards and to investigate linkage disequilibrium of HLA-DR and hsp70 alleles. An improved understanding of the underlying mechanisms contributing to tuberculosis susceptibility may open new avenues in the development of novel therapeutic approaches.

P0827. ACE Polymorphism in National Turkish Athletes

F. Ciloglu[1], *I. Peker*[2], *N. ine*[3], *A. Demirkan*[3]
[1]*GENLAB Medical Diagnostics and Research Laboratory; Istanbul, Turkey;* [2]*Marmara University; Istanbul, Turkey;* [3]*DETAM; Istanbul, Turkey*
fciloglu@hotmail.com

Cardiovascular performance is a key element in athletic success and certain genes are thought to be candiates for regulating cardiac and vascular physiology. One of the candidate genes is is in the renin-angiotensin system. The angiotensin converting enzyme (ACE) insertion allele (ACE I) is a genetic marker that might be associated with improved endurance performance. In this study ACE polymorphism was studied in past and present Turkish National male athletes who have been competing in national and international level for several years.. The athletes were seperated into three groups consisting of 40 long distance runners, 25 sprinters and 30 fotball players who are felt to have characteristics of both long and short distance athletes. The findings were compared to the results of 100 randomly selected males from the sedentary Turkish population. Genomic DNA was obtained from white blood cells and and the I and D variants of the ACE gene were identified by PCR amplification of the polymorphic region. The findings show that compared to the normal population, the long distance runners have an excess of the ACE I allele ($p<0.05$)and the ACE II genotype ($p<0.05$). On the other hand the sprinters show results very similar to the normal population. The fotball players show a slightly increased ACE I allele and ACE II genotype but it is not enough to be statistically significant. These findings suggest that atleast in the Turkish population, there does seem to be a positive association between endurance performance and ACE I allele.

P0828. Folic acid prevents more than neural tube defects; a registry-based study in the Northern Netherlands

H. E. K. de Walle, *J. Reefhuis, M. C. Cornel*
Department of Medical Genetics, University of Groningen; Groningen, The Netherlands
H.E.K.de.Walle@medgen.azg.nl

Folic acid (FA) is not only protective for neural tube defects (NTDs) but could be for other defects as well. Using data from the EUROCAT Northern Netherlands registry, we have investigated the possible preventive effect of FA in five selected groups of malformations; NTDs, heart defects, oral clefts, urinary tract defects, and limb reduction defects. A case-control analysis was performed using the years 1981 through 1998. Information on FA use was collected via the physician, pharmacist and mother. Odds ratios (OR) with 95% confidence intervals (CI) were calculated using chi-square tests. Cases were defined as infants with FA sensitive defects (n=2,751), defects part of chromosomal or monogenic disorders were excluded. Controls were all children and foetuses with anomalies other than the sensitive anomalies (n=3,647). Use of FA during the periconceptional period was known for 69.5% (n=1,914) of the cases and for 70.0% (n=2,553) of the controls. Of the FA sensitive cases 67 (3.5%) were periconceptionally supplemented with FA, while this was true for 144 (5.6%) controls. Significantly less case mothers than control mothers took folic acid periconceptionally. More specifically, this study showed a significant reduction in risk of heart defects (OR=0.52, 95% CI; 0.35-0.78) and a strong indication for a reduction in the prevalence of urinary tract defects (OR=0.54, 95% CI; 0.29-1.02). The OR for NTDs are indicative of a protective effect (OR=0.68, 95% CI; 0.35-1.30). Recall bias is an unlikely explanation, since sick controls were used as controls. The explanation probably lies in the power of this study; there were not enough cases to detect a significant protective effect of FA for NTDs. Birth defect registries will continue to monitor the future effect of FA on birth defects. Therefore these registries will remain important tools for determining effects of primary prevention on other severe congenital anomalies besides NTDs.

P0829. Postal collection of buccal wash samples for DNA; A successful method for expansion of the ALSPAC DNA resource.

S. Ring[1], *E. Spanakis*[2], *T. Beavan*[1], *B. Barratt*[3], *R. Jones*[4], *I. Day*[2], *M. Pembrey*[4], *L. Tyfield*[1], *A. Study Team*[5]

[1]*Department of Molecular Genetics, Southmead Hospital; Bristol, United Kingdom;* [2]*Division of Human Genetics, Southampton University Hospital; Southampton, United Kingdom;* [3]*Department of Medical Genetics, Wellcome Trust Centre for Molecular Mechanisma in Disease.; Cambridge, United Kingdom;* [4]*Institute of Child Health, London; London, United Kingdom;* [5]*Institute of Child Health, University of Bristol; Bristol, United Kingdom*

S.M.Ring@Bris.ac.uk

The Avon Longitudinal Study of Pregnancy and Childhood is a large epidemiological study of approximately 14,000 children designed to investigate the interplay between genes and environment. A DNA resource has been created using blood samples from approximately 10,000 children and 9,000 mothers and is being used for several genetic studies. Cell lines will be created from the study participants in order to ensure long-term provision of DNA. Paternal blood is currently unavailable. Therefore a new postal buccal wash collection and extraction method was piloted to create mother;father;child trios for transmission tests and imprinting studies. This method was developed for trio studies by the Southampton group and independently validated for ALSPAC in Bristol. Mouthwash kits were sent to 1386 mothers who were asked if they and their partners would return a mouthwash sample for genetic studies. 63% of mothers and 55% of partners returned a sample. Samples were normally less than 48 hours old when returned although some mothers took up to 3 months to collect the samples. DNA is extracted using a phenol-free method and concentration measured using picogreen. The mean yield is 62 g of DNA (range 2-300 g) per 10ml sample. In initial genotyping studies a PCR success rate of 94.6% has been obtained. Studies are underway to confirm sample identity. We expect to generate trios from 52% of the targeted families when parent samples are associated with existing child samples. In conclusion this is a successful method of collecting DNA for epidemiological studies by post

P0830. Whole Genome Amplification; A Means of Providing DNA from Limited Biological Sources

P. K. Bender[1], *M. Schmidt*[1], *W. Beggs*[2], *W. Beggs*[2], *J. C. Beck*[1], *R. T. Johnson*[1]

[1]*Coriell Institute for Medical Research; Camden, NJ United States;* [2]*Coriell Cell Repositories; Camden, NJ United States*

benderpk@umdnj.edu

Whole genome amplification offers a means to prepare sufficient DNA for detailed genetic analysis from biological materials of limited availability. Samples such as biopsies that cannot be cultured, forensic samples, hair and cheek cell samples, and animal scat represent valuable sources of genetic material if sufficient DNA can be obtained. The WGA method is based on a technique called primer-enhanced-preamplification (PEP). PEP relies on fully degenerate primers to target the PCR amplification of sequences throughout the genome without bias for sequence content. Biological samples including a sliver of skin tissue from a deceased centenarian, keratinocytes from foreskin, blood, and primate scat were subjected to WGA in order to evaluate the method. The WGA method yielded up to a 2,000-fold amplification of the genomic DNA providing up to 2.0 micrograms of WGA DNA from a 1.0 ng aliquot of genomic DNA. Several molecular assays were undertaken to determine the allelic bias in the samples and to assess the utility of the material for genetic analysis. For the human samples, the allelic representation of the X- and Y-chromosome amelogenin alleles and five microsatellites on different autosomes was compared between the WGA products and the original template genomic DNA. The DNA samples from primates similarly are being assayed using genomic and mitochondrial markers. The results with the human samples indicate that WGA generates DNA that is representative of the original genomic material at many loci, and potentially offers a method for amplification of DNA from animal samples collected in the field.

P0831. SNP-based genotyping using arrayed primer extension on the oligonucleotide array.

E. L hmussaar[1], *A. Lushnikov*[1], *N. T nisson*[2], *T. Nikopensius*[1], *I. Kask*[1], *A. Kurg*[1], *A. Metspalu*[1]

[1]*Inst. of Molecular and Cell Biology, University of Tartu; Tartu, Estonia;* [2]*AsperBio Ltd.; Tartu, Estonia*

elin@ebc.ee

SNP-s offer a number of advantages with respect to population-based analysis of the human genome. We present Arrayed Primer Extension (APEX) technology for SNP scoring all over the genome. The method is based upon an array of oligonucleotides immobilized via a 5 -end amino linker onto amino-silanized glass slide. Oligonucleotides are selected from the sense and antisense genomic sequence so that 3 -ends are one base upstream of the SNP. The amplified DNA template, containing the SNP, is digested enzymatically and then annealed to the immobilized primers, which promote sites for template-dependent DNA polymerase extension reactions. Four unique fluorescently labeled dye terminators are used to extend each primer by only one base. As a result of this reaction each primer identifies one base in the target sequence. The Genorama“ imaging system and software package is used for SNP scoring. In the present study APEX technology was successfully used for SNP-based genotyping. We have selected 68 SNPs over the whole genome and estimated the allele frequencies and heterozygosities of these SNPs in Estonian population analyzing 240 chromosomes. From the 68 analyzed SNPs 58 were polymorphic according to the allele frequencies data. This current oligonucleotide array with 68 SNPs can be successfully used for paternity testing and forensic analysis. Oligonucleotide design, quality, DNA Polymerase, dye terminators, template DNA quality and special software tools are all critical for the optimal results. APEX method is a reliable tool in SNP studies, which seem to have a great potential for large-scale genotyping in the near future, although method has utility already now in paternity and forensic testing, mutation analysis and SNP scoring.

P0832. Evolution and population genetics of the H-ras minisatellite

J. A. Langdon, J. A. L. Armour

Institute of Genetics, Queen s Medical Centre, University of Nottingham; Nottingham, United Kingdom

mrxjal@nottingham.ac.uk

Studies of the H-ras minisatellite have shown that 94% of all alleles in native Europeans and Americans of European descent consist of 4 common alleles. The remaining alleles are designated as rare. Rare alleles have been reported to be associated with cancer in several studies.

In this study 11 substitutional polymorphisms within the sequence flanking the minisatellite have been detected in 40 Castillian and 20 Pygmy chromosomes, and used to construct an allele tree for this locus. The ancestral state has been inferred by analysing several non-human primates, allowing the allele tree to be rooted. The internal structures of these minisatellite alleles have been deduced by minisatellite variant repeat (MVR) mapping. This information has been integrated with the tree constructed from flanking haplotypes, providing additional information regarding lineage evolution.

In addition a survey of allele lengths has been carried out in a UK control population, demonstrating a higher rare allele frequency than was reported in previous studies. The rare allele frequency of 17% detected is significantly greater than that of 6% reported by Krontiris et al. (1993). This is likely to be due to the increased power of the methods used in this study to detect rare alleles, and may have implications for the interpretation of the previously published case-control studies.

P0833. Classical association and TDT-type methods; power for detecting candidate genes influencing quantitative traits.

D. A. Tregouet, *C. Pallaud, C. Sass, S. Visvikis, L. Tiret*

INSERM U525; Paris Cedex 13, France

tregouet@idf.inserm.fr

Association studies based on candidate genes are one of the major strategies for identifying genes involved in complex traits. However, they suffer from the fact that they do not overcome the risk of spurious association due to uncontrolled stratification of the population. Therefore, the use of Transmission Disequilibrium Test (TDT)-based methods has been advocated by several authors for checking that an observed association is actually due to linkage and not to other uncontrolled phenomenon. In this paper, an estimating equation procedure is proposed to assess the power and the cost efficiency of a classical association and of two TDT-type analyses for quantitative phenotypes in sibship data. It is shown that an association analysis is generally more powerful, i.e. requires less informative sibs, than a TDT-type method when the allele frequency of the studied marker is close to 0.5. Conversely, TDT methods become more powerful when the allele frequency is low or high, all the more since the sibship size is large and the residual sib-sib correlation is strong. However, TDT methods always require more sibs to be sampled, regardless whether or not they are informative, than an association analysis to get the appropriate number of informative sibs, differences between methods tending to decrease as the sibship clustering increases.

P0834. Regression-based inference for association of haplotype frequencies with the phenotype.

D. V. Zaykin[1], P. H. Westfall[2], S. S. Young[3]
[1]Statistical Genetics, GlaxoWellcome Inc.; Research Triangle Park, NC United States; [2]Texas Tech University; Lubbock, TX United States; [3]GlaxoWellcome Inc.; Research Triangle Park, NC United States
dvz90620@glaxowellcome.com

There are increasing efforts to relate clinically important phenotypes, such as disease predisposition, drug efficacy or glucose level to single nucleotide polymorphisms (SNPs). Recent research gives examples where several SNPs in haplotypes affect the phenotype so there are biological and statistical interactions. It is important to develop statistical methods to identify and deal with these interactions. We give statistical tests for association of haplotype frequencies with discrete and continuous traits in samples of unrelated individuals. There is a need to deal with unrelated individuals as many data sets, e.g. clinical trials, will not have pedigree data. We present conditions for asymptotic equivalence of regression-based methods with methods that double the sample size in the case of known haplotypes. Then we extend these models to the case of statistically inferred haplotypes. We confirm that our methods have excellent power while maintaining type I error. We present applications to gene mapping using epidemiologic data with adjacent markers and show that our methods improve the efficiency of genome scans by incorporating information from consecutive markers.

P0835. Does accounting for gene-environment interaction increase the power to detect the effect of the gene ?

E. Genin[1], J. M. Norris[2], H. Selinger-Leneman[1], M. Khlat[3]
[1]INSERM U535; Le Kremlin Bicetre, France; [2]University of Colorado, Health Sciences Center; Denver, CO United States; [3]INED; Paris, France
genin@kb.inserm.fr

Despite tremendous efforts, few genetic risk factors involved in the susceptibility for complex disorders have been identified so far. One reason for this may be that ignored gene-environment interaction conceals effects of genetic factors on disease. To investigate whether this could be an explanation, we have studied the power to detect gene effects of family-based tests of association in the presence of a gene-environment (GXE) interaction. Family-based association tests that use case-parent trios can allow for the detection of a gene effect and a GXE interaction by the comparison of the allele transmissions from parents to exposed and unexposed cases. We have compared the power of the test that account for the GXE interaction and of the test that does not under different exposure frequencies and several models of GXE interaction. We show that the gain of power due to accounting for GXE interaction is highly dependent on the population exposure frequency and on the exposure effect; it is higher when the exposure is rare and when the exposure effect is low. When the exposure frequency is high (>30%) and/or the exposure effect is strong, there may even be a decrease in power when accounting for the GXE interaction. For given exposure frequency and effect, we found that the gain in power is usually stronger for dominant as compared to recessive models.

P0836. Quantifying genetic diversity in a putative German isolate by multivariate feature vector analysis

K. Hoffmann[1], H. H. Stassen[2], C. Planitz[3], R. P. Bochmann[3], M. Skorna[4], B. Lucke[4], L. Haucke[4], W. Gunia[4], M. Zschornack[5], A. Reis[6], F. C. Luft[7], C. Scharfetter[2], T. Wienker[8]
[1]Gene Mapping Center at the MDC and Franz Volhard Clinic at the Humboldt University; Berlin, Germany; [2]Psychiatric University Hospital; Zuerich, Switzerland; [3]University Clinic Carl Gustav Carus Dresden, Physiology, TU Dresden; Dresden, Germany; [4]Gene Mapping Center at the MDC; Berlin, Germany; [5]Malteser Hospital; Raeckelwitz, Germany; [6]Institute for Human Genetics at the University Nuernberg-Erlangen, and Gene Mapping Center at the MDC; Erlangen and Berlin, Germany; [7]Franz Volhard Clinic at the Humboldt University; Berlin, Germany; [8]Institute of Medical Biometry, Informatics, and Epidemiology, University of Bonn; Bonn, Germany
khoffma@mdc-berlin.de

Purpose; Population isolates are important for mapping both monogenic and complex traits. The success of genetic studies in isolated or founder populations depends on the effective number of founders, and the population history. Since reliable information on population history are not always available, the genetic diversity should be estimated in the present generation. In an isolated population, we would expect a reduced heterogeneity. Ethnological studies on populations use the genotypes of mitochondrial and Y-chromosomal polymorphisms. However, these methods are not absolutely representative for autosomal loci, where we would expect genes responsible for most diseases. Method; To address this issue, we applied a multivariate feature vector approach to quantify genetic diversity. Genotypes of 18 highly polymorphic microsatellite loci were analysed by a genetic similarity function. The inter-individual genetic distances d(xi,xj) between the 18-dimensional feature vectors xi,xj (made up by the allelic patterns of any two subjects i, j) represent the level of genetic diversity within a given population. Population; In an ongoing hypertension study, the population of interest are the Sorbs, a putative slavic population isolate in Eastern Germany (sample n=326). Normalized data from 257 subjects from Kanton Zuerich, and an earlier study of US-American samples and of Hutterites (as a proved isolated population) served as reference values. Results; The US-American population displayed a significantly higher genetic variation compared to the Sorbs and the Kanton Zuerich sample (increase of 50 %). In the Hutterites, the genetic diversity was even more reduced and displayed only 30 % of the variation observed in the Sorbs. Surprisingly, the variability is similar in the Sorbs and the Zuerich sample. Conclusion; As expected, the heterogeneous US population showed the highest and the Hutterites the lowest genetic variability. A significant reduction was observed both in the Swiss and the Sorbs sample, indicating a reduced genetic diversity.

P0837. POPULATION GENETICS; Proposed Guidelines for the Ethical Conduct of Genetic Research in Populations

M. Deschenes[1], G. Cardinal[1], B. Knoppers[1], C. Laberge[2]
[1]University of Montreal; Montreal, PQ Canada; [2]Quebec Network of Applied Genetic Medicine (RMGA); Montreal, PQ Canada
mylene.deschenes@umontreal.ca

Now that the human genome is mapped, genetic research will focus on understanding the function of each gene. The genetic profile of an individual is shaped by geographical, cultural and socio-economic considerations, all of which are underpinned by biological evolution. Hence, the renewed interest in population research. The genetic profile of small isolated populations may ease the discovery of gene function. Indeed, population genetics holds the key to individual genetic medicine. Genetic epidemiology in heterogenous populations is also of interest to health authorities. Consequently, we observe emerging national DNA banks in Iceland, Estonia, England, Sweden, Newfoundland (Canada) and Quebec (Canada). Genetic research has been framed by a wide set of legislation and international guidelines. However, genetic research the scale of a population brings issues which are not addressed by current frameworks. The Quebec Network of Applied Genetic Medicine is working on the creation of a population bank to study SNP variation within the general Quebec population. To do so, it is developing guidelines for population genetic research. Our presentation will first discuss the ten fundamental principles on which the guidelines are founded. Second, we will focus on certain themes elaborated in the guidelines such as population assent, communication of research results, commercialisation and benefit sharing.

P0838. Optimal combination of marker-specific statistics in a two-stage SNP selection method for disease association data

A. Wille[1,2], J. Hoh[1], J. Ott[1]
[1]Rockefeller University; New York, NY United States; [2]IMBIE; Bonn, Germany
wille@linkage.rockefeller.edu

Although single nucleotide polymorphisms (SNPs) provide a good setting for the fine mapping of complex traits, their density and abundance burden data analysis more than ever. In order to decide which SNP loci in a large pool synergistically influence a particular disease, the mere marker-by-marker method is not efficient because it ignores correlations among markers and requires conservative multiple testing adjustment. Large-scale approaches in which information on multiple markers is bundled to a test statistic should overcome these difficulties. Earlier, we proposed a two-stage analysis of association data in which, first, a subset of markers is selected, and secondly, interactions between these markers and the disease are modeled. Here, we address a question that arose in the first stage of the analysis; how to combine marker-specific statistics in order to achieve the optimal detection power. In principle, each SNP is initially assigned an index that measures the association strength between the disease status and its genotypes. For example, this index can be the usual Chi-square statistic. Then information is to be combined either by simply summing those indices or by the empirical Bayesian method. In doing so, it leads us to explore the whole class of the James-Stein estimators.

P0839. Logistic Regression analysis for case/control genetic association studies; combining genetics and epidemiology

F. Macciardi[1], R. Adolfsson[2], D. Blackwood[3], G. N. Papadimitriou[4], R. Kaneva[5], M. Noethen[6], L. Oruc[7], A. Serretti[8], C. Van Broeckhoven[9], J. Mendlewicz[10], B. Lerer[11]

[1]Centre for Addiction and Mental Health (CAMH); Toronto, ON Canada; [2]University of Umea, Dept. of Psychiatry; Umea, Sweden; [3]University Department of Psychiatry and MRC Human Genetic Unit; Edimburgh, United Kingdom; [4]Athens University Medical School, Department of Psychiatry and University Mental Health Research Institute; Athens, Greece; [5]Laboratory of Molecular Pathology, MaternityUniversity Hospital of Obstetrics, Medical University; Sofia, Bulgaria; [6]Institute of Human Genetics, University of Bonn; Bonn, Germany; [7]Department of Psychiatry, University Hospital Rebro , University of Zagreb; Zagreb, Croatia; [8]Instituto Scientifico Ospedale San Raffaele, Department of Neuropsychiatric Sciences; Milan, Italy; [9]Neurogenetics Laboratory, Flanders Interuniversity Institute for Biotechnology (VIB), Born-Bunge Foundation (BBS), University of Antwerpen (UIA), Department of Biochemistry; Antwerp, Belgium; [10]Dept. of Psychiatry, Erasme Hospital, Free University of Brussels; Brussels, Belgium; [11]Biological Psychiatry Laboratory, Hadassah Medical Organization; Jerusalem, Israel
fabio_macciardi@camh.net

The ultimate goal of genetic association studies, which should be viewed within the larger framework of epidemiological studies of risk factor/disease associations, is to identify gene(s) responsible for a given disease. To detect a genetic association the easiest design is the case-control approach, which usually apply a chi-square analysis to look at the significance of an association. The test is simple and does not depend on any population genetic theory or model, other than that which supposes the strength of an association between genes increases when physical and genetic distances between them decrease. The major problem with the case-control design is the identification of an appropriate control sample, to avoid spurious associations due to potential confounding factors related to population admixture or stratification. To deal with these issues, various methods have been proposed, as, for example, using a set of unrelated polymorphisms to evaluate the extent of random association (Devlin & Roeder, Biometrics, 1999). We propose an alternative strategy for genetic association in a case-control sample by applying a logistic regression (LR) model, which allows for the simultaneous consideration of other risk and/or confounding factors. Using a data set from a case-control multicenter European Collaborative study, with a response variable (i.e., the diagnosis), a predictor (i.e., a risk allele for the 5HT2C gene variant) and a confounder (i.e., the ethnical origin of cases and controls), we show that the LR outcome is equivalent to the Mantel-Haenszel test (MH) for multiple contingency tables. Both the statistical approaches lead to the same result, showing a positive association despite substantial population variability across the participating centers. The advantages of this alternative model will be discussed.

P0840. Advances in Genetic Epidemiology

N. E. Morton
University of Southampton; Southampton, United Kingdom
nem@soton.ac.uk

After two decades developing statistical methods applicable to DNA markers, genetic epidemiology is emerging into the sunlight of modern biology. Advances can be grouped into four categories; mapping, positional cloning, nonclassical inheritance, and complex genetic systems. Physical mapping is now sequence-based, constraining order on the linkage map and interpolating for loci without linkage information. It will be shown that this gives dramatic reduction of error. Further improvement will come from ways to define recombination hot spots and cold spots in small regions that cannot be reliably mapped by linkage. These approaches include the decline of allelic association between SNPs and identification of recombination - prone repeat sequences. Positional cloning depends on linkage and linkage disequilibrium (LD) with markers localised on integrated maps, LD providing the greater resolution. Examples will be given for both major loci and oligogenes, using the Malecot equation for allelic association, together with evidence that the latter is substantially more efficient than alternative metrics. Nonclassical mechanisms include imprinted disease loci, dynamic mutation, and possibly concatenated mutation. Complex inheritance has been approached by weakly parametric (nonparametric) methods including variance components and sib pairs that do not separate gene frequency and effect, as well as by parametric methods that combine segregation, linkage, and association with ascertainment correction. Both approaches allow synthesis of evidence over multiple studies, as illustrated by asthma and (at higher resolution) by SNPs within the ACE locus. Finally, brief consideration will be given to current ethical, legal, and social issues raised by population genetics.

P0841. Inflation of type I error when model-free statistics are computed between markers

M. C. Babron, *E. Genin, P. Margaritte-Jeannin, M. H. Dizier, F. Clerget-Darpoux*
INSERM U535; Le Kremlin Bicetre, France
babron@ccr.jussieu.fr

Several model-free methods of linkage analysis compare the observed allele sharing of pairs of affected individuals with the one expected under no linkage given only the genealogical relationship. In multipoint analyses, the allele sharing is estimated taking into account the joint information provided by all the genetic markers in a region. Different statistics, such as the MLS or the NPL, have been proposed. There is evidence for linkage if the value of the statistics at a given marker exceeds a given threshold, function of the number of markers tested. However, the statistics are often evaluated at different locations on and between the markers. When looking at the curves that give the value of the statistics as a function of the position in the map of markers, one striking feature is the little bumps that can be seen between markers indicating that the value of the statistic is higher between than on the markers. The maximum value of the statistics over a region is indeed often found between two markers rather than on the markers themselves although the information content is smaller between the markers. In order to investigate whether this could lead to an inflation of the type-one error, we have performed simulations under the null hypothesis of no linkage and compare the type-one errors under two analysis strategies ; including or excluding intermarker locations. The results are reported for different marker map densities and between-marker steps. The interest of the two strategies is discussed.

P0842. Genetic variation and linkage disequilibrium in 174 human genes

T. Acharya, *J. Schneider, R. Jiang, A. Wood, D. Tanguay, S. Stanley, W. Newell, B. Koshy, R. S. Judson, G. F. Vovis, J. C. Stephens*
Genaissance Pharmaceuticals; New Haven, CT United States
t.acharya@genaissance.com

2,549 SNPs were discovered in approximately 465 kb DNA sequence from 174 human genes with known genomic organization. DNA sequencing was performed on one chimpanzee and 82 unrelated individuals including African-Americans, Asians, European-Americans, and Hispanic Latinos. The data were collected from several gene regions including exons, exon-intron boundaries, untranslated regions and 5 flanking sequences. The average nucleotide diversity for 166 autosomal genes was p=0.063% and q=0.103%, while the average nucleotide diversity for eight X-linked genes was p=0.052% and q=0.070%. Of the four ethnic groups considered, the African-American sample had the highest average nucleotide diversity followed by the Hispanic-Latino, European-American and Asian groups. Haplotypes were inferred using a variation of the Expectation-Maximization algorithm, and linkage disequilibrium (D) was calculated between pairs of SNPs within each gene. Because allele frequencies affect linkage disequilibrium calculations, only SNPs that occurred at least five times in each population were considered in these analyses. The ~8,000 remaining pairwise comparisons were stratified by ethnic group and physical distance between SNPs. In general, the European-American sample had the highest levels of linkage disequilibrium and the African-Americans the lowest, although the median D value for all ethnic groups was 1, suggesting high overall levels of intragenic linkage disequilibrium. The observed relationship between linkage disequilibrium and physical distance was highly unpredictable; however, most of our inter-SNP physical distances were less than 10 kb. The complex relationship between linkage disequilibrium and physical distance within genes highlights the need to construct detailed, population-specific, empirical linkage disequilibrium maps of the human genome.

P0843. SNPs in the apo(a) unique kringles-population spectra and effect on the Lp(a) plasma levels

M. S. Ogorelkova[1], *H. Kraft*[2], *C. Ehnholm*[3], *G. Utermann*[4]
[1]*Institute of Medical Biology and Human Genetics, Univ. of Innsbruck, 6020 Innsbruck, Austria; Montreal, PQ Canada;* [2]*Instute if Medical Biology and Human Genetics; Innsbruck, Austria;* [3]*Natl. Public Health Institute; Helsinki, Finland;* [4]*Institute of Medical Biology and Human Genetics; Innsbruck, Austria*
miraog@netscape.com

Lipoprotein(a) [Lp(a)] is a complex of apolipoprotein(a) [apo(a)] and low density lipoprotein which is associated with atherothrombotic disease. Lp(a) plasma levels are controlled to a large extend by the apo(a) gene locus. Known polymorphisms in the apo(a) gene including the kringle IV-2 variable number of tandem repeats explain only part of the large interindividual variability and do not explain the differences in Lp(a) concentrations

between major human ethnic groups. We here performed screening for single nucleotide polymorphisms (SNPs) in exons and flanking intron sequences of the apo(a) kringle IV types 6, 8, 9, and 10 which represent 1.3 kb of coding sequence in two African (Khoi San, Black South Africans) and one Caucasian (Tyroleans) populations and investigated whether they affect Lp(a) levels. Together 768 alleles were analysed. We identified fourteen SNPs including eleven non synonymous, eight of which involved conserved residues, one splice site and two synonymous base changes. No sequence variants common to Africans and Caucasians were found. Some of the newly identified SNPs showed significant effects on Lp(a) plasma concentrations, e.g. the substitution G17R in K IV-8 was associated with Lp(a) levels significantly below average in the two African samples. On the contrary the R18W substitution in K IV-9 which occurred with a frequency of ~8% in Khoi San resulted in a significantly increased Lp(a) concentration. Together our data show that several SNPs in the coding sequence of apo(a) affect Lp(a) levels.

P0844. CYP21 gene SNPs and C4B Taq I RFLP disclose 54 haplotypes for the steroid 21-hydroxylase deficiency alleles in Brazil

M. P. De Mello, *M. de Araujo, S. H. V. Lemos-Marini, G. Guerra-Jr, M. T. M. Baptista*
Universidade Estadual de Campinas; Campinas, Brazil
mmello@unicamp.br

Congenital adrenal hyperplasia (CAH) due to steroid 21-hydroxylase deficiency is a common inherited defect of adrenal steroid hormone biosynthesis. Unusually for a genetic disorder, the majority of mutations causing CAH result from recombinations between the CYP21 gene encoding the 21-hydroxylase enzyme and the closely linked, highly homologous pseudogene CYP21P. The CYP21 and CYP21P genes are located in the major histocompatibility complex class III region on chromosome 6p21.3, a region that undergoes high recombination rates. Studies on the molecular basis of steroid 21-hydroxylase deficiency in Brazil have revealed the presence of fourteen mutations distributed among 117 chromosomes. The percentages of individual mutations does not differ from those found in different populations. We have performed haplotype analysis on a total of 111 chromosomes using Taq I CPY21 and C4 RFLP/Southern blotting, mutation-specific PCR and PCR/RFLP for two intragenic SNPs in order to evaluate the chromosomal background of ten recurrent mutations and four new mutations. Fifty four different haplotypes were identified.
Number of CYP21 gene affected alleles and haplotypes

CYP21 Mutation	Number of alleles	Number of haplotypes	CYP21 Mutation	Number of alleles	Number of haplotypes
Gene deletion	10	4	Gene conversion	15	8
A/C656 G	– 27	10	F306T+	3	2
D8	3	2	Q318X	9	7
I172N	19	12	R356W	5	3
V281L	8	3	Q318X/ R356W	4	4

The number of haplotypes varied from five to nine for each the five most frequent mutations. These data reflect the wide heterogeneity of the Brazilian population, and show that most recurrent mutations on the CYP21 gene are from various independent origins and do not present founder effect. Supported by Grants from; CAPES, CNPq and FAPESP

P0845. Origin of parkin gene mutations in Europe

M. Periquet[1], *C. B. L cking*[1], *V. Bonifati*[2], *A. D rr*[1], *G. De Michele*[3], *T. Gasser*[4], *M. M. B. Breteler*[5], *N. W. Wood*[6], *A. Brice*[1]
[1]*INSERM U289; Paris, France;* [2]*Universit La Sapienza; Rome, Italy;* [3]*Universit Federico II; Naples, Italy;* [4]*Klinikum Grobhadern; Munnich, Germany;* [5]*Harvard School of Public Health; Rotterdam, The Netherlands;* [6]*Queen Square; London, United Kingdom*
periquet@ccr.jussieu.fr

A wide variety of mutations in the parkin gene, including exon deletions and multiplications, as well as point mutations, result in autosomal recessive early-onset parkinsonism. Interestingly, several of these anomalies were found repeatedly in independent patients and may therefore result from recurrent (de novo) mutational events or founder effects. In the present study, haplotype analysis with 10 microsatellite markers covering a 4.7 cM region containing the parkin gene, was performed in 48 families with early-onset autosomal recessive Parkinson s disease, mostly from European countries. The patients carried 14 different mutations of the parkin gene that were detected more than once. Our results support the hypothesis that exon rearrangements occurred independently whereas some point mutations, found in families of different geographical origins, may have been transmitted by a common founder.

P0846. Analysis of polymorphisms in the human beta-defensin 1 and 2 genes

A. L. E. Vankeerberghen[1], *O. Scudiero*[1], *G. Castaldo*[2], *H. Cuppens*[1], *F. Salvatore*[2], *J. J. Cassiman*[1]
[1]*University of Leuven; Leuven, Belgium;* [2]*University of Naples, Federico II; Naples, Italy*
anne.vankeerberghen@med.kuleuven.ac.be

The consequences of pulmonary disease are the main death cause of cystic fibrosis patients. Nevertheless, no complete association between the severity of lung disease and the CFTR genotype has been found. Therefore, it is hypothesized that other genetic factors, like defensins, components of the innate immune system, might influence the sensitivity of CF patients to pulmonary infections. Mutations in these peptides could increase or decrease their activity and thereby influence the sensitivity to bacterial infections. In order to analyze the possible modulatory role of human beta-defensin (hBD)1 and 2 in pulmonary diseases associated with CF, the hBD1 and 2 intron boundaries and exons of 60 Italian homozygous ˇF508 CF patients, showing different levels of pulmonary disease, and of 90 Belgian and 90 Italian control individuals were amplified by PCR and sequenced on the ABI 377. Most of the nucleotide changes found in the 5 UTR, the 3 UTR and the introns are very frequent (more than 20%). However, no frequency differences were found between the control and the affected populations and between the Italian and the Belgian controls. Also, no correlation with the age of first colonization of CF patients by Pseudomonas aeruginosa was observed. In hBD2 intron1, multiple insertion/deletion polymorphisms were found. The coding regions of both genes are prone to rare nucleotide changes with some of them causing amino acid changes, four in hBD1, G22C and C67S in Belgian controls, and H34R and V38I in Italian CF patients, and only one in hBD2, M16I (Italian CF patient and Italian control). The promoters are very polymorphic but these nucleotide changes are mostly very rare. We can conclude that both genes are very polymorphic. No association with CF has been found up to now. Further analysis could, however, increase our insight into the role that defensins play in the innate immune system, our first defense against microbia.

P0847. Homogeneous Phase Assays Utilizing Novel Fluorescent Primers for Detection of Single Nucleotide Polymorphisms in Allele Specific Amplification Reactions.

J. Solus, *M. Darfler, A. Gallego, I. Nazarenko*
Life Technologies; Rockville, United States
jsolus@lifetech.com

Homogeneous phase fluorescence-based assays provide powerful tools for detection of specific nucleic acid sequences. We have developed assays based on the use of primers labeled with a single fluorescent dye which do not require a specific quenching moiety. These fluorescent primers exhibit a large increase in intensity when incorporated into double stranded DNA. This property provides a platform for real-time or endpoint detection of nucleic acids in a closed tube and requires no additional steps subsequent to PCR. We demonstrate the use of fluorescein-labeled primers to detect product in allele specific amplification (ASA) reactions on SNP targets. The effect of changes in the design of the ASA primer for improved discrimination is shown. We also demonstrate the use of a novel DNA polymerase which improves discrimination. Homogeneous phase detection of the allele specific products is demonstrated by real-time analysis during the PCR or by endpoint analysis in a fluorescence plate reader. Alternatively, multiplex PCR of several SNP targets of different sizes can be resolved on an automated fluorescence sequencer. This approach, although not utilizing the potential for homogeneous phase detection, offers the advantage of higher throughput for analysis of multiple SNP targets. This system is shown to be readily adaptable to a universal allele format where the ASA primer contains a 5 tail identical to the 3 portion of the labeled primer. The identification of alternate labels to fluorescein permits single tube genotyping. The combination of improvements to allele specific amplification with a flexible homogeneous phase fluorescent detection system provide a simple and reliable method for genotyping of SNP targets.

P0848. A demogenetic analysis of lipoprotein lipase D9N mutation carriers in the Saguenay population (Quebec, Canada)

M. Tremblay[1], *J. Lambert*[1], *D. Gaudet*[2]
[1]*University of Quebec at Chicoutimi; Chicoutimi, PQ Canada;* [2]*Lipid Research Group, Chicoutimi Hospital; Chicoutimi, PQ Canada*
marc_tremblay@uqac.uquebec.ca

D9N mutation in the lipoprotein lipase gene (LPL) is associated to a dominant risk of coronary disease, with variable expression and penetrance, and to arterial hypertension susceptibility. Clinical studies performed at the Chicoutimi Hospital (Quebec, Canada) suggest a relatively high prevalence of this mutation in the Saguenay population. The present study aims at explaining the distribution and origins of the D9N mutation in this population with the use of genealogical data. A sample of 39 carriers was drawn from files at the Chicoutimi Hospital Lipid Clinic. Genealogical data was obtained from the BALSAC population register. This register was also used to choose control individuals and reconstruct genealogies. These extended and deep-rooted genealogies go back to the 17th century (up to 16 generations), with an average depth of 10 generations. More than 12000 distinct ancestors were identified in both groups (carriers and controls). Measures of kinship, inbreeding and ancestors concentration showed significantly higher levels within the carriers group. Intergroup kinship coefficients were also higher than controls intragroup kinship coefficients. However, no great difference was observed between the two groups as regards to the ancestors geographical origins. Most regional founders (19th century) came from the adjacent region of Charlevoix, while early founders (17th century) came mainly from the French regions of Normandie, Ile-de-France, Poitou and Aunis.

P0849. Establishment of a DNA bank from the European population as a resource for studies of genetic variability

Y. Hua, *S. Cichon, N. Kluck, P. Propping, M. N then*
Institute of Human Genetics, University of Bonn; Bonn, Germany
y.hua@uni-bonn.de

A DNA bank is compiled including 96 samples of 30 European countries representing different population size. Cell lines have been established from every individual to ensure an unlimited DNA source. The DNA bank will be available on request to interested research groups from 2001. The DNA bank forms the basis for the second part of the project that aims at a systematical survey of genetic variability in target proteins of CNS-active drugs. Receptor genes of neurotransmitters as well as genes that are located downstream in the intracellular signaling pathways are considered primary candidate genes. We are using a high-throughput sequencing approach to identify genetic variants.

P0850. TaqMan Genotyping of 64 drug metabolizing enzyme polymorphisms and their allelic frequencies in 4 different populations

S. R. Asquith[1], *A. Yeo*[1], *D. J. Dow*[1], *T. M. Saunders*[1], *R. Hussein*[1], *B. Bahari*[1], *D. S. Montgomery*[2], *S. X. Pillai*[3], *M. Dickins*[1], *E. Foot*[2], *I. J. Purvis*[1], *M. J. Stubbins*[1]
[1]*GlaxoWellcome R&D; Ware, United Kingdom;* [2]*GlaxoWellcome R&D; Greenford, United Kingdom;* [3]*GlaxoWellcome R&D; Research Triangle Park, NC United States*
ms85772@glaxowellcome.co.uk

The study of polymorphisms in enzymes associated with the absorption, distribution, metabolism and excretion (ADME) of xenobiotics is a critical factor in our ability to rationalise an individual s response to different medicines. To date, there has been very little data presented on the frequency of these so-called metabolic polymorphisms in specific poplulations, the most detail being available currently for the cytochrome P450 isozymes CYP2D6 and CYP2C19. Here we have attempted to expand our knowledge in this area by developing a panel of TaqMan assays for the routine genotyping of over 60 metabolic polymorphisms. This study will also enable us to begin to answer a question which is perhaps more important for the Pharmaceutical Industry - how representative are clinical trials of the general population? Further statistical analyses may enable us to correlate the genotype data with various phenotypic parameters (measured during clinical studies), thus enabling us to move towards the prediction of an individual s metabolic capability with respect to a medicine, by virtue of their genotype. In conclusion, this report describes the design and development of low volume TaqMan genotyping assays for 64 potentially functional polymorphisms in various ADME-associated genes. We have subsequently used these assays to determine the allele frequencies of these polymorphisms in four defined populations. The information presented here will be a valuable aid to the further understanding of population differences in this important area of research.

P0851. Association of Human IL-1b (-511) Polymorphism with early Onset Periodontal Disease (EOP)

M. R. Bazrafshani[1], *W. E. R. Ollier*[1], *M. H. Thornhill*[2], *A. H. Hajeer*[1]
[1]*ARC Epidemiology Research Unit; Manchester, United Kingdom;* [2]*Oral Disease Research Center, The Royal London School of Medicine and Dentistry; London, United Kingdom*
Mohammad.R.Bazrafshani@fs1.ser.man.ac.uk

Background; Early onset periodontal diseases (EOP) are a group of inflammatory disorders characterised by a rapid rate of periodontal tissue destruction, in young individuals. There is now substantial evidence to suggest that genetic factors play a role in the pathogenesis of EOP. Periodontal disease is a major public health issue of worldwide significance. It is a chronic inflammatory disease of the supporting tissues of the teeth, starting with gingivae and progressing to gradual destruction of the bony support and peridontal attachment of the teeth. This results in significant morbidity, with loosening and loos of teeth the ultimate outcome. Indeed, improved prevention and treatment of tooth decay, has resulted in peridontal disease becoming the most common cause of tooth loss in Europe and the USA. Polymorphisms in cytokine genes, which may underpin inter individual differences in cytokine synthesis and secretion have been associated with other diseases which have an inflammatory pathogenesis, Genetic variations found within candidate genes in EOP patients may represent a mechanism by which individuals are rendered susceptible to disease. Objective; To investigate whether a biallelic polymorphism (A or G) occurring within the promoter region of the IL-1b gene (position -511) is associated with EOP. Methods; The -511 polymorphism was detected using a PCR-RFLP method. IL-1b polymorphism was examined in 97 patients with EOP and 91 healthy matched UK controls.
Result

IL-1ß-511 polymorphism allel and genotype distribution in EOP patients

	Controls		EOP patients	
IL-1ß genotype	n	%	n	%
Allele frequency				
-511*G	53	29.1	83[1]	42.8
-511*A	129	70.9	111	57.2
Genotype				
G/G	7	7.7	17[2]	17.5
G/A	39	42.9	49	50.5
A/A	45	49.4	31	32.0

[1]O.R.=1.8 C.I. 95% 1.2-2.8, P<0.05
[2]O.R.=2.6, C.I. 95% 1.0-6.3, P<0.05

Conclusion; Several polymorphisms exist in the IL-1 cluster that influences the IL-1b biological activity. Our results demonstrated a possible role for IL-1b gene in the development of EOP. Key Words; 1- EOP 2- Polymorphism 3- IL-1b 4- RFLP

P0852. The Polymorphism Detection of Human Genomic AA-NAT Gene

W. Ying
National University of Singapore; Singapore, Singapore
medp9087@nus.edu.sg

The role of melatonin in normal sleep-wake regulation has been inferred from the temporal relationships between its rhythmic synthesis and the 24 hours cycle. The biochemical basis of this hormone rhythm is the penultimate rate-limiting enzyme in melatonin synthesis, Arylalkylamine N-acetyltransferase (AANAT). [Axelrod, 1974; Klein, 1985; Namboodiri et al., 1985]. Specifically, our current study focuses on characterizing the AANAT gene in a local population and looking for possible genetic variability that may explain the widely documented variation in sleep pattern among normal human individuals. A self-assessed sleep pattern survey was conducted among 210 healthy, young and drug-free human subjects based on a whole week practice. Data pertaining to normal sleep onset, offset, and total nocturnal sleep duration was obtained. Circulating blood samples were collected from 4 individuals with early onset and long sleep length, and 5 individuals with late onset and short sleep length. Polymerase chain reaction (PCR) amplification of the genomic DNA containing all of the four exons of AANAT gene was done. Analysis of PCR products by direct sequencing demonstrated that all the 36 sequences were identical with the published data. Therefore, there is no evidence of genetic variability in the coding region of AANAT gene that may explain the extreme differences in sleep pattern. More studies are being conducted to examine the promoter regions for AANAT.

P0853. Segregation Analyses of Asthma and Respiratory Allergy; The Humboldt Family Study

Y. Chen[1], *A. Schnell*[2], *D. Rennie*[3], *R. Elston*[2], *L. Lockinger*[3], *J. Dosman*[3]
[1]*University of Ottawa; Ottawa, ON Canada;* [2]*Case Western Reserve University; Cleveland, United States;* [3]*University of Saskatchewan; Saskatoon, Canada*
chen@zeus.med.uottawa.ca

We performed segregation analyses of asthma and respiratory allergy based on data from 309 nuclear families comprising 1,053 individuals living in the town of Humboldt, Saskatchewan in 1993, using the REGD program of the S.A.G.E. program package. For adults, information on asthma and history of respiratory allergy was provided by the subjects themselves, and for children by their parents. When asthma was considered as the trait in segregation analysis, models of no major effect, with or without familial effects, were rejected, but were not rejected after adjusting for history of respiratory allergy. The major gene hypothesis was not rejected before adjusting for history of respiratory allergy. When respiratory allergy was analysed as the trait, both major gene and multifactorial models fitted the data well regardless of whether there was adjustment for asthma or not. Other covariates adjusted for in the segregation analyses were age, sex, number of household smokers, current smoking, number of household members, generation, and house type. The data suggest that a major gene related to respiratory allergy may explain the familial aggregation of asthma.

P0854. GLN223ARG SNP in the leptin receptor gene; studies in anorexia

A. I. F. Blakemore[1], *N. D. Quinton*[1], *D. W. Meechan*[1], *L. F. Pieri*[2], *K. M. O. Brown*[3], *H. Eastwood*[3]
[1]*Sheffield Hallam University; Sheffield, United Kingdom;* [2]*Yorkshire Centre for Eating Disorders; Leeds, United Kingdom;* [3]*University of Leeds; Leeds, United Kingdom*
a.i.blakemore@shu.ac.uk

Leptin, a cytokine expressed and secreted by the adipose tissue, is involved in the regulation of body weight. Leptin levels in subjects with anorexia nervosa are low, although the correlation with body mass index is still evident. We, and others, have shown an association between the leptin receptor SNP, GLN223ARG, and fat mass, body mass index (BMI) and leptin levels. The aim of this study was to determine if this association could be found in young female, Caucasian control subjects and if differences in allele frequency existed in female Caucasian subjects with anorexia nervosa. 175 subjects with anorexia nervosa (divided into bingeing/purging and restricting anorexics) were recruited from the Yorkshire Centre for Eating Disorders at Seacroft Hospital. 145 controls were recruited from the University of Leeds. In both cohorts, allele frequencies for GLN223ARG, did not differ significantly from published frequencies (A allele 0.61 and 0.56, G allele 0.39 and 0.44, in anorexia and control cohorts respectively). There was no significant difference in genotype frequency between the control and the anorexic cohorts or between restricting and bingeing subjects with anorexia. The previously published association between BMI and genotype was not observed in either cohort. This may strengthen the existing evidence that this association is only observed in older middle-aged males and postmenopausal subjects.

P0855. Segregation Analysis (sa) Of Heart Rate (hr)

M. B. Bravo-Luna, *C. A. R. Marcon, I. R. Tunkieviez, B. M. Perez*
Faculty of Medicine; Rosario, Argentina
mbravoluna@hotmail.com

Genetic variation, important feature regarding any trait is desirable. In hypertension loss of heart rate (HR) variability leads to poor prognosis. HR, semiquantitative discrete trait, regulated by the ANS, is involved in blood pressure (BP) regulation, which is admitted that is genetically determined, although the mechanism is controversial, due to the many regulation components. There are papers supporting the BP Mendelian inheritance (MH) hypothesis, though mixed models admitting the involvement of polygenes and environmental influences are more appropriate. We have published (Med Hypotheses(2000)54(2)307-9 a hypothesis considering the probable MH of HR. Objective; To demonstrate through SA methods HR-MH. Methods; 544 individuals (148 pedigrees) were monitored, in basal and comparable conditions, during 5 minutes in 3 different occasions. Data were entered in an ad hoc software to perform SA, mixed model of Morton-Maclean, taking into account 15 parameters to be analysed through 11 proposed models, estimating [-2Ln (LH)] (log. likelihood ratio), in a most parsimonious model. Heritability (H2) (regression coefficient) and D2 [D2=H2+(1-H2)R2], variance proportion of the trait measuring genetic and non-genetic factors, were estimated. Results [X2;d.f.;p;H2;D2], Mendelian model, transmissibility set free; 3.586;2;n.s.;0.60;0.70, proved the best model for this trait admitting MH without ruling out polygenes and environmental influence.

P0856. Analysis of the modifying effects of TAP 1 and TAP 2 genes on Cystic Fibrosis phenotype

F. Ozbas Gerceker[1], *U. Ozcelik*[2], *D. Anadol*[2], *N. Kiper*[2], *A. Gocmen*[2], *E. Yilmaz*[3], *H. Erdem Yurter*[3], *M. Ozguc*[1]
[1]*TUBITAK DNA/Cell Bank & Gene Research Laboratory; Ankara, Turkey;* [2]*Hacettepe University Medical School Pediatric Chest Disease Unit; Ankara, Turkey;* [3]*Hacettepe University Medical School Department of Medical Biology; Ankara, Turkey*
ozbas@hacettepe.edu.tr

Cystic Fibrosis is an autosomal recessively inherited genetic disease which results from the mutations in the gene encoding Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)protein. It is important to analyze the modulating and modifying effects on phenotype since we have genotypic and phenotypic heterogeneity among patients. In our study, we investigated TAP 1 and TAP 2 gene polymorphisms in CF. The TAP genes (transporter associated with antigen processing) encode two membrane-spanning ABC proteins that translocate antigenic peptides from the cytoplasm into the endoplasmic reticulum. TAP 1 and 2 genes are localized within the MHC class II region. Polymorphisms in the TAP genes are associated with the specificity of the binding of antigenic peptides. Comparison of 58 CF patients whose CFTR mutations were determined previously and 100 Turkish controls revealed a significant increase in the Ala/Ala dimorphism at position 665 of TAP 2 gene in CF patients as compared to controls ($p<0.025$). Furthermore, Gly phenotype at position 637 of TAP 1 gene was seen in CF patients in a significantly higher ratio ($p<0.05$).

P0857. Apolipoprotein E genotypes in a Turkish Population with renal disease; Preliminary findings

A. Be?er[1], *H. Sarı*[2], *R. Bircan*[1], *S. Manay*[1], *M. Ko* [2], *I. G ney*[1], *. zener*[2], *B. ırako?lu*[1]
[1]*Marmara University Faculty of Medicine Dept. of Medical Biol. & Genetics; ?stanbul, Turkey;* [2]*Marmara University Faculty of Medicine Internal Medicine Nephrology Department; ?stanbul, Turkey*
arbeser@hotmail.com

Background; Apolipoprotein E plays an important role in regulation of cholesterol and lipid metabolism. This study is performed to investigate the APO E allele genotypes in Turkish population and to determine the association among the APO E genotype, serum cholesterol levels, blood lipid profile and renal disease in a population of 286 people including 186 end stage renal disease patients (100 on hemodialysis and 86 on chronic ambulatory peritoneal dialysis (CAPD))and 100 control cases. Methods; The APO E genotypes are investigated with polymerase chain reaction. Amplified DNA fragments are digested with Hha I enzyme and analyzed with %4 agarose gel electrophoresis. Trigliseride and total cholesterol levels are determined by enzymatic colorimetric assay and HDL by homogenous enzymatic colorimetric assay. VLDL, LDL and body mass index are calculated. Results; The results of apolipoprotein E genotyping obtained in 55 patients are; 20 analyzed in the CAPD group;4 E2/E2, 12 E3/E3, 1 E4/E4, 1 E2/E3, 1 E2/E4, 1 E3/E4; 15 analyzed in the hemodialysis group;11 E3/E3, 4 E3/E4; 20 analyzed in the control group;15 E3/E3, 3 E2/E3, 2 E3/E4. Conclusion; Recording of the biochemical data is complete but the number of subjects whose APO E genotypes have been determined is not sufficient to make statistical calculations. After completion of genotyping, the APO E genotypes will be correlated with the cholesterol levels, lipid profile and the body mass index. Finally the distribution of APO E alleles between the normal population and the patients with ESRD will be evaluated.

P0858. Glutation S-transferase polymorfism in populations with different ethnisity.

S. N. Popova, *P. Slominsky*
Institute of Molecular Genetics; Moscow, Russian Federation
popova@img.ras.ru

The glutation S-transferase (GST) are widely expressed in mammalian tissues and involved in phase II detoxification reactions. The GST form a supergene family consisting of four distinct families, named alpha (GSTA), mu (GSTM), theta (GSTT) and pi (GSTP). Several of GST are polymorphic in humans. Among the GST genes we examined GSTT1 and GSTM1 polymorphism in some populations from Russian Federation; Russians from Holmogory and Oshevensk (Arkchangelsk region) and three populations with different level of Mongoloid component; Chant, Kalmyk and Buryat. We observed that frequency of GSTM1 null genotype are significantly higher in Chant, Kalmyk and Buryat than in Russians from Arkchangelsk reg. For GSTT1 gene null genotype frequency was statistically higher for

Kalmyk (p=0,03), but in Chant and Buryat it was similar as in Russians from Arkchangelsk region. Frequency GSTM1 (0/0), GSTT1 (0/0) in sum for Kalmyk was 18%, there as in other populations it wasn t bigger than 5%. Comparing Mongoloid populations with Russians using Chi squred-criterium and G-statistic one can see that Kalmyk population mostly varies from Russians populations, and differences between Chant, Buryat and Russians are less expressed. We can note that population differences are more conditioned by difference in GSTT1 genotype frequency. Gene GSTM1 has less marked divergence in populations, and this gene frequencies hardly vary for Oshevensk, Holmogory and Chant populations.

P0859. The TNFRSF6 gene is not implicated in familial early-onset Alzheimer s disease

J. Theuns[1], *L. Feuk*[2], *M. Cruts*[1], *J. Del-Favero*[1], *B. Dermaut*[1], *G. Roks*[3], *D. Van den Bossche*[1], *E. Corsmit*[1], *M. Van den Broeck*[1], *C. M. van Duijn*[3], *A. Brookes*[2], *C. Van Broeckhoven*[1]

[1]*Department of Molecular Genetics, Flanders Interuniversity Institute for Biotechnology (VIB), University of Antwerp (UIA); Antwerpen, Belgium;* [2]*Center for Genomics Research, Karolinska Institute; Stockholm, Sweden;* [3]*Department of Epidemiology & Biostatistics, Erasmus University Medical School; Rotterdam, The Netherlands*

theuns@uia.ua.ac.be

The tumor necrosis factor receptor superfamily member 6 (TNFRSF6) gene encoding FAS, a cell-surface receptor involved in apoptosis initiation, was recently reported to constitute a genetic risk factor for early-onset Alzheimer s disease (EOAD). Dynamic allele-specific hybridization (DASH) analysis of the —670 G/A polymorphism showed enrichment of the homozygous GG-genotype in Scottish sporadic EOAD cases, almost completely attributable to enrichment within the subset of APOE4 carriers (Feuk et al.,2000). In this study, we analyzed the promoter polymorphism in a Dutch population-based EOAD case-control sample, using the same technique, but could not detect a significant disease association. Thus our study does not reinforce the hypothesis of an independent involvement of the TNFRSF6 -670 G/A polymorphism in AD risk. In this study we also compared the reliability of DASH and pyrosequencing analysis and demonstrated the robustness of the two techniques by genomic sequencing.

P0860. The frequencies of chemokine receptor CCR5 polymorphisms affecting susceptibility to human immunodeficiency virus (HIV) and the rate of progression to AIDS in Kuwaitis

A. Voevodin

Faculty of Medicine; Kuwait University, Kuwait

voevodin@hsc.kuniv.edu.kw

Chemokine receptor CCR5 serves as the major co-receptor for HIV-1. Several polymorphisms in the CCR5 gene are known to affect the susceptibility/resistance to HIV-1 and/or the rate of progression to AIDS. The frequencies of these polymorphisms as well as their infection/disease modifying effect vary in different populations. Most of the data available in this field were obtained in the studies of Americans of various ethnic/racial origin. Very little in this respect is known about Arabic populations. We studied the frequencies of two CCR5 gene polymorphisms; m303 and 59029-A/G in Kuwaitis. The genotyping for both polymorphisms was carried out by PCR-RFLP tests. The m303 allele was not found in any of 230 Kuwaitis genotyped for this marker (the allele frequency is less that 0.002). The frequency of protective allele 59029-G in Kuwaitis (0.66, no. of chromosomes = 266) was higher than that in other populations for which the frequencies of the allele have been reported. The modifying effect of 59029-A/G genotype as well as two other genetic markers associated with slower progression to AIDS (CCR2-64I and SDF-3 A) is being investigated in HIV-infected Kuwaitis. Acknowledgement. This study was supported Research Grant MI 120 from Kuwait University.

P0861. Association of paraoxonase Gln -Arg 192 polymorphism with late-onset sporadic Alzheimer s disease and coronary artery disease in ultraoctuagenarians.

R. Corbo[1], *G. Gambina*[2], *T. Vilardo*[3], *L. De Bernardini*[4], *R. Scacchi*[5]

[1]*Dept. of Genetics and Molecular Biology, University La Sapienza ; Rome, Italy;* [2]*Division of Neurology, Hospital; Verona, Italy;* [3]*Surgery for the Prevention of the Vascular and Metabolic Diseases, S. Giovanni Hospital; Rome, Italy;* [4]*Geriatric Hospital Villa delle Querce ; Rome, Italy;* [5]*CNR Center of Evolutionary Genetics; Rome, Italy*

Corbo@axcasp.caspur.it

Recent advances suggest a possible relationship between Alzheimer s disease (AD) and atherosclerosis. This makes interesting to examine the coronary artery disease (CAD) candidate gene polymorphisms in AD too. In the present investigation we examined a polymorphism in the paraoxonase gene (PON1) due to an A -> G substitution at amino acid position 192, which has been found associated with cardiovascular diseases, though with conflicting results. A group of late-onset sporadic AD patients and a group of patients with CAD were studied in order to compare the results between them and with normal subjects. Most of the patients were ultraoctuagenarians. Significant differences in the allele frequencies between patient groups and correspondent controls were observed. In particular the AD patients aged ? 80 (n= 152) showed a frequency of the PON1*Q allele significantly higher than the control group (0.832 vs 0.741, p = 0.003). Also CAD patients aged ? 80 (n=56) had a PON1*Q allele frequency (0.848) higher than their controls (0.743) (p=0.04). A CAD patient group aged < 80 (n= 144) showed an opposite pattern with an PON1*R allele frequency (0.260) significantly higher than their controls (0.154) (p=0.01), confirming the previously reported association of PON1 *R allele with CAD. In summary our data suggest that the association of PON1*Q with both AD and CAD is peculiar to ultraoctuagenarian patients.

P0862. The dynamics of congenital malformations in Tomsk region according the results of 20-year s epidemiological research

N. Kricunova, *L. Nazarenko*

Institute of Medical Genetics; Tomsk, Russian Federation

kricunova@img.tsu.ru

The variation of structure and frequencies of congenital malformations (CM) in Tomsk region during the 20- year s period of monitoring (1979 — 1998) has been analysed. Epidemiological study has revealed the cyclic character of the CM frequency fluctuation in range from 13,9 to 35,2 , with the mean 23,65 . Significant increasing in the frequency of overall CM spectrum has been disclosed (from 22,8 up 26 , P<0,01). The summary frequency of the isolated forms of CM has increased significantly, but the frequencies of multiple CM and Down s syndrome have decreased. The average frequency of 19 forms of CM registered according the European register EUROCAT has been estimated as 14,2 , not differing from the basic level of CM. In CM structure, the most frequent were the defects of osteomuscular system, multiple CM, nervous system, cardiovascular system, face and neck. Regarding the separate CM forms, the most frequent were congenital heart defects, Down syndrome, hydrocephaly and hypospadia.

P0863. Epidemiological data about birth defects in Ural s region (Russia, Ekaterinburg)

M. Devaikina[1], *N. Demikova*[2], *G. Pavlov*[1], *E. Nikolaeva*[1], *I. Sklyar*[1], *N. Nikitina*[1]

[1]*Medico-Genetic Center; Ekaterinburg, Russian Federation;* [2]*Institute of pediatria and child surgery; Moscow, Russian Federation*

gvp@online.ural.ru

Since 1992 birth defects of newborns have been registered in Ural s region.

Since 1999 International Clearinghouse for Birth Defects Monitoring System was introduced into practice. 21 birth defects are due to be registered. Multiple sources of information are registration forms from maternal houses, child clinics, pathology departments.

From 1992 to 2000 348075 newborns were borne in the region, and 6161 of them had birth defects. Frequency of birth defects was 17 in 1000 births (1,7%).

The structure of birth defect s diagnoses; heart s defects — 21%, neural tube defects —13%, defects of musculoskeletal system — 10%, defects of urinary system — 9%, multiple congenital anomalies — 8%.

In 1999 35877 newborns were borne, and 1161 of them had birth defects. Frequency of birth defects was 32 in 1000 births (3,2%). The structure of birth defect s diagnoses; heart s defects — 25%, defects of musculoskeletal system - 15%, neural tube defects — 12%, multiple congenital anomalies —11%, defects of urinary system —8%.

Frequency of some birth defects in 1999; anencephaly — 0,6 in 1000, spina bifida — 1,2 in 1000, hydrocephaly — 1,6 in 1000, cleft lip with or without cleft palate — 1,2 in 1000, polydactyly — 1,6 in 1000, anotia and microtia — 0,8 in 1000, total Uml reduction defects — 0,3 in 1000, Down syndrome — 1,3 in 1000 births.

For drawing well- grounded conclusion about epidemiology of congenital anomalies, it s necessary to monitor birth defects during next two years.

P0864. Polymorphism of Apo AI-CIII Gene Cluster in Korean Essential Hypertensives

J. Shin[1], *B. Kang*[2], *K. Kim*[2], *C. Lee*[1]

[1]*School of Biological Sciences, Seoul National University; Seoul, Republic of Korea;* [2]*Seoulin Bioscience Institute, Seoulin Bioscience, Co., Ltd.; Seoul, Republic of Korea*

nariann77@yahoo.com
The apolipoprotein AI and CIII (apo AI and CIII) play an important role in the metabolism of plasma lipoproteins and lipids. The apo AI-CIII gene cluster is located in chromosome 11q23 and more than 20 different RFLPs have been described in this gene cluster. To search for a useful genetic marker for the essential hypertension in Korean population, the distribution of two restriction fragment length polymorphism (RFLPs) of the apo AI-CIII gene cluster and their association on essential hypertension was investigated in total 163 Korean individuals. The distribution of the genotypes of all the RFLPs was in Hardy-Weinberg equilibrium in this population. the G-75?A polymorphism of the apo AI gene was significantly associated with essential hypertension in Korean population (P<0.05). Therefore, this result suggest that the G-75?A polymorphism of the apo AI gene may be useful as a genetic marker for essential hypertension in Korean population.

P0865. Apolipoprotein E Genotypes And Diabetic Retinopathy In Iddm Patients

N. S. Shcherbak[1], *A. Stroykova*[2], *E. Schwartz*[1]
[1]*St.Petersburg State Medical University; St.Petersburg, Russian Federation;* [2]*St.Petersburg Diabetic Center; St.Petersburg, Russian Federation*
nshch@yahoo.com
Apolipoprotein E (apoE) was discovered as a plasma protein involved in lipoprotein metabolism. There are three common variants of apoE, resulting from common genetic variation, called E2, E3 and E4. Three-allelic variation in the apolipoprotein-E (APOE) gene have been suggested as risk factors for the development of diabetic micro- and macrovascular complications. We investigated the association between APOE genotypes and development diabetic retinopathy by case-control study in a Russian population. We studied 76 type 1 diabetic patients with diabetic retinopathy (34/42 m/f, age (mean+/-SD) 30+/-10 years, diabetes duration 18+/-8 years) and 96 patients without diabetic retinopathy (50/46 m/f, age 25+/-8 years, diabetes duration 16+/-6 years). APOE polymorphisms were detected by the restriction fragment lenght polymorphism method after a polymerase chain reaction. No did APOE allele frequencies (epsilon2/epsilon3/epsilon4) differ between diabetic patients with and without retinopathy; 0.138/0.776/0.086 vs 0.099/0.802/0.099, respectively. Genotype distributions were also similar, n.s. No associations between diabetic retinopathy and APOE polymorphisms were observed. These results suggest that APOE genotype are not assosiated with the development diabetic retinopathy in patients with IDDM in a Russian population.

P0866. Genetics of Human Female Pelvic Morphology and Menarcheal Status; a Twin Study

K. Sharma
Department of Anthropology, Panjab University; Chandigarh, India
neelamkuki@usa.net
To examine the relative role of genetic and environmental factors on pelvic morphology and menarcheal status, data on 60 pairs of female twins (30 MZ and 30 DZ) were analyzed. Various pelvic mesurements were normally distributed except for two of sixteen instances. Association of twin type with the mean value was found in only one out of eight traits, which might be attributed to type 1 error. Heterogeneity of variance between zygosities was observed for four pelvic traits (50%) suggesting considerable amount of hidden environmental influence. No evidence of stronger environmental covariance for MZ than DZ twins was observed. Significant genetic component of variation was observed for pelvic height, bi-ischeal breadth, age at menarche and pelvic area. In instnces where inequality of variances between zygosities were demonstrated, total among pair and within pair mean squares were larger for dizygotic than for monozygotic twins. This is interpreted as evidence of greater environmental influence between zygosities. Environmental modification shawing phenotypic plasticity was not of same magnitude in various pelvic traits thus indicating differential selection pressures. Similar differences were observed in the magnitude of cultural inheritance.

P0867. CC - chemokine receptor CCR5 gene polymorphism analysis in diabetes type I and type II patients in Estonia.

I. Kalev[1], *K. Oselin*[1], *P. P rlist*[1], *T. Podar*[2], *A. Mikelsaar*[1]
[1]*University of Tartu; Tartu, Estonia;* [2]*Clinicum of Tartu University, Department of Endocrinology; Tartu, Estonia*
ingridk@ut.ee
Chemokines are small chemotactic proteines, that mediate innate and adaptive immune responses and inflammatory processes through the G-protein-coupled receptors. Previous studies have shown, that the temporal expression of certain CC-chemokines and the CCR5 chemokine receptor in the pancreas is associated with the development of the insulitis and spontaneous type I diabetes. It is known, that some time before onset of diabetes type I, inflammatory process takes place in the pancreas. In the present study we have determined the frequency of the ccr5delta32 allele in diabetes type I (n=38; age 15-62, average 34,5y) and diabetes type II (n=113; age 42-77, average 63,3y) patients. In control group of healthy, unrelated native Estonians (n=504; age 14-94; average 54y) the ccr5delta32 allele frequency was 0,148. In diabetes type II cohort and in diabetes type I group the deleted allele frequency was 0,106 and 0,141 respectively (p>0,05). Results indicated, that the deleted allele of the CCR5 receptor gene has no significant association with disease frequency in both types of diabetes. In the case of type I diabetes there was a significant (p<0,05) difference in the onset time and duration of the disease being earlier and longer in the wild-type homozygotes compared to ccr5delta32 heterozygotes. In the type I diabetes the analysis of the correlation between clinical signs and ccr5delta32 genotype is in progress.

P0868. Genetic Susceptibility To The Evolution Of Liver Disease; Chronic Hepatitis C Model

C. Gritti[1], *L. Sonzogni*[1], *L. Silvestri*[1], *L. Rossi*[1], *E. Civardi*[1], *E. M. Silini*[2]
[1]*Associazione Studio Avanzato Epatiti Virali (ASAEV); Bonate Sotto (BG), Italy;* [2]*Department of Pathology, University and IRCCS S. Matteo; Pavia, Italy*
asaev@tin.it
The association between gene polymorphisms affecting drug metabolizing enzymes (DMEs) activity and chronic hepatitis C severity was studied by univariate and multivariate analysis. Two classes of DMEs genes were considered; phase I enzymes involved in oxidative metabolism (CYP2E1, CYP2D6) and phase II enzymes involved in conjugation of toxic compounds (GSTM1, GSTT1, Epoxide Hydrolase-EPHX). Polymorphisms were screened on cohort of 400 anti-HCV+ Italian patients divided into four groups; asymptomatic carriers, chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC) patients. A cohort of 100 healthy subjects was considered as reference category. Inherited differences in several DMEs activities were found to be significantly associate with liver disease expression. In particular, high activity phase I enzymes variants and low activity phase II enzyme alleles occurred more frequently among subjects with advanced liver disease. This effect was more pronounced in males and considering extreme patient categories (carriers and cirrhosis/HCC). Two multivariate analysis techniques (logistic regression and correspondence analysis) were used to model the unconfounded contribution of these genetic traits and their interaction, controlling for known variables of liver disease progression (age, sex, HCV genotype and alcohol consumption). An independent and synergistic effect in predicting cirrhosis and/or HCC was observed for two characters, EPHX T113H homozygosity, and CYP2D6 EM phenotype. Genetic epidemiology techniques may provide clinically meaningful information useful for the dissection of complex disease traits such as those involved in chronic hepatitis C progression.

P0869. Risk of osteoporosis in carriers of 9G>C polymorphism in osteoprotegerin (OPG) gene.

R. Slomski[1], *R. Kalak*[2], *W. Horst-Sikorska*[3], *D. Baszko-Blaszyk*[3]
[1]*Institute of Human Genetics; Poznan, Poland;* [2]*Department of Biochemistry and Biotechnology; Poznan, Poland;* [3]*Endocrinology Clinics, Medical School; Poznan, Poland*
slomski@au.poznan.pl
Scanning of the 5 exons of OPG gene was performed for 85 patients with osteoporosis or osteopenia. PCR-HD and PCR-SSCP analyses of genomic DNA revealed 6 polymorphisms localized in the intronic region and one polymorphism in exon 1. Five of these different polymorphic variants were substitutions; IVS1+15C>T, IVS2+4C>T, IVS3-5C>T, IVS4-24C>A, IVS4+8A>C and one was a 2 bp deletion; IVS3+46delTC. One polymorphism, 9G>C, detected in exon 1 resulted in a change in the encoded amino acid from positively charged lysine to neutral asparagine and was previously observed in cDNA clones. Because this polymorphism affects conserved region of the osteoprotegerin signal peptide and could influence of secreted protein, we studied the polymorphic allele frequencies in the patient and control population and looked for evidence of association between the polymorphism and osteoporosis. The change 9G>C does not create a new restriction site, and therefore PCR-SSCP analysis was used to determine allele status, by comparison with sequenced control samples. First, we observed that genotype distribution was in Hardy-Weinberg equilibrium for control population (CC=23/97, GC=45/97, GG=29/97, c2=0.458, df=2, p=0.79) we then determined OPG genotype distribution for patients with severe osteoporosis; CC=13/56, GC=36/56, GG=7/56. The genotype and allele frequencies in a combined group of 85 patients with osteoporosis or osteopenia revealed CC=25/85, GC=47/85 and GG=13/85. There were no significant differences in lumbar spine BMD for groups of osteoporotic women and men with different genotypes but genotypes with allele

C were over-represented in patients with severe osteoporosis as compared with population group (c2 =5.69, df=1, p<0.02). This is equivalent to odds ratio for osteoporosis of 2.99 for individuals who carry allele C (95% confidence interval 1.20-7.42). Significant differences in combined group of patients with severe osteoporosis or osteopenia, as compared with population group (c2 =5.31, df=1, p<0.025), was also observed (odds ratio 2.36, 95% confidence interval 1.13-4.94).

P0870. Familial Aggregation of Quantitative Traits for Insulin Resistance; The IRAS Family Study

S. S. Rich[1], *J. Norris*[2], *M. Saad*[3], *S. Haffner*[4], *L. Henkin*[1], *D. Zaccaro*[1], *L. Wagenknecht*[1], *R. Bergman*[5]
[1]*Wake Forest University School of Medicine; Winston-Salem, NC United States;* [2]*University of Colorado Health Sciences Center; Denver, CO United States;* [3]*UCLA School of Medicine; Los Angeles, CA United States;* [4]*University of Texas Health Sciences Center; San Antonio, TX United States;* [5]*USC School of Medicine; Los Angeles, CA United States*
srich@wfubmc.edu

Type 2 diabetes mellitus is a complex disease with strong familial aggregation but relatively low genetic risk (?s ~ 2). The strongest risk factors for type 2 diabetes include family history, obesity and factors associated with insulin resistance and §-cell dysfunction, each of which may be mediated in part by genetic factors. The IRAS Family Study characterizes important diabetes-related traits with respect to their familial aggregation and, ultimately, linkage to polymorphic DNA markers, in African-American (AA) and Hispanic-American (HA) families.

A total of 61 families (22 AA and 39 HA) comprising 650 individuals with phenotypic data were used for these analyses. Frequently sampled intravenous glucose tolerance tests (FSIGT) were performed on eligible subjects and analyzed using the minimal model to obtain estimates of insulin sensitivity (S_I), glucose effectiveness (S_G) and insulin secretion (acute insulin response to glucose, AIR). The disposition index (DI), a measure of insulin resistance-corrected § -cell function, was also derived (D_I = AIR*S_I), as was the HOMA measure (HOMA=fasting insulin*fasting glucose/22.5). Variance components analyses were employed to estimate familial aggregation (heritability, h^2) using the SOLAR software package.

After adjustment for age, sex, race and BMI, h^2 estimates were S_I; 19 $\pm$ 8% ($p < 0.001$); S_G; 19 $\pm$ 11% ($p < 0.02$); AIR; 5 $\pm$ 8% ($p = 0.26$); D_I; 4 $\pm$ 7% ($p = 0.29$); and HOMA; 15 $\pm$ 9% ($p < 0.03$). These results suggest that there is modest heritability for S_I and S_G as diabetes-related quantitative traits in this population.

P0871. Hsp70 polymorphism and HLA-DR diversity; Implications for tuberculosis susceptibility in the Cape Coloured population of South Africa

T. Boshoff[1], *A. E. Brune*[1], *E. G. Hoal van Helden*[2], *L. Bornman*[1]
[1]*Biochemistry Division, Rand Afrikaans University; Auckland Park, South Africa;* [2]*Department of Medical Biochemistry, University of Stellenbosch; Tygerberg, South Africa*

Tuberculosis (TB) being one of the leading causes of death from infectious disease in the world, is also a major problem in the Cape Coloured population in the Western Cape of South Africa. Multiple genes influence susceptibility to TB. HLA class II polymorphisms have been associated with susceptibility to inflammatory diseases, but linkage disequilibrium of alleles in the major histocompatibility complex (MHC) complicates identification of disease-associated genes. Genes encoding members of the 70 kDa heat shock protein (HSP)family (Hsp70) are localised within the MHC. In the light of the protective role of Hsp70 in infection and immunity it is hypothesized that Hsp70 polymorhisms may contribute to disease susceptibility. Possible linkage disequilibrium of hsp70 alleles with HLA-DR alleles might lead to extended haplotypes which might act as additive TB susceptibility markers. Hsp70 gene polymorphism (hsp70-1, hsp70-2, hsp70-Hom and the polymorphic PstI site within the coding region of hsp70-2) and HLA class II DR polymorphisms were investigated in the Cape Coloured population inhabiting the Western Cape of South Africa. Polymorphic analysis of hsp70 and HLA-DR genes was performed on genomic DNA from patients suffering from TB (n=60) and matched control subjects (n=61) using PCR-RFLP and PCR-SSP respectively. Preliminary results showed no evidence for an independent role of hsp70 gene polymorphism in susceptibility to TB while DR3 (DRB1*0301-0302) and DR53 (DRB4*0101) were present at a higher frequency (p=0.068 and p=0.034 respectively) in TB patients, and DR12 (DRB1*1201-1203) in control subjects (p=0.028). An additional number of 300-400 cases and controls are currently analysed to meet statistical standards and to investigate linkage disequilibrium of HLA-DR and hsp70 alleles. An improved understanding of the underlying mechanisms contributing to tuberculosis susceptibility may open new avenues in the development of novel therapeutic approaches.

P0872. Factor VII promoter decanucleotide insert polymorphism not associated with decreased Factor VII activity in African American

A. Ratti[1], *R. Kudaravalli*[1], *T. Tidd*[1], *R. Santacroce*[2], *M. Margaglione*[2], *M. Devoto*[3], *P. Fortina*[1], *E. S. Pollak*[1]
[1]*University of Pennsylvania; Philadelphia, PA United States;* [2]*IRCCS Casa Sollievo della Sofferenza; San Giovanni Rotondo, Italy;* [3]*duPont Hospital for Children; Wilmington, DE United States*
fortina@mail.med.upenn.edu

Factor VII is a procoagulant protein that initiates blood clotting with its cofactor Tissue Factor. Polymorphic variations in the Factor VII (FVII) gene correlate with cardiovascular disease risk in both familial and non-familial studies. The FVII A2 allele contains a 10 nt insert (CCTATATCTT at -323 in the FVII promoter where +1 is the start of translation) and occurs in 10-20% of the population. The A2 allele is associated with a 15-30% decreased FVII level per allele. Two additional SNPs at -122(C/T) and -401(G/A) have been reported to occur in complete linkage disequilibrium with the decanucleotide insert in the Italian population. In vitro assays have previously shown a significantly decreased FVII promoter strength of the A2 compared to the A1 allele and have further shown that this requires the concurrent presence of the -122 and -401 polymorphisms. Using both DNA sequence analysis and the Nanogen NanoChipTM, we analyzed 28 African-American individuals for the presence of the -401/ -323/ and -122 polymorphisms. Twenty-eight percent were found to have a new allele containing the -401 and -323 but not the -122 polymorphisms. The average FVII level in individuals with this allele was similar to that with the A1 allele whereas the combined -401/-323/-122 allele showed the expected significant 30% decrease in Factor VII levels. In summary, we have shown in vivo that the decanucleotide insert in the absence of the -122 polymorphism does not alone cause a decrease in Factor VII coagulant levels.

P0873. Mutations in Kringle IV-2 of the human apolipoprotein(a) gene

H. G. Kraft[1], *W. Parson*[2], *H. Niederst tter*[2], *A. Lingenhel*[1], *S. K chl*[2], *F. Fresser*[1], *G. Utermann*[1]
[1]*Institute of Medical Biology and Human Genetics; Innsbruck, Austria;* [2]*Institute of Legal Medicine; Innsbruck, Austria*
hans-georg.kraft@uibk.ac.at

The identification of mutations in one of many large identical repeated sequences requires special strategies because the detection of the mutation is hindered by the background of a high number of unchanged sequences. We have applied a brute force approach to detect mutations in the 5.6 kb Kringle IV type 2 domain of human apolipoprotein(a) which is present in 2 up to > 40 almost identical copies in individual alleles and is responsible for the size polymorphism of the protein. The procedure consisted of isolation of a single apo(a) allele from a heterozygote by PFGE, two independent amplifications by PCR, cloning of the amplicons and finally sequencing of a number (93) of clones high enough to represent by chance most individual repeats. The application of this strategy to an apo(a) allele with 26 K-IV-2 repeats yielded the following results; i) K-IV types 2A and 2B were both detected in 74 % of the analyzed clones, ii) the ratio of 2A;2B was different from the published cDNA sequence, iii) an additional K IV type 2 (designated K IV 2C) was detected in 5 % of the clones iv) in addition different mutations (4 silent, 2 nonsense and 5 missense) were detected, and v) all mutations were detected in the first exon of the K-IV structure and none in the second. The bona fide nature of one non-sense mutation was supported by the finding that the corresponding isoform was smaller than predicted by K IV-2 repeat number and by an independent PCR procedure based on genomic DNA. These results demonstrate a higher than anticipated sequence heterogeneity in K IV-2 of apo(a).

P0874. Heritability of Coronary Artery Calcium in Families with Type 2 Diabetes

D. W. Bowden, *L. E. Wagenknecht, J. J. Carr, C. D. Langefeld, B. I. Freedman, S. S. Rich*
Wake Forest University School of Medicine; Winston-Salem, NC United States
dbowden@wfubmc.edu

Type 2 diabetes is a major risk factor for atherosclerotic cardiovascular disease. Subclinical atherosclerosis, measured as coronary artery calcification (CAC), depends on both genetic and environmental factors. To evaluate evidence for genetic factors (familial aggregation) contributing to variation in CAC, we studied 122 type 2 diabetics and 13 nondiabetics (median age 59 years; range 38-80) in 56 families. CAC was measured by fast-gated helical computed tomography. Other measured atherosclerotic risk factors included blood pressure, body size, lipids, HbA1c, and medical his-

tory. Generalized estimating equations were used to test for association between CAC and the risk factors while accounting for underlying correlation due to family membership. CAC was detectable in 80% of patients with diabetes (median score = 84, range 0 - 5776). CAC, adjusted for age, was associated with male gender (p=0.0003), reduced HDL (p=0.02), albumin-creatinine ratio (p=0.008), and cigarette pack-years (p=0.03). CAC was associated with history of angina, myocardial infarction, stroke and vascular procedure (all p<0.005). HbA1c (p=0.14) and fasting glucose (p=0.08) were positively, but non-significantly associated with CAC. After adjustment for age, sex, race and diabetes status, CAC was significantly heritable (h2 = 0.50; p=0.009). In multivariate analysis with additional adjustment for HDL, BMI, hypertension, and smoking, the residual heritability remained significant (h2 = 0.40; p=0.038). These results suggest that genetic factors, independent of known risk factors for atherosclerosis, contribute to the variance of CAC in type 2 diabetes and that searching for QTLs for CAC will be an important phenotype for mapping genes for atherosclerosis and type 2 diabetes.

P0875. Estimation of individual admixture in Trinidad; application to a case-control study of systemic lupus erythematosus (SLE)

M. Molokhia[1], *A. L. Patrick*[2], *M. Shriver*[3], *E. Parra*[3], *J. Ye*[3], *A. J. Silman*[4], *P. McKeigue*[1]
[1]*London School of Hygiene & Tropical Medicine; London, United Kingdom;* [2]*Kavanagh Street Clinic; Port of Spain, Trinidad and Tobago;* [3]*Dept Anthropology, Penn State University; Philadelphia, PA United States;* [4]*ARC Epidemiology Research Unit; Manchester, United Kingdom*
mariam.molokhia@lshtm.ac.uk

Objective: To distinguish between genetic and environmental explanations for high risk of SLE in west Africans compared with Europeans by studying the relation of risk to individual admixture.

Methods: Cases of SLE and controls were sampled from an admixed population in Trinidad. The first 63 cases and 90 controls were typed with a set of 26 SNPs chosen to have large frequency differentials between the parental populations (west African, European and Native American). Individual admixture was estimated in a Bayesian analysis using Markov chain simulation.

Results: Mean admixture of the population was estimated as 51% west African, [95%CI 46%-56%]; 31% European [95%CI 27%-36%]; and 17% Native American [95%CI 14%-20%]. The estimated proportions with >75% and <25% African ancestry were 27%[95%CI 24-34%] and 31%[95%CI 25%-37%], respectively. Marker-based estimates of individual African admixture correlated [r= 0.86] with estimates based on reported grandparental ancestry, which were available for 70% of the sample. The slope of the relationship of risk ratio for SLE and renal SLE to African admixture were estimated as 2.3 (95% CI 0.7-7.2) and 3.2 (95% CI 0.5-19.1) respectively. The confidence interval for these slopes are wide because the current marker set extracts only 23% of the information that we would have if admixture were measured accurately.

Conclusion: Individual admixture varies widely within Trinidad so that the relationship of disease risk to admixture can be studied, but for accurate estimation of this relationship it will be necessary to type more markers informative for ancestry.

P0876. Apolipoprotein E Polymorphism In Patients With Alzheimer Disease In Iranian Population .

M. R. Keikhaee[1], *P. Pasalar*[2], *F. Sahebjam*[1], *H. Najmabadi*[1,3]
[1]*University of Welfare and Rehabilitation; Tehran, Islamic Republic of Iran;* [2]*Biochemistry dept., Tehran University of Medical Sciences; Tehran, Islamic Republic of Iran;* [3]*Karimi-Nejad Pathology and Genetics Center; Tehran, Islamic Republic of Iran*
maryamneishabury@yahoo.co.uk

Alzheimer s Disease (AD) is a progressive neurodegenerative disease, which is one of the most common forms of dementia in the age of 65 years and older. Diagnosis is based upon evaluation of cognitive skills, elimination of other dementia associated diseases and cranial CT examination. However, definitive diagnosis is proved only by neurohistological findings. The etiology of the disease is unknown and effective treatment is not available. Intensive research in the pathomechanism of the disease revealed a connection between increased frequency of apolipoprotein E4 allele and late onset of AD. The objective of this study was to characterize apolipoprotein E polymorphisms in late onset AD patients in Iranian population. Therefor, in this study 100 AD patients were examined. The polymorphic area was amplified by PCR, subjected to 8% polyacrylamide gel electrophoresis, and visualized by silver staining. The results shows that E4 allele was significantly more frequent in both early and late onset groups compared to controls (p<0.001). This data indicate that the Apo E4 is also a risk factor for AD in Iranian population.

P0877. CCR5 and CCR2 gene polymorphisms in essential hypertension

V. Romano-Spica[1], *M. Mettimano*[2], *A. Ianni*[1], *A. Migneco*[2], *D. Arzani*[1], *L. Savi*[2]
[1]*Inst. of Hygiene and Public Health (Dir. Prof. G.C. Vanini), Catholic University Medical School; Rome, Italy;* [2]*Hypertension Center, Dept. of Internal Medicine, Catholic University Medical School; Rome, Italy*
vrs@rm.unicatt.it

Blood pressure is a common trait of multifactorial origin. Evidence indicates that the immune system plays a role in the development of hypertension in both animal models and humans. Genes for CC-chemokine receptor (CCR) 5 and CCR2 map to human chromosome 3p21.3, within a cluster of 350 kb. A 32-bp deletion in the CCR5 gene (CCR5D32) and a G-to-A point mutation in the CCR2 gene (CCR264I) have been first associated to resistance to HIV-1 infection and recently involved in several immuno-related disorders. In the present study we evaluated allelic distribution of CCR5 and CCR2 genes in essential hypertension through a case-control study. Genotype frequency was compared between a group of essential hypertensive patients (stage I-II; n=53) and a group of Caucasian unrelated healthy subjects (n=340). Case selection was made by means of strict clinical evaluation and careful laboratory investigation, according to international guidelines. Genomic DNA was isolated from peripheral blood cells, and gene polymorphisms were analyzed by polymerase chain reaction (CCR5) and restriction enzyme analysis after amplification (CCR2). Chi-square analysis and Fisher s exact tests were used to compare data between cases and controls and test for conformity to the Hardy-Weinberg equilibrium. A statistically significant difference was observed for CCR5 and CCR2 mutant alleles in essential hypertensive patients in comparison with controls (p<0.01). Both CCR5D32 and CCR264I alleles showed a 0.11 frequency among cases. Genotype distribution was in equilibrium among cases and controls, according to the Hardy-Weinberg equilibrium. Our results suggest an association between polymorphisms at CCR genes and essential hypertension.

P0878. Interaction between allelic variants of Cystathionine beta synthase (CBS) and Metilenetetrahydrofolate reductase (MTHFR) genes as risk factor for Neural Tube Defects (NTD) in Argentina.

R. Cortese[1], *N. Buzzalino*[1], *E. Goldschmidt*[2], *G. Mercado*[1], *L. Dain*[1]
[1]*Centro Nacional de Gen tica M dica; Buenos Aires, Argentina;* [2]*Servicio de Gen tica. Hospital de Ni os R.Guti rrez ; Buenos Aires, Argentina*
renecortese@s6.coopenet.com.ar

Allelic variants of CBS and MTHFR have been suggested as risk factors for NTD. We have demonstrated an increased risk factor for the allele T of MTHFR in our population. The aim of this study was to assess the presence of a 68 bp insertion (68bp ins) in the CBS gene with the development of NTD, isolated or in association with the mutation C677T in MTHFR gene. We have studied both mutations in a group of 79 Argentine patients with NTD, 78 mothers and 67 fathers. Control group consisted of 105 healthy adults and 39 healthy children. DNA from blood samples was genotyped by PCR and BsrI and HinfI digestion for CBS or MTHFR respectively. We found an allelic frequency of 0.05 for the 68bp ins in our population. No statistical differences were found when the presence of the 68bp ins in the control group and the affected one were compared (0.11 vs. 0.15, OR= 1.39, 95% CI; 0.59-3.21, p>0.1). However, when comparing the 68bp ins in association with the C677T MTHFR mutation with the absence of both allelic variants, a significant difference was found (0.15 vs. 0.33, p<0.05). A frequency of 0.14 (n=145) for both allelic variants in parents of affected children and 0.15 for parents of non-affected ones (n=40) was observed. Our results suggest that the 68bp ins is not a risk factor for NTD in the studied population unless combined with the thermolabile mutation in MTHFR gene.

P0879. Study of two candidate loci to susceptibily for leprosy (NRAMP1 and MBP)

H. Buchm ller, *P. H. Cabello*
Funda o Oswaldo Cruz; Rio de Janeiro, Brazil
buchlima@infolink.com.br

Genetic factors can contribute to susceptibility to infectious diseases. The Mannose Binding Protein (MBP) is a seric protein that recognizes patogen microorganisms by carbohydrates present on its cell wall. The MBP acts as an opsonic agent inducing the fagocitosis by macrophages. Low concentrations of this protein are considered to be the most common immunodeficiency in humans. In mice, natural resistance to intracellular infectious disease is associated to the gene Nramp1. The effects of the human gene

homologue, NRAMP1, are not well known yet. In this study we genotyped the gene MBP and the locus D543N of the gene NRAMP1 of 204 leprosy patients and 211 blood-donors controlling sample, to evaluate the genes effects on infection or disease development by the Mycobacterium leprae. No significant differences were noticed of the genic and genotypic frequencies of these systems, between the affected group and the normal control, neither the haplotypes MBP/NRAMP1 between the different types of leprosy. Therefore, the genetic variations studied do not seem to be related to susceptibility to leprosy, as suggest by some authors in studies done on other populations.

P0880. Inheritance study of hand osteoarthritis (HOA) in Iceland

I. Manolescu[1], *H. Jonsson*[2], *S. Stefansson*[1], *T. Ingvarsson*[3], *H. Jonasson*[1], *A. Manolescu*[1], *J. Gulcher*[1], *K. Stefansson*[1]
[1]*DeCode Genetics Inc.; Reykjavik, Iceland;* [2]*University Hospital; Reykjavik, Iceland;* [3]*General Hospital; Akurery, Iceland*
ileana@decode.is

Objective; To investigate the genetic contribution in a large scale study of HOA in Iceland using an extensive genealogy database of the Icelandic population. Patients and methods; As part of an ongoing study, patients with HOA have been actively registered through nationwide clinical and surgical registers, radiology clinics and ergotherapy centers. At present 2950 HOA patients, comprising 1% of the Icelandic population, have been registered through the above channels. The patient phenotypes used are mild and severe finger OA and mild and severe thumb base OA based on clinical and radiological findings. Three methods were used to investigate the genetic contribution; (1) the Minimum Founder Test (MFT), to estimate the minimum number of ancestors (founders) needed to account for all patients at various years in the past, (2) the average pairwise kinship coefficient (KC) of the patients, and (3) the relative risk of HOA for relatives. In each case the results were compared with 1000 control sets of similar composition with regard to number, age and sex, generated from the genealogy database. Results; The MFT indicated that the study group had descended from fewer ancestors than controls ($p<0.001$). The KC was significantly higher than in the control sets ($p<0.001$). The relative risk of HOA for siblings and parents was higher than in spouses. The KC obtained for patients with severe finger OA was notably high whereas that for thumb base OA without finger involvement was not significantly different from controls. Conclusions; Patients seeking medical services for HOA are more related to each other than matched controls, supporting the role of a genetic component in the disease. Our methods may also contribute to the understanding of the inheritance of different subsets of hand OA.

P0881. Risks for Relatives of Patients with Multiple Sclerosis in Central Sardinia, Italy

I. Prokopenko[1], *L. Bernardinelli*[1], *C. Montomoli*[1], *B. S. Murgia*[2], *R. Ferrai*[2], *A. Ticca*[2], *A. Caria*[2], *L. Musu*[2], *M. Piras*[2], *A. Mander*[3]
[1]*Dep. Applied Health Sciences, University of Pavia; Pavia, Italy;* [2]*Divisione di Neurologia, Ospedale San Francesco; Nuoro, Italy;* [3]*MRC Biostatistics Unit; Cambridge, United Kingdom*
inga@fisher.unipv.it

Objectives: We calculated age-adjusted relative risks for different categories of relatives of Multiple Sclerosis (MS) patients from the case register of Central Sardinia (Italy) and examined some covariates that may influence the risk in sibs.
Background: MS is a multifactorial disease determined by genetic-environmental interactions. A genetic component to MS is indicated by an increased relative risk in siblings, by an increased concordance rate in monozygotic compared to dizygotic twins, by studies from adoptees, half-siblings and by familial recurrence risk data.
Design: Pedigrees were routinely obtained on prevalent patients of the MS case register. As a consequence most of the cases were independently ascertained.
We used life tables techniques to calculate age-adjusted recurrence risks. We estimated the effect of the studied covariates by fitting a proportional hazard model.
Results: We included in our analysis 313 probands and a total of 14173 relatives. The mean size of a pedigree was 47 family members. The age-adjusted risk in parents was 1.26 (0.60-2.63), in children 9.44 (9.32 - 25.25), in sibs 4.76 (3.57-6.32), in second-degree relatives 0.71 (0.41-1.21), and in third-degree relatives 1.75 (1.24-2.45).
The relationship of the studied covariates to the sibling risk was examined using Cox proportional hazards model. Sex of the proband and proband onset age were significant. More specifically, the hazard ratio for siblings of a female MS proband compared with a male proband was 0.62 ($p=0.05$). The risk for siblings of the oldest onset patient was lower than for siblings of the early onset patients (hazard ratio = 0.50, $p=0.023$).
Conclusion: We report the results of the first population-based study in Italy in which recurrence risks are computed within a sufficiently large population to provide figures for patient counselling. Due to the ascertainment scheme we used where each case is ascertained independently on the others, our study is not likely to be affected by ascertainment bias (families with a higher number of MS cases are more likely to be ascertained).
The higher risk observed in sibs of the less susceptible sex (male) can be interpreted as evidence in favour of a liability threshold model for MS.
The recurrence risks for different categories of relatives can be used to counsel patients and their families.
Our results agree with those obtained by similar studies.

P0882. The association alleles of VDR3, COL1A1, CALCR genes and severe osteoporosis in women from Russia.

M. Moskalenko[1], *M. Aseev*[1], *I. Zazerskaya*[2], *V. Baranov*[1]
[1]*Ott s Institute of Obs.& Gyn.; St-Petersburg, Russian Federation;* [2]*Pavlov s State Medical University.; St-Petersburg, Russian Federation*
mvmos@yahoo.com

The pathogenesis of osteoporosis is controlled by genetic and environmental factors. Although genetic predisposition seems to be a factor in the pathogenesis of osteoporosis, the precise cohort of genes that may be involved is not well defined. We investigated the association of VDR3, COL1A1 and CALCR genes polymorphism with osteoporosis in two groups of osteoporosis patients and unrelated controls of north-west Russia. The first group of patients consisted of 64 women with osteoporosis complicated with fractures, while the second group (78 women) contained patients with osteoporosis in postmenopausal (surgical or physiological). The statistical analysis proved preponderance of allele t of Taq I polymorphism in 9 intron of VDR3 in the first group of patients (53,1 %) compared to the control group (31,1 %)(?<0.01).The frequency of allele s of Apa I polymorphism in a recognition site for the transcription factor Sp1 of COL1A1 gene was three times higher in patients (48,4%) compared to the controls (16,7%) and this difference appears to be significant (?<0.01). The frequency of allele T of Alu I polymorphism in CALCR gene was also authentically higher (?<0.01) in this the group of patients (91,7%)(72,5% in control). In the second group of patients we had not elucidated any authentic differences in frequencies of alleles of these three genes, compered to the control group. The frequencies of alleles of these genes were 33,9% (VDR3), 21,8% (COL1A1) and 77,6% (CALCR). Our date indicate that different alleles of VDR3, COL1A1 and CALCR genes correlate with severe osteoporosis in women.

P0883. Association of polymorphisms in the NRAMP1 gene and susceptibility to tuberculosis in Chinese

Y. Lam, *S. W. K. Im, W. C. Yam*
The University of Hong Kong; Hong Kong, China
krissylamyin@yahoo.com.hk

Evidence for the genetic factor, human natural-resistance-associated-macrophage-protein 1 (NRAMP1) gene to have a role in susceptibility to tuberculosis was found in West Africans, Koreans and Japanese. The objective of the study is to investigate whether the polymorphisms in the four regions of the NRAMP1 gene; 5 microsatellite, Intron4, D543N and 3 UTR are associated with the host susceptibility to tuberculosis among Chinese population in Hong Kong. Polymorphisms in NRAMP1 gene were investigated in a case-control study of tuberculosis in Hong Kong, China. Polymerase Chain Reaction Restriction Fragment Length Polymorphism (PCR-RFLP) analysis was used to type the polymorphisms and to determine the allelic frequencies of the different regions of the gene among paitents and controls. Patients suffering from tuberculosis were diagnosed by positive findings in chest X-ray and sputum culture, while the controls were healthy blood donors with no history of tuberculosis. Relationship of the polymorphisms in the 4 regions of NRAMP1 and the host susceptibility to tuberculosis among Chinese population in Hong Kong will be highlighted and discussed.

P0884. BRCA1 and BRCA2 founder mutations account for 5% of 233 unselected Finnish ovarian carcinoma patients

L. M. Sarantaus[1], *P. Vahteristo*[2], *E. Ostermeyer*[2], *A. Tamminen*[2], *L. Unkila-Kallio*[2], *R. B tzow*[2], *H. Nevanlinna*[2]
[1]*Department of Obstetrics and Gynecology, Helsinki University Central Hospital; Helsinki, Finland;* [2]*Dept. of Obstetrics and Gynecology; Helsinki, Finland*
Laura.Sarantaus@hus.fi

Germ-line mutations of BRCA1 and BRCA2 predispose to hereditary breast-ovarian cancer syndrome. In Finland, 20 different BRCA1/2 mutations have been identified and 13 of the mutations are founders and

account for the vast majority of Finnish BRCA1/2 families. The purpose of our study was to determine the prevalence of BRCA1/2 mutations in unselected Finnish ovarian carcinoma patients and to evaluate the relationship between mutation status and personal/family history of cancer. Two hundred and thirty-three patients were screened for all BRCA1/2 mutations known in the Finnish population. Additionally, a subgroup of patients with personal history of breast cancer and/or family history of breast and/or ovarian cancer was screened for novel BRCA1/2 mutations. Thirteen patients (5.6%) had mutations; eleven in BRCA1 and two in BRCA2. Seven of the 13 known Finnish BRCA1/2 founder mutations were identified in this study, and they accounted for 12 of the 13 mutations detected. A logistic regression analysis was used to determine the odds of mutation for ovarian carcinoma patients. The most significant predictor of a mutation was the presence of both breast and ovarian cancer in the same woman, but family history of breast cancer was also strongly related to positive mutation carrier status. Although BRCA1/2 mutation testing is not warranted in the general ovarian cancer patient population, patients with personal/family history of breast cancer could benefit from referral to genetic counseling and mutation testing.

P0885. Prostaglandin H synthase 2 (PTGS2/COX-2) variant in African Americans and a case-control study of colorectal adenomas.

H. J. Lin[1], *K. M. Lakkides*[2], *T. O. Keku*[3], *H. D. Frankl*[4], *E. R. Lee*[5], *R. M. Garavito*[2], *R. S. Sandler*[3], *R. W. Haile*[6], *W. L. Smith*[2]

[1]*Harbor-UCLA Medical Center; Torrance, CA United States;* [2]*Michigan State University; East Lansing, MI United States;* [3]*University of North Carolina; Chapel Hill, NC United States;* [4]*Kaiser Permanente; Los Angeles, CA United States;* [5]*Kaiser Permanente; Bellflower, CA United States;* [6]*University of Southern California; Los Angeles, CA United States*

henry_lin@humc.edu

Prostaglandin H synthase 2 (PTGS2; also known as cyclooxygenase-2) is thought to take part in prevention of colorectal cancer by nonsteroidal anti-inflammatory drugs, which are inhibitor of the enzyme. We used DNA heteroduplex analysis to screen the PTGS2 gene for naturally-occurring enzyme variants, in order to obtain further information on a biochemical mechanism of prevention. We found a single-base substitution in 10% of African Americans (g.5939T>C; GTT>GCT; Val511Ala). The amino acid change is predicted to open a large cavity near the PTGS2 active site and may change conformations of key residues. No changes in Vmax, Km, or thermal stability were observed for the mutant enzyme in Cos-1 cell assays with arachidonic acid as substrate. However, the conditions of the assay were expected to differ substantially from the normal intracellular environment. Case-control analyses of 380 African Americans from 2 study populations showed odds ratios (and 95% confidence intervals) for colorectal adenomas of 0.50 (0.18-1.44) and 0.56 (0.13-2.44) among subjects with the mutation. The results are potentially consistent with a protective effect of the mutation, mimicking nonsteroidal anti-inflammatory drugs. Larger sample sizes are needed to confirm.

P0886. A Unique Resource for Breast Cancer Research; The Cooperative Family Registry for Breast Cancer Studies

R. T. Senie[1], *I. Andrulis*[2], *M. Daly*[3], *J. Hopper*[4], *S. Buys*[5], *D. West*[6], *H. Anton-Culver*[7]

[1]*Columbia University; New York, NY United States;* [2]*Cancer care Ontario; Toronto, ON Canada;* [3]*Fox Chase Cancer Center; Cheltenham, PA United States;* [4]*University of Melbourne; Carlton, Australia;* [5]*Huntsman Cancer Research Center; Salt Lake City, UT United States;* [6]*Northern California Cancer Center; Union City, CA United States;* [7]*University of California, Irvine; Irvine, CA United States*

rts15@columbia.edu

Although increased susceptibility to breast and ovarian cancer has been associated with genetic mutations of BRCA1 and BRCA2, risk may also be modified by lifestyle factors, health behaviors, environmental exposures,and other genetic components. To provide resources for studies of these complex interactions, the National Cancer Institute funded the Cooperative Family Registry for Breast Cancer Studies [CFRBCS], a consortium of six international research centers with an Informatics Support Center. Family recruitment was initiated in 1997 at the three population-based and three clinic-based sites. As of December 2000 more than 6,000 families including 15,000 individuals are participating. Data and biospecimens are collected from probands and their relatives using common instruments and protocols. Coded personal health information, dietary intake, treatment for breast and/or ovarian cancer, and pedigree data are routinely transmitted to the Informatics Center. Biospecimens including blood and tumor tissue samples are banked at each collaborating site following rigid quality control procedures. Genetic analyses are being conducted; currently 350 carriers of BRCA1 or BRCA2 have been identified. The primary purpose of the CFRBCS has been the development of a unique resource for interdisciplinary studies of genetic and environmental risk of breast and ovarian cancer. Several hypothesis-driven research projects are being conducted by CFRBCS investigators and international collaborators using the data and biospecimens; additional investigators are encouraged to develop research proposals for submission to the external Advisory Committee. Information about the CFRBCS and application procedures are available on the NCI website www-dccps.ims.nci.nih.gov/CFRBCS.

P0887. Glutathione S-transferase GSTM1 and GSTT1 polymorphisms and the risk of oral and laryngeal cancer.

A. Hatagima[1], *C. F. S. Marques*[1], *F. S. Rajoy*[1], *R. J. Koifman*[1], *J. S. Capelli*[1], *M. B. Kneipp*[1], *P. Boffetta*[2], *P. Brennan*[2], *N. Munoz*[2], *R. Herrero*[2], *S. Koifman*[1]

[1]*Funda o Oswaldo Cruz; Rio de Janeiro, Brazil;* [2]*IARC; Lion, France*

hatagima@gene.dbbm.fiocruz.br

Glutathione S-transferases are important xenobiotic metabolizing enzymes and several studies have showed that their genetic polymorphisms may contribute to individual susceptibility for some cancers. Oral and laryngeal cancers are important due to their incidence in several countries. Genetic and environmental factors are involved in the development of these cancers. Many of GST enzymes are involved in the desintoxication of active metabolites from tobacco smoke, what makes them important on modulating the susceptibility to tobacco-related cancers. As part of a wide case-control study conducted in Latin America by the International Agency for Research on Cancer, we studied GSTM1 and GSTT1 genetic polymorphisms in 200 oral and 73 laryngeal cancer patients from Rio de Janeiro (Brasil). Polymorphisms were analyzed in DNA from peripherical blood extraction by Polymerase Chain Reaction and restriction enzymes digestion. The null homozygotes frequencies were 45% (GSTM1) and 24% (GSTT1) in oral cancer patients and 44% (GSTM1) and 21% (GSTT1) in laryngeal cancer group. The observed differences among these frequencies and the ones from a sample of healthy individuals were not statistically meaningful. No associations were observed concerning laryngeal cancer and polymorphisms studied here. However, for the GSTM1 polymorphism both healthy and oral cancer samples were not under the Hardy-Weinberg equilibrium ($p<.001$ and $p=.003$, respectively). Among healthy controls it happens due to the excess of AB heterozygotes, but in the oral cancer group this genotype was absent, suggesting its protective role in oral cancer in our population. Support; CNPq, FAPERJ-FIOCRUZ, European Community DG XII.

P0888. Frequency distribution of ATM gene alterations in German breast cancer patients and in the general population

P. Yamini, *K. Kl pper, R. Bendix, M. Bremer, D. Rades, J. H. Karstens, T. D rk*

Medizinische Hochschule; Hannover, Germany

doerk.thilo@mh-hannover.de

The ATM gene that is mutated in the autosomal recessive syndrome ataxia-telangiectasia (A-T) encodes a 350 kDa protein kinase which controls radiation-induced DNA damage responses via regulation of downstream targets such as p53, c-abl or BRCA1. Heterozygosity for ATM germline mutations has been implicated as a genetic predisposition in breast cancer and other age-related disorders. We have sought to determine the relative frequencies of common ATM gene alterations in a hospital-based series of 1000 consecutive breast cancer patients and in 500 random individuals from the general population of Lower Saxony in order to gain more insight into the potential clinical relevance of these genetic variations. We have developed a hexaplex ARMS based method for a rapid and specific screening of the most frequent ataxia-telangiectasia mutations in Germany, and we have screened for an additional six common amino acid substitutions using restriction-enzyme or SSCP-based methods. The most frequent ATM truncating mutation in Germany is the leaky splicing mutation IVS10-6T->G that we have identified in seven (0.7 %) breast cancer patients but also in three (0.6 %) control individuals. The most common amino acid substitution, D1853N, was present at an allele frequency of 0.13 in both the breast cancer and control cohorts. However, the rarer ATM amino acid substitutions were, in a composite test, more frequent in the breast cancer patients than in the general population (7.9% vs. 5.3% of alleles, $p<0.01$). These results suggest that ATM missense substitutions could play a role in the genetic predisposition towards cancer.

P0889. Evaluating the APEX-based resequencing assay of p53 tumour suppressor gene

N. T nisson[1,2], *J. Zernant*[1], *A. Kurg*[2], *H. Pavel*[1], *A. Metspalu*[2,1]
[1]*Asper Ltd.; Tartu, Estonia;* [2]*Estonian Biocentre / IMCB, University of Tartu; Tartu, Estonia*
neemet@asper.ee

Mutation data of the p53 gene have relevant prognostic and therapeutic value in several types of cancer. We are developing and evaluating an APEX (Arrayed Primer Extension) - based test for the gene with goal of getting the full mutation data at both DNA strands in a single assay. A patient DNA sample is amplified, digested enzymatically, and annealed to arrayed primers, which promote sites for template-dependent DNA polymerase extension reactions using four fluorescently labelled dideoxynucleotides. The Genorama" imaging system and genotyping software are used for imaging and semiautomatic sequence analysis. The p53 gene chip is scanning exons 2 to 9 plus two introns from both strands (1218 bases). An average of 98 % of the arrayed p53 gene sequence can be analysed from either sense or antisense strand and 85 % from both strands. In best cases the readable sequence is up to 99.8 % and 96 %, respectively. Tumour samples with known mutations were analysed in a blind test. Predominantly the results were concordant with TTGE (Temporal Temperature Gradient Electrophoresis) plus dideoxy sequencing. One case showed the presence of double mutation in codon 290. In addition, one heterozygous SNP and an SNP with minor allele were found. The entire resequencing procedure can be completed in less than six hours and at 5 to 10 times less cost compared to ABI sequencing per sample. Once fully developed, the p53 gene chip should become a medium for accurate and efficient DNA sequence analysis of this or other frequently mutated genes.

P0890. Introduction and diffusion of the BRCA1 mutation R1443X in the French Canadian population

H. Vezina[1], *F. Durocher*[2], *L. Houde*[1], *C. Szabo*[3], *S. Delos*[4], *M. Dumont*[4], *M. Jomphe*[1], *M. Plante*[4], *R. Laframboise*[4], *J. Chiquette*[5], *D. Stoppa-Lyonnet*[6], *D. Goldgar*[3], *D. Easton*[2], *P. Bridge*[7], *J. Simard*[4]
[1]*Universite du Quebec a Chicoutimi; Chicoutimi, PQ Canada;* [2]*CRC Genetic Epidemiology Unit, University of Cambridge; Cambridge, United Kingdom;* [3]*Unit of Genetic Epidemiology, International Agency for Research on Cancer; Lyon, France;* [4]*Centre hospitalier universitaire de Quebec, Laval University; Quebec City, PQ Canada;* [5]*Hopital St-Sacrement; Quebec City, PQ Canada;* [6]*Oncology Genetics, Institut Curie; Paris, France;* [7]*Alberta Children s Hospital, University of Calgary; Calgary, AB Canada*
hvezina@uqac.uquebec.ca

In the 17th century, about 5 000 immigrants, coming mostly from France, settled in Canada. Among them, 3 500 to 4 000 have descendants in the contemporary Quebec population and they account for the major part of the gene pool of the 5 million Quebecers of French descent. The present study was designed to analyse the role of this founder effect and the resulting population demogenetic structure in the introduction and diffusion of the BRCA1 recurrent R1443X mutant allele. The highly conserved haplotype observed in 46 R1443X carriers from 11 French Canadian families and generated using seventeen microsatellite markers spanning a 9.3 cM region surrounding the BRCA1 locus confirms that the R1443X mutation is a founder mutation in the Quebec population. Ascending genealogies of one carrier individual per family (n=10) and of controls (n=30) were reconstructed using the BALSAC population register. These genealogies have an average depth of ten generations but many lineages go as far back as 13 generations. We identified the founder couple with the highest probability of having introduced the mutation in the population. Genetic contributions of this founder couple to the contemporary regional populations of Quebec were measured in order to understand the spread in time and space of the mutation and to detect the presence of spatial stratification in the diffusion pattern of the mutation. Finally, we also performed haplotyping analysis of R1443X carriers from 10 French families and although the results are consistent with a common origin for this mutant allele in both populations, a distinct haplotype was obtained in two French families, thus suggesting multiple origins for the R1443X mutation.

P0891. Interaction between BRCA1 and BRCA2 genes and environmental risk factors in early-onset breast cancer. Results from a French population-based study.

V. Bonodona[1], *O. Sinilnikova*[2], *A. Bremond*[1], *H. Mignotte*[1], *P. Mathevet*[3], *A. Martin*[4], *C. Zinzindohoue*[3], *J. Bobin*[3], *P. Romestaing*[3], *D. Raudrant*[3], *R. Rudigoz*[3], *G. Lenoir*[2], *C. Lasset*[1]
[1]*Centre L on Berard; Lyon, France;* [2]*International Agency for Research on Cancer; Lyon, France;* [3]*Hospices Civils de Lyon; Lyon, France;* [4]*Clinique Jeanne D Arc; Lyon, France*
bonadona@lyon.fnclcc.fr

Studies have suggested the role of gene-environment interactions on breast cancer risk, since the effects of known risk factors differ according to the existence or not of a family history of breast cancer. The recent identification of susceptibility genes for hereditary breast cancer provides further insight into the evidence of such interactions. The purpose of this study was to evaluate the prevalence of BRCA1 and BRCA2 mutations in early-onset breast cancer cases and to analyse gene-environment interactions. From a population-based cohort of women with breast cancer diagnosed before age 46 years, we collected information about environmental and familial risk factors and blood sample for genetic testing. We used a case-only design to study gene-environment interactions. A total of 269 women were included and genetic analyses were performed for 232 women. Twenty-one BRCA1 and BRCA2 mutations carriers were identified. Mutation prevalence was higher in women with breast cancer before age 41 years than the other women (12.8% versus 5.2%). We found a non-significant interaction of some reproductive factors; an increased breast cancer risk was associated with a late age at menarche (>15 years) (odd ratio=2.5; p=0.2) and parity (odd ratio=1.8; p>0.2) in carriers of BRCA1 and BRCA2 mutations. No effect of age at first-full term pregnancy and oral contraception were found. These results indicate a possible interaction between BRCA1 and BRCA2 susceptibility genes consistent with the previous studies. However, they need confirmation with further larger studies. The implications of our findings for the high prevalence of BRCA1 and BRCA2 mutation in women with breast cancer before 41 years will be also discussed.

P0892. Characterization of a subcloned fragment (pBA0.6) of pCMM86 located on 17q21 and its potential use in generating an individual-specific DNA profile and detection of genetic variability in Indian population and sporadic breast cancer tissues

A. Saha, *R. Bamezai*
Jawaharlal Nehru University; Delhi, India
anjana14@hotmail.com; bamezai@hotmail.com

Sequence analysis was carried out of a human clone pBA0.6 generated after ExoIII/S1nuclease digestion and subcloning of pCMM86 (GDB; 168382, D17S74), which was not available in the database. It revealed the presence of a reiterating core motif of 24mer GTGGGTGTGTTGGAGGGGGTGAGG present 23 times (Accession no. AF079321), which was GC-rich and minisatellitic in nature. Genomic blots of HaeIII-digested human DNA when hybridized to pBA0.6, generated a ladder of polymorphic bands which were individual specific in nature .The probability of identity ranged from 5.07X10-14 to 2.64X10-16 in different population groups. Out of three isomorphic bands of 29.0 kb, 2.4 kb, 2.1 kb, 29.0 kb band was observed to be Homo sapiens specific (Saha et al., 2000, DNA and Cell Biology, vol. 19 no. 4; 219-226). Dendrograms based on UPGMA method with Jaccard s coefficient values suggested high genetic diversity in all the population groups suggesting that the samples taken were random. Maximum likelihood estimates through bootstrap sampling method showed that Punjabis, Bengalis and UPites formed one cluster whereas South Indians formed a separate cluster altogether thus showing the proximity of these three population groups as compared to that from South India. Further with another minisatellite sequence (Accession no. AF157691) dendrogram at the individual level led to the formation of several small clusters which were interleaved; also, the subgroups for each of the populations were intermingled with the subgroups for the other populations (Saha and Bamezai, 2000, J Hum Genet vol. 45; 207-211). Further, pair studies comparing the lymphocyte and tumor DNA of 19 sporadic breast cancer patients indicated a genetic variation in 32% of the cases studied, pointing towards its utility in screening for somatic changes in the breast cancer tissues. A preliminary study of Northern hybridization with pBA0.6 resulted in two transcripts of 0.63 kb and 0.29 kb. This was further corroborated with RT-PCR results where 2 amplicons, matching with the expected size of two reading frames within the minisatellite sequence, were obtained. Interestingly, amplicons were also generated when tumour samples of breast cancer patients were analyzed. The role of the two transcripts from the minisatellite sequence is not clear as yet.

P0893. A common variant of the methylenetetrahydrofolate reductase gene (MTHFR, 1p36) is associated with an increased risk of cancer

B. T. Heijmans[1,2], *J. M. A. Boer*[3], *C. J. Cornelisse*[4], *D. Kromhout*[3], *R. G. J. Westendorp*[5], *E. J. M. Feskens*[3], *E. Slagboom*[2]
[1]*TNO Prevention and Health; Leiden, The Netherlands;* [2]*Dept. of Medical Statistics, Leiden University Medical Centre; Leiden, The Netherlands;*

[3]Dept. of Chronic diseases, National Institute of Public Health and the Environment; Bilthoven, The Netherlands; [4]Dept. of Pathology, Leiden University Medical Centre; Leiden, The Netherlands; [5]Gerontology and Geriatrics, Leiden University Medical Centre; Leiden, The Netherlands
bt.heijmans@pg.tno.nl

Folate metabolism is thought to play an important role in carcinogenesis through its involvement in both DNA methylation and nucleotide synthesis. A common Ala/Val variant in the methylenetetrahydrofolate reductase gene (MTHFR) leads to a mildly disturbed folate metabolism. We previously reported that the MTHFR Val/Val genotype was associated with increased cancer mortality in men aged 85 years and over (Eur J Hum Genet 1999;7;197-204). To further explore the deleterious effects of the MTHFR genotype, we studied the association of the genotype with overall cancer risk and the risk of cancer of specific organs in 860 men aged 65-84 years who were followed over 10 years. During follow-up, 150 new cases of cancer occurred among the 793 men without cancer at baseline. The risk of cancer was 1.81-fold (95% CI, 1.09-3.00) increased among men with the Val/Val genotype as compared to men with the Ala/Ala genotype. The higher incidence of cancer could be attributed to an increased risk of cancer of the prostate (RR, 3.48; 95% CI 1.05-11.6), the colorectum (RR, 3.65; 95% CI, 1.07-12.5) and the kidney and bladder (RR, 5.48; 95% CI, 1.67-18.0), but not to an increased risk of lung cancer. The risk of cancer appeared to be particularly increased among men with lower folate intake, higher alcohol consumption and of an older age. In conclusion, our current and previous studies in two independent populations indicate that a common Ala/Val variant in the MTHFR gene may have a deleterious effect on the risk of cancer in the general population.

P0894. Thiopurine methyltransferase polymorphism; genotype-phenotype correlation analysis in a Portuguese sample

S. Alves[1], A. Amorim[1], F. Ferreira[2], M. Prata[1]
[1]IPATIMUP; Porto, Portugal; [2]Servi o de Hematologia Cl nica, Hospital Geral S. Jo o; Porto, Portugal
sandra.alves@ipatimup.pt

Thiopurine methyltransferase is a polymorphic enzyme that catalyses the S-methylation of thiopurine drugs used in immunosupressive and anticancerigenous therapy. Therefore, characterisation of all the variation at TPMT gene which can produce changes in TPMT activity should be conducted. We set out to perform genotype-phenotype correlation analysis in 143 Portuguese individuals for several TPMT SNPs (including silent and intronic substitutions) and for a VNTR located in the promoter region of the gene. The SNPs and the VNTR were studied by PCR based methods and the phenotype quantification of TPMT was performed using a HPLC method. As expected a statistical significant association was found between the presence of TPMT mutant alleles and reduced TPMT activity. For the other silent or intronic substitutions analysed, no signs that they could influence TPMT activity were detected. The VNTR at the promoter region is characterised by a composite internal structure due to the presence of 3 different types of repeats - A, B and C. Therefore each VNTR allele is defined by a particular pattern of repeat arrangement. We found a statistical significant association between VNTR*6 and reduced levels of TPMT activity. Since VNTR*6 is the allele with more B type repeats, we can hyphotesize that the high number of B repeats is the responsible for the association registered. However the question needs further support. Finally we reported linkage disequilibrium between VNTR*6 and TPMT*3A and between VNTR*4 and TPMT*2.

P0895. Evidence For Deviation From Hardy-weinberg Equilibrium In Brca1 And Brca2

C. S. Healey[1], A. M. Dunning[1], F. Durocher[1], D. Chase[2], J. Burn[3], B. A. J. Ponder[1], D. F. Easton[1]
[1]University of Cambridge; Cambridge, United Kingdom; [2]Westlakes Research Institute; Cumbria, United Kingdom; [3]University of Newcastle; Newcastle, United Kingdom
katie@srl.cam.ac.uk

We have investigated a number of common polymorphisms in both BRCA1 and 2 for an association with low penetrance breast and ovarian cancer susceptibility. Two of the most common variants in BRCA1 (P871L and Q356R) and six variants in BRCA2 (a-26g, N289H, N372H, T1915M, R2034C and K3326X) have been studied in population-based series of ~2000 breast cancer cases from the ABC study, 500 ovarian cancer cases and 1500 controls from the EPIC cohort. We have found a significant increase in risk of breast cancer associated with the BRCA2 N372H polymorphism (OR=1.3 [95%CIs; 1.07-1.61]) The BRCA1 polymorphisms show no association with breast cancer risk. We have also observed a significant deviation from Hardy-Weinberg equilibrium (HWE) in adult females for both the BRCA2 N372H and BRCA1 P871L polymorphisms, with a deficit of homozygotes and an excess of heterozygotes for both variants ($p<0.01$). In a set of ~2400 new-born individuals, new-born girls also showed a deficit of homozygotes and an excess of heterozygotes for both genes, consistent with the adult females. The new-born boys, however, showed the opposite effect, with an excess of homozygotes, which was significant in BRCA2 ($p=0.001$). This suggests that common variants of BRCA1 and 2 are subject to selection, which appear to affect foetal survival in a sex-dependent manner.

P0896. Folate and breast cancer; the role of polymorphisms in methylenetetrahydrofolate reductase (MTHFR)

A. C. Schofield, L. Sharp, E. Pavlidov, Z. Miedzybrodzka, S. D. Heys, J. Little, N. E. Haites
University of Aberdeen; Aberdeen, United Kingdom
a.schofield@abdn.ac.uk

Breast cancer is one of the most common malignancies affecting women in the UK. Recent evidence has highlighted the importance of nutrition in the aetiology of breast cancer. In particular, diets with higher intakes of fruit and vegetables are considered protective. Vegetables are a major source of dietary folate, which is involved in DNA synthesis and methylation. An association between higher folate intake and reduced risk of breast cancer has been observed. Functional polymorphisms in MTHFR, an enzyme involved in folate metabolism, have been reported (C677T and A1298C). We undertook a case-control study to investigate associations between breast cancer, dietary folate and MTHFR polymorphisms. Sixty-five breast cancer cases were recruited at Aberdeen Royal Infirmary. Age-sex matched controls (without breast cancer) were selected from general practitioner registers. Subjects completed a food-frequency questionnaire and provided a mouthwash sample to enable MTHFR genotyping. A trend of decreasing breast cancer risk with increasing dietary folate intake was observed (OR=0.49, 95% CI 0.2-1.2). For the C677T polymorphism, the TT genotype had a protective effect (OR=0.38, 95% CI 0.12-1.24). A similar protective effect was observed for women with the CC genotype for the A1298C polymorphism (OR=0.24, 95% CI 0.06-0.97). A significant protective effect was observed for women with homozygosity for the A1298C variant only with higher folate intake (OR=0.09, 95% CI 0.01-0.82). A1298C homozygote individuals with high folate intake appear to have the strongest protective effect against breast cancer. Both dietary and genetic variation in folate status may be implicated in the pathogenesis of breast cancer.

P0897. Relation between childhood leukemia and ABO and Rh blood groups in Serbian population

R. Papovic[1,2], S. Cvjeticanin[2], B. Tri?ic[2]
[1]Medical Faculty University of Belgrade; Belgrade, Yugoslavia; [2]Institute for Biology and Human Genetics; Belgrade, Yugoslavia
bebrapap@eunet.yu

In the sample of 214 children with leukemia(ALL-acute lymphoblastic N=163;ANLL-acute nonlymphoblastic N=43 and HGL-chronic granulocite leukema N=8)the frequencies of ABO and Rh blood types were similar to the average value of the Serbian population. Comparation of the frequencies of ABO blood types between samle of affected individuals with ALL and control sample we can see that O blood type is slightly increased and A,B and AB blood types are slightly decreased. But, in the sample of affected children with ANLL the frequencies of A,O and AB blood types are slightly increased and B blood type is statisticaly decreased (X2=3.8, $p<0.05$)compared with incidence of ABO blood groups in Serbian population.If we compared frequencies of ABO blood groups between affected children of ALL and ANLL we can see that percentages are similar for A,O and AB blood types but frequences of blood type B is statisticaly decreased in the groop of ANLL affected (X2=3.9,$p<0.05$). Taking all this into account we may conclude that most frequently affected individuals in whole sample and group of ALL are with blood types O;A;B;AB. But,in the sample of ANLL the most affected children are with blood types in this order A;O;B=AB.

P0898. Cytochrome P450 (CYP1A1 and CYP2E1) Genetic Polymorphisms and Susceptibility to Oral and Laryngeal Cancer.

C. F. S. Marques[1], F. S. Rajoy[1], S. Koifman[1], R. J. Koifman[1], J. S. Capelli[1], M. B. Kneipp[1], P. Boffetta[2], P. Brennan[2], N. Munoz[2], R. Herrero[2], A. Hatagima[1]
[1]Funda o Oswaldo Cruz; Rio de Janeiro, Brazil; [2]IARC; Lion, France
tiane.ntg@terra.com.br

Oral and laryngeal cancers are responsible for about 7% of new cancer cases worldwide. Many studies have provided evidences of their association with tobacco and alcohol intake, while others suggest the role of

genetic factors contributing to carcinogenesis. Polycyclic aromatic hydrocarbons (PAH) and other chemicals present on tobacco smoke and environmental pollutants are submitted to a biotransformation process. Its first part is mostly attributed to P450 superfamily enzymes, which function is to transform these chemicals into intermediates that will be metabolized by other enzymes into the excretion way. The disability to eliminate toxic products due to an enzymatic deficiency may contribute to individual risk for chemical-induced carcinogenesis. Enzymes encoded by polymorphic CYP1A1 and CYP2E1 genes were previously associated with oral, esophagus and lung cancer. In this case-control study we genotyped 277 patients with oral and laryngeal cancer and 200 healthy controls from Rio de Janeiro (Brasil) in order to compare genotypic and allelic frequencies between the groups. Using Polymerase Chain Reaction and restriction enzymes techniques we analyzed this sample, which is part of a greater study coordinated by the International Agency for Research on Cancer. The observed allelic frequencies between cases and controls were similar for CYP2E1 polymorphism and we found no association with the cancers studied. However, the CYP1A1 polymorphism seems to be associated with an increased risk for oral cancer, as this group had twice more mutant homozygotes (GG) than the control one, although the allelic frequencies were similar between them. Support; CNPq, FIOCRUZ-FAPERJ, European Community DG XII.

P0899. Molecular-Genetic Study of CFTR Gene Mutation delF508 in Cystic Fibrosis Families from Bashkortostan

G. Koritina[1], *T. Viktorova*[1], *E. Khusnutdinova*[1], *G. Baykova*[2]
[1]*Institute of Biochemistry and Genetics; Ufa, Russian Federation;* [2]*Bashkir medical university; Ufa, Russian Federation*
ekkh@anrb.ru

Cystic fibrosis (CF) is the most common lethal autosomal recessive disease among Caucasians. It is caused by defects in the CF transmembrane counductance regulator (CFTR) gene. More then 900 molecular defects have been reported to date. The distribution of these mutations is modify among different human populations. Fifty five patients with CF from Bashkortostan were tested for mutation delF508. We have analyzed 110 unrelated CF-chromosomes (53 - Slavians, 39 - Tatars,12 - Bashkirs,4 - Chuvashes, 2 - Udmurts). The common mutation delF508 was found only on 31,8% of tested CF-chromosomes; 30 were homozygous and 5 were compound heterozygous. It is one of the lowest incidence of this deletion reported in Russia. Taking into account, that CF-patients from Bashkortostan characterised by high ethnical heterogeneous, we have analyzed frequency of delF508 in different ethnical groups of CF patients. Among Slavians probands (Russians, Ukrainians, Belorussians) delF508 was detected on 45% CF-chromosomes, among Tatars - on 28% and among Bashkirs, Chuvashes, Udmurts delF508 was not found. We suppose, that the absence of delF508 in Bashkirs CF-families could be explained by the features of formation of this population, resulting from mixing of different ethnical components (Turkic, Mongolian and probably Finno-Ugric and Indo-European). By the data of archeology and anthropology the Turkic and Mongolian components in Bashkirs gene pool is highest among other investigated populations. The delF508 practically does not meet in Turkic and Mongolian populations. So our results showed the little portion of Slavians component in Bashkirs gene pool.

P0900. The major cystic fibrosis mutation in Latvia; frequency, origin and age of the ˇF508 mutation

A. Krumina[1], *V. Kroshkina*[1], *V. Svabe*[1], *Z. Krumina*[1], *L. Krumina*[1], *I. Tamane*[1], *M. Lazdinsh*[2], *V. Baumanis*[2]
[1]*Medical Academy of Latvia; Riga, Latvia;* [2]*Latvian State University; Riga, Latvia*
Astrida.Krumina@rsu.lv

CFTR gene mutation ˇF508 is the most frequent cystic fibrosis (CF) mutation, accounting for 70% of CF chromosomes. Strong linkage disequilibrium found between ˇF508 and different polymorphic DNA markers have substantiated the hypothesis of a single origin of this mutation. The aims of our study were 1) to characterize the prevalence of mutation ˇF508 among normal and CF chromosomes;2)to estimate the age and origin of the ˇF508 mutation in Latvia. 31 CF patients,30 their healthy family members and 136 healthy unrelated Latvians were subjected to analysis of mutation ˇF508, three extragenic and three intragenic polymorphic DNA markers. The ˇF508 allele frequency in the overall CF patient population was 59.7%.An average incidence of ˇF508 mutation carriers among healthy Latvians was 1;42.Absolute linkage disequilibrium was found between ˇF508 and loci IVS6a and XV-2c,highly significant -between ˇF508 and loci TUB18 and KM-19.The results of extended haplotype analysis showed different distribution of haplotypes between normal,ˇF508 and non-ˇF508 CF chromosomes.These results are consistent with the data published for most European populations and confirms the hypothesis that the ˇF508 mutation has derived from a single mutational event.Taking into consideration the values of recombination rates between ˇF508 and extragenic polymorphic markers,the estimate of the number of generations elapsed since the mutation ˇF508 was first introduced into Latvia, approximates 101 (2020 years).These data are in contrast with those published for other populations.

P0901. Why is cystic fibrosis so common?

M. Super[1], *J. Chadwick*[2], *T. Roberts*[1], *N. Andrew*[3]
[1]*Royal Manchester Children s Department of Clinical Genetics; Manchester, United Kingdom;* [2]*Medical Student, Manchester University; Manchester, United Kingdom;* [3]*Molecular Genetics Laboratory; Royal Manchester Children s Hospital; Manchester, United Kingdom*
Maurice.Super@man.ac.uk

It is widely accepted that a heterozygote advantage must be operating to maintain a carrier frequency of 1 in 25 associated with the 1 in 2500 incidence children born with cystic fibrosis. This could operating prenatally, through meiotic drive, after birth or both. We have explored meiotic drive by counting the number of carriers born to couples where only one parent is a carrier proven on DNA testing and the partner negative. In 519 offspring born to such couples 313 have been found to be carriers instead of the expected 259 (chi squared =11.4, p<0.01. This phenomenon is seen most strongly in female offspring born to female carriers. Implications and possible mechanisms will be discussed.

P0902. The FV Leiden and prothrombin G20210A mutations in healthy and thrombophilic Yugoslav population

L. B. Rakicevic[1], *D. Mikovic*[2], *M. Kovac*[2], *V. Djordjevic*[1], *D. Radojkovic*[1], *A. Savic*[1]
[1]*Institute of Molecular Genetics and Genetic Engineering; Belgrade, Yugoslavia;* [2]*Hemostasis and Hemophilia Center, National Blood Transfusion Institute; Belgrade, Yugoslavia*
qwert@eunet.yu

Purpose; The development of thrombotic disorders in humans is one of the most common causes of morbidity and mortality in the Western world. The risk of venous thrombosis is increased in individuals who carry specific genetic abnormalities in blood coagulation proteins.The FV Leiden, and prothrombin G20210A mutations are the most prevalent genetic defects which increase risk of venous thrombosis. Their allelic frequencies in Caucasian populations vary between 1 and 8%, and 0.35 and 2% respectively. For Yugoslav population there are no data for either one. Purpose of this study was to establish the prevalence of the FV Leiden and prothrombin G20210A mutations in normal and thrombophilic subjects inYugoslavia. Methods and matherials; A study was carried in a group of 100 unrelated blood donors and in 87 unrelated thrombophilic patients. Criteria for selection of patients for this study were; the occurrence of the first episode of thrombosis at the age below 50 years and at least one clinical feature of the inherited thrombophilia. FV Leiden and prothrombin G20210A mutations were detected by polymerase chain reaction, followed by digestion with allele-specific restriction enzymes. Normal and mutated genes were distinguished by the sizes of the restriction fragments. Results; In 100 control subjects 4 carriers of FV Leiden and 3 carriers of prothrombin G20210A mutations were detected, wich gives the frequencies of mutated alleles 2% and 1.5% respectively. Among 87 trombophilic patients, the frequencies of mutated alleles were higher. For FV Leiden mutation, 23 carriers (21 heterozygous and 2 homozygous) were detected, giving the allelic frequency of 14%. For prothrombin G20210A mutation, 8 heterozygotic carriers were discovered, i.e. the allelic frequency of 4.5%. Conclusions; The frequencies of FV Leiden and Prothrombin G20210A mutations in our population are within the range for general Cucasian populations. The prevalences of these mutations in thrombophilic patients, show the usual higher frequencies than in control.

P0903. The correlation of the genotype and phenotype in Slovak Huntington‘s population.

M. Kvasnicov [1], *L. Kad ?i*[2], *M. Luk sov* [3], *F. Cis rik*[4], *J. Baro?ov* [5], *E. Nebes kov* [6]
[1]*Hospital Roosevelt; Bansk Bystrica, Slovakia;* [2]*Institut of Molekular Biology Scientific Faculty; Bratislava, Slovakia;* [3]*Centrum of Medical Genetics Faculty Hospital; Bratislava, Slovakia;* [4]*Department of Medical Genetics; ?ilina, Slovakia;* [5]*Department of Medical Genetics; Nitra, Slovakia;* [6]*Department of Medical Genetics Faculty Hospital; Ko?ice, Slovakia*
fdr@isternet.sk

Since Huntington disease (HD) mutation was identified a direct molecular

test has become an integral part of diagnosis of the disease. The aim of our study was to find out the relationship between number of CAG repeats in IT-15 gene and some phenotype signs of disease in Slovak Huntington's patients. Since molecular test has been available in our country diagnostic positive molecular test has been confirmed in 63 individuals. In accordance with the range of mutation these individuals were divided into 3 groups; I. With the number of CAG repeats 40 - 45 II. - - 46 - 50 III. - - over 50 The majority of our patients belong to the group I. The mean age at onset HD in this group was 40 years (with the range 20 - 59 years). The less patients belong to the group III with the mean age at onset of disease 22 years (with the range 15 - 29 years). Except the relationship between the size of mutation and the age of onset HD we have found also the relationship between the mutation and the first observed symptoms of the disease. While in the I. and II. group majority of patients showed neurological symptoms at the onset of the disease in the III. group that were the changes in behavior and cognition. Our study was the first study of the Slovak Huntington's population. We confirmed correlation of the size of mutation in HD gene with the age at onset disease and with some of the clinical symptoms of the disease.

P0904. Congenital Cataract — Autosomal Recessive Form — in An Ethnic Isolate, Intensely Inbred

D. Bembea[1], *L. Spineanu*[1], *M. Bembea*[2], *O. Vancsik*[2], *C. Jurca*[2], *R. Spineanu*[2]
[1]*Clinical Adults Hospital, Department of Ophtalmology; Oradea, Romania;* [2]*Clinical Children Hospital; Oradea, Romania*
diabem@yahoo.com

Introduction; The most common lens defect in the neonate is cataract. It may occur as an isolated defect or it may be associated with other ocular or systemic abnormalities. Cataract may be inherited or sporadic. The most common type of inheritance is autosomal dominant; autosomal recessive inheritance occurs less frequently and it is sometimes found in populations with high rates of consanguinity. Objective; The study of congenital cataract in an isolate, in order to identify the aetiology, the type of inheritance and the genetic and clinical characteristics. Material and method; The study group comprises an isolate population of approx. 200 individuals, with 8 cases of congenital cataract identified so far. The pedigree, segregation study and the probability calculation using the Bayes formula have been accomplished for each family. Results; Endogamy in this isolate is of 80%. The consanguinity coefficient of the population forming the isolate is 0.004373 and the frequency of consanguineous marriages is of 20%. Congenital cataract has been noticed in 8 patients of 4 siblings, which yields a prevalence of the disease of 7.4% inside the isolate (1/14). The calculated risk for the 4 families varies between 1/513 and 1/5. The frequency of heterozygous carriers is 32%. The chances of maintaining the frequency of the disease in the isolate and particularities of genetic counselling in this case are discussed. Conclusions; Congenital cataract, usually rare, can reach an unusually high incidence in isolates. Its study becomes more interesting today, as isolates are disappearing.

P0905. Geographic clustering of left-right axis abnormalities in Finland - evidence of a founder mutation?

E. Kajantie[1], *A. Eerola*[2], *T. Boldt*[2], *K. Virkola*[3], *M. Eronen*[2], *K. Aittom ki*[4]
[1]*Department of Clinical Genetics and The Hospital for Children and Adolescents, Helsinki University Central Hospital; Helsinki, Finland;* [2]*The Hospital for Children and Adolescents, Helsinki University Central Hospital; Helsinki, Finland;* [3]*Department of Radiology, Helsinki University Central Hospital; Helsinki, Finland;* [4]*Department of Clinical Genetics, Helsinki University Central Hospital; Helsinki, Finland*
eero.kajantie@hus.fi

Backgorund; The population history of Finland with regions of old (around 2000 years ago) and new (about 500 years ago by a small number of founders) settlement has created an unique model to study genetic disorders on the basis of their geographical distribution across the country. Left-right axis abnormalities constitute a clinically well-defined but genetically heterogeneous disorder manifesting as congenital defects in the sidedness of the heart, great vessels, bronchi, spleen, liver and intestine. Objective; To determine whether left-right axis abnormalities in Finland occur more frequently within the new founder settlement than the old settlement. Methods; The database of the Helsinki University Central Hospital was searched for suggestive diagnoses which were confirmed by chart review. In Finland, almost all patients having left-right axis abnormalities with cardiovascular malformations are evaluated in this hospital. To exclude the effect of modern migration, the birthplaces of the patients grandparents, collected from population records, were analysed. Results; 42 patients were found, among them 2 sibpairs (total number of siblings 131). An excess, 94 (59%), of the grandparents was born in provinces corresponding to the region of new settlement, which comprise of 37% of the Finnish population in the 1930s (khi square = 15.4, $p < 0.001$). Clustering of the grandparents birthplaces with in a few smaller areas was also observed. Conclusions; Founder mutation(s) predisposing to left-right asymmetry abnormalities are likely to be present in the region of new settlement of Finland. Given the small number of sibpairs found, a complex inheritance pattern is more likely than monogenic.

P0906. CAG/CTG repeat polymorphism of IT-15 and DMPK genes and prevalence of myotonic dystrophy and Huntington disease in different populations

O. Malysheva, *T. Ivaschenko, V. Baranov, V. Baranov*
Institute of Obstetrics and Gynecology; St.Petersburg, Russian Federation
omal99@mail.ru

It is considered that in many cases the frequency of alleles with tandem trinucleitide repeat number more than 20 correlates with prevalence of corresponding expansion diseases in population. We studied distributions of CAG/CTG repeat alleles of DMPK and IT-15 genes in four populations; North-West Russia (St-Petersburg), Moldavians, Georgians and Uzbeks. The frequencies of more than 20 CTG-repeat alleles were in a good correlation with prevalence of MD in Russians, Uzbeks and Europeans (literature data). There weren t a statistically significant differences between frequencies of IT-15 gene alleles with more then 20 CAG repeats in Russians and in Uzbeks compared to East England (Rubisztein et al., 1994). HD is the most common in England, 2-5 times more rare in Russia and practically absent in Uzbeks. We also compared the distributions of long normal CAG/CTG repeat alleles of IT-15 and DMPK genes in many populations. In some populations there weren t alleles with more then 16-17 CTG-repeats in DMPK gene. In IT-15 gene alleles with 20-22 CAG repeats were common in all populations, while longer alleles were represented only in part of them. Taken together, these facts indicate that the alleles groups which are a reservoir for de novo mutations could differ in their repeats number in two genes. According to our suggestion, the threshold value between stable alleles and alleles predisposed to expansion is 17-18 repeats for DMPK gene and 22-23 repeats for IT-15.

P0907. Genetic epidemiology of mendelian diseases in Yakutia

L. Nazarenko, *A. Nogovicina, O. Saljukova*
Institute of Medical Genetics; Tomsk, Russian Federation
lnaz@img.tsu.ru

We have studied the incidence and prevalence of monogenic disorders in 100000 inhabitants of native population of Yakutia, situated at nothern-east part of Siberia. The maximal morbidity rate of autosomal dominant (AD) diseases was defined for eastern Yakutian subpopulations (2.4 x 10-3) and minimal — for western subpopulations (1.4 x 10-3). The values of morbidity rate of autosomal recessive (AR) diseases ranging from 0.4 to 0.9 x 10-3. The mean values of morbidity rate of mendelian diseases for Yakutian population were 1.7 x 10-3 for AD, 0.7 x 10-3 for AR and 0.4 x 10-3 for X-linked diseases. The total morbidity rate of monogenic diseases is 2.8 per 1000 person. In Yakutian population we observed a high frequency of spinocerebellar ataxia I (SCA1 - MIM 164400), dystrophia myotonica (DM - MIM 160900) and methemoglobinemia (NadH-Cytochrome b5 Reductase deficiency - MIM 250800). The study of hereditary diseases will give us the opportunity to create a prophylactic register of hereditary diseases and come to the prospective medico-genetic consultations.

P0908. Haplotype Analysis of Apolipoprotein B Gene in Korean Essential Hypertensives

B. Kang[1], *K. Kim*[1], *J. Shin*[2], *C. Lee*[2]
[1]*Seoulin Bioscience Institute, Seoulin Bioscience, Co., Ltd.; Seoul, Republic of Korea;* [2]*School of Biological Sciences, Seoul National University; Seoul, Republic of Korea*
kby_99@yahoo.com

Essential hypertension is considered to be a multifactorial disease that is influenced not only by environmental factors but also by genetic factors. Alterations of lipid metabolism in plasma have been reported to be related to an increased risk of essential hypertension. The purpose of this study was to estimate haplotype frequencies of apolipoprotein B(apo B) gene in Korean population and investigate the relationship between haplotypes of this gene and essential hypertension. In order to estimate the haplotype frequencies, Pvu II, Xba I and Eco RI RFLPs of apo B gene were used as genetic marker. There were no significant differences in allele, genotype or haplotype frequencies between normotensives and essential hypertensives. However, Xba I and Pvu II RFLPs of apo B gene were significantly associated with plasma total cholesterol and triglyceride levels in essential hypertensive groups, respectively($P<0.05$). Therefore, our result suggest

that these two RFLPs of apo B gene may be genetic components of cardiovascular risk factors in Korean essential hypertensives.

P0909. High Incidence of 550delA Mutation in Limb-Girdle Muscular Dystrophy Type 2A (LGMD2A) in Croatia

A. Milic[1], *B. Kovac*[2], *F. Leturcq*[3], *N. Zurak*[1], *N. Canki-Klain*[1]
[1]*Croatian Institute for Brain Research and Department of Neurology, Zagreb University Medical School; Zagreb, Croatia;* [2]*Department of Neurology, University of Osijek; OSIJEK, Croatia;* [3]*Laboratoire de Biochimie et GenetiqueMoleculaire, Hopital Cochin; Paris, France*
stella24hrv@yahoo.com

We report preliminary data concerning 28 LGMD2A patients from Croatia s population of 4,8 million people. Sequence analysis of patients from six apparently non related families from a small rural community revealed homozygocity for a 550delA. Analysis of additional 4 families from different parts of the country revealed homozygous 550delA patients in two families, compound heterozygotes 550delA / Y537X in one family, and compound heterozygotes for a 550delA /one unknown mutation. These results motivated us to develop rapid screening method for 550delA. The fact that 550delA creates restriction site for Bsa AI enzyme was used to distinguish wild type PCR product (210bp) unchanged after the incubation with the enzyme, from mutated type (cut in two bands of 120 and 90 bp). Application of this method on 28 patients from 18 families permitted us to identify; homozygous 550delA patients in 8/18 families; compound heterozygotes 550delA in 7/18 families. Second mutated allele is not identified in 4 families. In five typical LGMD2A patients from 2 families both alleles are unknown. In conclusion our preliminary study shows that 550delA mutation accounts for 63,8 %(23/36) of CANP3 chromosomes.

P0910. Distribution the most common cystic fibrosis mutations in Balkans, Central & Eastern European populations.

M. Macek Jr.[1], *M. Macek*[1], *T. Doerk*[2], *E. Dequeker*[3], *J. Cassiman*[3]
[1]*CF Center-Institute of Biology and Medical Genetics; Prague 5, Czech Republic;* [2]*IHG, MNH; Hannover, Germany;* [3]*Cnt. Med. Genet. Univ.; Leuven, Belgium*
pavel.roubic@lfmotol.cuni.cz, milan.macek.jr@lfmotol.cuni.cz

We have analyzed 151 CFTR mutations in representative groups of CF patients from 20 Balkans, Central and Eastern European countries (BCEC; 73 AL, 290 A, 94 BY, 216 BG, 138 HR, 247 CZ, 30 EE, 359 EL, 488 HU, 18 LV, 47 LT, 113 RM, 863 PL; 28 RO, 1,281 RU, 117 SK, 66 SI, 335 TR, 260 UA, 199 YU; a total of 5262 cases). All centers screened for the 12 most common CF mutations, while the entire CFTR coding region was scanned in A, BG, CZ, EE, EL, PL, RM, RU, SI and TR. The 25 most common CF alleles comprise; deltaF508 (n=5636/53.55% of all CF chromosomes), G542X (227/2.16), N1303K (164/1.56), CFTRdele2,3/21kb/ (120/1.14), 3849+10kbC->T (67/0.64), 621+1 G->T (63/0.59), R553X (62/0.59), W1282X (56/0.53), 1677delTA (47/0.45), G551D (44/0.42), 1717-1G->A (34/0.32), 2184insA (32/0.30); 2143delT, R334W (28/0.27 each); R347P (27/0.26); 2183AA->G (24/0.23), 2789+5 G->A (21/0.20), R1162X (19/0.18), 394delTT (17/0.16), 1898+1G->A (16/0.15), R1070Q (15/0.14), G85E (13/0.12); 457TAT->G, E822X (13/0.12 each) and R1158X (11/0.1). The spectrum and distribution of mutations markedly differs from that observed in Western Europe (Hum Mutat 10;135,1997 extrapolated data) with statistically significant differences being observed in deltaF508, G542X, CFTRdele2,3 /21kb/ and G551D mutations (p<0.001). Inclusion of common BCEC mutations into screening panels will considerably improve the genetic diagnosis of CF. Supported by grants;#6250-3, 6411-3, 000000064203; #111300003, ME258, OK192; LN00A079; ERB IC20 CT96 0058. (*)We acknowledge contribution of members of the INCO-BIOMED CF Mutation Analysis Consortium that could not be listed by their names due to abstract space constraint.

P0911. The mutation spectrum of Hyperphenylalaninaemia in Southern Ireland; the population genetics of the Irish revisited

D. T. Croke
Royal College of Surgeons in Ireland; Dublin, Ireland
dtcroke@rcsi.ie

Phenylketonuria (PKU), the most severe form of hyperphenylalaninaemia (HPA), has an incidence in the Republic of Ireland of approximately 1 in 4,500 live births. The purpose of this study was to investigate the HPA mutation spectrum in the Republic, to determine mutation-haplotype associations and to investigate genotype-phenotype correlations. Mutational analysis was carried out on a cohort of 279 unrelated hyperphenylalaninaemic patients (558 independent alleles) by standard methods. Mutations were detected in 92% of alleles revealing a total of 29 mutations. A large proportion of alleles (63.6%) were accounted for by three mutations; R408W (41.0%), F39L (12.1%) and I65T (10.1%). The predominant haplotype associations for these common mutations were; R408W-1.8-242, F39L-1.8-238 and I65T-1.8-246. The R408W-1.8 mutation exhibits an East to West gradient of increasing relative frequency across Europe peaking in Ireland. Our data demonstrates that this gradient continues across Ireland and peaks in Connacht, the most Westerly province. Combining our data with that from Northern Ireland (Zschocke et al., Am J Hum Genet 1995; 57; 1311-1317) we have produced a merged data-set for the island of Ireland. Our analysis of this data concurs with previous suggestions that the English contribution to the Irish genepool was small and that gene flow between Ireland and Scandinavia was unidirectional. It further demonstrates that the province of Ulster has been a zone of population admixture between Ireland and Scotland.

P0912. Study of the mutations causing familial hypercholesterolemia in St.-Petersburg

J. Tatisheva, *F. Zakharova*
Institute for Experimental Medicine; St.-Petersburg, Russian Federation
July@postbox.spb.ru

Familial hypercholesterolemia (FH) is an inherited metabolic disease with a dominant mode of inheritance. It is quite common in most human populations (1;500) and results in drastical reduction of low-density lipoprotein (LDL) catabolism followed by premature coronary heart disease (CAD). The disease is caused by mutations in the LDL receptor gene. In order to develop presymptomatic diagnostic tools for management of the FH we aimed to study the LDL receptor gene mutation spectrum in St.-Petersburg (Russia). We have created a DNA bank from 100 unrelated patients with clinical picture of FH. Separate exons of the receptor gene were amplified by polymerase chain reaction (PCR) and screened for presence of the mutations via combined single-strand conformation polymorphism-heteroduplex analysis (SSCP-HA). The fragments showing shifted mobility in polyacrylamide gel electrophoresis were sequenced by method of Sanger. Up-to-date, we have identified six mutations - C74X, 347delGCC, ?130?, ?146R, C188Y, G578E, all of them besides C74X were novel. Rapid methods for mutation detection were developed. Cosegregation of mutations and high cholesterol levels proves the role of mutations in disease development. St.Petersburg population was found to be polymorphic in many postions of the LDL receptor gene - 447 ?/?, 750C/T, 1170A/G, 1413 G/A, 1545 ?/?, 1773 T/C, 2177 C/T, 2231 G/A (exons 4, 5, 8, 10, 10, 12, 15, 15 correspondingly). Using DNA methods we have confirmed or set diagnosis of FH in 34 patients and confirmed absence of FH in 14 relatives of patients.

P0913. International variation in the prevalence of the C677T variant of the 5,10 methylenetetrahydrofolate reductase (MTHFR) gene among well-defined populations.

R. Tenconi[1], *P. Mastroiacovo*[1], *-. MTHFR study group*[2], *-. MTHFR study group*[2]
[1]*International Center for Birth Defects; Rome, Italy;* [2]*International Clearinghouse Birth Defects Monitoring Systems; -, Italy*
icbd@icbd.org

MTHFR encodes a critical enzyme in folate and homocysteine metabolism. The C677T allele of the MTHFR gene is reportedly common in some populations and has been associated with an increased risk for spina bifida and for adult cardiovascular diseases. Although many reports the C677T prevalence are available, few were based on well-defined populations. We report data from 12 countries in Europe (Finland, France, Hungary, Italy, Netherlands, Spain), Israel, Russia, China, and the Americas (Canada-Alberta, United States-Georgia, Mexico). We attempted to sample populations that were well defined; our approaches included using newborn blood spots from specific geographic areas or from consecutive liveborn babies from hospitals. Typically these populations were also under birth defect monitoring. We found a high prevalence of C677T homozygotes in Mexico (32%), Northern China (20%), and Southern Italy (20% in Catania, 26% in Campania). Such prevalence was intermediate in Northern Italy, France, and Spain (15, 13, and 12%, respectively), and it was low in Finland, the Netherlands, and Russia (4,6, and 7%, respectively). In the sample from the United States (Georgia), we noted racial variations; prevalence was high among Hispanics (18 percent), low among African Americans (3 percent) and intermediate among Whites (11 percent). These findings underscore the geographical and racial variability of the C677T variant. Such data should be useful to better design studies of gene-environment interactions involving the MTHFR gene, as well as for future applications in population-based prevention of conditions related to such gene.

P0914. Two novel mutations in the HEXA gene found in TSD carriers of Iraqi Jewish origin and haplotype analysis suggesting a founder effect.

L. Peleg[1], *M. Karpati*[1], *B. Goldman*[1], *E. Gazit*[2], *A. Frisch*[3]
[1]*Danek Gertner Institute of Human Genetics, Sheba Medical Center; Tel Hashomer, Israel;* [2]*Department of Pediatrics, Sheba Medical Center; Tel Hashomer, Israel;* [3]*Felsenstein Medical Research Center; Rabin Medical Center, Petah-Tikva, Israel*
lea_peleg@hotmail.com

Among Jews, Tay-Sachs disease (TSD) was thought to be restricted to individuals of Ashkenazi (carrier frequency of 1;29) and Moroccan (1;110) descent. However, an elevated carrier frequency of 1;140 was found also among Iraqi Jews (IJ). A G749 to T change resulting in the substitution of Glycine250 to valine was recently identified by us in 41% of the TSD carriers (24/58). We now report an additional novel mutation; a C1351 to G transversion resulting in a change of Leucine451 to valine in 29% (17/58) of IJ carriers. Neither of the mutations was found in 100 non-carriers of the same ethnic group. Haplotype analysis was conducted using 6 markers closely linked to the HEXA gene. Mutation G749 to T was associated with allele #1 of D15S131 in 70% of heterozygotes compared with 10% in ethnically matched controls (chi square=46.9, p=10-5) and with allele #2 of D15S1025 in 68% of heterozygotes compared with 43% in controls (chi square=4.4, p=0.03). The calculated linkage disequilibrium was 0.66 and 0.44 respectively. Mutation C1351 to G was also associated with allele #1 of D15S131 (chi square=7.0, p=0.008) yielding a linkage disequilibrium of 0.3 yet, no significant linkage was found with D15S1025. These results suggest a possible common ancestor for each of the HEXA mutations in Iraqi heterozygotes. The C1351 to G mutation had probably occurred earlier than G749 to T, as it shows less association with the adjacent markers. Since these mutations have not been documented in Ashkenazi Jewish carriers, they were probably introduced after the 70 AD Exile.

P0915. Notification of Cystic Fibrosis as primary cause of death in South and Southeast Brazil, from 1981 to 1995

C. L. A. Paiva*, L. Janotti, C. Naurath, A. Essinger, R. Gon alves, E. Baltazar, S. R. Middleton, S. R. dos Santos*
Universidade do Rio de Janeiro; Rio de Janeiro, RJ, Brazil
clapaiva@prolink.com.br

Cystic Fibrosis (CF) is an autossomal recessive chronic disease mapped to 7q31-q32. Lung disease accounts for ~95% of its morbidity and mortality. The lungs of CF patients are compromised by persistent bacterial infection from early age. Of the congenital disorders CF is one of the most common with ~1 in 2000 Caucasians affected, with carrier frequency as high as one in 25 in Caucasians of northern European ancestry. There was ample motivation to investigate the notification of CF as primary cause of death in South and Southeast Brazil, since these regions have a multiracial mixed population including Caucasians from European origin. Our hypothesis was that there was under estimation of deaths from CF, since the disease has not been currently recognised mainly in the inland parts of the country. CF symptoms, such as pulmonary infection, and diarrhoea,as well as malnutrition (caused by lack of pancreatic enzymes secretion) are also common causes of death of non-CF infants in Brazil. Our data were extracted from the Brazilian CD-rom entitled Sistema de informacao sobre mortalidade/1979-1997 which contained data of all death declarations of the country.The frequencies of CF death notifications per 100,000 inhabitants per year for Brazil and for each south or southeast state were calculated for comparison. Chi-square test was performed by comparing the values of each state against that of Brazil and of Sao Paulo(SP),the more developed state. Our results may indicate that SP has a more precise notification of CF as primary cause of death than other states of Brazil

P0916. Intragenic recombination events and de novo mutations in the DMD gene; rapid detection by analysis of microsatellite markers.

M. Kaczmarek[1], *D. Napierala*[1], *M. Szalata*[1], *R. Slomski*[1,2]
[1]*Institute of Human Genetics; Poznan, Poland;* [2]*Department of Biochemistry and Biotechnology; Poznan, Poland*
slomski@au.poznan.pl

Studies of allele frequencies of three microsatellite markers revealed few new alleles of each marker in Polish population. All new alleles are very rare. The number and frequency of alleles are similar for Polish and Caucasian population. Only one discrepancy occurred between our studies and previous data and it concerned size of the most common allele of the DMD(CA)5 II marker. The three markers were run in a multiplex reaction to determine carrier status for sisters of affected child from 36 DMD/BMD families. In two families carrier analysis concerned an aunt of an affected child and in one case a cousin. At least one marker was informative in all studied families. All three markers were informative in seven families. In seven cases deletion of the DMD(CA)45int marker in affected children occurred. It gave possibility of direct identification of mutations in female relatives. Additionally direct carrier detection was possible in one family with large deletion including the DMD(CA)5 II marker. However, in order to distinguish homozygous and hemizygous females, DNA samples of both parents are necessary. Three cases of homo-/hemizygous mother occurred in our investigation. Unfortunately, grandparents DNAs were not available and in these cases carrier status of mothers was not determined. Direct approach failed also in two families, in which both mother and father had the same allele and it was impossible to estimate whether a sister is homo- or hemizygous. New mutations were observed in seven families. In four of them deletion of marker occurred in an affected child while mother was heterozygous. In three other cases the same haplotype occurred in an affected child and a healthy brother. Additionally, seven cases of intragenic recombination events were detected. Five of them occurred in DNA obtained from sisters. Only two markers were informative in all these cases, so it was impossible to determine which marker is linked with a mutation.

P0917. (AC) repeats of 5 beta globin gene and its relation with sickle cell anemia in Mexican population.

R. I. E. Pe aloza[1], *P. Delgado*[1], *T. Sedas*[2], *C. Hernandez*[3], *L. Buentello*[4], *D. Arenas*[1], *F. Salamanca*[1]
[1]*Unit of Medical Investigation in Human Genetics; Mexico City, Mexico;* [2]*Regional Hospital; Veracruz, Mexico;* [3]*Regional Hospital; Acapulco, Mexico;* [4]*Institute of Antropological Investigations, UNAM; Mexico City, Mexico*
rosenda@servidor.unam.mx

The (AC) repeats are been found in the human genomic very frequently. Some of them are related with pathologies. The (AC)n of 5 beta globin gene end was studied in normal Mexican population. We founded three alleles. The first A was the most common, B was intermidiat frequency and C only in Pur pecha and Mestizo population was found . Now we reported its relation with sicle cell anemia of Mexican patients. The allele B it was found more frequente in beta-S mutation. Moreover, two news alleles there are in the Mestizo Mexican population studied, that have high African component. This finding are useful for better knowling of this human group.

P0918. The Distribution of Bardet-Biedl Syndrome Loci in the Newfoundland Population

W. S. Davidson[1], *M. O. Woods*[2], *J. S. Green*[2], *P. S. Parfrey*[2]
[1]*Simon FRaser University; Burnaby, BC Canada;* [2]*Memorial University; St. John s, NF Canada*
wdavidso@sfu.ca

Bardet-Biedl Syndrome (BBS) is a rare, genetically heterogeneous (>6 loci), recessive disorder characterized by dysmorphic extremities, retinal dystrophy, obesity, male hypogenitalism, and renal disease. We have identified 22 families with BBS in Newfoundland, 17 of whom participated in a genetic linkage study. A founder effect was associated with BBS1 in 5 families, 2 families have BBS2, and 1 is linked to BBS3. Another Newfoundland family positioned BBS5 on chromosome 2q. No BBS4 families have been identified. A genome wide scan of family B13 (excluded from BBS loci 1-5) suggested that the locus for BBS6 was at 20p12. Fine mapping reduced the critical interval to 1.9cM encompassing a chaperonin-like gene associated with McKusick Kaufman Syndrome (MKKS). Mutation screening of the MKKS gene identified combinations of 3 mutant alleles in five BBS families and confirmed that MKKS and BBS6 result from mutations in the same gene. Of the 3 unassigned families, one has been excluded at BBS1-6, implying that another disease locus exists. The remaining 2 families are uninformative due to pedigree structure. We conclude that, unlike the rest of North America and Europe, BBS6 accounts for approximately one third of BBS in Newfoundland. We have no explanation at this time for the high incidence of BBS in this relatively small, isolated population or for the presence of such a diversity of loci (at least 6) or the number of different mutations (at least 8).

P0919. Analysis of autosomal dominant polycystic kidney disease (ADPKD) in the Newfoundland population.

D. S. Compton[1], *W. S. Davidson*[1], *J. S. Green*[2], *E. L. Dicks*[2], *P. S. Parfrey*[2]
[1]*Simon Fraser University; Burnaby, Canada;* [2]*Memorial University of Newfoundland; St. John s, Canada*
dcompton@sfu.ca

The Newfoundland population is ideal for examining founder effects and for studying the incidence of hereditary disease in what is essentially a closed community. Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common genetic diseases in humans. Muta-

tions in any one of at least two different genes are thought to be responsible for this disease and two genes, PKD1 and PKD2, have been mapped to 16p13.3 and 4q21-23, respectively. Fifteen Newfoundland families with ADPKD have been examined using microsatellite markers flanking and within the PKD genes on chromosome 16 and chromosome 4. Eight of the fifteen families showed linkage to PKD1 whereas four were linked to PKD2. The other three families were uninformative due to the structure of the family. Comparisons of the haplotypes of the families linked to PKD1 revealed that four families share a partial haplotype suggestive of an ancestral founder disease chromosome. The other four PKD1 haplotypes suggest that these all resulted from independent mutation events. Comparisons of the PKD2 haplotypes revealed that two families shared a haplotype and sequencing of exon 6 showed that both families shared the same R464X mutation. The other two PKD2 haplotypes suggest that they resulted from independent mutation events. Mutational analysis is currently underway to determine the exact number of founder mutations for ADPKD in the Newfoundland population. (Supported by the Kidney Foundation of Canada and the Medical Research Council of Canada)

P0920. Frequency of the C282Y mutation of the HFE gene in Ukraine.

V. Pampuha[1], *A. Cherchenko*[2], *V. Rozumenko*[3], *I. Lubyanova*[4], *L. Livshits*[5]
[1]*Institute of Molecular Biology & Genetics National Academy of Science of Ukraine; Kyiv, Ukraine;* [2]*Institute of Neurosurgery Academy of Medical Science of Ukraine; Kyiv., Ukraine;* [3]*Institute of Neurosurgery Academy of Medical Science of Ukraine; Kyiv, Ukraine;* [4]*Institute for Occupational Health Academy of Medical Science of Ukraine; Kyiv, Ukraine;* [5]*Institute of Molecular Biology and Genetics National Academy of Science of Ukraine; Kyiv, Ukraine*
livshits@imbg.org.ua

Hereditary hemochromatosis (HH) is a common autosomal recessive genetic disorder in Caucasians. Hemochromatosis is characterized by increased gastrointestinal iron absorbtion in excessive uptake and persistant accumulation of iron in the body. Classical symptoms of hemochromatosis include liver disease, diabetes mellitus, myocardiopathy, arthralgia, skin siderosis. The gene of hemochromatosis HFE is located on the short arm of the chromosome 6 telomeric to major histicompatibility complex. A single missence mutation C282Y is responsible for hemochromatosis in approximately 80% of all cases. C282Y frequencies of 1-10 % are observed in Europeans. We analysed the frequency of the C282Y in 260 individuals with Slavic origin from three Ukranian regions (West, Central , East) and in 100 individuals from isolated Crimean tartars. Frequency of C282Y mutation in different regions of Ukraine ranged from 2% to 3%; frequency of this mutation in pooled Ukrainians was 2,5% and in Crimean tartars was 1%. The high prevalence of the C282Y mutation in Ukraine suggests that population screening for the C282Y mutation could be highly advantageous in terms of preventive health care.

P0921. Detection of the CFTR gene mutations in cystic fibrosis patients and in men with azoosperma from Estonia.

M. Teder[1], *T. Kahre*[2], *K. Kaasik*[3], *M. Punab*[4], *A. S ritsa*[5], *A. Metspalu*[6]
[1]*Estonian Biocentre; Tartu, Estonia;* [2]*Centre of Molecular Diagnostics, Tartu University Clinics; Tartu, Estonia;* [3]*Institute of Cell and Molecular Biology, University of Tartu; Tartu, Estonia;* [4]*Surgery Clinic, Tartu University Clinics; Tartu, Estonia;* [5]*T he Private Clinic; Tartu, Estonia;* [6]*Institute of Molecular and Cell Biology, Centre of Molecular Diagnostics, University of Tartu; Tartu, Estonia*
mteder@ebc.ee

Since the identification of the cystic fibrosis transmembrane conductance regulator (CFTR) gene more than 900 mutations, responsible for CF have been described. Involvement of CFTR gene mutations has also been confirmed in otherwise healthy but infertile men with obstructive azoospermia from congenital bilateral or unilateral absence of vas deference, and epididymal or ejaculatory duct obstruction. The aim of the study was to identify the spectrum of CFTR gene mutations in CF patients and in infertile men with azoospermia and oligozoospermia in Estonia. Thirty families with CF patients were studied. All 27 exons and their flanking sequences were scanned by SSCP and DGGE analyses. Two CFTR gene mutations were found to be common in Estonia; ˇF508 in 31 (51.7%) alleles and 394delTT in 8 (13.3%) alleles. Eight rare mutations were detected, all described earlier in other populations. We did not find any of the mutations more common in the European populations like G542X(2.6%), N1303K(1.6%), G551D(1.5%) or W1282X(1.0%). In conclusion, about 80% of mutations were identified in CF patients. The study group of men with fertility problems included 20 individuals with obstructive and 29 individuals with nonobstructive azoospermia and 107 men with severe oligozoospermia. Patients were tested for three CF mutations ˇF508, 394delTT, R117H and a IVS8-T variant. The patients with obstructive azoospermia were additionally tested for more mutations by INNO/LipaTMCFTR12 and INNO/LipaTMCFTR17+Tn kits. In the result of these tests only ˇF508 mutation and 5T allele were revealed. We identified two heterozygotes ˇF508/5T and two heterozygotes with 5T allele in one chromosome in obstructive azoospermia group. One compound heterozygote ˇF508/P508C, one 5T homozygote and one patient with only one 5T allele were detected in nonobstructive azoospermia group. Among the oligozoospermic men 14 patients revealed 5T in one allele and one carried ˇF508 muation on one chromosome. In comparison with the control group of normal men, the frequency of ˇF508 mutation and 5T allele was considerably higher only in patients with obstructive azoospermia; 5% versus 0,6% for ˇF508 and 10% versus 4,2% for 5T.Therefore CFTR gene mutation screening and genetic counselling should be recommended to males with obstructive azoospermia and there partners to reduce there risk for CF child by assisted reproduction procedures.

P0922. Factor V Leiden and Factor II Mutation frequencies in Turkish pregnant women ; preliminary findings

R. Bircan[1], *C. Erzik*[1], *F. Akbas*[2], *I. G ney*[1], *E. Oral*[2], *B. Cirakoglu*[1]
[1]*Marmara University Faculty of Medicine Dept of Medical Biol&Genetics; Istanbul, Turkey;* [2]*Istanbul University Cerrahpasa Medical Faculty Dept of Obstetrics and Gynecology; Istanbul, Turkey*
rifatbircan@yahoo.com

Objective; The study s objective was to evaluate association between hereditary coagulation abnormalities including factor V Leiden and prothrombin G20210A and obstetrical complicalitions such as preeclampsia, abruptio placentae, intrauterine fetal growth retardation (IUFGR)and stillbirth in Turkish population. Material and Methods ; DNA was extracted from whole blood of 84 women -42 complicated pregnancies and 42 control cases. The polymerase chain reaction was used to amplify exon 10 of factor V gene and untranslated end of prothrombin gene followed by enzymatic digestion with Mnl I and Hind III for mutation detection respectively. Results ; The mutation at nucleotide 1691 in factor V gene was detected in 8 of the women with obstetrical complication and in 3 of the women with normal pregnancies (19% and 7.1% respectively; $p<0.005$). This mutation was detected 5 of the 28 women who had preeclampsia (17.9% $p<0.005$), 2 of the 5 women who had stillbirth (40%) and 1 of the 7 pregnant women who had IUFGR (14%). Prothrombin G20210A polimorphism detection results under evaluation. Conclusions ; The Leiden mutation is relatively common in pregnant women with preeclampsia. Although the mutation was detected in women who had stillbirth and IUFGR, the number of subject is not meaningful for statistical evaluation. It is suggested that factor V Leiden and prothrombin G20210A mutation detection screening can be used for pregnant women under risk.

P0923. Variable expression of presenilin 1 is not a major determinant of risk for late-onset Alzheimer s Disease

R. Rademakers[1], *G. Roks*[2], *M. Cruts*[1], *J. Theuns*[1], *J. Houwing-Duistermaat*[2], *S. Serneels*[1], *A. Hofman*[2], *M. Breteler*[2], *C. Van Broeckhoven*[1], *C. van Duijn*[2], *B. Dermaut*[1]
[1]*Flanders Interuniversity Institute for Biotechnology (VIB); Antwerpen, Belgium;* [2]*Erasmus Medical Center; Rotterdam, The Netherlands*
rrademak@uia.ua.ac.be

We have demonstrated a significant association between early-onset Alzheimer s disease (EOAD) and an allele in the promotor of presenilin 1 (PSEN1),significantly decreasing PSEN1 expression in vitro. For late-onset Alzheimer s disease (LOAD), numerous studies have reported inconsistent associations with a polymorphism in intron 8 of PSEN1. We hypothesized that these conflicting reports in LOAD might be due to linkage disequilibrium between this intronic PSEN1 polymorphism and the functional promotor polymorphism. To determine the genetic contribution of the PSEN1 intronic and promotor variations to LOAD we analyzed both variations in 356 LOAD patients and 230 controls in a population-based case-control study. In addition, we reanalyzed all published literature on the PSEN1 intronic polymorphism in a meta-analysis. No significant association was found with the PSEN1 intronic or promotor polymorphism in our case-control sample. In the meta-analysis no major differences between patients and controls were found for the PSEN1 intronic variation. Together our results do not support a major role for variable expression of PSEN1 in LOAD.

P0924. Genotype and gene frequency analysis of beta thalassemia in Chaharmahal Bakhtiary Province (Iran)

M. Hemmat
Shahrekord University; Shahrekord, Islamic Republic of Iran
MO_hemmat@yahoo.com

The beta thalassemia is an autosomal ressesive genetic disease, caused

by a loss in activity in one or both alleles of the beta globin gene. The beta thalassemia is well known as one of the major genetic disease in ten provinces located in north and south of Iran. It has been estimated that more than 3000000 gene carriers exist in these regions. The present study has been focused on beta thalassemia in Chaharmahal Bakhtiary province located in south of Iran. Hardy-Weinberg principle was utilized in order to determine the genotype and gene frequencies and estimation of the percentage of homozygote and heterozygote carriers in this population. The frequency of all the affected genes (q)and normal gene (p)were estimated as 0.016 and 0.984 respectively. The frequency of the number of people expected to be homozygous normal, heterozygous carriers (minor thalassemic) and homozygous affected (major thalassemic) were determined as p2=0.9682, 2pq=0.0315 and q2=0.0003 respectively. From these data it was concluded that, 96.82 percent of human population in this province were homozygous normal, 3.15 percent were heterozygous carriers (minor thalassemic)and 0.03 percent were homozygous affected and demonstrated major thalassemia.

P0925. Incidence of XmnI RFLP among Iranian Phenylketonuria Patients as a Marker Contributing to Carrier Detection

M. seifati[1], *M. Hosseini Mazinani*[1], *N. Hosein Pur*[1], *M. Koochmeshgi*[2], *B. s. Behboudi*[3]

[1]*national research center for genetic engineerig and biotechnology; tehran, Islamic Republic of Iran;* [2]*Iran medical university; Tehran, Islamic Republic of Iran;* [3]*Tehran university; Tehran, Islamic Republic of Iran*

mseifati@nrcgeb.ac.ir

Phenylketonuria (PKU) is the most common error of amino acid metabolism. It is an autosomal recessive disorder caused by a variety of mutations in the Phenylalanine Hydroxylase (PAH) gene. These mutations exhibit a high degree of association with specific haplotypes in different populations. These haplotypes are composed of different kinds of genetic markers. An Extended- haplotype is a combination of different RFLPs (EcoRV, XmnI, MspI, EcoRI, PvuIIb, PvuIIa, BglII,), while a mini-haplotype consists of one specific STR, one specific VNTR and XmnI RFLP of the PAH gene. Since STR and VNTR are polymorphic markers, they have higher polymorphism information contents (PICs) in comparison with RFLPs (each having only two forms), and thus they represent to be more suitable markers in carrier detection procedure done by segregation analysis. However, some RFLPs are needed beside these polymorphic markers to yield highly efficient carrier detection results. One of the several markers we have studied in order to identify their degree of heterozygosity and PIC is XmnI, which is the only common marker in extended- and mini-haplotype. For this purpose we extracted DNA from PKU patients blood samples and amplified exon 8 of PAH gene in them. Among 124 alleles, only 8 alleles (6.5%) were digested with related enzyme (ASP 700) . Thus the degree of heterozygosity and PIC of XmnI RFLP in Iranian PKU patients are 0.12155 and 0.11416 respectively. Combining this RFLP with STR and VNTR polymorphic markers in segregation analyses will increase the efficiency of carrier detection procedure.

P0926. Identification of the Major Mutation in PAH Gene among Iranian Phenylketonuria Patients

N. Hosein Pur, *M. Hosseini Mazinani, J. Koochmeshgi, K. Moshiri, Z. Khazaee, K. Anisi, M. Seifati*

National Research Center for Genetic Engineering and Biotechnology; Tehran, Islamic Republic of Iran

nasim@nrcgeb.ac.ir

Phenylketonuria (PKU), the most common inborn error of amino acid metabolism, is an autosomal recessive disorder caused by more than 400 mutations in Phenylalanine Hydroxylase gene (PAHdb). This gene, 90 kilo bases long, includes 13 exons and is located in 12q22-q24 region. Distribution patterns of mutations in the PAH gene are specific to each population. In this study,the incidences of IVS10nt546, R261X, R261Q and R408Q mutations among a group of Iranian PKU patients from all over the country were determined. IVS10nt546, occurring in intron 10 of PAH gene, is the most common mutation among Mediterranean populations. The next two ones have a good chance to occur since they belong to a hotspot in exon 7 of this gene,and R408Q mutation,existing in exon 12, is one of the only two reported mutations among Persian ethnicity in PAHdb to date. In order to study these mutations, we amplified exons 7, 11 and 12 of PAH gene, extracted from blood samples of PKU patients. PCR products were then digested with DdeI, HinfI, DdeI and HaeIII restriction enzymes for detecting R261X, R261Q, IVS10nt546 and R408Q mutations respectively. Among 130 alleles studied, 40 alleles (33.3%) were positive for IVS10nt546 mutation and among 80 alleles, 5 alleles(6.25%) were positive for R261Q and 2alleles(2.5%) for R261X mutations. No allele was positive for R408Q mutation. According to this data, IVS10nt546 mutation seems to be the major mutation in Iranian PKU patients. This result is in accordance with the prevalent mutation in Mediterranean populations.

P0927. Molecular diagnosis of Rett syndrome; Detection of the prevalent mutations in MeCP2 gene in Czech and Slovak patients.

R. Rosipal[1], *J. Zeman*[1], *J. Hada?*[2], *N. Mi?ovi?ov* [3], *S. Nev? malov* [1], *P. Mart sek*[1]

[1]*Charles University, Faculty of Medicine I; Praha, Czech Republic;* [2]*Thomayer Hospital; Praha 4, Czech Republic;* [3]*Faculty Hospital; Martin, Slovakia*

rrosipal@hotmail.com

Rett syndrome is an X-linked dominant neurodevelopmental disorder affecting 1 in 10,000 to 15,000 females worldwide (1) and is caused by mutations in X-linked MECP2 gene, encoding for methyl-CpG-binding protein 2 (2,3). It plays an important role in the regulation of gene expression. There are 5 prevalent mutations in methyl-CpG binding protein 2, causing Rett syndrome. Four of them are detectable by restriction analysis. In this study we present the results of the search for four prevalent mutations in the gene for methyl-CpG binding protein 2 in Czech and Slovak patients with Rett syndrome. Twenty two females with clinical diagnosis of Rett syndrome from Czech and Slovak Republics were investigated. Restriction analysis and direct sequencing of PCR products of methyl-CpG binding protein 2 gene revealed 3 different mutations [473 C to T (T158M) in 3 patients, 502 C to T (R168X) in 2 patients, and 808 C to T (R270X) in 1 patient] in six unrelated patients with Rett syndrome. The results of restriction analysis were confirmed by bi-directional direct sequencing. The first study of Rett syndrome in Slavic population shows that three from hot spot mutations in exon 3 of MECP 2 gene are present in 27% of the patients and dictates therefore the strategy in molecular diagnosis of Rett syndrome. Mutation R306C, frequent in Swedish patients (4), was not found in 22 Czech and Slovak patients. 1. Rett, A.; Wien Med. Wochenschr., 116, 1966, 723-726. 2. Amir, R.E., et al. ; Nature Genet., 23, 1999, 185-188. 3. Sirianni, N., et al.; Am. J. Hum. Genet., 63, 1998, 1552-1558. 4. Xiang, F., et al.; J. Med. Genet., 37, 2000, 250-255. Supported by Czech Granting Agency (GACR 301/01/P068 and 302/99/0648).

P0928. Haplotype Analysis of the Most Common Beta-Thalassemia Mutation Intervening Sequence II-1 (IVSII-1) in Iran

T. Parsian[1], *N. Ghobadian*[1], *A. Javadian*[1], *S. Teimourian*[2], *F. Pourfarzad*[1], *S. Amjadi*[1], *H. Najmabadi*[1,2]

[1]*University of Welfare and Rehabilitation; Tehran, Islamic Republic of Iran;* [2]*Karimi-Nejad Pathology and Genetics Center; Tehran, Islamic Republic of Iran*

maryamneishabury@yahoo.co.uk

IVSII-1 is considered the most common beta-thalassemia mutation in Iran. In the North of Iran by the Caspian Sea, over 50% of affected individuals carry this mutation and the overall frequency in Iran is 30%. Restriction enzyme mapping for beta-globin gene cluster is important in systematic studies of single copy genes causing beta-thalassemia. The most frequent mutation in the Iranian population, which leads to beta-thalassemia is the substitution of G to A in the intervening sequence II, nucleotide number 1. We have studied ten affected homozygous for IVSII-1. After restriction digestion for six polymorphic loci (XmnI, HindIII/Ggamma, HindII3 /wb, AvaII/b,RsaI/b, HinfI/b) . We constructed chromosomal haplotype that are related to this mutation. Our result shows that the IVSII-1 mutation is essentially in company with two types of haplotypes about 40% and 30% respectively. These haplotypes are quite different from Mediterranean haplotype for IVSII-1.

P0929. MEFV gene mutations and high carrier rates for familial Mediterranean fever in Turkish FMF patients and healthy population

B. Balci, *E. Yilmaz, S. Ozen, R. Topaloglu, N. Besbas, A. Bakkaloglu, M. Ozguc*

Hacettepe University; Ankara, Turkey

banubalci@hotmail.com

Familial Mediterranean fever (FMF) is a recessive disorder characterised by episodes of fever and neutrophil-mediated serozal inflammation. The most serious complication of FMF is the development of amyloidosis. Colchicine has been shown to be effective in preventing the attacks of FMF as well as the development of amyloidosis1. The disease affects primarily ethnic groups living around the Mediterranean basin; Ashkenazi Jews, Armenians, Turks, and Arabs. The gene causing FMF has been cloned in 1997, and several mutations characterised in MEFV gene. The aim of this study was to determine the carrier rate in the Turkish population and the mutation frequency in the clinically diagnosed FMF patients. We found a

high frequency of carriers in the healthy Turkish population (20%). One healthy asymptomatic individual was found to carry mutations in both alleles (V726A/E148Q). The distribution of the five most common MEFV mutations among healthy individuals (M694V 3%, M680I 5%, V726A 2%, M694I 0% and E148Q 12%) was significantly different (p <0.005) from that found in patients (M694V 51.55%, M680I 9.22%, V726A 2.88%, M694I 0.44%, and E148Q 3.55%). The high incidence of MEFV gene mutations in the Turkish population (gene frequency of 0.011; 95% CI = 0.06-0.15, and carrier rate of 0.20; 95% CI = 0.12-0.28) indicate that new-born screening may be discussed in the future.

P0930. Carrier frequencies of DHCR7 mutations in West-Austria indicate that Smith-Lemli-Opitz syndrome is among the most common autosomal recessive disorders

***J. Loeffler**, M. Witsch-Baumgartner, H. J. Menzel, H. G. Kraft, G. Utermann*

Institute of Medical Biology and Human Genetics; Innsbruck, Austria

witsch-baumgartner@uibk.ac.at

Smith-Lemli-Opitz syndrome (SLOS, MIM 270400) is an autosomal recessive multiple congenital anomaly/mental retardation syndrome of variable severity. Its incidence has been estimated to be approximately one in 20 000 to 60 000 based on case frequency surveys in populations of Caucasian origin. Identification of the underlying gene defect has paved the way for molecular methods to identify carriers. Using allelespecific PCRs we screened 640 anonymous and random blood donor samples from Tirol (part of West-Austria) for the presence of the three most common caucasian SLOS mutations (IVS8-1G>C, W151X, V326L). For the mutation IVS8-1G>C a carrier frequency of 1 in 50 (1;26-1;75, 95% CI) was identified. W151X was much less frequent (1;141-0, 95% CI). V326L was not found in the sample analysed. Based on this data an incidence of SLOS of about 1;10.000 (1;1666 — 1;22 201, 95% CI) is expected. Remarkably, we know only of two unrelated Tirolean SLOS patients. The discrepancy between the expected and observed incidence could be due to misdiagnosed severe cases, death prior to diagnosis, or fetal loss. The high frequency of the functional null mutation IVS8-1G>C suggests a founder effect and/or heterozygote advantage.

P0931. The Genealogy of Fanconi Anaemia Patients Homozygotic for the Type I and Type II Afrikaner Mutations

***T. Pearson**[1], N. V. Morgan[2], A. J. Tipping[2], L. P. Kuyt[3], C. G. Mathew[2], S. Jansen[1]*

[1]Division of Human Genetics, University of the Orange Free State; Bloemfontein, South Africa; [2]Division of Medical and Molecular Genetics, GKT School of Medicine; London, United Kingdom; [3]Department of Human Genetics, Free University of Amsterdam; Amsterdam, The Netherlands

gnmgtp@med.uovs.ac.za

Fanconi anaemia (FA), a rare autosomal recessive genetic disorder, is known to occur at a higher than normal incidence among the white Afrikaans speaking population of South Africa. The disease is characterised by progressive bone marrow failure, starting predominantly in childhood, various phenotypic abnormalities as well as an increased sensitivity to the clastogenic agents diepoxybutane (DEB) or mytomycin C (MMC), resulting in chromosomal aberrations. Initial genealogical investigations on 12 families affected by FA but with unknown mutational status, identified GN and/or his spouse JDLB as common ancestors. A founder effect, the cause of the high incidence of FA among this population group, was further substantiated by results obtained through complementation studies and mutation screening, showing the predominance of complementation group A and mutation types I, II and III. The existence of three mutation types among the descendants of GN and JDLB raised the possibility of more than one founder pair or family being involved. For this reason an additional genealogical investigation was carried out, limited to seven and six FA parents carrying respectively the Afrikaner types I and II mutations. Apart from GN /JDLB, HDP and wife CD also featured prominently as possible founders for the FANCA Afrikaner type I mutation, whereas either PV/MDP are candidate founders of the FANCA Afrikaner type II mutation.

P0932. Analysis of CTG polymorphism in untranslated 3 region of SCA8 gene in Polish control group

***A. Sulek**, D. Hoffman-Zacharska, M. Bednarska-Makaruk, J. Zaremba*

Institute of Psychiatry and Neurology; Warsaw, Poland

sulek@ipin.edu.pl

Autosomal dominant spinocerebellar ataxias (SCA) are a group of neurodegenerative disorders characterized by progressive ataxia, dysartria and other neurological signs. Genes responsible for different types of SCA (SCA1, 2, 3, 6, 7) have been cloned, showing in coding regions an expanded CAG repeats in mutant alleles. In 1999 a novel CTG trinucleotide expansion in 3 untranslated region of SCA8 gene on chromosome 13q21 was identified. Studies established on controls and patients with linkage to chromosome 13q21 showed tetramodal distribution of CTG repeat number. The prevalence of three non-pathogenic ranges with different frequency was shown. The pathogenic range was estimated as approximately 100 and more CTG repeats and was observed in patients with clinical signs of SCA (we observed such alleles in 3 families). However, alleles with more than 100 CTG repeats were also observed rarely in healthy controls and in unaffected patients relatives. This phenomenon may be the result of reduced penetrance of the gene. To establish a non-pathogenic range of CTG repeats for Polish population we performed an analysis of CTG polymorphism in a group of 250 Polish healthy controls. In one subgroup consist of 100 persons aged 18-69 years two alleles were found with number of CTG repeats 82 and 100, respectively. In the second subgroup 150 Polish controls age of 65-82 we observed one allele with 95 CTG repeats. The results of our study suggest that only CTG repeat number above 100 may relate to neurological signs.

P0933. Sensoryneural Hearing Loss (snhl); A Complex Trait

***A. Z. Spadoni**[1], M. T. Trincavelli[1], G. E. Dotto[2], M. C. Fumagalli[1], M. V. Dalmaso[1], M. V. Chinellato[1], A. Jannelli[2], M. B. Bravo-Luna[1]*

[1]Faculty of Medicine-UNR; Rosario, Argentina; [2]Faculty of Medicine; Rosario, Argentina

mbravoluna@hotmial.com

SNHL is a mono or multifactorial hearing deficit of great significance in our culture, that can be prevented in some cases. Objective; to assess, in a first stage, the prevalence of SNHL etiology in a qualified population aged 0-15 yr. in order to analyse the involvement of the genetic component. Methods; Retrospective study through the analysis of professional records of children and adolescents (N= 250) with hearing disorders, attending public and private specialised institutions of the city of Rosario, that were diagnosed of hearing impairment by audiometry and evoked potentials. Results (%) are presented according the following summarised etiological classification; I) Congenital [32%]; 1) Genetic [35]; 2) Non genetic [65]; a) infections; [96]; b) toxics; [4]. II) Acquired [19]); 1) infections [66]; 2) toxics [17; 3) others [17]. III) Indeterminate [40]; and IV) multifactorial [9]. Conclusions; It is striking the high prevalence of the indeterminate and multifactorial groups, within these groups it is probable the occurrence of genetic disorders, either syndromic or no syndromic, that have not been adequately diagnosed. Presently, we are studying this population, considering SNHL as a complex trait, which is our hypothesis, through segregation analysis, and improving diagnosis regarding its etiology.

P0934. Mutation Spectrum of Connexin 26 in Iranian Patients with Autosomal Recessive Non-syndromic Sensorineural Hearing Loss

***S. Sahebjam**[1], N. Kouchakian[1], S. Arzhangi[1], M. Daneshmandan[1], M. Javan[1], B. Cucci[2], M. Menezes[2], R. Smith[2], H. Najmabadi[1]*

[1]Genetics Research Center,Welfare Sciences and Rehabilitation University; Tehran, Islamic Republic of Iran; [2]Molecular Otolaryngology Research Laboratories,Department of Otolaryngology Head and Neck Surgery,University of Iowa; Iowa, IA United States

sahebjam.s@angelfire.com

Hereditary deafness is one of the most common inherited sensory disorders, which affects 1;2000 newborns. About 70% of hereditary deafness is non-syndromic. Autosomal recessive forms (DFNB) make up about 85% of these cases. Mutations in the connexin 26 (Cx26) gene are associated with a type of autosomal recessive non-syndromic sensorineural hearing loss (ARNSHL) known as DFNB1. DFNB1 accounts for about 50% of congenital severe to profound ARNSHL. The most common mutation 35 del G is found in over two-thirds of individuals with DFNB1. The present study investigates the mutations of connexin 26 found in patients with ARNSHL in the Iranian population. Fifty-five unrelated patients with congenital ARNSHL were studied. DFNB1 mutations were found in 9 patients. Four patients were homozygotes for 35 del G mutation. Compound heterozygousy of 35 del G/ W24X and R184/ splice site mutation G-to-A 5 ss was the cause of ARNSHL in 2 patients. Three patients carried only a single 35 del G mutation. A second mutation is under sequencing. Mutations of other patients are under study. The frequency of 35 del G allele was much lower than reported studies from other countries. These results suggest that the different types of mutations affect ARNSHL according to ethnic background. The study is ongoing.

P0935. Why are Mutations at the Connexin 26 Locus Such a Frequent Cause of Genetic Deafness?

***W. E. Nance**, A. Pandya, X. Liu*
Virginia Commonwealth University; Richmond, VA United States
nance@hsc.vcu.edu

Heterosis, founder effects and mutation hot spots, have been suggested to explain the high frequency of Cx26 deafness. Alternatively, we have proposed that Cx26 deafness may have been specifically amplified during the past 200-300 years by relaxed selection and assortative mating. In the past, the genetic fitness of the deaf may have approached zero. However, the social, economic and educational circumstances of the deaf have improved dramatically, and have been accompanied by an increase in fertility, and the onset of assortative mating in some but not all countries. Conventional wisdom holds that curing a rare recessive disease will not affect the phenotype frequency appreciably for many generations. However, this conclusion assumes random mating, and with phenotypic or genotypic assortative mating, the approach to a new equilibrium can be much more rapid. Since Cx26 marriages account for a disproportionate share of non-complementary matings and can only produce deaf offspring, intermarriage among the deaf has preferentially increased this phenotype. With data from the 19th century, we have shown that the frequency of Cx26 deafness has doubled during the past 100 years. Furthermore, the current prevalence is lower in countries without a long tradition of intermarriage among the deaf. If our hypothesis is correct, the availability of premarital genetic testing to either avoid or ensure the birth of deaf children could have even greater effects on the phenotypic frequency, but only if genotypic mate selection became a universal and uniform practice among the deaf.

P0936. Spectrum of Connexin 26 gene (GJB2) mutations in Turkish families with inherited non-syndromic deafness and determination of the GJB2 35delG mutation carrier frequency in Turkish population

***O. Uyguner**[1], M. Emiroglu[2], G. Hafiz[2], M. Yuksel-Apak[1], A. Ghanbari[1], N. Basaran[2], B. Wollnik[1]*
[1]Division of Medical Genetics, Child Health Institute, Istanbul University; Istanbul, Turkey; [2]Ear, Nose, and Throat Department, Istanbul Medical Faculty, Istanbul University; Istanbul, Turkey
muyguner@superonline.com

Congenital deafness occurs in about 1 in 1000 infants and approximately 80% of the cases are non-syndromic and inherited in an autosomal recessive pattern. Mutations in the gene encoding connexin 26 (Cx26), GJB2, are the most common cause of recessive non-syndromic hearing loss (NSHL). Cx26 mutations account for 30-60% of recessive NSHL in European and American populations. A specific mutation in the GJB2 gene, known as 35delG, represents a [a]hot spot mutation and is found in approximately 2/3 of the detectable Cx26 mutations. Nothing is known yet about the spectrum of Cx26 mutations in Turkish population. In the present study, we have studied 37 index patients from consanguineous Turkish families with autosomal recessive NSHL. The entire coding region of the Cx26 gene was sequenced and a homozygous mutation was found in 27% of the cases. The 35delG mutation was present in 78% of all Cx26 mutations identified. One new mutation and two polymorphisms were identified. To determine the prevalence of the 35delG mutation in Turkish population we established a recently described screening method using PCR-mediated site-directed mutagenesis, followed by a BsiYI digestion. We tested 359 unrelated individuals and found 3 heterozygous carriers giving a carrier frequency of 0.84% (1 in 120). Our data show that both, frequency of Cx26 mutations in recessive NSHL and the carrier rate of Cx26 35delG mutation in normal Turkish population is much lower than described for other Mediterranean countries. This finding reflects the mixed origin of Turkish population.

P0937. Determination of the carrier frequencies of two common Cx26 mutations (35delG and 167delT) in the Hungarian population

***A. Bors**[1], H. Andrikovics[1], L. Kalmar[1], Z. Liska[1], A. Borbely[1], S. Galambos[2], A. Losonczi[2], S. Furedi[3], I. Balogh[4], C. Szalai[5], A. Tordai[1]*
[1]National Institute of Haematology and Immunology; Budapest, Hungary; [2]Jewish Community Hospital; Budapest, Hungary; [3]Institute for Forensic Sciences; Budapest, Hungary; [4]University of Debrecen; Debrecen, Hungary; [5]Heim Pal Paediatric Hospital; Budapest, Hungary
bors@biomembrane.hu

The most common form of non-syndromic neurosensory autosomal recessive deafness (NSRD) is caused by mutations in the gene GJB2 located on chromosome 13, encoding the gap-junction protein connexin 26. One mutation, which represents a deletion of a guanine within a strech of six Gs between nucleotide positions 30 and 35 of the GJB2 cDNA (35delG) is found in 40-70% of NSRD cases, and was shown to have a high allele frequency (2-5%) in several randomly selected populations. Another deletion, the loss of a timine in position 167 was found in the Ashkenazi Jewish population in 4% frequency. A simple polymerase chain reaction (PCR)-based test was used to detect both mutations in order to establish the allele frequencies in the Hungarian control, Gypsy, and Ashkenazi populations. We screened 173 randomly selected Hungarian first time blood donors and 351 unrelated, unaffected Gypsy individuals for the Cx26 35delG mutation. We found 2 heterozygous in the control population, which gives a carrier frequency of 1,17% (0%-2.81%) which is lower, but does not differ significantly from the published data for Caucasian populations. The heterozygous carrier rate in the Gypsy population was 1.4% (0.1%-2.7%) (5 heterzygous from 351). We identified 2 heterozygous individuals for mutation 167delT among 48 Ashkenazi samples analysed so far. Our data support the assumption that the G track containing the deletion may represent a region with higher mutation rate, since we detected similar carrier frequencies in two populations with distant origin.

P0938. A carrier frequency of the 35delG deafness mutation in several populations of Russia

***A. Anichkina**[1], T. Kulenich[1], I. Shagina[1], T. Viktorova[2], A. Polyakov[1], E. Khusnutdinova[2], E. Ginter[1], O. Evgrafov[1]*
[1]Research Center for Medical Genetics; Moscow, Russian Federation; [2]Ufa Research Center; Ufa, Russian Federation
dnalab@online.ru

The connexin-26 (Cx26) gene has been shown to be a major cause of non-syndromic recessive deafness. Although many mutations have been found, a single mutation is responsible for the majority of alleles in different populations (35delG). A high frequency of this mutation was revealed in populations of Northern, Central and Southern Europe with higher prevalence in Southern Europe, and the highest percentage in Estonia. We decided to expand this study further to the East, and investigate several populations of Russia. First, we consider this investigation to be important for defining the strategy of diagnosis and genetic counseling of congenital deafness in Russia. Second, this study could clarify the origin and the history of 35del mutation. Taking into account high frequency of this mutation in Estonia, we paid a special attention to related Finno-Ugric populations, namely Mari and Komi. Three other populations, Bashkirs, Chuvashs and Yakuts, belong to Turkish speaking populations. In ethnogenesis of Bashkirs and Chuvashs participated not only Turkish but also some other populations including Finno-Ugric populations. Altogether 560 persons from 5 ethnic groups of Russia were analyzed for 35delG mutation. Twelve mutations were found, resulting in average carrier frequency 1/46,7. It corresponds to the frequency of more than 1% chromosomes with mutation.

P0939. Hereditary Deafness - Genetic And Epidemiological Aspects -

***S. Bembea**[1], M. Bembea[2], O. Vancsik[2], C. Skrypnyk[2], C. Jurca[2], S. Ciuchina[2]*
[1]Clinical Adult Hospital, ORL Department; Oradea, Romania; [2]Clinical Children Hospital; Oradea, Romania
diabem@yahoo.com

Introduction; The relative incidence of different types of neuro-sensorial deafness has clearly changed for the last 25 years. Maternal rubella, postnatal ototoxicity and meningitis are much better controlled today. Meanwhile, there s only little achievement in what prevention or reduction of hereditary deafness (HD) incidence is concerned. Therefore, the tendency in the present and probably for the coming years, is an increase in the HD relative incidence, with a lowering of acquired deafness of any kind. Objective; Determining the incidence and the genetic characteristics of the HD in Bihor county (with a population of 630000). Material and method; A number of 59 cases have been studied. The pedigree, following the segregation and establishing the type of heredity have been studied for each case. Results; The incidence of HD is approx. 1/10000. The model of inheritance is autosomal recessive in 55% of cases, autosomal dominant in 40% of cases and undetermined in 5% of the cases. Most of the times, HD is neuro-sensorial. Syndromic and non-syndromic HD are described, with examples from the personal experience of rare cases of deafness, like Deafness + Sinphalangism S., Waardenburg S., Deafness + Oculo-cutaneous albinism S., Alport S., Treacher-Collins S. Conclusions; Any case of deafness should be suspected as having a genetic cause. Therefore, the family history, a meticulous clinical examination, audiogram and a try to situate the case in one of the genetic mechanisms are all recommended.

P0940. Connexin26 mutation 35delG in non-syndromic hearing loss in Northern Finland

T. L pp nen[1], *M. V is nen*[1], *M. Allinen*[1], *P. Lindholm*[2], *M. Luotonen*[2], *H. L pp nen*[2], *E. M ki-Torkko*[2], *M. Sorri*[2], *M. V yrynen*[2], *J. Uusimaa*[3], *J. Leisti*[1]

[1]*Department of Clinical Genetics, Oulu University Hospital; Oulu, Finland;* [2]*Department of Otorhinolaryngology, Oulu University Hospital; Oulu, Finland;* [3]*Department of Pediatrics, Oulu University Hospital; Oulu, Finland*

tuija.lopponen@oulu.fi

OBJECTIVE; The aims of the study were to evaluate the role of the most common mutation reported, 35delG, of the connexin26 gene (Cx26/GJB2) in children with non-syndromic sensorineural hearing loss and to evaluate the carrier frequency of this major mutation in Northern Finland. STUDY DESIGN; The study population comprised children, who were referred to the Oulu University Hospital and who had non-syndromal congenital sensorineural hearing loss. A total of 70 patients were analyzed. The estimation of carrier frequency was carried out in a random sample of 313 control persons. METHODS; Detection of the 35delG mutation was performed by PCR amplification of the Cx26 sequence followed by direct sequencing of purified PCR products. Simultaneous sequencing to both directions was carried out by automated sequencer (Li-Cor 4200) using forward and reverse primers labeled with IRD800 and IRD700, respectively. Conformation sensitive gel electrophoresis (CSGE) of PCR products was applied to detect the heterozygous carriers in control population and positive samples in this screening were confirmed by direct sequencing. RESULTS; Of the 70 patients with the hearing loss 11 were found to be homozygous for the 35delG mutation. These belonged to 7 families, three from one, two from two and a single patient from four different families. Out of these 11 patients three had severe and 8 profound hearing loss. In the control population four individuals heterozygous for the 35delG mutation were found, which donotes a carrier frequency of 1/78 in Northern Finland. The screening of the Cx26 coding sequence for other mutations is under study. CONCLUSIONS; The present results indicate that the Cx26 mutation 35delG is an important contributor to recessive inherited non-syndromic hearing loss also in Finland. The carrier frequency of this mutation was found to be of the same magnitude as reported previously in Central and Northern Europe.

P0941. Statistical analysis of non-syndromic genetic deafness in Iranian population

N. Kouchekian[1], *N. Daneshmandan*[1], *H. Sheikh-Al-Eslamy*[1], *H. Najmabadi*[1,2]

[1]*University of Welfare and Rehabilitation; Tehran, Islamic Republic of Iran;* [2]*Karimi-Nejad Pathology and Genetics Center; Tehran, Islamic Republic of Iran*

maryamneishabury@yahoo.co.uk

Genetic deafness affects 50% of patients. A great portion of this kind of disorder is Non-syndromic which respect to the mode of inheritance is categorized to autosomal recessive (AR),autosomal dominant (AD) ,X-linked and mitochondrial. In order to illuminate these ratios in Iranian population, we started a retrospective study. A total of 180 patients were selected based on genetic background. The genetic counseling and pedigree analysis were performed for each individual. Our results demonstrate 155 families (86.11%) AR, 21 families (11.67%) AD and for 4 families (2.2%) we could not determine the mode of inheritance exactly. The statistical analysis of data showed that distribution of mendelian inheritance of non - Syndromic genetic deafness in Iranian population is similar to other studies. In our study the results showed that 38% of AR had moderate to severe and 61.9% had profound hearing impairment. More over among AD cases 61.9% had moderate to severe while 38% had profound hearing loss. The results also revealed that 57% of AD and only 17% of AR cases had progressive hearing loss. The investigation of consanguine marriage of the parents was revealed as 79.4% of AR and 7.4% of AD cases. From all the patients 68.6% had consanguineous parents who 99.2% of them were AR and 0.8% were AD. In the future most of these families can be used for linkage analysis.

P0942. The prevalence of Usher syndrome in Sweden; A nation - wide epidemiological and clinical study.

C. G. M ller[1], *M. Sadeghi*[2], *W. Kimberling*[3], *L. Tranebj˘rg*[4]

[1]*dep. audiology Gothenburg Univ sweden; 41345 Gothenburg, Sweden;* [2]*Dept. of Audiology, Sahlgrenska University Hosptial; Gothenburg, Sweden;* [3]*Dept. of genetic; Omaha, NE United States;* [4]*Dept. Of Medical Genetics; Tromsoe, Norway*

claes.moller@audiology.gu.se

BACKGROUND. Usher syndrome (USH) is characterized by hearing loss, vestibular dysfunction and Retinitis Pigmentosa (RP). Three clinical types of USH are recognized. USH I; profound congenital sensorineural deafness, vestibular areflexia and RP. USH II; congenital moderate to severe hearing loss, normal vestibular function and RP. USH III; progressive hearing loss, progressive vestibular deficiency and RP. MATERIALS AND METHODS. The study was an epidemiological, population-based survey, based on examination of data from medical researches, schools and organizations. A large number of the patients underwent clinical and genetic assessment by the authors. RESULTS. A total of 366 deaf-blind subjects were assessed. 21% (77 subjects) previously diagnosed as having USH were excluded because of incorrect diagnosis. In total, 140 patients with USH I, 122 patients with USH II and 27 patients with USH III were diagnosed. The prevalence of USH I was1.6/100 000, USH II 1.4/100 000 and USH III 0.3/100 000. The prevalence rate varied in different counties. USH I was most common in the three northernmost counties, (7.4 /100 000). CONCLUSION. The total prevalence rate of USH is lower then previously reported. A large variation was found in USH I . While the three major types of USH are clearly different, the subtypes within type I, II are so far believed to be clinically indistinguishable.Thus, there is a critical need to further study phenotype-genotype correlation in USH. This study of USH is the most complete ascertainment that so far has been undertaken.

P0943. Haplotypic determinants of instability in the FRAX region; concatenated mutation or founder effect?

S. M. Ennis[1], *A. Murray*[2], *N. E. Morton*[1]

[1]*Wessex Human Genetics Institute, Southampton General Hospital; Southampton, United Kingdom;* [2]*Wessex Regional Genetics Laboratory, Salisbury District Hospital; Salisbury, United Kingdom*

se@soton.ac.uk

The fragile X triplet repeat expansion at Xq27.3 has been shown to be associated with mutation or instability 600 kb distal at the FMR2 repeat locus. Concatenated mutation, whereby a mutation at one locus somehow interacts with mutation, recombination, deletion or transposition at another locus is a possible explanation. In this study we examine evidence from a sample of over 7000 independent haplotypes from the FRAX region. We adopt the use of cladistic groups to more thoroughly define the properties of these haplotypes, and in doing so isolate one group of haplotypes which may be predisposed to the phenomenon of concatenated mutation. Distinguishing concatenated mutation from founder effects is difficult within a single population. We present our evidence for and against concatenated mutation, and in the process describe a previously undefined mutation at FRAXE.

P0944. Different distribution of DXS548 and FRAXAC1 haplotypes between normal and fragile X population in Croatia

R. Bago[1], *S. Hecimovic*[1], *I. Barisic*[2], *V. Culic*[3], *K. Pavelic*[1]

[1]*Rudjer Boskovic Institute; Zagreb, Croatia;* [2]*Children s Hospital Zagreb; Zagreb, Croatia;* [3]*Clinical Hospital Firule; Split, Croatia*

bago_r@yahoo.com

The fragile X syndrome is caused by expansion of the (CGG)n repeat in 5 end of the FMR1 gene. In order to look for linkage disequilibrium between the fragile X locus and its flanking markers, we analyzed the DXS548 and FRAXAC1 microsatellite markers in normal and unrelated fragile X males of Croatian origin. Different distribution of alleles and haplotypes was found between these two samples. A significant increase in frequency of DXS548 allele 2 was found among fragile X patients when compared to normal individuals (31,3% vs. 2.86%). We also noticed a different distribution of FRAXAC1 allele A (18.8% in fragile X group vs. 10.0% in normal population). Haplotype 7-C was the most represented in normal population (57.14%), while haplotypes 2-C, 8-C and 2-A were more frequent in fragile X group (accounted for 43.75% of all fragile X chromosomes and less than 4% of normal population). This difference may suggest the existence of linkage disequilibrium between the two loci and/or selective advantage of this haplotypes among fragile X affected individuals in Croatia.

P0945. Epidemiological Study Of Some Sentinel Anomalies and Down s Syndrome

E. Nikula, *O. Tymchenko, O. Turos, T. Pokanevich, L. Piotrovich, O. Makaruk*

Institute of hygiene and medical ecology; Kyiv, Ukraine

elena@ushc.kiev.ua, otimch@usch.kiev.ua

It is known that the sentinel anomalies (SA) and Down s syndrome (DS) make a considerable contribution into infant morbidity and mortality. These defects are diagnosed well and can provide important clues in the detection of teratogenic agents. An anencephalia, spina bifida, limb reduction, oesophageal atresia and traheo-oesophageal fistula, ano-rectal atresia, cleft of a labium with / or without cleft of a palate, multiple congenitals anomalies and Down syndrome were taken into account. Objective. To

estimate the relative risk (RR) of the live-borns with SA and DS in different Ukrainian regions. Methods. Data on live-borns only were received from the annual reports of seven medical genetic centres of Ukraine (Southern, Western, North, Northern-Eastern, Central, South-Eastern, South-Western). Period of the observation - from 1993 to 1999. The precise data about still-borns with birth defects are unknown in Ukraine. RR was calculated according to Epi Info program for each selected defects. Results. It was established that RR evenly disseminated among the new-borns of the country. RR was lower in the Northern and the South-Eastern regions (0,86 at the 0.81-0.90 confidence interval; 0.88; CI 0.84-0.93). RR was the highest in the Southern (1.10; CI 1.02-1.19) and the Northern-Eastern regions (1.19; CI 1.14-1.25). Conclusion. The distinctions in the rates of observed defects can reflect not only the difference of incidence in population, but mainly the difference in the levels and the quality of medical care and other socio-economic factors.

P0946. Epidemiology and Relative Incidence of Rare Metabolic and Genetic Disorders in IRAN and the results of 50 Prenatal Testings

Y. Shafeghati[1,2], *R. Karimi-Nejad*[1], *F. Azimi*[1], *M. Karimi-Nejad*[1], *J. Huijmann*[3], *W. J. Kleijer*[3], *O. VanDiglen*[3]
[1]*Karimi-Nejad Pathology and Genetics Center; Tehran, Islamic Republic of Iran;* [2]*University of Welfare and Rehabilitation; Tehran, Islamic Republic of Iran;* [3]*Genetics and Metabolic Department of Erasmus University; Rotterdam, The Netherlands*
maryamneishabury@yahoo.co.uk

Since 10 years ago, our Genetics Center has been evaluating 128 families including 196 cases with the collaboration of the Genetic and Metabolic Department of Erasmus University based in Rotterdam, the Netherlands. The result has been the diagnosis and discovery of these rare but very important disorders in the families under scrutiny. Out of the above number of families mentioned, the final diagnosis in 41 was MPS, the most common type being MPS III. Also, 48 families with 77 patients were suffering from Lipid Storage Diseases. The most common disorder was Metachromatic Leukodystrophy followed by Niemann-Pick, Gauchers and Tay-Sachs diseases respectively. 39 families with 67 cases were diagnosed to have micromolecular Metabolic Diseases. Organic Acidemias was the most common type. Totally, in 50 families prenatal testing was carried out showed that 11 (22%) of the fetuses were affected. Details of different clinical subtypes would be presented in this article. In dealing with this patients especially while planning for prenatal testings, genetic counselling was carried out to provide the families with detailed information about their suffering children. In doing so, Ethical principles also fully recommended in Islam for different contexts such as Autonomy, Beneficence, Non-maleficence, Justice and confidentiality were carefully observed.

P0947. Screening for fragile X syndrome among mentally retarded children in Greece

C. Sofocleous[1], *H. Fryssira*[1], *L. Thomaidis*[2], *A. Kolialexi*[1], *A. Mavrou*[1], *C. Metaxotou*[1]
[1]*Medical Genetics, Athens University, School of Medicine; Athens, Greece;* [2]*1st Dept. of Pediatrics, Athens University; Athens, Greece*
csofokl@cc.uoa.gr

Fragile X syndrome (FXS), second most common cause of mental retardation (MR) affects 1; 4000 males and 1;7000 females. The CGG repeat of FMR-1 gene varies from 6 to 54 triplets in normal individuals, whereas in FXS families prermutations (PM) of 55 to 200 repeats and full mutations (FM) of more than 230 repeats are observed. Hypermethylation of CpG island, upstream the CGG repeat, causes the transcriptional shutdown of FMR-1 gene resulting in the absence of FMRP protein in FXS patients. 1687 individuals, 590 MR boys and 88 MR girls, their parents and other family members (759) were tested for FXS and 250 normal individuals were used as controls. DNA extraction from peripheral blood lymphocytes followed by modified non radioactive Southern blot and PCR (CGG sizing) analysis were performed. Immunocytochemical detection of FMRP on blood smears was also performed for 300 individuals. The results confirmed the clinical diagnosis of FXS in 28 males and 2 females with FM and 1 male with PM. In the remaining individuals an allele distribution of 7 to 58 CGG repeats with a mean of 28-30 triplets was observed. The FMRP antibody test confirmed the diagnosis in 15 patients and revealed 3 more FXS families with no CGG expansion. The combination of non radioactive Southern and PCR analysis, enhanced by the FMRP test was highly efficient for diagnosis and thus supportive of large scale population screening especially since a rough estimation, revealed an FXS frequency at about 1;4250 boys in Greece.

P0948. Social selection against X-linked intelligence?

N. V. Kovaleva
Institute of Obstetrics and Gynecology; St. Petersburg, Russian Federation
kovaleva@robotek.ru

Social selection against X-linked intelligence? Natalia V. Kovaleva Institute of Obstetrics and Gynaecology RAMS, Mendeleev line, 3, 199034, St.Petersburg, Russia, e-mail; kovaleva@robotek.ru During the past decade, several different genes involved in learning capacity were identified on human X-chromosome. They were discovered because of mutations causing nonspecific mental retardation in males. On the other hand, these genes are reasonably considered to be the genes for higher intelligence and even have been discussed as genes by which Homo may have become sapiens [Turner, 1996]. As the pattern of X-chromosome inheritance is very specific (men transfer X-chromosome to their daughters only), the process of transmission and spreading of such genes in population may be influenced by social factors. In times when there was no effective birth control, and even women with high intellectual potency had no or little chance of self-realization, outstanding and successful men and their daughters had many offspring, which ensured maintenance of X-linked genes for intelligence in population. At present, intelligent women who had inherited excellent genetic material from their father tend to have few children because childbearing hampers their social development. This leads to a decrease in frequencies of the clever alleles in population. Thus, social selection against genes for higher intelligence has been putting into effect through successful women. The genetic drift of the clever alleles away from initial values is expected to be stronger in societies with traditional attitude to gender roles in household.

P0949. Trinucleotide repeat polymorphism of some X-linked loci in a normal West Siberian Slavic population

E. N. Tolmacheva, *V. N. Evdokimova, S. A. Nazarenko*
Institute of Medical Genetics; Tomsk, Russian Federation
kate@img.tsu.ru

AR, FMR1 and FMR2 genes have been mapped to the X-chromosome. The expansion of CAG (AR), CGG (FRAXA/FMR1) and GCC-repeats (FRAXE/FMR2) is the mechanism associated with Kennedy disease and fragile X-syndromes correspondingly. The distribution of these loci is varied and characterized by the positive asymmetry among the world populations. This means they tend to accumulate the repeats over time. A hypothesis does exist that the transition from normal-sized to expanded alleles is a result of a mutational bias towards larger repeat sizes of the repeat arrays in the normal population. The distributions for AR, FRAXA and FRAXE loci were analyzed in a West Siberian Slavic population. Automated genotyping of these loci was perfomed by PCR amplification with fluorescently labeled primers on the ABI PRISM 310 Genetic Analyzer and Genescan softwere. We detected among 113 unrelated healthy individuals; 12 alleles for the AR locus, ranging in size from 21 to 32 repeat units; 31 alleles for the FRAXA locus, ranging in size from 8 to 56 repeats; and 18 alleles for the FRAXE locus, ranging in size from 9 to 27 repeats. The allele s frequency distribution for AR and FRAXE loci was unimodal with the size of modal allele in 27 repeats (18%) and 17 repeats (19.9%) correspondingly. The FRAXA locus showed a bimodal distribution with the modal alleles in 18-20 repeats (11.5%) and 28-29 repeats (24.6%). Allele s distribution for AR locus in Slavic population had a narrower spectrum and a higher size of modal allele compared with another studied populations. The FRAXA locus showed very high frequency of gray zone alleles. Alleles containing more than 40 repeats share 12.4% and more than 45 - 8.0% of all alleles in population. In contrast, the allele s distribution for FRAXE locus was characterized by the predominance of the short-size alleles (<15 repeats). Intriguingly, each investigated locus has the special features in allele s distribution compared with another world populations.

P0950. Etiology of moderate to profound mental retardation ; a retrospective study of 1242 patients

A. Destree[1], *A. Vokaer*[2], *Y. Gillerot*[1], *L. Van Maldergem*[1]
[1]*Centre de Genetique Humaine, Institut de Pathologie et de Genetique; Loverval, Belgium;* [2]*CHU Brugmann, Centre de P rinatalogie; Brussels, Belgium*
secret.genet@ipg.be

Objective ; To carry out a retrospective epidemiological survey of the etiology of moderate to profound (IQ £ 50) mental retardation in a population of 1242 patients and to compare this data with those from previous studies. Method ; We analysed the files of 729 moderately to profoundly retarded patients evaluated in our centre for Human Genetics. Most of these patients underwent karyotyping, brain imaging and a search for inborn errors of metabolism. The files of 513 additional patients from institutions for the mentally retarded were also studied. In these cases, the work-up

was often minimal. The study of this heterogeneous population gave the following results ; Constitutional disorders ; Down Syndrome ; 193 cases, partial chromosomal duplication and/or monosomy ; 32 cases, apparently balanced reciprocal translocations ; 5 cases, sex chromosomes aneuploidy ; 6 cases, mosaic triploidy ; 1case, partial tetrasomy ; 2 cases, microdeletion syndromes ; 22 cases, autosomal dominant disorders ; 31 cases, autosomal recessive disorders ; 79 cases, X-linked MR syndromes ; 39 cases, Known MCA/MR syndromes ; 18 cases, undiagnosed MCA/MR ; 281 cases, CNS-malformations ; 5 cases. An acquired etiology was likely in 171 cases. No etiology was found for 349 patients. Conclusion ; The study of a heterogeneous population of 1242 moderately to profoundly retarded patients indicates a proportion of 28.1% for which no etiological clue is found. This population includes 513 patients with a minimal workup.

P0951. Consanguinity and Mental Retardation in North India

M. Afzal[1], *M. S. Ahmad*[2], *B. Shadab*[3], *G. G. H. A. Shadab*[1]
[1]*Section of Genetics, Department of Zoology, AMU; Aligarh, India;* [2]*Section of Genetics, Department of Zoology, AMU; India;* [3]*Department of Human Genetics, Guru Nanak Dev University; Amritsar, India*
hep_amu@usa.net

We have studied mental retardation from different States of North India viz. Bihar, Uttar Pradesh, Delhi and Punjab. Consanguinity is prevalent widely among muslim groups, the first cousin unions being most common. From our field survey (Ansari and Qureshi groups) and hospital samples (Jawahar Lal Nehru Medical College and Hospital, Aligarh & All India Institute of Medical Sciences, New Delhi), we tried to find out the genetic load due to mental retardation classified as chromosomal disorders, fragile sites, single gene disorders and low IQ in polygenic variation. Among chromosomal disorders, trisomy 21 was most conspicuous, again free trisomy 21 being the most prevalent one, followed by G/G and D/G translocation. Turner s, Klinefelter s and intersex cases showed lower IQ levels. Fragile site cases comprised Xq.27 cases being most common. Amino acidopathies, and enzymatic disorders were more common among consanguineous offsprings than non-consanguineous ones. Surprisingly age of the mother show highest number of defectives at around 40 years, but the downship invariably was toward the last/later parity. Verbal, performance and total IQ, scores on WISC R-74 (Hindi/Urdu rendition by the author) shows lowering of IQ, the effect being statistically significant. Loss of mental age due to consanguinity, percentage inbreeding depression and genetic load have been calculated. We are looking ahead for molecular markers, and pedigree analysis for non-specific aetiology of mental retardation, and work out a model for genetic epidemiology for North India.

P0952. Autosomal allele frequencies and the search of ancestors in Hungarian populations

C. R. Guglielmino[1], *A. De Silvestri*[2], *J. Beres*[3]
[1]*Dipartimento di Genetica e Microbiologia, Universit di Pavia, & Istituto di Genetica Biochimica ed Evoluzionistica- CNR; Pavia, Italy;* [2]*Dipartimento di Genetica e Microbiologia, Universit di Pavia; Pavia, Italy;* [3]*National Center for Epidemiology, Dep. of Human Genetics and Teratology; Budapest, Hungary*
guglielmino@ipvgbe.igbe.pv.cnr.it

Autosomal gene frequencies allow to describe the genetic structure of populations, in particular to reveal the effects of drift or of admixture. Applying specific models to population samples, it is also possible to infer a rate of admixture of a population with respect to hypothetical parent populations. Some Hungarian ethnic groups claim to be descendant either of Turks , Iranian or of the ancient Huns. Hungarian nation was founded in the 9th century from people caming from eastern regions close to the Ural mountains and since then people speak an Uralic language belonging to the Finno-Ugric language group. We compared the gene frequencies of eight ethnic groups and seven hypothetical ancestral populations, including Uralics, applying a model of admixture. The results, most of which confirm historical hypotheses or the oral tradition, show that only one ethnic group highly resembles the Uralic population.

P0953. Occurance of ABO and Rh blood groups in three samples of Serbian population

S. Cvjeticanin[1,2], *B. Tri?ic*[3], *R. Papovic*[3]
[1]*Medical Faculty University of Belgrade; Belgrade, Yugoslavia;* [2]*Institute of Biology and Human Genetics; Belgrade, Yugoslavia;* [3]*Institute for Biology and Human Genetics; Belgrade, Yugoslavia*
bebrapap@eunet.yu

In the group of patients with congenital hip dislocation (N=93) the frequencies of ABO blood types were similare to the average value of Serbian population, while the percentage of blood group A is slightly increased.Comparing frequencies of Rh blood groups we can see that there is no differences between tested samples. In the sample of children affected with acute lymphoblastic leukemia (N=163)the frequencies of ABO blood types was similar to the average value of Serbian population, while the percentage of blood group O is slightly increased.The percentages of Rh blood types are not showing any differences between tested samples. It is interesting that in the group of top sportsment, including 61 winners of international champions, the frequencies of A and B blood groups were decreased,AB was absent and O group was about twice more frequent (71%)than an average value of Serbian population.The percentage of Rh negative individuals is significantly lees (10%)in the group of top sportsmen comparing with percentage of those individuals in Serbian population(17%). Taking all this into account we may see that variation in physical abilities can bee correlated with their immunogenetics properties.

P0954. Haptoglobin (Hp) and Transferrin (Tf) polymorphism - a population genetic study of Romanian population

G. G. Meianu[1], *V. Stoian*[2], *A. Rodewald*[3], *C. Glavce*[4]
[1]*Biology Faculty Bucharest Universyti; Bucharest, Romania;* [2]*Laboratory of Genetics - Faculty of Biology, Bucharest University; Bucharest, Romania;* [3]*Institute of Human Biology, University of Hamburg; Hamburg, Germany;* [4]*Center of Anthropological Research - Academy of Science; Bucharest, Romania*
pisica_ii@hotmail.com

The aim of this study is to present the distribution of the Hp subtypes (by means of the polyacrylamide gel electrophoresis - method by Linke, 1984) and of the Tf subtypes (by polyacrylamide gel isoelectric focusing at pH 4-6, method described by Weidinger et al., 1984) in the Romanian population. EDTA - plasma samples were obtained from 120 unrelated donors from a premountain Carpatian region of Romania (Prahova - Valley), and only this individuals were considered as autochthonous. X2 - tests and genetic distance analysis were used to compare the data with those of other studies of Mediterranean and European populations. The number of the phenotypes observed in both systems showed no significant differances to the data expected according to the Hardy-Weinberg equilibrium. The comparison with the other European and Mediterranean populations shows that the genes and the phenotype frequencies of the tested samples lines nearer to those of Southeuropean -Mediterranean population than to Balkan or Slavic groups. Our results are interpretable in the background of historical and ethnical events, which have played an important role in changing the genepool of the Romanian population. Linke R.P.; Analytical Biochemistry, 141, 55-61 (1984) Weidinger et al.; Human Genet., 66, 356-360 (1984)

P0955. Mesolithic-Neolithic population relationships in Portugal; the evidence from mtDNA

F. J. Bamforth, *K. Albertson, A. O Neill, M. Jackes, D. Lubell*
University of Alberta; Edmonton, AB Canada
FBamfort@cha.ab.ca

Analyses of Mesolithic and Neolithic samples from Portugal have led to new methods for the investigation of paleodemography and to the formulation of an hypothesis that the population increased during and after a protracted period of shift from hunter-gatherer to agricultural subsistence. While culturally the two populations are distinct, there is evidence on non-metric analysis of dental traits that the two populations may be genetically continuous. We report on the analysis of mitochondrial DNA (mtDNA) from Mesolithic (Moita, Arruda, Amoreira, Melides) and Neolithic (Casa da Moura, Feteria, Furninha) skeletal samples and the implications of this data in understanding the prehistoric population relationships in this region. The analysis of mtDNA haplogroups in ancient remains provides a means of mapping human population divergence and adds a new tool to the investigation of biocultural evolution. We will examine the prevalent mtDNA haplogroups from Neolithic and Mesolithic remains from Portugal to establish the degree of relatedness between individuals from different burial sites. The haplogroups selected for the study are based on data from modern Iberian populations. These are H, I, J, K, T, U3, U4, V, W and X. Fourteen Neolithic dental and skeletal samples from five sites and fifteen Mesolithic samples from three sites are available for analysis. Our work to date has shown absence of haplogroups V and I in this population. While haplogroup I is predominantly a Northern European haplogroup, it is interesting that haplogroup V is not represented as this is a significant haplogroup in modern Portugal. Work on haplogroup K is in progress and we have identified a novel polymorphism in one Neolithic and one Mesolithic sample.

P0956. Portuguese influence in Atlantic islands using 5 STRs (CD4, FES/FPS, VWA31/A, TH01 and TPO) and CD4/Alu haplotype

R. Velosa[1], *A. T. Fernandes*[2], *H. Sp nola*[2], *A. Brehm*[2]
[1]*Departamento de Biologia, Universidade da Madeira; Funchal, Portugal;* [2]*Centro de Ci ncias Biol gicas e Geol gicas, Universidade da Madeira; Funchal, Portugal*
atgf@uma.pt

This work presents a survey of 5 STRs and a polymorphic marker based on the association between an STR and an Alu element. The survey includes the Madeira archipelago, composed by two inhabited islands, the Azores and Cabo Verde by nine islands each (the former divided into two groups, Windward and Leeward) and S o Tom e Principe by two inhabited islands. Madeira was discovered in 1419, the Azores in 1427, Cabo Verde in 1460 and S o Tom e Pr ncipe in 1470. All these Atlantic islands were colonized by the portuguese but with different populational inputs. Madeira and the Azores were populated mainly by Portuguese from the mainland although the former island received a non-negligible amount of slaves in the 15th and 16th centuries originated from Cabo Verde islands, then a portuguese colony. Cabo Verde was essentially populated with slaves brought from Guin (West African coast) and relatively few europeans, and S o Tom and Principe received their black population from the slave trade with Angola. STRs (Short Tandem Repeat) are polymorphic markers mainly used for forensic and population genetics. We used 5 STRs (CD4, TPO, FES/FPS, TH01 and VWA31/A) and a haplotype CD4/Alu. Our aim is to verify the genetic input of the European Portuguese population in the Atlantic islands they colonized. We used DNA from unrelated individuals originating from 3 archipelagos with known ancestors from the same island for at least 3 generations; Madeira (N=210); Azores (N=135); Cabo Verde Leeward (N=202); Cabo Verde Windward (N=110) and from 3 areas in mainland Portugal; North (N=120); Center (N=127); South (N=151). DNA extraction followed the chelex method and PCR amplification was done with specific primers for each STR. PCR products were separated in polyacrilamide gels and visualized by silver staining. All populations were found to be in Hardy-Weinberg equilibrium. Frequences and heterozigozity values were estimated according to standard methodologies. Values were compared with different populations including one from S o Tom islans, and the results are discussed.

P0957. Polymorphism of the HLA-DRB1 gene in Volga-Ural region Populations

I. Khidijatova, *R. Fatkhlislamova, E. Khusnutdinova*
Biochemistry and Genetics; Ufa, Russian Federation
ekkh@anrb.ru

HLA-DRB1 gene polymorphism is investigated in population of Bashkirs (n=60), Tatars (n=60), Chuvashs (n=55) belonging to Turkic brunch of Altai language family by linguistic classification, and in population of Komis (n=50) belonging to the Finnich-Ugric branch of the Uralic language family using PCR technique. 11 alleles were detected in Bashkir population and till 10 identical ones in remaining, with frequency from 2% to 27%. An allele DRB1*09 was detected only in Bashkirs. Among identified DRB1 gene alleles the most frequent are *07 (26%) and *17 (16,5%) in Bashkirs, *04 (16%) and *15 (14%) in Tatars, *04 (27%) and *11 (17%) in Chuvashs, *04 (18%) and *11 (22%) in Komis. The Bashkir population is significantly differ from Chuvash and Komi ones in allele distribution. Allele distribution in Tatars, Chuvashs and Komis is the some as in East Europe populations. Later on these alleles will be included in pattern of genetic markers for analysis of genetic structure and ethnogenesis of the Volga-Ural region populations, and also for medical genetic aims for HLA-associated diseases prevalence forecasting.

P0958. The Twinning and Triplet Rates by Zygosity in Japan, 1975-1998

Y. Imaizumi
Hyogo University; Kakogawa City, Japan
Hyogo University, Hiraoka, Kakogawa City, Hyogo Prefecture

Twinning and triplet rates by zygosity in entire Japan were analyzed using vital statistics for 1975 to 1998. These rates by zygosity were estimated using Weinberg s method for twins and Allen s method for triplets. The monozygotic (MZ) twinning rate in Japan had remained nearly constant from 1975 (3.74 per 1,000 births) to 1998 (4.25), whereas the dizygotic (DZ) twinning rate had remained nearly constant from 1975 (1.86) to 1986 (2.27), and had gradually increased up to 1998 (4.64). As for triplets, the DZ triplet rate increased during the period from 1975 (14 per million births) to 1998 (39), reflecting the increase in the DZ twinning rate in the period. MZ triplet rates remained constant from 1975 (28) to 1998 (23). The trizygotic (TZ) triplet rate gradually increased from 1975 (18) up to 1985 (29), and rapidly increased to 1994 (202), and remained nearly constant thereafter. Then, during the last 23 years, the DZ twinning raate increased 2.5-fold, 2.8-fold for DZ triplet rates, and 11.2-fold for TZ triplet rates. The higher DZ twinning and triplet rates, and the TZ triplet rate since 1987 have been attributed to the higher proportion of mothers treated with ovulation-inducing hormones and partially attributed to in-vitro fertilization in Japan.

P0959. Phylogeography of the human mitochondrial haplogroup L3e; a snapshot of African prehistory and Atlantic slave trade

J. Alves-Silva[1], *P. E. M. Guimar es*[1], *A. Brehm*[2], *A. Torroni*[3], *V. F. Prado*[1], *S. D. J. Pena*[1], *H. Bandelt*[4]
[1]*Universidade Federal de Minas Gerais; Belo Horizonte, Brazil;* [2]*Universidade da Madeira; Funchal, Portugal;* [3]*Universit La Sapienza; Roma, Italy;* [4]*Universit t Hamburg; Hamburg, Germany*
jumps@icb.ufmg.br

The mtDNA haplogroup L3e, identified by the restriction site +2349 MboI within the Afro-Eurasian superhaplogroup L3 (-3592 HpaI), is omnipresent in Africa but virtually absent in Eurasia (except for neighbouring areas with limited genetic exchange). L3e was hitherto poorly characterised in terms of HVS-I motifs, as the ancestral HVS-I type of L3e cannot be distinguished from the putative HVS-I ancestor of the entire L3 (differing from the CRS by a transition at np 16223). An MboI screening at np 2349 of a large number of Brazilian and Caribbean mtDNAs, now reveals that L3e is subdivided into four principal clades, each characterised by a single mutation in HVS-I, with additional support coming from HVS-II and partial RFLP analysis. The apparently oldest of these clades (transition at np 16327) occurs mainly in central Africa and was probably carried to southern Africa with the Bantu expansion(s). The most frequent clade (transition at np 16320) testifies to a pronounced expansion event in the mid-Holocene and seems to be prominent in many Bantu groups from all of Africa. In contrast, one clade (transition at np 16264) is essentially restricted to Atlantic western Africa (including Cabo Verde). We propose a tentative L3e phylogeny that is based on 197 HVS-I sequences. We conclude that haplogroup L3e originated in central or eastern Africa about 46,000 (+/-14,000) years ago, and was a hitchhiker of much later dispersal and local expansion events, with the rise of food production and iron melting. Enforced migration of African slaves to the Americas translocated L3e mitochondria, the descendants of which in Brazil and the Caribbean still reflect their different regional African ancestries.

P0960. Mitochondrial DNA Analysis of Populations of Iran, Turkmenistan and Southern Russia

B. A. Malyarchuk[1], *M. V. Derenko*[1], *G. A. Denisova*[1], *E. I. Rogaev*[2], *I. A. Zakharov*[3]
[1]*Institute of Biological Problems of the North; Magadan, Russian Federation;* [2]*Center of Mental Health; Moscow, Russian Federation;* [3]*Vavilov Institute of General Genetics; Moscow, Russian Federation*
ibpn@online.magadan.su

Mitochondrial DNA (mtDNA) polymorphism in populations of Iran, Turkmenistan and southern part of Russia was examined by RFLP analysis of haplogroup-specific sites. The Iranians, Turkmenians and Russians were found to have a large portion of mtDNAs belonging to haplogroups observed in West Eurasian populations. However, the relative frequencies of this continental fraction of the mtDNA pool vary considerably over the populations studied. In Iranians and Turkmenians, the majority of the mtDNA lineages belong to haplogroups T, J, U, K, W, I. However, haplogroup H, which encompasses 19% of Turkmenian and 42% of Russian mtDNAs, is virtually absent in Iranians. The East Asian-specific fractions of mtDNAs were observed in 12% of Iranians (haplogroups M*, D, A, B) and in 20% of Turkmenians (haplogroups C, D, E), but were not found in the Russian mtDNA pool. Therefore, the Russians exhibited a high portion of identical haplotypes in relation to those observed in other Europeans. Based on the mtDNA haplogroups composition analysis, the Turkmenians were found to be the closest population to the Caucasus populations. This work was supported by the Russian Fund for Basic Research (grant 00-06-80448) and the State Program ˙Frontiers in Genetics¨ (grant 99-4-30).

P0961. The analysis of CTG/CAG repeats in populations of Volga-Ural region

R. I. Fatkhlislamova[1], *I. M. Khidijatova.*[1], *I. A. Kutuyev*[1], *P. Slominsky*[2], *E. K. Khusnutdinova*[1]
[1]*Institute of Biochemistry and Genetics; Ufa, Russian Federation;* [2]*Institute of the Molecular Genetics; Moscow, Russian Federation*
ekkh@anrb.ru

Trinucleotide repeats are the class of highly polymorphic STRs, expansion of which leads to several of human diseases. The expansions of CAG in IT

15 and CTG repeats in DMPK are associated with Huntington s disease and myotonic dystrophy respectively. The aim of the present research was to study polymorphism of CAG and CTG repeats in 10 populations of the Volga-Ural region and test the hypothesis about unstable transmission within normal range. 24 CTG allelic variants with bimodal frequency distribution and 19 CAG ones were detected. CTG/CAG repeats distribution is characterized with a high range of variations; from 5 to 34 repeats for CTG and from 10 to 32 for CAG repeats. A majority of alleles in these populations contain 5 and between 11-14 for CTG and 13-15 for CAG repeats. Investigated populations reliably differ, that is evidence of subsistence of populations heterogeneity. Heterozygotes frequency varies from 70% to 92% for CTG and from 51% to 59% for CAG repeats. CTG repeats transmission within 50 healthy families showed change CTG22 to CTG26 and CTG32 to CTG20. Within normal repeat range frequency of new mutations is 4% and passage unstability occurs in alleles CTG>19. So it has been demonstrated that the populations of Volga-Ural region are between European and Asian populations, and are more close to the Asian.

P0962. Molecular characterisation of two Mongoloid populations(Khasi and Garo) of Meghalaya, India, using Y chromosome and mtDNA markers

B. T. Langstieh[1], *K. Thangaraj*[2], *B. M. Reddy*[1], *L. Singh*[2]
[1]*Anthropology and Human Genetics Unit, Indian Statistical Institute; Calcutta, India;* [2]*Centre for Cellular and Molecular Biology; Hyderabad, India*
banrida_2000@yahoo.com

India is a country with rich diversity in language, caste, religion, culture, socio-economic structure, food and marriage practices. The Northeastern part of India is distinct in being a home to many indigenous ethnic mongoloid populations, which are not found elsewhere in the country. The region itself is described as a narrow bridge or passage between the east and west of the Asian continent. Two major language groups divide this region one with an ancient history and the other with a recent past. The former is represented by the only MonKhmer Austro Asiatic speaking tribes of India while the other involves many other migrant tribal populations of Tibeto Burman origin. Geographically they inhabit the young fold mountain belts of the eastern Himalayas and the Austrics is particularly located in the geologically oldest plateau of the region. It is postulated that the present land mass was an island which drifted away and got attached to the Indian continent. Hence, our interest is to find the possible origin and possible migration of these populations (Khasi and Garo) using Y chromosome and mitochondrial DNA markers, as they are uniparentaly transmitted and does not undergo recombination. Recent findings suggest that this language group represents one of the oldest inhabitants of this country and most probably the origin of the language itself. We substantiate our findings with tracing the paternal and maternal lineages using Y specific markers and mitochondrial DNA on two matrilineal population group of Mon Khmer Austro Asiatic Khasi and the recent migrant Tibeto Burman speaking Garo tribes from the state of Meghalaya in North east India. Of the 310 individuals studied 4 showed a 9 bp deletion in the mtDNA between COII and tRNA gene. The frequency is very less when compared to other Mongoloid populations. In the HVRI sequence these population showed a very unique haplotype. Analysis of Y Alu polymorphism (YAP) in these populations did not show any YAP+ alleles, however YAP related M15 showed 9bp deletion, which is an ancestral allele. In addition analysis is also being carried out with 400 unrelated blood samples with Y chromosome STRs (DYS19, 389I and II, 390, 391 AND 393), Biallelic markers, SNPs and Southern based markers. Results obtained from the above mentioned markers will hopefully throw more light in tracing the origin of this linguistic group.

P0963. HLA-DRB1 alleles and longevity in Macedonian population

R. S. Jankova[1], *L. J. Simjanovska*[2], *M. Z. Spiroski*[3], *G. D. Efremov*[4]
[1]*Institute of Immunology,Faculty of Medicine; Skopje, The former Yugoslav Republic of Macedonia;* [2]*Macedonian Academy of Sciences and Arts; Skopje, The former Yugoslav Republic of Macedonia;* [3]*Institute of Immunology, Faculty of Medicine; Skopje, The former Yugoslav Republic of Macedonia;* [4]*Macedonian Academy of Science and Arts; Skopje, The former Yugoslav Republic of Macedonia*
renjankova@yahoo.com

Aging involves many factors and processes. Several hypotheses have been suggested, such as the effect of non specific free radical damage of macromolecules, their repair which includes genetically determined factors, or the theory that aging is programmed in cellular genome. The genetic effects on human longevity were found at a few loci. The most interesting is the association between HLA genes and longevity directly connected to their physiological function. Numerous studies have indicated that the longevity is associated with HLA DRB1 locus. With an aim to reassess the contribution of HLA DRB1 polymorphisms to inter-individual variation of human longevity we have compared their genotype distribution between older and young control group of Macedonian population. We studied 170 unrelated healthy aged subjects, between 65 and 93 years (55 man and 115 women) selected according to Senieur protocol. The control group consisted of healthy individuals, age 25-35 years. The HLA typing was performed by dot-blot analyses using 24 nonradioactively labeled probes. In the group of aged subjects we found that the most frequent alleles are HLA DRB1 *11, *04 and *01. Comparing the frequency of alleles found among the group of older people with the alleles in the control group of young people significant difference of the DRB1 *11 allele was observed ($x2=3.54$, $p<0.05$). These results support the finding of other authors that the DRB1 *11 allele is associated with survival advantage.

P0964. Genetic Diversity and Distance Among the Hill-Tribes of Northern Thailand

D. Kangwanpong[1], *M. Srikummool*[1], *S. Suttisarakorn*[1], *M. T. Seielstad*[2]
[1]*Chiang Mai University; Chiang Mai, Thailand;* [2]*Harvard School of Public Health; Boston, MA United States*
scidkngw@chiangmai.ac.th

In Thailand the term hill tribes , or more recently used highland Thai , designates ethnic minorities, most of whom live in the remote mountainous areas of the north and the southwestern parts of the country. These groups of people migrated from neighboring countries and from south China within the last century. Great varieties of languages and traditions have been practiced, depending on where they came from. For better understanding of the population structure of these ethnic minorities, we aim to investigate the degree of genetic variation within and between these populations and to analyze their phylogeny. Y chromosome tetranucleotide repeat microsatellites at 3 loci, DYS19, DYS389I and DYS393 were employed for genetic diversity investigation of 6 hill-tribe populations in Northern Thailand. DNA was extracted from white blood cells of 107 males; 21 Karen and 15 Lahu (matrilocal groups); 29 Hmong, 15 Iu Mien, 15 Akha and 12 Lisu (patrilocal groups) by inorganic salting out procedure, amplified by polymerase chain reaction (PCR) technique using Ampli Tag Gold (Perkin-Elmer). Polyacrylamide gel electrophoresis and silver stainning were used to identify allele sizes. Five alleles were observed at the locus DYS19, while four alleles were detected at both DYS389I and DYS393 loci, with different frequencies among populations. The genetic diversity is 0.264, 0.313. 0.5323, 0.5769, 0.6503 and 0.6623 in Akha, Lisu, Hmong, Karen, Lahu and Iu Mien respectively. A neighbor-joining tree based on Fst distance reveals that the genetic distance between Karen and Iu Mien is the nearest while Akha and Lisu are furthermost apart.

P0965. Analysis of Alpha-1 Antitrypsin deficiency allele PI S in healthy Latvian population

I. Tamane[1], *L. Krumina*[1], *B. Maliseva*[1], *V. Baumanis*[2], *A. Krumina*[1]
[1]*Medical Academy of Latvia; Riga, Latvia;* [2]*University of Latvia, Biomedical Research Centre; Riga, Latvia*
ievuska@inbox.lv

AAT deficiency is an inherited disease, which usually manifests as COPD and early pulmonary emphysema. The most common mutations associated with lowered serum AAT are PI type Z and PI type S. Allele PI S being in homozygous state does not result in disease but in compound heterozygosity with allele PI Z causes clinical manifestation. The frequency of allele PI Z in Latvian population is 0,040 which is the highest frequency reported so far (Beckman L. et al.,1999;Maliseva B. et al.,2000). The aim of this research was to detect prevalence of allele PI S in Latvian population. DNA was extracted from venous blood of 236 healthy Latvians whose relatives for at least 3 generations are Latvians living in Latvia. The analysis was performed by PCR. The products were digested by TaqI, and analysed on 6% PAA gel. From 472 chromosomes analysed 15 PI S alleles were found. Consequently the allele frequency in Latvian population is 0,032. The PI S allele frequency is the highest in southern part of Latvia. Finding the reason of such geographical distribution differences might be the subject of following studies.

P0966. Some Gene Frequencies Of GSTM1, GSTT1 And CYP2D6 In The Population Of South - West Poland

T. Dobosz, *M. Sasiadek, K. Schlade - Bartusiak, A. Beszlej, M. Grzesiak, M. Zakrzewska*
Medical University of Wroclaw; Wroclaw, Poland
tdobosz@forensic.am.wroc.pl

The supergene family of glutathione S-transferases is divided into four classes; the classes mu (GSTM1) and theta (GSTT1) showed genetic polymorphism, that involves a gene deletion. This deletion has been shown to be associated with the susceptibility to mutagen - induced cyto-

genetic damages. The P450II supergene family comprises five subfamiles, one from this, the reduced activity of P450IID subfamily has been related to Parkinson disease and responsible for poor metabolism of more than 30 commonly prescribed drugs. The aim of the paper was to calculate the null gene frequency in GSTM1 and GSTT1 systems and the frequencies of the two most occurred defective alleles of CYP2D6 systems, *2 and *3. The DNA samples were prepared by Chelex methods and phenotypes were established using PCR method. In GSTM1 the population sample was 160, 53% of tested persons have the phenotype GSTM1(-), and the gene frequencies were GSTM1+ = 0.27 and GSTM1- = 0.73. In GSTT1 the population sample was 170, the GSTT1(-) phenotype occurred in 26% of tested persons, and the gene frequencies were GSTT1+ = 0.49 and GSTT1- = 0.51, respectively. The frequencies of two main ªdefective alleles of CYP2D6 gene, named CYP2D6*3 and CYP2D6*4 are under calculation in the moment. All obtained results were compared with results of other Polish or Central Europe population samples.

P0967. The distribution of D1S80 (pMCT118) and D17S5 (YNZ22) alleles in a Vietnamese population

N. H. Giang
Center of Biotechnology, Vietnam National University; Hanoi, United States
nhgiang@netnam.org.vn

For the first time, Vietnamese population in the North was studied for the D1S80 (pMCT118) locus at a large amount. Blood, bloodstain, hai and saliva samples of 217 individuals were tested by PCR technique.

Those samples were extracted by chelex, then using directly as DN templates for PCR with the specific primers of D1S80 locus. After amplification, PCR products were separated by 6% of polyacrylamide gel electrophoresis (PAGE) and stained by silver staining.

From 217 samples amplified on D1S80 locus; 57 different genotypes and 24 alleles were observed. Alleles 15, 35, 37, 40 and 42 had not bee found yet. Allele 24 (14,3%) and allele 22 (14,1%) were the most common alleles, whereas alleles 14, 33, 34, 36 and 39 were the less popular ones (0,2%) in Vietnamese population. The D1S80 locus demonstrated a heterozygosity of 80,18%. All mother/child pairs (42 cases) shared at least one D1S80 allele.

Similar method was applied for D17S5 locus. The study shows that 12 alleles were observed and 2 ones (B12 and B13) not found yet in Vietnamese population. B8 was the most spread allele (14,7%). Heterozygosity of D17S5 locus is 83,76%.

Those data show that D1S80 and D17S5 loci are highly polymorphic in Vietnamese population and can be used as the important markers for forensic, medical analyses and paternity tests.

P0968. Genetic diversity in Central Asian populations assessed by eight autosomal microsatellite loci

D. Luiselli[1], *S. Pelotti*[2], *F. Facchini*[1], *S. Fuselli*[1], *E. Tarazona Santos*[3], *D. Pettener*[1]
[1]*Dipartimento di Biologia, Area di Antropologia, Universit di Bologna; Bologna, Italy;* [2]*Dipartimento di Medicina Legale e Sanit Pubblica, Universit di Bologna; Bologna, Italy;* [3]*Departamento de Bioquimica e Immunologia, ICB. Universidade de Minas Gerais; Belo Horizonte, Brazil*
luiselli@alma.unibo.it

Historical, archaeological and anthropological data show that Central Asian populations are admixed groups which received cultural and biological contributions from Europeans and Eastern Asian. To test the impact of these historical events on the genetic pool of Central Asian populations we have analysed eight autosomal microsatellites (DM, DRPLA, AR, TH01, TPOX, CSF1PO, VWA, LPL) in more than 200 individuals from the following four populations settled in a vast region at the crossroads of different cultures and habitats; Kazakhs from the Kegen mountain valley (Kazakhstan), Huigurs from Penjim (Kazakhstan), Kirghiz from the Pamir mountains and Kirghiz from the Talas plains (Kirghizstan). The same individuals were previously studied for D-loop mt-DNA sequences, RFLPs, Y-chromosome markers (STR and SNPs) and classical markers. The statistics measuring internal variation within populations evidence high level of diversity, as expected in the case of admixed populations. Analysis of molecular variance (AMOVA) showed a very low degree of genetic differentiation among the four populations using the autosomal markers (Fst=0.01), in agreement with the results given by HVR-I mtDNA sequences (Fst=0.05), but in discordance with the Y-chromosome markers results (Fst=0.22). Non-metric multidimensional scaling was used to represent genetic distances between Euro-Asiatic populations. The Central Asian populations present an intermediate position between Europeans and eastern Asians, in agreement with the historical record. Eastern Asian and European sex-specific contributions to the current gene pool of Central Asian populations differ significantly.

P0969. Genetic Diversity Of Volga-ural People; Dna Markers Of Nuclear And Mitochondrial Genome

E. Khusnutdinova*, T. Victorova, I. Khidiatova, M. Bermisheva*
Institute of Biochemistry and Genetics; Ufa, Russian Federation
ekkh@anrb.ru

The Volga-Ural region, occupying the central place on a map of Euroasia. With the aim of knowledge of the factors of gene pool formation, genetic differentiation, evolutionary development and genetic interaction of the modern peoples of the Volga-Ural region the study of three types DNA-polymorphisms of nuclear genome, restriction fragments length polymorphism and analysis of nucleotide sequences of HVSI mtDNA, polymorphism of seven Y-chromosomal microsatellite loci were carried out. The results of claster analysis on the data of polymorphism of autosomal DNA-loci has allowed to reveal the greatest similarity of gene pool of Bashkirs with Tatars, least with gene pools of Mordvinians and Komi. Other matrix of genetic distances between populations comes to light on mtDNA mitotypes; correlation between genetic distances that revealed by two independently evolutioning genomes - nuclear and mitochondrial - is absent. We think that these differences is possible to explain by different type of inheritance of nuclear and mtDNA. Genetic distances between populations of the Volga-Ural region received by test with phage 13, will most be coordinated to the complex information about ethnogenesis of these peoples. It testifies to efficiency of use of this marker for the population-genetic analysis of modern population structure. The genetic distances received on a basis of polymorphism of seven Y-chromosomal microsatellite loci reflect philogenetical relation between the Volga-Ural peoples on father s line. The data of claster analysis only in general will be coordinated to linguistic classification, according to which these peoples belong to different language families. So, phylogenetic analysis shows their genetic connection by father s line, that is explained by centuries-old ethnical and cultural communications and territorial interactions of close ethnic components.

P0970. Polymorphic Markers to Assessment of Ecogenetic Adaptation of the Population of Large Chemical Centre

S. N. Petrova[1], *A. P. Goloshchapov*[1], *E. K. Khusnutdinova*[2]
[1]*Laboratory of Medical and Biological Problems, Bashkir Academy of Science, Research Genetic Laboratory; Sterlitamak, Russian Federation;* [2]*Institute of Biochemistry and Genetics of Ufa Scientific Centre, Russian Academy of Science; Ufa, Russian Federation*
genlab@strl.bashmail.bashnet.ru

In conditions of toxic effect of the factors of environment of the large chemical centres it is vital important to evaluate character of interrelations between the acting of influence of environmental and distribution of polymorphic markers of blood the adaptive importance of which is known in cohorts of inhabitants. Character of distribution of five biochemical markers including transferrin (Tf), group-specific component, proteinase inhibitor, haptoglobin (Hp), AB0 blood groups and its significance for ge-netic-biochemical adaptation were studied by isoelectricfocusing in 75 families (14-16 years children and their parents, which represent two generations) and in two national groups (79 Bashkirian and 129 Russian) of constant inhabitants of large indus-trial centre of the Republic Bashkortostan — Sterlitamak. The peculiarity of the gene pool of citizens was studied by way of a comparison of features of the distribution frequencies of phenotypes, alleles and genotypes in the investigated groups both in some other urban and rural populations of Russia - about Russians of Egorievsk city (Moscow range) and Asbest (Sverdlovsk range) and Bashkirians of rural districts of Republic Bashkortostan. The characteristic feature of every investigated group is de-tected, namely - the high density of alleles Tf*C3 and Hp*2. The redistribution of concentrations of alternative alleles is ob-served; for the locus Tf are peculiar lowered frequency Tf*C1 and increased for the gene Tf*C2, for the locus Hp reduction of the allele Hp*1 is featured. The considerable density of the alleles Tf*C3, Tf*C2, Hp*2 in the investigated cohorts on one hand is being represented rather of adverse, in view of a correlation of the given alleles with the formation of an increased number of the hydroxyl free radicals (Eckfeldt, 1985) and with the intensive current of the lipoperoxidation (Mironov, 1992). It allows to an-ticipate that a share of individuals inclined to the damage of subcellular structures is growing, and as the consequence they are more subjected to diseases. However, the earlier carried out researches on health of the citizens within frame works of biochemi-cal monitoring, have established of more than in twice the activation of processes of the lipoperoxidation along with high inten-sity of the microsomal oxidation, which has a positive influence at a stage of the fast detoxification of considerable amount of xenobiotics. Probably persons with the alleles TfC2 and Hp2 have the increased level of detoxification, in a connection with this are more adapted to the influence of a chemical environmental pollution, however, the given assumption is only preliminary, by virtue of a limited proportions of investigated groups. The

analysis of the genetical attitudes between investigated cohorts was carried out on a complex of the data about frequencies of sixteen alleles out of five independent locuses researched. In a course of clustering it is revealed that three investigated cohorts are very genetic similar. Most detached from all other groups of the towns-people was cohort of bashkirians of Sterlitamak. This group was enough removed from cohort of bashkirians of rural districts, also group of Russians of Sterlitamak occupies the detached position in comparison with cohorts of Egorievsk and Asbest, which unite into one cluster. Use of procedure of Maximum Likelihood Factor has allowed to lead research of joint variation of frequen-cies of alleles of the investigated populations and to frame mathematical model which describe differentiation of groups in the whole with the help of sizes of more common order - of the factors. Two factors (1 and 2) with dispersion 3,54 and 3,01, which totally depict 93,56% of total variance of frequencies of alleles were considered. It is revealed that all four cohorts of the towns-people are subject to influence of the factor 1 and occupy in expanse of factors unified compact range. Whereas specified cohorts unites residing in city with a high toxic load, it is possible to interpret conditionality of the factor by 1 specific influence of me-dium of city with the developed chemical industry. The locating in expanse of factors of groups of the townspeople differs essen-tially from peasants of the Bashkir nationality. The load of the factor 2, at our glance, is attributable to absence of unsuccessful toxic influence in cohort of Bashkirians of a countryside. The cohort of the Russians of Sterlitamak in expanse of two factors also is removed from compared cohorts of Russians of Asbest and Egorievsk. Apparently the found shifts in frequencies of alleles are demonstrating two parties of the genetic processes occurring among the Sterlitamak citizens; on the one hand - adaptive plasticity of the population, and on the other hand - adaptive reorganisation with the essential payment for the adaptation.

P0971. Polymorphism of minisatellite loci ApoB and D1S80 in Russian populations.

D. A. Verbenko[1], *O. P. Balanovsky*[1], *T. V. Pogoda*[1], *L. V. Bets*[2], *A. V. Stepanova*[2], *V. P. Ivanov*[3], *M. A. Solodilova*[3], *N. A. Bebyakova*[4], *S. A. Limborska*[1]

[1]*Institute of Molecular Genetics, Russian Academy of Sciences; Moscow, Russian Federation;* [2]*Moscow State University; Moscow, Russian Federation;* [3]*Kursk Medical University; Kursk, Russian Federation;* [4]*Arkhangelsk State Medical Academy; Arkhangelsk, Russian Federation*

dav@img.ras.ru

Genetic diversity in native peoples from six Russian areas (Arkhangelsk, Kostroma, Kursk, Novgorod, Ryazan and Smolensk regions) has been analysed. We examined the polymorphism of minisatellite loci D1S80 and 3 ApoB because of their high discrimination power. More than 700 unrelated individuals from 13 population samples were studied. Allele distributions of these loci have been obtained. Allele typing was performed using PCR technique and PAAG electrophoresis followed by silver staining.For precise determination of 3 ApoB alleles specially designed allelic ladder was used. Observed allele distributions were found to be similar with that shown for other Caucasoid populations. Both loci revealed high degree of polymorphism - we detected more than 20 alleles for each - and geterosygosity level above 76 per cent. These data make us possible to suppose that differencies between Russian populations are statistically insignificant. Comparative study of Mongoloid group (Jakut population sample) has educed statistically confirmed difference with Russians.

P0972. Patterns of Genetic Diversity at the 9 Forensically Approved STR Loci in the Indian Populations

M. Battini[1], *R. Datta*[2], *P. Chattopadhyay*[2], *G. Sun*[3], *V. Kashyap*[2], *R. Deka*[3]

[1]*Indian Statistical Institute; Calcutta, India;* [2]*Central Forensic Science Laboratory; Calcutta, India;* [3]*University of Cincinnati; Cincinnati, United States*

bmr@isical.ac.in

Genetic diversity at the 9 STR loci, which are universally approved and widely used for forensic investigations, has been studied among the 9 Indian populations representing diverse ethnic linguistic and geographic backgrounds. The 9 STR loci were profiled on 902 individuals using fluorescent detection methods on ABI377 System with the aid of Profiler Plus Kit. The studied populations represent two upper castes, Brahmin and Kayastha, and a tribe (Garo) from West Bengal, Meitei, a Hindu caste with historical links to Bengal Brahmins, a migrant Muslim group and three tribal groups-Naga, Kuki, and Hmar- from Manipur State in Northeastern India, and a middle ranking semi-nomadic herders caste called Golla from Andhra Pradesh. Gene diversity analyses suggest that the average heterozygosity is uniformly high (>0.80) in the studied populations and the coefficient of gene differentiation highest so far observed for any class of markers among Indian populations at any hierarchy- local, regional, or national. Both NJ and UPGMA trees bring out distinct clusters that are consistent with ethnic, linguistic and/or geographical affiliations of the studied populations. The fit of Harpending and Ward model of regression of average heterozygosity on the gene frequency centroid (Rii) is good and the observed outliers in line with the known history and population structure of the studied groups. Our study suggests that the 9 STR loci, employed thus far mostly in forensic investigations, can be fruitfully used in microevolutionary studies as well, and for reconstructing phylogenetic histories of the human populations, at least at the local level.

P0973. Genetic Variation at the STR Loci vWA, THO1, D21S11 and HPRT in Three Bulgarian Population Groups

B. Zaharova[1], *A. Gilissen*[2], *J. Cassiman*[2], *R. Decorte*[2], *I. Kremensky*[1]

[1]*Laboratory of Molecular Pathology; Sofia, Bulgaria;* [2]*Laboratory for Forensic Genetics and Molecular Archaeology, Center for Human Genetics; Leuven, Belgium*

zaharova@ns.medfac.acad.bg

Over 400 unrelated individuals from the three largest population groups in Bulgaria (Bulgarians, Bulgarian Turks and Gypsies) have been analyzed for the highly polymorphic STR loci vWA, THO1, D21S11 and HPRT. The study was performed by quadruplex amplification of the loci followed by denaturing electrophoresis on ALF DNA sequencer (Amersham Pharmacia Biotech). No deviations from Hardy-Weinberg expectations were observed for studied loci. Forensic efficiency values (H, MEC and DI) of the studied STR set indicate its applicability for identity and paternity testing in the Bulgarian population. Significant differences in allele frequency as well as in genotype distributions across all loci except vWA have been observed between studied population groups. The observed heterogeneity supports our previous suggestion based on Y-chromosome STR data that population differentiation should be taken into account in forensic case analysis and paternity testing in Bulgaria.

P0974. Paternity Testing experience and Genetic Data Analysis for nine STR loci in 5th Region Population, Chile.

S. Smalley, *G. Molina, R. Almarza, F. Gonz lez*

Laboratorio de Biolog a y Gen tica Molecular. Centro de Investigaciones M dicas. Escuela de Medicina. Universidad de Valpara so; Valpara so, Chile

Lab.Biomolecular@uv.cl

Background; In October 1999, the country«s new filiation law begun, and paternity testing by DNA profiling has increased in importance. Genetic data analysis of STR loci in the chilean population has only been done in the Metropolitan region. Chilean population is mainly the result of the admixture of Spaniards and Amerindian women during the XVI and XVII centuries. Later inmigrations never reached over 4%. Aim; Describe our experience in paternity testing and present the results of genetic data analysis of CSF1PO, TPOX, TH01, F13A01, FESFPS, vWA, D16S539, D7S820, D13S317 loci in the 5th region population. Subjects and methods; 36 non-related subjects attending our paternity testing service were analyzed for the nine loci. PCR amplification and analysis was done as described by Promega Corp. Results; CSF1P0, TPOX, F13A01, vWA loci, presented a departure from Hardy Weinberg equilibrium. Only in four pairs of alleles of 3 loci, show linkage dissequilibrium. The allele frequency was alike to other population of similar ethnic origin. The power of exclusion of the nine loci is 0.9998. We discarded paternity in 4 cases and confimed in 13 cases with an average probability of 99.97637 (99,89-99,99999). Conclusions; Despite that the alleles frequency is similar to the observed in other populations, the power of exclusion of these loci is higher than the reported for hispanic populations. To confirm these preliminary results and get a better knowledge of our population, we are going to sample it by screening and typing. DIPUV 20/2000.

P0975. Genetic characterization of Afro-derived Brazilian populations based on Y STRs and classic markers

S. F. Oliveira[1], *R. R. Lima*[1], *C. E. V. Wiezel*[2], *A. L. Sim es*[2], *E. M. B. Beira*[2], *E. J. S. Pilla*[1], *M. Klautau-Guimar es*[1]

[1]*Universidade de Brasilia; Brasilia, Brazil;* [2]*Universidade de S o Paulo; Ribeir o Preto, Brazil*

silviene@unb.br

In Brazil there are several rural, afro-derived populations (dubbed as quilombo remnants) founded, in their majority, at the end of the 19th century by fugitive and/or freed slaves. These populations maintain substantial genetic and cultural African derived characteristics, in spite of migratory contributions by other neighboring populations. We have been working with three main quilombo remnant populations from northeastern Brazil (Rio das R s, Riacho de Sacutiaba and Mocambo). We have used Y-chromosome STRs markers (DYS 390, DYS 391, DYS 392, DYS393 and DYS

394) as well as protein markers (ESD, CAII, GLO, PGM 1 HP and Hb) in order to study gene diversity and interpopulational heterogeneity. Protein analysis revealed mean heterozigosity levels of 0.26 (s.e.=0.074) for Riacho de Sacutiaba (n=41), 0.26 (s.e.=0.073) for Mocambo (n=99) and 0.28 (s.e.=0.059) for Rio das R s (n=176). Mean gene diversity for the STRs was, respectively, 0.62 (s.d.=0.177), 0.53 (s.d.=0.273) and 0.66 (s.d.=0.078). There is very significant heterogeneity between these populations as estimated by protein markers (Chi-square=54.65; df=14; P<0.001). Pairwise comparisons between the three populations indicates that the Rio das R s population is distinct from the two others, due mainly to the presence of the HB*C allele and to the high frequency of the CAII*2 allele. STRs markers also revealed significant heterogeneity between the three populations (Chi-square=89.53; df=38; P<0.05). Pairwise comparisons indicated that all three populations are distinct from one another. Therefore, we conclude that the Y-specific markers are more informative and show greater gene diversity between these populations. Acknowledgments; Funda o Cultural Palmares

P0976. Frequencies of Y-Chromosomal Haplogroups in several European Populations

M. Adojaan
Estonian Biocentre; Tartu, Estonia
maarja@ropka.ut.ee

There are several features that make the Y-chromosome a very valuable tool in human population studies. Unlike other human chromosomes, the Y-chromosome is haploid and most of it does not recombine in meiosis, so it is passed as a whole from father to son and in principle it is possible to reconstruct the histories of paternal lineages by comparing modern Y-chromosomes. The aim of this study was to find out about population histories by measureing the frequencies of different Y-chromosome types in different populations of eastern Europe and the Caucasus area by using unified protocols of the European Y-chromosome Diversity Project. A total of 980 DNA samples were screened for 8 unique biallelic Y-chromosome markers. The set of markers included SRY-1532, SRY-8299, DYS 257, DYS 287, M9, 12f2, Lly22g and Tat. All populations differed considerably and every population had an unique pattern of haplogroup occurrencies. Distinct clinal distribution of HG9, HG1 and HG3 was detected, allowing to speculate in terms of founding populations. A general demographic conlusion of this study suggests, that none of the studied populations underwent recent Y-chromosomal bottleneck. Further progress of this study depends on studying as many as possible uncharacterised populations, enlargement of the repertoire of the informative SNP-s and parallel analysis of microsatellite variation.

P0977. Analysis of human Y-microsatellite haplotypes in Ukraine

S. Kravchenko*, L. Livshits*
Institue of Molecular Biology & Genetics NAS of Ukraine; Kiev, Ukraine
livshits@imbg.org.ua

Five Y-chromosome-specific human STR-loci (DYS393, DYS392, DYS391, DYS390 and DYS19) were analysed in 222 samples of Ukrainians with Slavic origin from different regions of Ukraine (West, East, North and Central) and in 68 samples from isolated Crimean tatars population. DNA typing of STRs loci was performed by fluorescent PCR followed by high-throughput fragment analysis on a single-wavelength automated DNA sequencer. The allelic variation analysis has not revealed differences in all loci between four Ukrainian populations. Allele frequency distribution showed high significant differences between pooled Ukrainian and the Crimean tatars populations for all loci. Gene diversity index (D), average number of repeats index for each locus (R) and genetic distances between Ukrainian and Crimean tatars population were estimated. Five loci (DYS393/DYS392/DYS391/DYS390/DYS19) were found to generate 222 different haplotypes; 78 - in Ukrainian population and 46 in Crimean tatars populations, but only 14 of which were shared by these two populations. The most common haplotype (13/11/11/24/16) was found in 28 out of 222 (13%) Ukrainians (D = 0.94). Assuming a stepwise mutation model, haplotype tree was constructed. Haplotype analysis in Crimean tatars revealed a high level of heterogenity - D = 0.97. It was hard to find ancestral Crimean tatars haplotype because of nine relatively common haplotype were observed. Such high level of diversity within Crimean tatars suggests that this population is likely to consists of a set of discrete lineages with different origin.

P0978. African influence in actual population of the atlantic islands Madeira and cabo Verde using an Y chromosome haplotype YAP/SY81

A. Rosa[1]*, A. T. Fernandes*[2]*, A. Brehm*[2]
[1]*Departamento de Biologia, Universidade da Madeira; Funchal, Portugal;* [2]*Centro de Ci ncias Biol gicas e Geol gicas, Universidade da Madeira; Funchal, Portugal*
atgf@uma.pt

Madeira and Cabo Verde archipelagos were discovered by the portuguese in 1419 and 1460 respectively. Madeira is composed of two inhabitated islands, Madeira and Porto Santo, while Cabo Verde has nine islands divided in Barlavento (Windward) and Sotavento (Leeward) groups. Both archipelagos were part of the slave trade route which flourished in the 15th and 16th centuries. Slaves originated from the West African coast (specially Guinea) were sold in Cabo Verde and then proceeded to Lisbon. They were also used to colonize the islands of Santiago and Brava in Cabo Verde. Here we used markers YAP/SY81 from the Y chromosome to characterize the populations from both archipelagos plus a population from Portugal. YAP polymorphism is due to Alu insertion in chromosome Y, while SY81 is a mutation from an A to G. It is believed that the insertion in YAP occurred only once in human history, and it appears to have happened after the out of Africa event. The SY81 mutation supposedly happened only after the migration from the South to the North of Africa. Thus, this marker allows the separation of subsaharians from other africans. Our aim is to verify the african genetic influence in the atlantic islands of Madeira due to the slave trade. We collected DNA from unrelated individuals which are originary from the same island at least for three generations. Sample sizes and origin were the following; Madeira (N=130); Cabo Verde Sotavento (N=120); Cabo Verde Barlavento (N=110) and Guinea (N=130). DNA extraction and amplification were done using the chelex method and specific primers for each Y marker. In case of SY81 marker, we used restriction enzyme NlaIII to digest the PCR product, and separated the fragments in polyacrilamide gels visualized by silver staining. Haplotype frequency values of all populations were compared and the results discussed.

P0979. Human genetic characterization of Madeira and Porto Santo islands using Y chromosome haplotypes

A. T. Fernandes[1]*, L. Gusm o*[2]*, A. Brehm*[1]*, A. Amorim*[2,3]
[1]*Centro de Ci ncias Biol gicas e Geol gicas, Universidade da Madeira; Funchal, Portugal;* [2]*IPATIMUP; Porto, Portugal;* [3]*Faculdade de Ci ncias, Universidade do Porto; Porto, Portugal*
atgf@uma.pt

The Madeira archipelago is composed of two inhabited islands, Madeira, with about 250 000 inhabitants, and Porto Santo with 5000. Colonised in 15th century by the Portuguese, it suffered many different influences through the centuries. In the 15th and 16th centuries, Madeira was part of the slaves route and received many of the slaves going to mainland Portugal. On the 18th century, there was a British commercial influence with entire families settling there. Known by its favourable climate, a lot of people came to recover from diseases such as tuberculosis. In this century tourism certainly contributed to the various inputs on its inhabitants. Porto Santo, unlike Madeira, was exposed to attacks by North African pirates, especially during early colonisation. Here we present a survey on the genetics of Madeira population using Y STRs (short tandem repeat) which are currently applied in forensic, genealogical and evolutionary studies. Our aim is to verify the different male influences in the existing population from these two islands. We collected blood samples from unrelated male individuals originated from Madeira (95) and Porto Santo (16) with known local ancestors at least until 3 generations back. The STRs (DYS19, DYS389I and II, DYS390, DYS391, DYS392 and DYS393) were determined by PCR multiplexing, using a Perkin Elmer ABI 370 sequencer. We compared the haplotype frequencies found with other population studies and discuss the results.

P0980. MtDNA and Y chromosome polymorphisms in Hungary; inferences from the palaeolithic, neolithic and Uralic influences on the modern Hungarian gene pool

J. Beres[1]*, O. Semino*[2]*, G. Passarino*[3]*, L. Quintana-Murci*[2]*, A. Liu*[2]*, S. Santachiara-Benerecetti*[2]
[1]*National Center for Epidemiology; Budapest, Hungary;* [2]*Univ. of Pavia; Pavia, Italy;* [3]*Univ. of Pavia, Dept. of Cell Biology Univ. of Calabria; Calabria, Italy*
beres.oek@antsz.hu

Magyars imposed their language on Hungarians but seem not to have affected their genetic structure. We analysed some mtDNA and Y chromosome polymorphisms in a sample of the Hungarian ethnic group, Pal c

who, for historical reasons, could have retained genetic traces of Magyars more than other groups. In addtion, we examined a mixed Budapest sample. 100 individuals were tested for the markers defining all the European and Asian mtDNA haplogroups and 50 individuals for Y chromosome markers, namely the 12f2 and 49a,f/TagI RFLPs, the YAP insertion, the microsatellites YCAIIa, YCAIIb, DYS19 and the Asian 50f2/C deletion. In the mtDNA analysis only two subjects belonged to the Asian B and M haplogroups. The Y chromosome analyses showed that the Pal c differed from the Budapest sample by the absence of YAP + allele and by the DYS19 allele distribution;that the proto-European 49a,f Ht 15 and neolithic 12f2-8Kb were rather uncommon in both groups;that there is a high prevalence of the 49a,f Ht11 and the YCAII a5-b1;and that the Asian 50f/2C deletion is absent. The influence of Magyars on the Hungarian gene pool has been low through both females and males and Hungarian language could be an example of cultural dominance. An expansion centred on YAP; 49a,f Ht 11 is revealed by the median network based on compound haplotyes. 49a,f Ht 11 could represent either a paleolithic marker of eastern Europe which underwent expansion after the last glacial period, or a marker of more recent spread of the Yamnaia culture from southern Ukraine.

P0981. Y Chromosome Haplogroups Detect A Sharp Genetic Boundary In Eastern Central Europe

A. Novelletto[1], *M. Stefan*[2], *G. Stefanescu*[3], *L. Gavrila*[2], *L. Terrenato*[4], *M. A. Jobling*[5], *P. Malaspina*[4]
[1]*University of Calabria; Rende, Italy;* [2]*Genetics Dept.; Bucharest, Romania;* [3]*Institutul Cercetari Biologice; Iasi, Romania;* [4]*University Tor Vergata ; Rome, Italy;* [5]*Department of Genetics; Leicester, United Kingdom*
novelletto@bio.uniroma2.it

Nine single nucleotide (SNP) or indel binary polymorphisms were used to determine the frequencies of 12 Y chromosomal haplogroups in 219 males belonging to 8 administrative districts of Romania and 70 males from the Republic of Moldova. The Romanian samples represented the country s three main geographical regions, i.e. Transylvania in the North-West (Samples 1-5), Walachia in the South (Sample 6) and Moldavia in the East (Samples 7-8) up to the right bank of the Prut river, setting the border with the Republic of Moldova (Sample 9). After carrying out a hierarchical grouping of population samples into two major groups, the different quotas of genetic diversity were evaluated by AMOVA. The five highest Fct were found when one of the groups included the Western Samples 1, 3 and 6 and the other included the Eastern Samples 7, 8 and 9. Thus the Carpathians turn out to be a breaking point in the gene geography of Eastern Central Europe, providing a finer definition of one of the possible sharp genetic changes between Western and Eastern Europe. We suggest that the association between the mountain ridge and this genetic discontinuity is not a mere coincidence, being the Carpathians also representative of an ecological boundary. This latter may have acted as a barrier to gene flow over long periods of time, allowing a divergence whose signature is still detectable in the population samples examined in this work. Work supported by PRIN MURST 1999, and C.N.R. grants 97.00712.PF36, 99.02543.CT04. M.A.J. is a Wellcome Trust Senior Fellow, grant n. 057559.

P0982. Autosomal and Y-linked microsatellites in Amerindians from the Brazilian Amazon.

A. L. Sim es, *L. M. W. Santos, C. T. Mendes Jr*
Dept.Genetics, Fac.Medicina, Univ.S o Paulo; Ribeir o Preto(SP), Brazil
alsimoes@rge.fmrp.usp.br

In 1976, about 2,000 blood samples pertaining to 7 Amerindian tribes was collected in the central-west portion of the Brazilian Amazon. It was studied for a great number of classic markers and recently, DNA was extracted from some of these samples. The amount obtained was enough to study microsatellites by PCR followed by PAGE (silver stained). Results from 10 autosomal and 5 Y-linked microsatellites in about 320 Amerindian distributed in 8 villages pertaining to 4 tribes (4 villages Tik na, 2 Kaxin wa, 1 Kanamar and 1 Ban wa) are here presented. Thirteen of the eighty exact tests performed indicates Hardy-Weinberg deviation. The heterozigosity for the ten autosomal loci ranged from 0.46 to 0.68, with an average of 0.58. Interpopulational diversity revealed by Y-linked microsatellites (Gst=0.333) is much higher than that revealed by autosomal STRs (Gst=0.045); intrapopulational gene diversity obtained from the first markers set (Hs=0.188) is much lower than that from the second one (Hs=0.593). These estimates, from both sets of markers, show that interpopulational diversity values are higher than those usually described for the major ethnic groups and lower than the observed for other Amerindians from Brazil. The intrapopulational diversity obtained here is lower than reported values for other populations, including South-American Amerindians. These findings corroborate previous reports that indicates (a) a higher interpopulational diversity among Amerindian populations and (b) high informativeness of Y-linked polymorphisms in populational studies. Financial support; FAPESP, FAEPA

P0983. Y chromosome haplotype diversity in populations of North Eurasia

V. A. Stepanov[1], *V. P. Puzyrev*[1], *M. G. Spiridonova*[1], *V. N. Kharkov*[1], *Z. O. Soltobaeva*[2]
[1]*Institute of Medical Genetics; Tomsk, Russian Federation;* [2]*Medical Academy; Bishkek, Kyrgyzstan*
vadimst@img.tsu.ru

Distribution of Y-chromosome haplotypes in 10 ethnic groups of Siberia and Middle Asia (Tuva, Buriat, Altai, Evenk, Kirghiz, Uzbek, Siberian Tatar, Tadjik, Russians, Ukrainians) was investigated in order to reconstruct the evolution of paternal lineages in North Eurasia. Compound haplotypes were constructed for more than 400 Y chromosomes using five biallelic loci (SRY3225, YAP, DYF155S2, Tat and DYS199) and seven microsatellites (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392 and DYS393). Automated genotyping of STR loci was performed with HEX-, TET- or FAM-labeled primers with the ABIPrism310 genetic analyzer and Genescan software. High level of gene diversity and substantial degree of genetic differentiation of male-specific gene pool (Fst = 0.212) in modern North Eurasian populations were found. Haplotypic lineages and their evolutionary relationships were reconstructed. It was shown that most haplotypes in modern North Eurasian population have common ancestry traced back to the Upper Paeleolithic period (about 15000 years ago). Using the molecular variance of microsatellites within the biallelic haplogroups the age and origin of ancestral haplotypes for DYF155S2 - (6900 years) and TatC (4200 years) lineages which are specific for population of North Eurasia were estimated.

P0984. Genetic Differentiation In South Amerindians Is Related To Environmental And Cultural Diversity; Evidence From The Y Chromosome

E. Tarazona Santos[1], *D. R. Carvalho-Silva*[1], *D. Pettener*[2], *D. Luiselli*[2], *G. De Stefano*[3], *O. Rickards*[3], *C. Tyler-Smith*[4], *S. D. Pena*[5], *F. R. Santos*[6]
[1]*Departamento de Bioqu mica e Imunologia, Universidade Federal de Minas Gerais; Belo Horizonte, Brazil;* [2]*Area di Antropologia. Dipartimento di Biologia e. s., Universit di Bologna.; Bologna, Italy;* [3]*Dipartimento di Biologia, Universit di Roma Tor Vergata ; Rome, Italy;* [4]*Department of Biochemistry. University of Oxford; Oxford, United Kingdom;* [5]*Departamento de Bioqu mica e Imunologia, Universidade Federal de Minas Gerais.; Belo Horizonte, Brazil;* [6]*Departamento de Biologia Geral, Universidade Federal de Minas Gerais.; Belo Horizonte, Brazil*
edutars@mono.icb.ufmg.br

The geographic structure of Y-chromosome variability has been analyzed in native populations of South America using the high-frequency Native American haplogroup defined by the DYS199-T allele and 6 Y-linked microsatellites (DYS19, DYS389A, DYS389B, DYS390, DYS391 and DYS393) typed in 169 individuals. The following pattern of within- and among- population variability emerges from the analysis of microsatellite data; 1. The Andean populations exhibit significantly higher levels of within-population variability than the Eastern populations of South America. 2. There is a weak but significant correlation between genetic and geographic distances, which explains 5-10% of the observed variability between populations. 3. The isolation-by-distance model for the geographical structure of variability in South America, tested by spatial autocorrelation analysis, was rejected. 4. Genetic distance analysis suggests higher homogeneity between Andean populations than non-Andean ones. Based on these results, we propose a model for evolution of the male lineages of South Amerindians, based on differential patterns of genetic drift and gene flow. In the West of the continent, associated with the Andean area, populations have relatively high effective sizes and gene flow levels among them, which creates a trend to homogenization of the gene pool. On the other hand, Eastern populations, settled in the Amazonian region, Central Brazilian Plateau and Chaco region, exhibit higher rates of genetic drift and lower levels of gene flow, with a resulting trend to genetic differentiation. This model is consistent with the linguistic and cultural diversity of South Amerindians, the environmental heterogeneity of the continent and the available paleo-ecological data.

P0985. Y chromosomal polymorphisms in two Lithuanian ethnolinguistic subgroups

D. Ambrasiene, *V. Kuzinskas*
Human Genetics Center, Vilnius University; Vilnius, Lithuania
Daiva.ambrasiene@mf.vu.lt

Polymorphisms in the Y chromosome have been already recognised to be highly valuable in human evolutionary studies and population genetics. We are presenting results of the study of Y chromosome haplotypes variation in two main Lithuanian ethnolinguistic subgroups; Aukstaiciai (A) and Zemaiciai (Z), using three biallelic markers; 92R7, SRY-1532 and R5. Blood DNA samples were extracted from 201 individuals (A; 107, Z; 94). The biallelic markers were amplified by PCR and the resulting 730-bp product was screened, by digestion with Hind III (C was cut; T was uncut) for the 92R7 polymorphism; for SRY-1532; 432-bp, AdeI (DraIII) (A was uncut, G was cut); for R5; 1450-bp, Hsp92II (T was cut, C was uncut). 201 individuals have been analysed, and fall into four haplotypes; 1, 2, 3 and 16. The frequency of the 1 haplotype among males is 5% (A; 3%, Z; 7%), 2 — 13% (A; 15%, Z; 11%), 3 — 46% (A; 46%, Z; 47%), and 16 - 36% (A; 36%, Z; 37%). Haplotype 3 shows a highest frequency of chromosomes, defined by A allele of SRY-1532 marker, and haplotype 16 shows a high frequency of chromosomes, defined by a T to C transition (these frequencies are different from dates of T. Zerjal et al., unpublished). The haplotype distribution frequency showed a very small variation between Lithuanian ethnolinguistic groups. Comparison of our results with the date of other European populations shows that Lithuanian population is old homogenous group residing on its territory for a long period and various migration forces influenced its formation.

P0986. Y Chromosome Polymorphism in Central Anatolian Region of Turkey

A. R stemov[1], *G. Gumus*[2], *H. G. Karabulut*[3], *A. I. Kaya*[2], *G. Pekkurnaz*[2], *A. Kadikiran*[1], *I. Bokesoy*[3]
[1]*Ankara University Graduate School of Natural and Applied Sciences, Department of Biology; Ankara, Turkey;* [2]*Ankara University School of Medicine, Department of Medical Biology; Ankara, Turkey;* [3]*Ankara University School of Medicine, Department of Medical Genetics; Ankara, Turkey*
karabul@dialup.ankara.edu.tr

Determination of frequency and distribution of human Y-chromosome short tandem repeats (STR) alleles and haplotype analysis plays an important role in forensic medicine, paternity testing and tracing back to genomic drift in human evolution. Though disadvantages due to it s sex-limited nature, Y-STRs also have some advantages since they present as single copy sequences in genome and do not necessitate complex molecular methods which differs from autosomal STRs. In this study, eight Y-STR loci including DYS19, DYS388, DYS389/I, DYS389/II, DYS390, DYS391, DYS392, DYS393 in males from Central Anatolian Region were investigated. Normal healthy males living in the same geographical region for at least three generations were included in the study. PCR analysis were carried out with Y-STR specific primers on genomic DNA from peripheral blood samples after obtaining informed consent. Size determination of PCR products were detected by silver staining following %6 polyacrylamide gel electrophoresis (PAGE). Distribution of alleles for each Y-STR locus was determined and haplotype analysis was carried out and compared with data in the literature from other geographical regions.

P0987. Y-linked Microsatellites in African-Derived Black Communities of Northeastern Brazil.

C. E. V. Wiezel[1], *S. M. B. Sousa*[2], *C. T. Mendes Jr*[3], *Z. Arpini-Sampaio*[4], *A. L. Sim es*[5]
[1]*Fac.Medicina, Univ. S o Paulo; Ribeir o Preto (SP), Brazil;* [2]*Depto.Ci ncias Biol gicas, Univ.Estadual do Sudoeste da Bahia, Brazil; Jequi (BA), Brazil;* [3]*Depto.Gen tica, Fac.Medicina, Univ. S o Paulo; Ribeir o Preto (SP), Brazil;* [4]*Univ.Uberaba (MG); Uberaba (MG), Brazil;* [5]*Depto.Genetica, Fac. Medicina, Univ. S o Paulo; Ribeir o Preto (SP), Brazil*
alsimoes@fmrp.usp.br

Historic data and population genetics studies demonstrate that the Brazilian population is tri-hybrid (composed by Amerindians, Africans and Europeans) and heterogeneous in it s composition. About 4 million Africans were brought to work as slaves between 1600 and 1870; in different regions of Brazil, slaves often fled their masters, founded independent communities in remote locations with difficult access, called quilombos or mocambos. Many of these communities remain isolated still preserving their identity today with little cultural influence from the surrounding communities. Because of that they become an important resource for studies of the origin and characterisation of Brazilian blacks. Results of 5 Y-linked microsatellites (DYS19, 390, 391, 392, 393) in 4 communities (northeastern Brazil) are here reported. Interpopulational diversity (Gst=0.044) is similar to those described for the major ethnic groups; however intrapopulational diversity (Hs=0.567) exhibits a very high value. Financial support by CNPq, CAPES, FAEPA.

P0988. The Y-chromosomal haplotypes diversity in Turkic and Finno-Ugric people of Russia

T. Viktorova[1], *M. Bermisheva*[1], *E. Khusnutdinova*[1], *O. Evgrafov*[2]
[1]*Institute of Biochemistry and Genetics; Ufa, Russian Federation;* [2]*Medical-Genetical Scientific Centre of Russian Acad. of Sciences; Moscow, Russian Federation*
tvict@imail.ru

Polymorphism of Y chromosomal microsatellite loci are very useful tool to investigate the origin and the history of human populations. We studied 9 ethnical groups from Volga-Ural region of Russia, that is the eastern part of Europe. It has been an area of constant genetic contacts between Finno-Ugric, Indo-Iranian, Turkic, Siberian and other ethnical for-mations during the long historical period. The Bashkirs, Tatars and Chuvashes are a Turkic-speaking populations. The Mari, Mordvinians, Udmurts and Komi are a Finno-Ugrian populations. Being horse breeders and cattle-herders, they are of great anthropological interest, because they differ in their lifestyle and language one from another. The haplotypes diversity based on Y-chromosomal short tandem repeat polymorphisms (STRs) and frequency of biallelic T/C transition (Tat-mutation) of RBF5 locus in Volga-Ural populations have been typed. The specific putative ancestral Y-chromosomal haplotypes were observed in everyone ethnical groups. Median-Joining microsatellite networks and neighbour-joining trees were constructed. Our results revealed high intrapopulation variation in the male gene pool. We demonstrate that the several ancestral Y-chromosomes taken part in formation of the modern Volga-Ural people on a male line. The age of ancestral haplotype in each population was estimated at 9000 or 10000 years. It will be coordinated to the archeological and historical data about occurrence of the first man in territory of Volga-Ural about 10000 years ago.

P0989. Y Chromosome Microdeletions in Infertile Males

R. Dada[1], *N. P. Gupta*[2], *K. Kucheria*[1]
[1]*Department of Anatomy, All India institute of Medical Sciences; New Delhi, India;* [2]*Department of Urology, All India institute of Medical Sciences; New Delhi, India*
rdada@hotmail.com

Infertility affects 15% attempting pregnancy and in 40-50% of these cases the male partner has qualitative or quantitative abnormalities of sperm production. In 60% of cases the origin of reduced testicular function is unknown. Microdeletion of the long arm of the Y chromosome (distal part of band 5 and 6) are associated with spermatogenic failure and have been used to define three regions on Yq (AZFa, AZFb and AZFc) which are critical for spermatogenesis and are recurrently deleted in infertile males.
Eighty infertile males males with oligozoopsemia and azoospermia were included in this study. Semen analysis was done in each case to determine the spermatogenic status. They were subjected to detailed clinical examination, endocrinological and cytogenetic study. 30 G-banded metaphases were analyzed and in all cytogenetically normal cases (n=60) microdeletion analysis was carried out using PCR. For this genomic DNA was extracted using peripheral blood. The STS primers tested on each subject were sY84, sY86 (AZFa); sY127, sY134 (AZFb); sY254, sY255 (AZFc). PCR amplifications found to be negative were repeated at least 3 times to confirm the deletion of a given marker. The PCR products were analyzed on a 1.8% agarose gel.
Seven of the sixty cases (11.7%) showed deletion of at least one of the STS markers. Three cases had AZFc deletion, three cases had AZFa and AZFb deletion and one case showed AZFb deletion alone. Correlation of phenotype with microdeletion was done in each case to determine any phenotype association with deletion of particular AZF locus. The overall frequency of microdeletions varies from 1-55%. In the present study the frequency of microdeletion was 11.7%. These differences in deletion frequency and loci may reflect genuine geographical and ethnic dfferences.

P0990. Isonymy and the genetic structure of Colombia.

A. Giraldo, *I. Bustos, C. E. Pardo*
Universidad Nacional de Colombia; Bogot , Colombia
alegiraldo@usa.net

Population genetic studies in Colombia have been scarce and limited to few regions. Surname studies can be used to approach the genetic structure of a population in a relatively simple and economic manner. We present the genetic structure of Colombia based on a sample of 48054 individuals and 4015 surnames, distributed in 20 regions, representing the whole Country. Different estimators of population structure were calculated, among them random isonimy (Iii) the coefficient of kinship (phi-ii), Fisher s alpha and the Karlin-McGregor s ni. The lowest alpha values were observed in Antioquia (156.33) and Viejo Caldas (136.31), regions sharing common culture. The highest alpha values were observed in Atl ntico

(313.76) and San Andr s (294.52), regions of relatively recent immigration. Consistently, the first two aforementioned regions showed the lowest ni values (0.036455 and 0.044484) and the other two, the highest ni values (0.189841 and 0.656652). Other estimators were calculated, estimator A, the proportion of population with single surnames, and estimator B, the proportion of population included in the twenty more common surnames. A highly significant correlation were observed between ni and the estimator A (0.98; test value 10.35), and between phi-ii and the estimator B (0.93; test value 7.39). Also, negatively, between alpha and phi-ii (-0.87; test value -5.9). Principal component analysis showed that the two first axes represent the 72.79% of the total variance. A dendrogram, according the Ward s method, showed six main clusters, representing geographic, cultural and ethnic similarities. The Euclidian and geographic distances among regions also showed significant correlation.

P0991. Report of the Postnatal Evaluation of the Program of Amniocentesis in Panama

C. S. Canton
Centro Nacional de Gennetica, Hospital Nacional; Panam , Panama
celiacanton@hotmail.com

Clinical and laboratory outcome was made that include metabolic neonatal screening of patient coming from 388 amniocentesis, made in the Social Security Hospital of Panama from August 1997 until November of the 2000. Until that moment 292 children was born. 9 pregnancies were interrupted for parents request. We find 88.1% fenotipically normal and 11.9% with alterations. Of these 10.3% of the cases had non cromosomic alterations and 1.6% with cromosomics alterations (1.0% in diagnostic pitfalls and 0.6% alterations detected prenatally). Additionally was made in all patients, neonatal metabolic screening, finding 1.0 % altered. The early detection of some alterations allowed to apply therapeutic measures according to the case for the improvement of the quality of the patients life.

P0992. Novel Maternal Lineages During the Early Peopling of South America; The presence of mtDNA Lacking the Classical Amerindian Haplotypes in Mummies from the North of Chile

M. P. Carvallo[1], *M. L. Moraga*[2,1], *D. A. Merriwether*[3], *F. Rothhammer*[2]
[1]*Department of Cell Biology, Facultad de Ciencias Biologicas, P. Universidad Catolica de Chile; Santiago, Chile;* [2]*ICBM, Facultad de Medicina, Universidad de Chile; Santiago, Chile;* [3]*Department of Anthropology, University of Michigan; Ann Arbor, MI United States*
pcarvall@genes.bio.puc.cl

Ancient DNA was successfully extracted from 35 mummy bones from the North of Chile, dated 4000 to 700 years BP. Our main objective was to compare the mitochondrial DNA (mtDNA) diversity of pre-Columbian populations with contemporary Chilean aboriginal lineages and to examine hypotheses about the peopling of South America. We determined the presence of the main four Amerindian haplotypes by RFLP analysis. We found 14 individuals belonging to haplogroup A, 7 to haplogroup B, 7 to haplogroup C, 3 to haplogroup D and 4 individuals who did not harbor any of the classical Amerindian haplotypes, representing 11.5% of the sample. Twelve DNA samples corresponding to different individuals were sequenced for the D-loop hypervariable region I between positions 16081 and 16380. Most of the haplotypes determined as A, B, C and D, through restriction analysis and 9 bp deletion determination, were confirmed by sequencing. Three of the samples not presenting any of the classical Amerindian haplogroups, neither show any of the described Amerindian nucleotide changes.The results indicate that haplogroups A, B, C, and D were the most common among Amerindians who peopled South America in the past. Interestingly other mtDNA lineages that were also carried to the continent at least 4000 years BP, remain until 1300 BP. In conclusion we suggest that early Amerindian aboriginal populations showed a greater diversity than living populations. The apparent loss of some mtDNA lineages over time may be explained by the extensive decrease in the aboriginal population size due to the European colonization.(Fondecyt 198-1111)

P0993. Presence Of Haplotype B In Human Skeletal Remains Dated To The Early Holocene From The Chilean Patagonia

M. L. Moraga[1,2], *O. Reyes*[3], *F. Mena*[3], *M. P. Carvallo*[2]
[1]*ICBM, Facultad de Medicina, Universidad de Chile; Santiago, Chile;* [2]*Department of Cell Biology, Facultad de Ciencias Biologicas, P. Universidad Catolica de Chile; Santiago, Chile;* [3]*Departamento de Antropologia, Facultad de Ciencias Sociales, Universidad de Chile; Santiago, Chile*
mmoraga@canela.med.uchile.cl

Skeletal remains from five human individuals in good stratigraphic sequence were discovered in 1996 at Ba o Nuevo cave site in the Chilean Patagonia. All of them have been dated between 9000-8000 years BP, thus being the earliest human skeletal remains found to date in Patagonia. DNA was extracted from bones of the two adults and two children, and the mtDNA haplotype determination was carried out through RFLP and mtDNA D-loop sequencing. The RFLP analysis revealed that three individuals, the female, the male, and one child found physically next to the female remains, belong to haplogroup B. The analysis of the hypervariable region I, showed the presence of characteristic Amerindian nucleotide changes for this haplotype in the female and in the child DNA. However the male adult mtDNA did not present any of these characteristic changes. The second child present haplotype C, either through RFLP, or D-loop sequence analysis. Additionally, we extracted and PCR amplified DNA from ground sloth (Mylodon) ossicles, estimated to be 11000 years BP, and from Camelidae bones dated to 9000 years BP. A fragment of the 12S rRNA gene was analyzed for these two samples and the human bones. The sequences obtained for these samples are different between them, and each one is homologue to the corresponding published sequence. These results strongly support an early arrival of haplogroup B to the south of South America and suggest that early colonizers carried other haplotypes besides the characteristic Amerindian haplogroups described for living populations.

P0994. ˇccr5 mutation in Afro-derived Brazilian populations

M. W. P. Carvalho[1,2], *A. P. M. Leboute*[2], *S. F. Oliveira*[3], *S. M. B. Souza*[2], *N. Klautau-Guimar es*[3], *A. L. Sim es*[2]
[1]*Universidade Federal de Pernambuco; Recife - PE, Brazil;* [2]*Universidade de S o Paulo; Ribeir o Preto - SP, Brazil;* [3]*Universidade de Bras lia; Bras lia - DF, Brazil*
mwpc@rge.usp.br

A total of 722 rural communities founded mainly by fugitive slaves at the end of the 19th century has already been identified in Brazil. Many of them remain similar to the founder populations in terms of their genetic and cultural characteristics. A deletion of 32 pb in the CCR5 gene (ˇccr5 allele) is considered to be an European genetic marker since it occurs in European populations (highest observed frequency = 10%) but it is absent in African, Asian and South American Indian populations. Thus, its presence in non-European populations must reflect European contribution in their origin or the occurrence of inter-ethnic admixture. In this work we investigated the incidence and distribution of the ˇccr5 allele in three Afro-derived populations from northeastern Brazil — Rio das R s (n=100), S o Gon alo (n=53) and Mocambo (n=71). The allele ˇccr5 was observed in these three communities, which indicates European contribution to all of them. However, its frequency in Mocambo (5,6%) is higher than in Rio das R s (1,0%) and in S o Gon alo (0,9%), which may reflect different origins or different histories of inter-ethnic contact. The frequency of ˇccr5 in Mocambo is similar to urban Brazilian populations, which have a high degree of European contribution. This corroborates our phenotypic observation of high frequency of mixed people (European and Amerindian contributions) in this community. On the other hand, the high frequency of ˇccr5 in Mocambo can also have resulted of founder effect. Acknowledgments; Funda o Cultural Palmares, CAPES and FAEPA

P0995. Mitochondrial DNA variability in native Andean populations

S. Fuselli[1], *E. Tarazona Santos*[2], *A. Conventi*[1], *D. Luiselli*[1], *D. Pettener*[1]
[1]*Dipartimento di Biologia. Area di Antropologia. Universit di Bologna.; Bologna, Italy;* [2]*Departamento de Bioqu mica e Imunologia. ICB. UFMG.; Belo Horizonte, Brazil*
luiselli@alma.unibo.it

The genetic variability in 100 individuals from three native Quechua populations of Northern-Central, Central and Southern Peruvian Andes was examined on the basis of mitochondrial-DNA by RFLP analysis and partly (n=65) by D-loop (first hypervariable control region) sequencing. The mitochondrial DNA haplotypes of these three South American populations clustered into the four main Amerindian haplogroups; A, B, C and D, with frequencies 13%, 53%, 9% and 25% respectively. Intra-population variability analysis evidences higher variability of Andeans in comparison with other Southamerindian populations (nucleotide diversity=0.02 +/- 0.01). The neutrality test of Fu (Fs) and mismatch distribution show that Andean populations conserve the sign of an ancient expansion, probably predating the peopling of South America. This result suggests that the peopling of the Andes has not been associated with a bottleneck-founder effect. The three Andean samples analysed are significantly differentiated (Fst=0.065, p<0.05, calculated by AMOVA). The analysis of mitochondrial variability gives further support to the Andean-Brazilian differentiation hypothesis (Fsc=0.11, p<0.01 by AMOVA) previously proposed using classical markers. This result is consistent in part with data from Y-chomosome variability. The east-west differentiation of Southamerindian populations probably constitutes the major continental pattern of genetic structure in South

America. However, little information is available up to now on mtDNA variation to perform a reliable and detailed phylogeographic analysis. More populations should be investigated to identify spatial patterns of genetic variability with higher resolution.

P0996. mtDNA diversity in the Antioquian population

L. G. Carvajal-Carmona[1,2], *I. D. Soto*[2], *C. Duque*[2], *J. Ospina-Duque*[3], *M. McCarthy*[4], *G. Bedoya*[2], *A. Ruiz Linares*[1,2]
[1]*Galton Laboratory, Department of Biology,University College; London, United Kingdom;* [2]*Laboratorio de Genetica Molecular, Universidad de Antioquia; Medellin, Colombia;* [3]*Departamento de Psiquiatria,Universidad de Antioquia; Medellin, Colombia;* [4]*Complex Trait Analysis Group, Imperial College Genetics and Genomics Research Institute,Imperial College; London, United Kingdom*
lgcarvajal@yahoo.com

In a previous attempt to investigate the maternal origins of Antioquia, we evaluated in this population 4 biallelic markers that identify the major Amerind mtDNA haplogroups. In that survey, 90% of the samples had Amerind diagnostic sites while the remaining 10% could have represented either other Amerind linages (e.g. by reversion of diagnostic sites) or non-Amerind linages introduced by the Spanish and African immigrants during colonial times. In order to have a more refined estimation of the origins and diversity of the Antioquian mtDNA; here we have sequenced the mtDNA control region hypervariable segment I in a sample of 87 Antioquians, including all those individuals previously identified as non-carriers of Amerind diagnostic sites. A comparison of mtDNA sequence diversity with published data for Amerind and non-Amerind Latin American populations was also done. Our results indicated that Africa has the second largest maternal contribution to the Antioquian gene pool , while Europe had an unexpectedly low contribution Diversity estimates seen in Antioquia are comparable to those seen in Amerind populations and lower than in other non-Amerind Latin American populations. The larger African female contribution to Antioquia could be explained by several factors including their earlier arrival, higher reproduction rate and higher number of immigrants during colonial times. These views are supported by historical data. The diversity analysis supports the genetic isolation of the pre-Columbian Antioquian population and a population expansion after the arrival of the Spanish and African immigrants.

P0997. Investigation Into The Genomic Meaning Of racial Phenotypic Traits In Brazil

F. C. Parra[1], *R. Amado*[2], *J. R. Lambertucci*[3], *C. M. Antunes*[2], *S. D. J. Pena*[1]
[1]*Depto. Bioqu mica e Imunologia - Universidade Federal de Minas Gerais; Belo Horizonte, Brazil;* [2]*Depto. Parasitologia - Universidade Federal de Minas Gerais; Belo Horizonte, Brazil;* [3]*Faculdade de Medicina - Universidade Federal de Minas Gerais; Belo Horizonte, Brazil*
fparra@mono.icb.ufmg.br

We classified 173 Brazilian individuals as White, Black or Brown, based on a standardized multivariate phenotypic analysis that included skin color, hair color and texture, and the format of the lips and nose. DNA samples were collected from the same subjects and typed with the 10 population specific polymorphisms defined by Parra et al (Am. J. Hum. Genet. 63; 1839-1851, 1998). As geographic controls we used 70 African, European and Amerindian individuals. Based on the DNA typing, we assigned to each individual an Index of African Ancestry (IAA) that consisted of the ratio of the likelihoods of observing the person s findings on the hypotheses of African or European origin. The logarithm of IAA (LIAA) proved to be an extremely reliable tool for differentiating African from Euro-Asiatic individuals, with absolutely no overlap. Although analysis of the Brazilians showed a small correlation between average LIAA scores and the phenotypic classification, individual analysis revues, in several instances, complete dissociation between the two parameters. For instance, one black individual had the fourth lowest score while one Brown individual had the highest observed. Our study clearly demonstrates that genetic markers permit an individual classification in respect to African versus Euro-Asiatic geographic ancestry. Moreover, in Brazil, phenotypic racial traits are several orders of magnitude worse as predictors of genomic origin than studies involving indigenous populations of Africa and Europe.
Financial support; CNPq of Brazil

P0998. A Study of Natural Selection In Tharus of Uttar Pradesh

S. Rastogi[1], *A. Srivastava*[2], *B. R. K. Shukla*[2]
[1]*NSN College,Lucknow University; LUCKNOW, India;* [2]*Lucknow University; Lucknow, India*
rastogiak@rediffmail.com

This paper examines the Genetical demography of Tharus, a tribal group of Uttar Pradesh of North India. This tribal group is divided into six endogamous divisions representing six genetic isolates viz. Rana,Gosain,Rawat,Katharia,Dangora,and Pachchimaha.Rana and Dangora are further regionally subdivided and are reproductively isolated.These endogamous groups inhabit areas having diverse social,geographic,occupational and economic conditions. These variations in habitat are reflected in their reproductive patterns. A study of opportunity of selection based on the reproductive pattern reveals that the index of mortality is high in all the endogamous groups except one,but variation can be observed in the index of fertility. Tharus practice family planning and the mean of live births and surviving children is less in groups which are educated. Thus the total index of selection has contribution of both , the fertility and mortality.Only in two groups the index of total selection has the contribution of the index of mortality and the index of fertility is very small. The effective population size depicts that genetic drift does not seem likely to be a significant force in the general history of these populations.

P0999. ALU Genetic Diversity in Indian Populations.

S. S. Mastana[1], *S. Papiha*[2], *P. Singh*,[3], *K. Das*[4], *A. Pacynko*[1], *P. Fisher*[1], *N. Malik*[5], *M. Das*[6], *P. Reddy*[7]
[1]*Loughborough University; Loughborough, United Kingdom;* [2]*University of Newcastle upon Tyne; Newcastle Upon Tyne, United Kingdom;* [3]*Panjabi University; Patiala, India;* [4]*ISI,; Calcutta, India;* [5]*Raipur University; Raipur, India;* [6]*ISI; Calcutta, India;* [7]*Neurological Sciences Institute, Oregon Health Sciences University; Portland, OR United States*
s.s.mastana@lboro.ac.uk

ALU polymorphisms provide a useful tool to population geneticists for understanding the population dynamics that have occurred over time. We report here a study of Six Alu insertion loci (TPA25, D1, APO, PV92, FXII-IB and ACE) from 18 endogamous caste and tribal populations (900 samples). The regions studied include North (5 populations), Central and South (9 populations) and Western India (4 populations). Overall spectrum of variation in these populations is very interesting at different geographical and cultural levels. High level of insertion frequencies was observed in some highly inbred groups. Average levels of heterozygosities were found to be relatively high in these populations (range 41% to 49.8%). The genetic diversity coefficient GST among this group of populations was observed to be high. Phylogenetic trees and principal components analysis (PCA) computed from Alu frequencies provide support for socio-cultural and geographical assignment of these populations in Indian population structure. Results are discussed with reference to population origins and human evolution in India.

P1000. Microsatellite variation in three endogamous groups of Uttar Pradesh,India

S. D. Agrawal[1], *B. Muller*[2], *U. Bharadwaj*[3], *S. Bhatnagar*[3], *A. Sharma*[3], *S. S. Agarwal*[4]
[1]*Sanjay Gandhi,PGIMS; Lucknow, India;* [2]*Bernhard Nocht Institute for Tropical Medicine; Hamburg, Germany;* [3]*SGPGIMS; Lucknow, India;* [4]*SGPGIMS; Luknow, India*
suraksha@sgpgi.ac.in

We have examined variation in twenty-four microsatellite markers amongst 150 individuals each from the three endogamous groups of Uttar Pradesh, India. DNA was amplified using fluorescently labelled primers, run on a standard 6% denaturing sequencing gel using ABI, 373 sequencer. Statistical packages used for data analysis were POPGENE and PHYLIP. The number of alleles varied from 4-10, with an average of 6, at each locus. Total number of alleles found for 24 STR markers under study were 145. Heterozygosity was found to be quite high at all the loci. The average heterogygosity varied between 0.44 to 0.84 among Bhargava, 0.44 to 0.80 among Chaturvedi and 0.42 to 0.85 among Brahmin. Populations under study were in genetic equilibrium and obeyed the null hypothesis. High heterozygosity was observed within these populations showing high diversity within these pupulations. Our results reveal that Bhargavas and Chaturvedis differ significantly from one another.There might have been an ancestral pupulation i.e. Brahmin which expanded rapidly and subsequently split into largely isolated (endogamous) pupulations viz Bhargavas and Chaturvedies. Centroid analysis revealed that maximum gene flow has taken place in Brahmins.However, Bhargvas experienced the lowest gene flow. From the present study it may be postulated that Brahmins may

be the ancient population as the genetic distance of Brahmins from the over all gene freuency centroid of the three populations was found to be lowest among the three populations.

P1001. Genetic diversity of Apolipoproteins in North Indian Populations.

P. P. Singh[1], *M. Singh*[1], *S. S. Mastana*[2]
[1]*Department of Human Biology, Punjabi University; Patiala, India;* [2]*Human Genetics Lab. Department of Human Sciences Loughborough University; Leeds, United Kingdom*
s_puneet@angelfire.com

Various epidemiological studies have showed that genes coding for apolipoproteins (APOB, APOE,APOAI-CIII-AIV and ACI-CII-E gene clusters, Lp(a)) are the candidates for determining genetic variation in plasma lipid levels and risk of atherosclerosis. Most of these studies evaluating the risk of atherosclerosis vis-a-vis apolipoprotein were carried out mainly in European populations.Such type of polymorphic studies are limited in Indian Populations.We have analysed APOE,APOCII,APOH and APOAIV in 9 endogamous populations from North Indian state of Punjab.1075 individuals belonging to Brahmin, Bania, jatsikh, Kahatri,Rajput,Scheduled Castes, Lobanas, Ramdasias and Ramgarhia castes were genotyped using isoelectric focusing and two-dimensional gel electrophoresis.The overall level of polymorphism in these populations is extensive and comaparable to many Caucasian populations.A number of interesting genetic features and clines emerged from our extensive analyses.The world s lowest APOE*4 allele frequency was observed in Ramgarhia, while Ramdasia had the highest APOA-IV*2 allele frequency (0.093). The frequency of APOCII-2 was observed to be the highest in Punjabi [populations.Multivariate analyses (Correspondence analysis, multidimensional scaling analysis) of the Punjabi and the world population highlights the potential of these markers for human genetic diversity studies in addition to medico-genetic implications.

P1002. Tribal Communities show Inverse Relationship between Sickle Cell Hemoglobinopathies and G-6-PD Deficiency in Central-East India

R. S. Balgir, *B. Murmu, B. P. Dash*
Division of Human Genetics, Regional Medical Research Center (ICMR); Bhubaneswar, Orissa, India
immunol@dte.vsnl.net.in

Tribal communities in India constitute the largest tribal population in the world. There are about 461 tribal groups which constituted 8.08% (about 68 million) of the total population of India as per 1991 Census. In India, tribal communities are highly vulnerable to hereditary diseases and have a high degree of malnutrition, morbidity and mortality. Tribal health is compounded by poverty, illiteracy, ignorance of causes of diseases, hostile environment, poor sanitation, lack of safe drinking water, exploitation, faith in traditional beliefs, etc.

Out of 461 tribes, 62 tribes live in Orissa alone forming about 10.8% of the tribal population of India. The Orissa state occupies an important place, being the 3rd in rank for high concentration of tribal population in the country.

Hemoglobinopathies and glucose-6-phosphate dehydrogenase (G-6-PD) enzyme deficiency are important genetic and public health problems in Orissa. In order to map out these genetic disorders among the tribal people, 15 major tribal communities were randomly screened from different parts of Orissa. High frequency of sickle cell hemoglobinopathy and G-6-PD deficiency, the range being 0-22.4% and 5-15.9%, respectively with §-thalassemia trait taking an intermediate position, was observed in 15 major tribes. For G-6-PD deficiency, males as well as females and both female heterozygote and homozygote cases were detected. Twelve cases showed double heterozygosity for sickle hemoglobinopathy and G-6-PD deficiency.

The state of Orissa is one of the hyper endemic areas for malaria especially for Plasmodium falciparum. Moreover, the inverse relationship between the sickle cell hemoglobinopathy and G-6-PD deficiency in major tribal communities of Orissa is interesting one. When the frequency of sickle cell disorders decreases in the population, the frequency of G-6-PD enzyme deficiency increases and vice versa. It seems that the Natural Selection has played a major role in favour of sickle cell and G-6-PD enzyme mutations so that they have probably evolved as a protective mechanism against the lethal effects of malaria in this part of the country. Intervention and genetic counselling strategies among these affected tribal communities are being planned and implemented for the control and prevention of hereditary hemolytic disorders. All the above related issues have been discussed in this paper.

P1003. Population Structure and opportunity of Natural Selection among the Bhoksa tribals of North India

B. R. K. Shukla, *U. P. Singh*
Lucknow University; Lucknow, India
rastogiak@rediffmail.com

The Bhoksas inhabit the Garhwal foot hills and the tarai areas of the Kumaon division of newly formed State of Uttaranchal,which was hither to part of State of Uttar Pradesh,North India.Tharu another tribal group lives in close vicinity and shares the same eco-zone and eco-niche. Bhoksas are divisible in two subgroups; the foot hill Bhoksas and the Tarai Bhoksas. Both these groups are maritally isolated. Tharus on the otherhand are divisible into five sub groups viz. Rana, Jogia,Dangaria,Kathuria and Pachchimaha.These sub-groups are also reproductively isolated. An attempt has been made to compare two tribal groups namely the Bhoksas and Tharus on the basis of population composition like age, sex ,marital status,education,occupation etc., and population dynamics on the basis of their fertility and mortality. Further, genetical considerations based on marriage pattern, mean marriage distance,reproductive pattern and the opportunity of Natural Selection have also been studied. Many similarities and differences have been noted on the basis of population competition, fertility, mortality and genetic considerations. Further, inter group variations,have been found on the basis of above parameters between the foot hill Bhoksas and Terai Bhoksas and also with the endogamous group of Tharus. The opportunity of total selection is slightly low in the Tarai Bhoksa as compared to Foot hill Bhoksa. However, the Index of Mortality is slightly higher in the Tarai group as compared to the foot hill group. The index of fertility is higher in Foot hill Bhoksas. With index of Mortality slightly higher in the Tarai Bhoksa group and index of fertility higher in the Foot hill group, the total Opportunity for Selection is low among the Tarai Bhoksas as compared to the foothill group.On the other hand the five groups of Tharu tribals have a slightly higher level of the Index of Opportunity for selection.Thus it is clear that both groups of Bhoksas exhibit low Opportunity of Selection as compared to the five groups of Tharus.

P1004. HLA Class II antigen distribution in Maratta population from Mumbai.Maharastra. Western India.

U. Shankarkumar
Institute of Immunohaematology; Mumbai, India
shankarkumar16@hotmail.com

The HLA DR and DQ antigen maximum likelihood gene frequency estimates in 95 marattas revealed that HLA DR2 and DR7 had highest frequency(0.27;0.15) while DR3 and DR9 had low frequency(0.04). HLA DR8 was absent. HLA DQ1 and DQ2 presented with high frequency(0.40;0.13).Two locus haplotype frequency analysis revealed that DR2-DQ1 and DR7-DQ2 had highhaplotype frequency with significant T value(>2) and in positive Linkage disequilibruim(LD).The haplotype DR4-DQ1 had less frequency with negative LD. Molecular Low resolution PCR-SSP typing for DRB1 and DQB1 gene revealed that DRB1*02;DRB1*07;DQB1*06 and DQB1*0203 had frequencies as 17.85%,14.28%,50% and 12.5%.

P1005. Biological Affinity of Brahmins of Uttar Pradesh (India)

V. Agnihotri[1], *B. R. K. Shukla*[2]
[1]*NSN Degree College; Lucknow, India;* [2]*Lucknow University; Lucknow, India*
arastogi@lw1.vsnl.net.in

In India the caste system is a living reality.The caste are characterized by hierarchy,economic specialization and endogamy. The caste are divided into endogamous groups.These endogamous groups vary in their gene frequencies even though they share the same econiche.Principles of endogamy regulate their gene pool and are normally not disturbed except by selection effect and mutation. In Uttar Pradesh like other parts of India there are several endogamous groups of Brahmins. Probably these endogamous groups are split product of a larger group. Therefore in order to examine the hypothesis that whether these endogamous groups are the product of fission of a common parental population or independent realities,which have evolved during the course of time and continue to exist as individual genetic isolates, studies were made on their demographic,morphological and genetic traits. These traits indicate similarities between the Brahmin groups, suggesting the belief of a common parental stock or original oneness . The statistical test for natural selection (of Crow),Morphological distance- T2(Of Sanghwi), and G2 for genetic distance(Sanghwi) were also used to assess quantitatively the biological affinities between these groups. On the basis of these statistics, it appears that probably the process of fission following adaptation to new ways of life has been at work (as historical evidence with various myths and legends also suggests), and resulted in the formation of different endogamous groups of Brahmins.

P1006. Incidence of haemoglobin-E in tribal population of Tripura especially among school children

M. De[1], *B. Sengupta*[2], *G. Talukder*[2]
[1]*Vivekananda Institute of Medical Sciences,; Calcutta, India;* [2]*Vivekananda Institute of Medical Sciences; Calcutta, India*
madhusnata_de@yahoo.com

Tripura - a small state in the Eastern India close to Bangladesh with a predominant tribal population (80%) showed a high incidence of haemoglobin-E in an unrelated cohort of age group between 7 to 70 years(1). This encouraged us to screen adolescent tribal school children with a view to start counselling at an early age, by agarose gel electrophores followed by PCR-ARMS confirmation. This study comprised of 196 school children aged 9 to 14 years of both sexes showed 57.65% HbE carriers, 20.41% homozygous E and normal 21.94%. The incidence of HbE has been established to be the highest in the indigenous Tripuri and Tripuri Deb Barman tribes who have migrated to this area at an early date and are supposed to be Tibeto- Burmans with admixture of tribes from the West. Other tribes like Chakma, Neotia and Jamatia also showed HbE carrier status but the numbers were not adequate. Study of a parallel cohort of 114 tribal and local populations have shown an increased incidence of E beta-thalassaemia cases (10.53%), which was absent in our previous study (1). This suggests evidence of racial admixture with the surrounding beta-thalassaemia carrier population, which may pose a public health problem in future. The HbE haplotypes found in Tripura has been established to be different from that found in West Bengal.2 This milder gene action, opens up possibilities of study of regulatory or interactive gene functions specially in the populations belonging to over 30-40 tribal groups populating North Eastern India. REFERENCES ; 1. De M, Chakraborty G,Bhattacharya DK, Talukder G. Molecular studies of haemoglobin-E in tribal populations of Tripura. Lancet 1997; 349; 1297. 2. Das SK, De M, Bhattacharya DK, Sengupta B, Das N, Talukder G. Interaction of different haemoglobinopathies in Eastern India with a view to establish genotype - phenotype correlation. Am. J. Human Biol. 2000; 12; 454-59.

P1007. Genetic Register of Nervous System Hereditary Diseases in Moscow Region

S. V. Kotov, *V. Y. Neretin, B. V. Agafonov, B. M. Geht, V. G. Tsuman, M. A. Lobov, O. P. Sidorova, S. G. Kalinenkova, A. E. Nalivkin, O. S. Nazarov, N. E. Shcherbakova*
Moscow Regional Research & Clinical Institute (MONIKI), Research Institute of General Pathology and Pathologic Physiology; Moscow, Russian Federation
kunpe@orc.ru

The register of hereditary diseases is intended to currently record all corresponding medical cases. The object of the present study is creation of a register of hereditary neuropathologic cases in Moscow region to widely apply up-to-day methods of diagnosis and treatment. Direct and indirect registration methods were used. Today our register includes the following numbers of families with hereditary neuropathology; essential tremor 29 families, Wilson-Konovalov disease 36, myotonia 82, Huntington chorea 111, hereditary ataxia 126, progressive dystonia 24, Strumpell disease 66, paroxysmal myoplegia 15, hereditary sensorimotor neuropathy 294, Duchenne myopathy 124, Becker s myopathy 10, distal myopathy 3, ophthalmic myopathy 18, limb-girdle myopathy 49, Landouzy-Dejerine myopathy 34, rigid spine syndrome 12, muscular dystrophy with Dreyfus contractures 2, non-progressing myopathy 12, Stark-Kaiser neural scapuloperoneal amyotrophy 1, spinal amyotrophy 138, undetermined cases of hereditary neuromuscular pathology 279, multiple sclerosis 1103, and myasthenia 60. The patients currently undergo medico-genetic consulting and DNA-analysis. Their relatives also undergo current examination. Prenatal diagnoses are ordered to prevent ill children birth.

P1008. Classical and complex segregation analysis in an isolated population; a study on Multiple Sclerosis in Sardinia.

C. Scapoli[1], *C. Montomoli*[2], *I. Prokopenko*[2], *B. S. Murgia*[3], *A. Ticca*[3], *R. Ferrai*[3], *A. Caria*[3], *L. Bernardinelli*[2]
[1]*Department of Biology, University of Ferrara; Ferrara, Italy;* [2]*Department of Applied Health Sciences, University of Pavia; Pavia, Italy;* [3]*Neurology Department, S. Francesco Hospital, ASL n¡ 3; Nuoro, Italy*
scc@dns.unife.it

Genetic epidemiological data strongly indicate that Multiple sclerosis is a complex trait. The most acceptable theory is that susceptibility to MS is inherited and environmental factors trigger the autoimmune process that leads to the disease. Since the Sardinians are an ethnically homogeneous population, having a genetic structure quite different from that of other Italian and European populations, we believed that this population could give an important contribution to the study of the disease s inheritance. Classical segregation analysis, carried out on data from the population based Registry of Nuoro, indicated that MS may be attributed to a possible autosomal recessive gene, with reduced penetrance. This concept of incomplete penetrance , can conceal a complex pattern; the presence of more genes with modifying effects may results in a reduced penetrance, especially when the analysis is performed on a small sample. This hypothesis seems supported by complex segregation analysis. In fact, after the splitting up of the sample, according to parental mating type, the polygenic model was the most suitable for crosses between unaffected parents, whereas when one parent was affected the best fitting model was a mixed model with a polygenic component and a major locus. Segregation analysis on MS may be considered only an exploratory tool, since MS is a complex entity. However our results based on classical and complex segregation analyses indicate that the possible genetic basis could be a polygenic system. This indication is in complete agreement with the well known complexity of the disease.

P1009. An Item Response Theory Approach to Phenotype Refinement for Genetic Studies

B. S. Maher, *M. M. Vanyukov, M. L. Marazita, H. F. Simkevitz, L. Kirisci, R. E. Ferrell, G. P. Kirillova, R. E. Tarter*
University of Pittsburgh; Pittsburgh, PA United States
maherb@cpc.pitt.edu

Substantial evidence suggests that the liability to attention deficit hyperactivity disorder (ADHD) is highly heritable. To disentangle the complex system of the determination of this latent quantitative trait, it is critically important to use informative phenotypic definitions. Item response theory is capable of providing unbiased trait estimates that take into account both the individual trait level and the properties of the trait s indicators (items). Segregation analysis in a sample of 602 families supported a transmissible, but non-Mendelian, sex-dependent effect for ADHD symptom count. In a clinical subgroup of 184 pedigrees, segregation analysis supported a dominant Mendelian model. Factor analysis of the ADHD symptoms yielded two factors representing the dimensions underlying ADHD liability (inattention and hyperactivity-impulsivity). Item response theory modeling was applied to derive latent trait estimates for the dimensions, using information from multiple instruments and raters. Measured genotype analysis (MGA) was conducted to examine the relationship between the latent traits and polymorphisms at several candidate genes of the dopamine system in a sub-sample of 169 Caucasian males. Significant association was detected between a polymorphism in the DRD2 gene and IRT-derived indices of attention (p=0.0249) and activity-impulsiveness (p=0.0066). Moderate association was found between the DRD4 gene and the IRT-derived attention index (p=0.0389).

P1010. Research on Genomic Polymorphisms in Patients with Abnormal Saliva

A. Yimit[1], *H. Upur*[1], *I. Sabit*[1], *O. Kasim*[2], *R. Sabir*[2]
[1]*Institute of Xinjiang Uighur Medicine; Urumqi, Xinjiang, China;* [2]*Hotan Uighur Hospital; Hotan, Xinjiang, China*
askaryimit@hotmail.com

Polymorphism in genomic fingerprints generated by arbitrarily primed PCR (AP-PCR) can distinguish between strains of almost any organism. We applied the technique to the four-patient group (n=116, in it Hypertensive=55, Diabetic=20, Tumor=15, Athma=26) and Normal group (n=50) DNA of Abnormal Saliva and obtained 42 fragments, among them 64.2% did not show gene polymorphism. In addition, on the 220-344bp regions we had detected six AP-PCR productions. Their figure are: 5/5,4/5,3/5,2/5 (the 5/5 and 4/5 figure of Tumor group and 5/5 figure of Asthma group P<0.05 that compare with normal group), which will be the molecular index of gene diagnosis for the patients of Abnormal Saliva.

P1011. Genoprotection Activity Of Blood Of Men And It s Mechanisms

E. S. Koshpaeva
Kazan State Medical University; Kazan, Russian Federation
medbiogen@mail.ru

Antimutagenic properties of plasma and proteins (albumin and gammaglobulin) of blood of men were studied using seeds of Crepis capillaris (chromosome aberration assay). Antimutagen p-aminobenzoik acid was used as a comparative reagent. Antimutagenic activity dependent on processing conditions of the biosubstratum used; for the pre-processing and combined processing antimutagenic effect was higher than for post-processing, the processing properties of the blood being higher than those of the blood s proteins. Antimutagenic potential of biosubstrata did not depend on mutation inductor. Complex-forming properties of plasma and

blood albumen have been revealed using spectrophotometry through the substantial spectral displacement — relative to the expected spectrum — for the mixture of biosubstrata and mutagens. All plasma, albumin and gamma-globulin concentrations have been shown using chemoluminescence to increase the generation of hydroxyl radical of the Fenton reagent, especially for albumin in 1.0 g/l concentration. The general trend for all experiments was that the said substances decreased the stimulating effect as their concentrations grew. Peroxidation of yolk lipoproteids showed that only high concentrations of blood s plasma and albumen have antioxidizing properties. gamma-Globulin did nor reveal any ability to inhibit lipid peroxidation of yolk lipoproteids. Complex-forming mechanisms of the blood s albumen and antioxidizing property of plasma and proteins of men have been proved to form the blood s amtimutagenic potential.

P1012. To the problem of genetics of ageing; programmed increasing of long-lasting cells death caused by low doses irradiation

***R. F. Fedortseva**, R. P. Stepanov, I. B. Bychkovskaya*
All-Russian Center of Emergency and Radiation Medicine Emercom of Russia, Institute of Experimental Medicine RAMS; St. Petersburg, Russian Federation
arcerm@spb.cityline.ru

After Chernobyl accident there are became accumulate the epidemiological data on remote determined (tissue, organs and organism) disturbances in persons received low-dose irradiation. This data can not be explained with classical radiation genetic positions as genetic damage appear in small quantity, undergo reparation and can not lead to such pathology
Purpose; Study of cellular mechanisms of determinated, nonstochastic consequences of the action of a low-dose irradiation. Material and methods;Using electron microscopy, myocardial capillary endothelium is studied in rats X-ray irradiated at a relatively low dose of 0.5 Gy and 2.25 and 4.5 Gy (total irradiation) and 9, 30, 48 and 100 Gy (irradiation of the cardiac area). The observation time was 18 months (control, 4.5 and 9 Gy), 12 months (0.5 and 2.25 Gy). 8 months (30 Gy), and one month (48 and 100 Gy).Determined was frequecy (%) of the occurrence of endotheliocytes with signs of edematous degeneration, intracellular lysis and isolated damage of mitochondria.
Results;The total irradiation of rats at a dose 0.5Gy as well as at higher doses, 2.25 and 4.5 Gy, produces in the population on vascular endotheliocytes a persistent increase of the yield of damaged cells as compared with the background. This effect was characterized by an early appearance, irreversibility, and dose-independency. The damage was revealed without association with mitosis.The mean level of the amount of damaged cells during the observation time with the confidence interval [P=0.05] in the control and after the total irradiation at the doses of 0.5, 2.25 and 4.5 Gy amounted to 1.29– 0.4, 18.5 – 2.8, 14.2 – 6.0 and 16.0 – 2.0, respectively. The same regularities have been also established after theirradiation of the cardiac area at doses of 9, 30, 48 and 100Gy.
Conclusion; In mammals and humans the destabilization of such type is assumed to can cause expressed departure from physiological norms in slowly renewling tissues since the stable increasing of frequency cells death can no compensate in its by proliferation processes. The hypothesis are suggested that these changes can play a significant role in the development of low doses radiation consequences.

P1013. Damage To The Dna Of Female Banana Plantation Workers Exposed To Pesticides.

***V. Ram rez**, P. Cuenca*
Instituto de Investigaciones en Salud. Universidad de Costa Rica; San Jos , Costa Rica
vramirez@cariari.ucr.ac.cr

Abstract; pesticides use in developing countries is extensive in quantity and in time. A great deal of what is applied remains in the environment and in the organisms inhabiting it. This brings contamination and health impairment of those in contact with them. Effects can be manifested at a short term and in the long run and symptoms range from a headache to cancer development. Much research has been directed to acute and chronic effects and more recently to the study of genotoxic effects of pesticides. The objective of this work was to assess the genotoxicity of those pesticides used in the packaging of bananas to which female workers are exposed, using the single cell electrophoresis (or comet assay) as a biological marker, in lymphocytes from their peripheral blood. The study population was 30 exposed females from 15 different banana farms and 28 unexposed females as normal controls. According to our results, there is a significant damage to single stranded DNA after five years of work(R2=0.1210, p>0.001). The question is whether this comes from the actual exposures or from past exposures to other kind of pesticides. Neither was it possible to correlate dose and frequency of exposure to pesticides with DNA damage, since the national registries of kind of products, the length of time of their use and the periods when they were used, are very deficient. Genetic monitoring to detect DNA damage through the comet assay is recommended in order to surveillance the health of workers in contact with pesticides.

P1014. Impact of wheat flour fortification with folic acid on the prevention of neural tube defects (NTD)in Chile; preliminary results.

***F. Cortes**[1], E. Hertrampf[1], C. Mellado[1], S. Castillo[2]*
[1]INTA, University of Chile; Santiago, Chile; [2]Clinical Hospital, University of Chile; Santiago, Chile
fcortes@uec.inta.uchile.cl

Starting in January 2000, the Chilean Ministry of Health legislated to add folic acid (FA) to wheat flour (2.2 mg/kg). Chilean women of low socioeconomic status (70% of the population) consume high amounts of bread (mean; 200 g/day) which would provide 330 ug/day of FA. To determine the effectiveness of this fortification we are monitoring folate status of child bearing age women and the rates of NTD in Chile. Folate status was assessed in 750 women of reproductive age from low socioeconomic level in Santiago, before and one year after fortification. Frequencies of NTD will be recorded from 1999-2000 (baseline) to 2002, in around 60.000 births/year occurring in the nine public maternity hospitals of Santiago. Serum and red blood folates values of chilean women before fortification are similar to those of US non supplemented women before grain FA fortification (NHANES II). Samples to measure folate levels after fortification in the same group were obtained recently. Results are being processed. The frequency of NTD in Santiago before fortification, including year 1999 up to September 2000, in 103.554 newborns (alive and stillbirths, over 500 g of birth weight) was 1,69/1000. Rates were 1,32/1000 and 49,93/1000 in alive newborns and in stillbirths respectively. Rates of each defect were anencephaly 0,56/1000; encephalocele 0,25/1000 and spins bifida 0,88/1000. All defects were more frequent in females (61,1% vs 37,7 %; p=0.001). According to folic acid supply from bread, we expect that babies born under the effect of the intervention at conception will start by January 2001, so we will be able to show the results from the first trimester of 2001 at the Congress. *Supported by March of Dimes, Center for Disease Control and Prevention, Chilean Ministry of Health, PAHO/WHO

P1015. The Prevalence of Connexin 26 Mutations Within the Palestinian Deaf Population

***H. Shahin**[1,2], T. Sobe[2], T. Walsh[3], M. King[3], K. Avraham[2], M. Kanaan[1]*
[1]Bethlehem University; Bethlehem, Palestine; [2]Tel Aviv University; Tel Aviv, Israel; [3]Department of Medical Genetics, University of Washington; Seattle, WA United States
hshahin@bethlehem.edu

In some Palestinian communities, the prevalence of inherited prelingual deafness is among the highest in the world. As an initial step towards understanding the genetic causes of hearing loss in the Palestinian population, 48 independently ascertained probands with bilateral non-syndromic hearing loss (NSHL) were evaluated for mutations in the connexin 26 gene. Connexin 26 encodes the gap junction protein GJB2 and is the most common cause of recessively inherited NSHL worldwide. Of the 48 Palestinian probands, 11 (23%) were homozygous or compound heterozygous for mutations in GJB2. Multiple mutations were identified, both among and within extended families; -3170 G >A, 35delG, 167delT, 229T >C 235delC. We show that GJB2 —3170 G >A disrupts splicing, yielding no detectable message. From genome sequence of chromosome 13, we determined distances between markers flanking connexin 26 on the BAC contigs. Distances between markers flanking connexin 26 are consistent with single origins in the Middle East for 35delG and for 167delT, with subsequent recombination between markers. Twenty-five deaf probands wildtype at GJB2 represent informative, extended families who may harbor mutations in as-yet-unknown genes for inherited hearing loss.

P1016. 40 Years later; The health related quality of life of women affected by Thalidomide

***I. Nippert**, B. Edler, B. Teige*
Westfaelische Wilhelms-Universitaet; Muenster, Germany
nippert@uni-muenster.de

Introduction; Almost 40 years ago the sudden rise of births of babies with a range of severe deformities shocked the world. It was caused by a drug; the tranquilizer Thalidomide. Pregnant women who took the drug in the first trimester of their pregnancy had children with a wide but distinctive range of malformations. Thalidomide claimed more than 10.000 victims worldwide. Those who survived are now reaching middle-age. How have they

fared in major areas of life and how is their health related quality of life today?
Methods; A questionnaire using the WHO QOL-BREF instrument for measuring the health related quality of life was distributed among 166 females affected by Thalidomide, living in Germany. A randomized control group matched by age and area of residence was asked to fill out a corresponding questionnaire.
Results; Women affected by Thalidomide have a significantly lower overall health related quality of life score as compared to their matched controls and have a marked lower physical health score. They are significantly less frequently married, have fewer children, have less household income and are less mobile. They suffer from chronic pain because of a steady progress of deterioration of bones and muscles. They rely heavily on medical treatment and are less satisfied with the availability of health services. Because of ongoing gradual impairment they face an uncertain future in regard to their mobility, their ability to remain in the workforce, and the availability of health services that are apt to meet their needs.

P1017. Efficacy of a Prenatal Screening-Diagnosis Program in Hispanic Patients Attending a USA Urban State University Medical Center.

K. Mancera[1], *F. Field*[1], *L. Cohen*[1], *E. Powell*[1], *J. Matheson*[1], *J. Santolaya*[2]
[1]*University of Illinois at Chicago; Chicago, United States;* [2]*Texas Tech Health Sciences Center at Amarillo; Amarillo, United States*
kmance1@uic.edu

OBJECTIVE; According to the 1997 U.S. Census Bureau, Hispanics are the fastest growing ethnic group in the USA. Despite this fact, they continue to be a relatively under-served population in respect to genetic screening and prenatal diagnosis. To assess this issue further and possibly improve their care, we studied the efficiency of our prenatal genetic screening program by evaluating the number of invasive prenatal tesing (Amniocentesis/CVS procedures) performed after non-directive genetic counseling of Hispanic women referred for advanced maternal age (AMA), abnormal maternal serum markers (MSM) or abnormal fetal ultrasound (AU) evaluation. MATERIAL AND METHODS; Over the last 4 years 2525 pregnant women were referred for genetic counseling. Of these, 1256 were Hispanic (50%), 871 African-American (34%), 305 Caucasian (12%), and 93 (4%) of other ethnicities. 1866 patients (74%) were on Medicaid/uninsured. 663 of the Hispanic patients (52.7%) had a positive genetic screening test; 327 AMA, 273 MSM, and 63 AU. The remaining 593 Hispanic patients were referred for other reasons including a family history of genetic disorder, exposure to teratogens, recurrent pregnancy losses, etc. RESULTS; Overall 36% of the prenatally screened poitive Hispanic patients elected to pursue further invasive testing to confirm the chromosomal status of their fetuses. This acceptance rate for invasive testing depended on the indication for referral; 57% for AU, 33% AMA, and 34.3% MSM. CONCLUSION; From a public heatlh perspective, the relatively low acceptance rate of prenatal diagnosis calls into question the efficiency of the genetic screening program in Hispanic women. It is possible that most of these women are not interested, or do not understand the overall objectives of the program. Further studies are needed to address these questions.

P1018. Screening For C283y Gamma-sarcoglycan Mutation In High Risk Group Of Bulgarian Gypsies

A. Todorova[1], *I. Tournev*[2], *N. Ninova*[1], *V. Georgieva*[3], *I. Kremensky*[1]
[1]*Laboratory of Molecular Pathology; Sofia, Bulgaria;* [2]*Department of Neurology; Sofia, Bulgaria;* [3]*Department of Medical Genetics; Sofia, Bulgaria*
todorova@ns.medfac.acad.bg

Limb-girdle muscular dystrophy type 2C (LGMD2C) is an autosomal recessive disorder, caused by mutations in the g-sarcoglycan gene. A private Gypsy mutation C283Y was detected in that gene. Recently, a number of LGMD2C affected families from Gypsy origin have been found in East Bulgaria and all these cases were due to the same mutation. We have screened 303 individuals in a reproductive age from high risk Xoroxane Gypsy group. The genetic tests revealed 8.3% C283Y heterozygote carriers. The screened sample was not ethnically homogeneous, but it was divided in ethnonym groups on the basis of a number of socio-anthropological criteria; social status, language characteristics, handicrafts, etc. We found that the mutation C283Y was not randomly distributed among the Gypsy subgroups. The disease seemed to be restricted to the group of Xoroxane Gypsies and geographically localized in East Bulgaria. Our results stress upon the importance to have a precise knowledge on the socio-anthropological structure of the group to be genetically analyzed, especially when it concerned isolated, endogamous populations (as it was in our case). A number of important questions such as the origin of the ethnic subgroups, the routs of migration, the demographic history, the subgroup s endogamy, isolation and admixture with surrounding populations, remain open.

P1019. Prenatal Detection Of Chromosomal Abnormalities By Fetal Ultrasonographic Examination Across Europe.

C. De Vigan[1], *M. Clementi*[2], *E. Cariati*[2], *N. Baena*[2], *C. Stoll*[2]
[1]*Medical Genetics; Strasbourg, France;* [2]*Service de G n tique M dicale, Hautepierre; Strasbourg, France*
Claude.Stoll@chru-strasbourg.fr

The objective of the EUROSCAN study was to evaluate the accuracy of the antenatal detection of chromosomal anomalies by routine fetal ultrasonographic examination in unselected populations. All congenital malformations suspected prenatally and all congenital malformations confirmed at birth were identified from Congenital Malformations Registries including 20 registries from 12 European Countries. These registries are following the same methodology. During the studies period 1996-98, 664.269 births were covered including liveborn, stillborn and terminations of pregnancy. The chromosomal abnormalities were classified into 12 groups; 1783 cases of chromosomal abnormalities were collected; the main groups were Down syndrome (n=1050), trisomy 18 (n=191), Turner syndrome (n=125), trisomie 13 (n=86) and triploidy (n=56). The detection rate by prenatal ultrasonography among the 1683 cases having had at least one scan was 38,9%, with variation by regions and according to the chromosomal categories. The lowest rate was observed in Klinefelter syndrome (5,7%), the highest one in polyploidy (78,6%), it was 26,4% for Down syndrome; 75,9% of the cases detected by ultrasonography were terminated. In conclusion this study shows that close to 40% of fetal chromosomal anomalies can be detected by routine prenatal ultrasonographic examination. However this detection rate varies according to the regional policy of prenatal diagnosis.

P1020. Molecular Genetic Testing In Phenylketonuria;a Model To Assess The Quality Control System For Monogenic Disease

V. Kucinskas[1], *V. Jurgelevicius*[1], *D. Steponaviciute*[1], *S. Giannattasio*[2], *P. Lattanzio*[2], *E. Marra*[2], *R. Lugovska*[3], *N. Pronina*[3], *J. Zschocke*[4]
[1]*Human Genetics Centre of Vilnius University; Vilnius, Lithuania;* [2]*Centre for Studies of Mitochondria and Energy Metabolism; Bari, Italy;* [3]*State Medical Genetics Center Children s Hospital; Riga, Latvia;* [4]*University Children s Hospital and Department of Human Genetics, Heidelberg University; Heidelberg, Germany*
Vaidutis.Kucinskas@MF.VU.LT

EU COPERNICUS joint research project Molecular Genetic Testing in Phenylketonuria; a Model to Assess the Quality Control System for Monogenic Disease (MOLGENT) is aimed to develop an external quality assurance scheme for molecular genetic testing in PKU (EQA-PKU) for diagnostic laboratories. Such scheme should be adequate to other EQA schemes already in action. On the 1st stage, a standardised strategy for PAH gene mutation identification based on DGGE and RED analysis and direct DNA sequencing was developed. Standardised protocols for (i) DGGE, (ii) DNA sequencing and (iii) routine identification of common PAH gene mutations were prepared (http;//www.geneticahumana.lt/MOLGENT/). Analysis of the spectrum of PAH gene mutations in South, West and East Europe revealed the sets of 10 most prevalent mutations for the EQA-PKU trials in definite populations; Italian — IVS10-11G>A, L48S, R158Q, R261Q, P281L, R261X, R252W, delT55, IVS12+1G>A, IVS7+1G>A; German — R408W, IVS12+1G>A, IVS1-11G>A, Y414C, R261Q, R158Q, P281L, L48S, I65T, E390G; Lithuanian — R408W, R158Q, G272X, A403V, R261Q, E280K, R261X, L311P, IVS10-11G>A, IVS12+1G>A. The 2nd stage was the first trial of the pilot EQA-PKU scheme. Coded DNA samples bearing four PAH gene mutations matching to clinical mock referrals and reply forms were sent to the laboratories participating in the pilot EQA-PKU scheme. The same samples were also sent to experts-reviewers for external expertise. On the basis of the analysis of the reports received from the laboratories involved in the first EQA-PKU trial, a scheme open for all European laboratories will be developed, as a result of the 3rd stage of MOLGENT implementation.

Also presented orally in Concurrent Session 15: Poster Discussion Genetic Testing and Screening

P1021. Parents Satisfaction With Medical And Social Help Provided To The Child With Down Syndrome

T. Reimand[1], *O. Uibo*[1], *R. Zordania*[2], *V. Palmiste*[1], *T. Talvik*[3]
[1]*University of Tartu, Medical Faculty; Tartu, Estonia;* [2]*Tallinn s Children Hospital; Tallinn, Estonia;* [3]*University of Tartu, Medical Faculty; Children s Clinic, Tartu University Clinics; Tartu, Estonia*
tiia.reimand@kliinikum.ee

The aim of this work is to investigate the extent of parents satisfaction with medical and social services provided for the families with a member with Down syndrome (DS families) and how they are coping with this situation. Material and Methods; Fifty questionnaires filled by the DS families were analysed. The age of persons with DS was 5 months-28 years (mean age 8.5 years) Results; The clinical diagnosis of DS in most cases was made at maternity hospitals by paediatricians who also gave the first information about the DS (in 82% of cases). Only 24% of parents were satisfied with this information, 76% of families would like to have more information and support. The cytogenetic diagnosis was made during the first month in 50% of cases. In 4 cases (8%) diagnosis was confirmed later than one year. Only 22% of families were completely satisfied with social benefits. The home atmosphere became worse during the years in 44% of cases. However, 70% of the families agreed to support other DS families. Conclusions; The DS families need more information about DS and support, especially right after the diagnosis have been established and more possibilities for habilitation and social help. Most of the parents are ready to support other families and share their knowledge and skills.

P1022. Iranian Human Gene Bank

A. Hajibeigi[1], *K. H. Javan*[1], *F. Pourfarzad*[1], *N. Nikzat*[1], *M. Neishabury*[1], *H. Najmabadi*[1,2]
[1]*University of Welfare and Rehabilitation; Tehran, Islamic Republic of Iran;* [2]*Karimi-Nejad Pathology and Genetics Center; Tehran, Islamic Republic of Iran*
maryamneishabury@yahoo.co.uk

For over a decade prevention of the common genetic disorders was a national programme in Iran. The result of our ten years study on carrier identification and prenatal diagnosis of genetic diseases, has revealed the existence of a wide spectrum of mutations for certain diseases in this country. This is a reflection of Iran s long history of foreign invasion, immigration, cultural exchange and also the high prevalence of interfamilial marriages, creating a multiethnic society with a highly heterogenous gene pool. During the last three years investigation we have established a DNA bank of all genetic diseases with Mendelian mode of inheritance studied in Iran. Some of these samples are assigned to common or novel mutations and some others belong to patients with clinical profiles associated with a particular genetic disease but unidentified mutation. This bank stores patient and his/her first degree relatives DNA together with a comprehensive pedigree and a clinical profile for each sample. To improve our diagnostics, we found it essential to establish a link between our findings and the other research projects elsewhere in the world by presenting our experimental projects and this DNA bank on line. Our web site as well as providing opportunities for us to collaborate with outside, offers a free of charge valuable sample resource to all the researchers in the world, who are working on various aspects of genetic disorders from prenatal diagnosis to gene structure and function. This article introduces the structure and the content of this DNA bank.

Also presented orally in Concurrent Session 15: Poster Discussion Genetic Testing and Screening

P1023. A 2 year postgraduate programme for training Clinical Scientists in UK Diagnostic Molecular Genetics Laboratories.

A. Seller[1], *J. Harvey*[2], *F. Macdonald*[3], *J. Whittaker*[4], *L. Meredith*[5], *A. Curtis*[6], *G. Taylor*[7], *S. Warburton*[3], *D. Baty*[8], *A. Dalton*[9]
[1]*Oxford Medical Genetics Laboratories; Oxford, United Kingdom;* [2]*Wessex Regional Genetics Laboratories; Salisbury, United Kingdom;* [3]*West Midlands Regional Genetics Laboratory; Birmingham, United Kingdom;* [4]*East Anglian Medical Genetics Service; Cambridge, United Kingdom;* [5]*Institute of Medical Genetics; Cardiff, United Kingdom;* [6]*Northern Genetics Service; Newcastle, United Kingdom;* [7]*Yorkshire Regional DNA Laboratory; Leeds, United Kingdom;* [8]*Ninewells Hospital; Dundee, United Kingdom;* [9]*Centre for Human Genetics; Sheffield, United Kingdom*
anneke_seller@hotmail.com

Diagnostic molecular genetics laboratories in the UK are directed and staffed mainly by Clinical Scientists with technical support provided by Medical Technologists. A two year, Department of Health approved graduate training programme for scientists in this speciality has been available since 1992 with supernumerary posts funded regionally. The aim is to provide a highly motivated and skilled workforce, fit for purpose, to work in any diagnostic clinical molecular genetics laboratory within the National Health Service. The programme also acts a foundation for statutory registration with the Health Professions Council. Training within the speciality is overseen by the profession s Training Accreditation Board (TAB). The programmes are modular, cover defined core topics and specialist areas with opportunities for research and development. Trainees must demonstrate competence in the following 7 areas; Clinical, Scientific, Technical, Observational, Management, Communication and Research & Development. Following assessment by the TAB at 6, 12 and 24 months, a postgraduate certificate of competence is awarded, qualifying trainees to apply for career grade posts. 14 laboratories have been accredited by the TAB for training. To date 69 graduates have registered and 5 have left before completion. 46 have received their certificates and of those 38 remain in the profession, 6 have gone into research,1 into industry and 1 is unemployed. 18 are partway through training. Experience to date indicates that this formal approach to training molecular geneticists has contributed significantly to the maintenance of a high standard of diagnostic service in Clinical Molecular Genetics Laboratories across the United Kingdom.

P1024. Mutation analysis in patients with osteogenesis imperfecta; identification of five novel mutations

E. Benusiene, *D. Kasperaviciute, V. Kucinskas*
Human Genetics Centre, Vilnius University; Vilnius, Lithuania
Egle.benusiene@santa.vu.lt

Osteogenesis imperfecta (OI) is heritable connective tissue disorder caused in >90% cases by dominant mutations in two genes; COL1A1 (17q21.31-22.05) and COL1A2 (7q21.3-22.1). The aim of the study was to improve postnatal and prenatal diagnosis of OI in Lithuania using molecular genetic testing. Lithuanian OI database consists of 110 case records. Thirteen familial and eight sporadic dominant OI cases were available for molecular genetic testing. The segregation of COL1A1 and COL1A2 was analysed using RFLP within or close to the genes. Comparison of phenotypic features with the concordant collagen locus showed that in three pedigrees Sillence OI type I segregated with the COL1A1 locus, while in two pedigrees Sillence OI type I and OI type IV segregated with the COL1A2 locus. In eight remaining pedigrees the data available were insufficient for the identification of a definite OI-linked COL1A locus. Probands from 11 OI families and eight sporadic OI patients were screened for mutations in 26 exons of the COL1A1 gene using DNA heteroduplex analysis. The results showed the presence of nucleotide sequence changes segregating with the OI phenotype in six probands. Subsequent direct DNA sequencing revealed six mutations in four exons of the COL1A1 gene; missense (1), nonsense (1), frameshift (3), and splice site mutation (1). Out of them, five mutations appeared to be novel; E500X, c.2165-2166insCTCTCTAG, c.1787delT, c.1786-1787insC, IVS19+1G>A. These findings confirm clinical diagnosis of OI and enable prenatal OI diagnosis based on direct identification of a COL1A1 gene mutation in six families.

P1025. Legal Issues in the Practice of Clinical Genetics in the US

L. D. Fleisher
Sidley & Austin; Chicago, IL United States
lfleishe@sidley.com

As scientists increase our ability to diagnose genetic conditions, preconditions, and susceptibilities, lawyers and ethicists increase our questions and concerns regarding the societal implications of these new capabilities. This presentation will address those questions and concerns as they arise in the practice of clinical genetics. For example, who decides when a new genetics test becomes the standard of care? What does the standard of care require; i.e., when must clinical cancer geneticists advise patients regarding the availability of a new test; must they offer the test? Who should/should not be tested? How do we protect the right not to know? What about children? What constitutes informed and voluntary consent to genetic testing? Who do the genetic test results belong to and what must be done to protect privacy and ensure security? Is there a duty to warn potential carriers of disease-predisposing genes and, if so, what if that duty conflicts with the duty of confidentiality? What obligations do we have to prior patients? What if something goes wrong?

P1026. A High-Throughput Screening Method for Wilson disease (ATP7B) gene in the highly heterogeneous Greek population.

E. Panagiotakaki[1], *M. Tzetis*[1], *N. Manolaki*[2], *G. Loudianos*[1], *E. Kanavakis*[1]
[1]*Athens University, Medical Genetics; Athens, Greece;* [2]*St Sophia s Children s Hospital; Athens, Greece*
mtzeti@cc.uoa.gr

To completely characterize the spectrum of mutations in the ATP7B gene in Greek Wilson disease (WD) patients, we screened 150 Greek WD chromosomes by SSCP. 18 mutations were identified accounting for 85% of WD chromosomes. H1069Q (29.3%), R969Q (17.3%), Q289X (9.3%), L936X (7.3%), 2530delA (5.3%), I1148T (3.3%), 2299insC (2.7%), 1708-1G>A (2.0%), 845delT (1.3%), Ter1466R (1.3%), and mutations 1703+3insC, G1099S, 777insC, 1782delT, 2101delAT, G1061E, R778G

and 3907-2A>G for 0.7% each, while 15% of the mutations remain unknown. Subsequently we developed a universal high-throughput double gradient denaturing gradient gel electrophoresis system (DG-DGGE) for the analysis of 80% of WD mutations in Greece. Exons 2, 4, 8, 10, 12, 13, 14 and 16 were PCR amplified. Heteroduplexes were generated at the end of each PCR session and amplified DNA samples were electrophoresed in a linear porosity gradient 6-12% polyacrylamide gel in the presence of the same linear gradient of denaturants, urea and formamide, as used for standard DGGE. The DG-DGGE conditions used were the following ; denaturants 20-70% for exons 2, 4, 10, and 12 and 30-80% for exons 14, 13, and 16. Running conditions were; 75V for 15 h. In addition exons 10 and 12 and 14 and 16 were double loaded. Following electrophoresis, gels were stained in ethidium bromide. Using DG-DGGE together with the highly informative microsatellite markers in linkage with the ATP7B gene (D13S301, D13S316, D13S314) we are able to offer genotype analysis and preclinical diagnosis to the vast majority of Greek WD families.

P1027. DHPLC for Germline Mutation Screening in the Analysis of the VHL Tumor Suppressor Gene; Usefulness and Limitations

B. Klein[1], *G. Weirich*[2], *H. Brauch*[1]
[1]*Dr. Margarete Fischer-Bosch-Institut for Clinical Pharmakology; Stuttgart, Germany;* [2]*Institut for Pathology, TU; Munich, Germany*
bettina.klein@ikp-stuttgart.de

In order to evaluate sensitivity and specificity of the recently introduced high throughput method DHPLC (denaturing high pressure liquid chromatograpy) for mutation screening in the VHL tumor suppressor gene, we subjected DNA from 43 unrelated VHL patients with previously sequenced VHL germline mutations to this method. In addition, 36 genomic DNAs of unrelated individuals suspected of being VHL carriers but unknown germline status were analyzed by DHPLC and sequencing. Aims of the present study were to compare mutation results obtained by direct sequencing and DHPLC, and the comparison of two different DHPLC systems. The sensitivity of DHPLC was tested with two commercial devices and protocols i.e. the Varian-HelixTM -System and the Wave¤ Nucleic Acid Fragment Analysis System. Both resolved all but one mutation in exon 2 and 3 of the VHL gene. In contrast, the GC rich exon 1 showed discrepancies in the rate of mutation detection. Whereas the Varian-HelixTM -System detected 10/15 (67%) mutations of the known mutations, the Wave¤ Nucleic Acid Fragment Analysis System detected 13/14 (93%). All three mutations of samples with unknown mutation status were called by both systems raising the mutation detection rate to 72% and 94%, respectively. Cases with different substitutions at the same nucleotide showed different elution profiles but similar elution profiles could be obtained from different mutations. The Wave¤ Nucleic Acid Fragment Analysis System detected most VHL mutations however when 100% detection rate is needed sequencing is still required and must therefore be the recommended VHL mutation procedure. Once a family specific mutation has been established, DHPLC may be suitable for a rapid and cost-effective determination of VHL carrier status in family members

P1028. Validation and Harmonisation in Genetic Testing

A. J. Woolford, *C. A. Foy, L. Birch, H. C. Parkes*
LGC; Teddington, United Kingdom
ajw2@lgc.co.uk

The growth of genetic testing over the last decade has created a need for quality control and external assessment of results. An ever increasing repertoire of genetic loci can be examined for association with genetic abnormalities, cancer predisposition, disease status and prognosis. This means that laboratories that are proficient in one area are assumed to be qualified in testing areas that may be very far removed from their area of previous experience.

Some important criteria for assessing a test will need to be addressed more formally. Issues of particular importance are fitness for purpose ; can the test yield the desired information? What are the limits of the test, in terms of specificity, selectivity, and detection? Does sample origin, complexity of the sample matrix or its storage have any effect on the result? Has the result been accurately interpreted? Can we assign a confidence limit based on our previous data? Statistical analysis of the performance of a method and calculation of an uncertainty budget could enable more informed genetic counselling and prevent the inappropriate application or extrapolation of some genetic test results.

In order to deliver the potential advantages of genetic testing to society and improve public perception of test results, it is important to demonstrate the quality and reliability of the test. Proficiency testing schemes and the use of reference materials or certified test samples are possible routes to harmonisation of testing procedures.

P1029. Validation Issues Surrounding Emerging Homogeneous, DNA-Based Technologies for Bioanalytical Measurements in the Clinical Laboratory

C. A. Foy, *A. J. Woolford, H. C. Parkes*
LGC; Teddington, United Kingdom
caf@lgc.co.uk

Advances in DNA analysis to develop methods which are increasingly specific, sensitive, fast, simple, automatable and cost-effective, are considered paramount. These demands are currently driving the rapid evolution of a diverse range of newer technologies. One such advance is the development of technologies with the ability to amplify, detect and quantitate DNA targets in a single closed-tube reaction as PCR proceeds. These real-time homogeneous systems offer many advantages over traditional methods including speed, reduced risk of contamination and the ability to more accurately quantitate the amount of starting material present. As rapid technological advances produce systems which are capable of increased sensitivity, throughput and quantitative potential, new sets of validation issues need to be addressed. These include the reproducibility of the techniques both intra- and inter-laboratory, sensitivity, accuracy, precision and the interpretation of data where arbitrary selection of cut-off points to separate positive and negative results are often employed. Under the DTI s Valid Analytical Measurement (VAM) programme, LGC has been identifying recent developments which may have an impact on the future of clinical DNA-based diagnostics. This presentation will highlight some of the most popular homogeneous technologies currently making the transition from the research laboratory to the clinical laboratory and discuss key aspects in their validation.

P1030. Screening for fraX-A, fraX-E and fraX-F syndrome by one multiplex methylation-sensitive polymerase chain reaction (MS-PCR)

A. Weinhaeusel[1], *P. Waldner*[1], *O. A. Haas*[2]
[1]*CCRI; Vienna, Austria;* [2]*Ludwig Boltzmann-Institute for Cytogenetic Diagnosis; Vienna, Austria*
weinhausl@ccri.univie.ac.at

Three fragile sites, FRAXA, FRAXE and FRAXF are located on the chromosomal region Xq27-28 that result from repeat-expansion. Phenotypic expression concurs with the methylation of the expansion and the promoters of the respective genes, FMR1, FMR2 and FRAXF sequences. Both FRAXA and FRAXE are significant forms of mental retardation, whereas the significance of the FRAXF expansion is unclear so far. In contrast to FRAXA, the most prevalent form of inherited mental retardation in males, whereas FRAXE and FRAXF are extremely rare. Therefore, it has been questioned whether screening for the two rare forms is warranted. Nevertheless, it is important to distinguish the one from the others. We have therefore devised a multiplex MS-PCR strategy that allows the simultaneous evaluation of the de novo promoter methylation of all three promoters. It consists of primer sets that are specific for the deaminated methylated promoter regions of FRAXA, FRAXE and FRAXF as well as a primer pair that is specific for the unmethylated FMR1 promoter. Our approach distinguishes between normal and affected FRAXA, FRAXE and FRAXF males solely based on the respective band patterns. Female full mutation carriers can be identified by a densitometric analysis of the PCR products on gels stained with ethidium-bromide. So far, we have screened more than 500 DNA samples from patients referred to for the diagnostic evaluation of FRAXA, Prader-Willi and Angelman syndrome. Amongst these samples, we identified seven patients with FRAXA, but none with FRAXE or FRAXF.

P1031. Identified Mutations for Genetic Testing

J. C. Beck, *C. M. Beiswanger, L. H. Toji, P. K. Bender, J. C. Leonard, R. T. Johnson*
Coriell Institute for Medical Research; Camden, NJ United States
jbeck@umdnj.edu

Currently more than 600 molecular genetic tests are offered by a variety of laboratories and institutions. There is a critical need for validated standards accessible to laboratories offering these tests. The Coriell Cell Repositories through the NIGMS Human Genetic Cell Repository and the NIA Aging Cell Repository have a collection of more than 600 cell lines and DNA samples representing 72 diseases with characterized mutations which could be used as standards. These include diseases caused by expansion of trinucleotide repeats, such as dentatorubral-pallidoluysian atrophy (for which three samples with known repeats are available); myotonic dystrophy (13); Friedreich ataxia (10); fragile X syndrome (26); Huntington Disease (13); SCA1 (2); and SCA3 (2). The collection also includes 32 different mutations in the CFTR gene, 20 unique mutations in the BRCA1 gene, 6 mutations in the BRCA2 gene, and 4 mutations in the APC gene. Samples from patients with hemochromatosis (19), muscular dystrophy (11), spinal mus-

cular atrophy (3), Angelman syndrome (4), and Prader-Willi syndrome (12) have also been molecularly characterized. In addition, specimens carrying the factor V Leiden mutation (2), the MTHFR thermolabile variant (2), and the 20210G-A polymorphism in the prothrombin gene (2) are also included in the collection. Standards are also available for apolipoprotein E and Rh D genotyping. These samples are valuable reagents for laboratories performing molecular genetic testing and may also be useful for quality assurance programs. Detailed information about these samples, including ordering instructions, is available in an electronic catalog (http;//locus.umdnj.edu/ccr).

Also presented orally in Concurrent Session 15: Poster Discussion Genetic Testing and Screening

P1032. Rapid detection of aneuploidies of chromosomes 13,18,21,X and Y with multiplex-fluorescence-polymerase-chain reaction in 800 amniotic fluids

***P. Kozlowski**, I. Kops, A. Ernsting, M. Oros*
Center for Prenatal Diagnosis and Genetics; Duesseldorf, Germany
institut@praenatal.de

In 85% of all fetal abnormalities aneuploidies of the chromosomes 13, 18, 21, X and Y are present. After invasive diagnostic the classical cytogenetic karyotyping of amniotic cell-cultures takes about 10-14 days. To get a quicker diagnosis detection-result of these aneuploidies there are two common methods; the FISH technique and the multiplex-fluorescence-PCR test. Using the multiplex-f-PCR test, results of the chromosomes 13,18,21 and XY are presentable in only 18 hours, in urgent cases in 5 hours after amniocentesis. Only 1ml of amnotic fluid is necessary for performing the test. It is possible to use the test on amniotic-cells after the 11th week. Three multiplex-f-PCRs include fifteen different STRs (tri-and tetranucleotid-repeats), four highly polymorphic STR-markers are used for the chromosomes 13, 18, 21, three for XY.The PCR products are analysed on the ABI 310. From 07.99 to 11.00 we carried out 800 tests in our laboratory. These 800 results were confirmed by the classical cytogenetic karyotypig. We detected 11 trisomies 21, 1 trisomy 13 and 2 trisomies 18, which were also confirmed by routine cytogenetics. In our laboratory the multiplex-f-PCR method is replacing the FISH technique more and more. In comparison to FISH the multiplex-f-PCR method is less expensive, less timeresuming and more sensitive. In addition it is more comfortable for our patients to get a result for the most common aneuploidies that fast.

P1033. Cost-efficient organisation of external quality assessments in molecular diagnostic laboratories

***E. Dequeker**, J. J. Cassiman*
Center for Human Genetics, KULeuven; Leuven, Belgium
els.dequeker@med.kuleuven.ac.be

External quality assessments (EQAs) have been organized for several genetic diseases, and demonstrated the importance of continued efforts to improve quality in molecular diagnostic laboratories, including regular participation in EQA. It can thus be expected that more EQA schemes will (need to) be organised in the future, also because the number of diseases which can be molecularly diagnosed is increasing rapidly. The objective of the present study was to evaluate the costs of organising an EQA. This analysis was based on the 5 years experience with European EQAs for cystic fibrosis (1996-2000; see also Nature Genet 2000, 25;259-260). The total cost was separated in personnel costs and material costs (laboratory reagents, administration, travel). A further separation was made between fixed costs (independent of participants number) and variable costs (cost per participant in addition to fixed cost). The results of the analysis show that the fixed personnel time required for the EQA organisation is high compared to the additional personnel time required per participant (the latter is less than 1% of the fixed time). Similarly, the variable material cost per participant is low (less than 0.5%) compared to the fixed material cost for the organisation of an EQA. Given the relative high fixed costs for the organisation of an EQA, we conclude that a high number of participants (e.g., >100) is required in order to enable cost-efficient organisations of EQAs. Furthermore, one might hypothesize that the organisation of EQA which cover several diseases simultaneously could further optimize this cost-efficiency.

Also presented orally in Concurrent Session 15: Poster Discussion Genetic Testing and Screening

P1034. Nosology Of Genetic Disorders.

***Y. Lacassie**, I. LaMotta, L. Florez*
LSU Health Sciences Center and Children s Hospital; New Orleans, LA United States
ylacas@lsuhsc.edu

In this time of extraordinary advances in molecular genetics, the clinical classifications and codifications of genetic disorders are still heterogeneous and confusing. In 1991 (8th ICHG meeting, Washington), the main author proposed the use of a multiaxial diagnostic system (MADS) in clinical genetics. For simplicity, only four major categorical axes (I-Phenotypic; II-Pathogenetic; III-Etiologic, and IV-Differential Diagnoses) were defined. The major advantages of such a system include; improved diagnostic accuracy; greater homogeneity of the diagnostic categories, enhancing the comparison of diagnostic achievements among different centers, regions or countries; more specific definitions for controversial terms; user friendliness, as it organizes the usual diagnostic information in a systematic and logical way and does not require further skills; ease of modification and improvement to keep it consistent with advances. We have also shown that the system is an excellent aid in teaching clinical genetics (Lacassie, Y. An International Multiaxial Diagnostic System in Clinical Genetics. In; Dysmorphology and Genetics of Cardiovascular Disorders. Zerbinis, Athens, 1994). Although it was not an original goal, the MADS also can be used to improve the current International Classification of Diseases (ICD-9). We recently reviewed the diagnoses on 4352 patients, representing 88% of the patients seen in our division between November 1986 and June 2000. We will discuss this experience regarding the classification and distribution of genetic diseases and birth defects due to environmental causes, advocating the international use of this system.

P1035. Quality control in a reference thalassemia center in Iran

***E. Keyhany**, M. Neishabury, A. Hatami, H. Najmabadi*
University of Welfare and Rehabilitation; Tehran, Islamic Republic of Iran
maryamneishabury@yahoo.co.uk

The necessity of quality control (QC) is not obscure especially for the reference laboratories that are the centers of refered doubtfull or broderline results. In our center, which is one of the two national referral centers for thalassemia (a&b) and the only reference lab, where routine hematological investigations are achieved in association with molecular diagnostics for prenatal diagnosis and carrier detection of haemoglobin disorders, QC has a great importance. QC in our laboratories is achieved by the presence of instrument booklets, laboratory manuals containing standard methods, use of high quality materials and kits, use of external and internal control material for hematology, biochemistry and genetics tests, control material for calibrating the instruments, regular analysis of instrument performances, the use of all available techniques in parallel if necessary and sequencing as the gold standard and daily, weekly & monthly QC data analysis. These have helped us to minimize the lab errors. Since the routine hematology procedures such as complete blood count (including RBC indices), Hb electrophoresis & HbA2 measurements are achieved for screening of thalassemia correct results sometimes prevent unnecessary expensive, time consuming & specific molecular diagnostic procedures. When the hematological investigation proved thalassemia then specific molecular techniques run to detect mutations, known and unknown. In our highly quality controlled environment less than five percent lab errors has been observed during the last six months.

P1036. Down syndrome screening protocol in Czech Republic

D. Stejskal
GENNET; Praha, Czech Republic
david.stejskal@gennet.cz

Antenatal Down syndrome screening protocol incorporating ultrasound nuchal translucency (NT) into established second trimester screening is proposed. Accredited centres measuring NT are equipped with screening software Obstetrician s Screening Guide — OSG with following features. Æ OSG calculates NT value in multiple of medians (MoM). Æ Assesses optimal terms for further antenatal screening procedures. Æ Prints screening reports and request forms for second trimester screening. Æ Evaluates ultrasound biometry parameters and minor Down syndrome ultrasound markers in the second trimester. Æ Monitors NT statistical parameters. Second trimester screening centres are equipped with another piece of software Integrator . After entering NT values and second trimester Down syndrome risk, Integrator prints an appendix to second trimester screening report with risk adjusted by NT. Proposed screening protocol can decrease false positive rate of established second trimester screening. It can be used at full combined test in the first trimester or integrated test using results from both first and second trimesters in the future.

P1037. Comparison of Biochemical and Molecular Testing for Tay-Sachs Disease

***T. L. Stockley**, J. Callahan, J. T. R. Clarke, P. N. Ray*
The Hospital for Sick Children; Toronto, ON Canada
tracy.stockley@sickkids.on.ca

Tay-Sachs disease is an inherited lysosomal storage disorder caused by deficiency of the enzyme hexosaminidase A and is most prevalent in the Ashkenazi Jewish population (carrier rate 1/30). Carrier screening for Tay-Sachs is available by biochemical analysis of hexosaminidase activity in blood. However, the biochemical assay is complicated by several difficulties, such as inaccurate results for women who are pregnant or using birth control pills. As well, individuals who carry non-pathogenic pseudodeficiency mutations are reported as Tay-Sachs carriers by use of the biochemical assay. Due to these difficulties, molecular analysis of mutations in the HEXA gene is an important compliment to biochemical screening in order to resolve these issues. We are conducting a large-scale study to compare the effectiveness of biochemical and molecular testing for Tay-Sachs disease. Individuals of Ashkenazi Jewish descent are tested by both standard biochemical testing and molecular testing for three common pathogenic mutations [1278insTATC, IVS12(+1)G>C and 805G>A (G269S)] and two pseudodeficiency alleles [739C>T (R247W) and 745C>T (R249W)]. In order to feasibly test this number of individuals, we have developed a rapid molecular diagnostic assay using allele specific amplification methodology. To date we have tested >1000 individuals by both the biochemical and molecular assay. In the carrier range by biochemical analysis (0 to 49.9% HexA), 80% of individuals have one of the five mutations identified by the molecular assay, while only 8.5% of individuals in the inconclusive range by biochemical analysis (50 to 55% HexA) have identified mutations. Sequencing of the hexosaminidase gene in individuals who are carriers by biochemical analysis but who do not have one of the five common mutations will be necessary to determine if these individuals are carriers of rare hexosaminidase A mutations. In the biochemical non-carrier range, (>55% HexA), 1 individual was found to be a carrier of the pathogenic 1278insTATC mutation, and 1 individual who identified as Ashkenazi Jewish was found to be a carrier of the pseudodeficiency mutation R247W. The identification of individuals in the non-carrier range by biochemical analysis who have pathogenic mutations suggests that molecular analysis may be a necessary adjunct to current biochemical testing programs. The results and implications of the study to date will be presented.

P1038. A simple, fast, low-cost screening method for the detection of (GAA)n repeat expansions in Friedreich s Ataxia.

***A. Georghiou**, M. Tsingis, K. Christodoulou*
The Cyprus Institute of Neurology and Genetics; Nicosia, Cyprus
roula@mdrtc.cing.ac.cy

Friedreich s ataxia (FRDA) is the most common of the autosomal recessive spinocerebellar ataxias with an estimated frequency of 1 in 50000 in the European populations. More than 95% of FRDA cases are caused by large homozygous expansions of a (GAA)n triplet repeat in the first intron of the frataxin gene. Expanded (GAA)n repeats associated with the FRDA phenotype may be detected by Southern blot analysis or PCR-based analysis. Southern blot analysis is reliable but requires large amounts of DNA and is a time consuming procedure. Long range PCR is not reliable for the detection of expansion carriers since preferential amplification of the normal (GAA)n repeat allele has been observed. Detection of large (GAA)n repeat expansions by fluorescent triplet repeat primed PCR (TP-PCR) has recently been introduced. We hereby present a modified TP-PCR method for launching a carrier population-screening program for FRDA in the Paphos district of Cyprus. The TP-PCR product of expanded (GAA)n repeat alleles gives a characteristic trace (smearing) on an agarose gel thus enabling the rapid identification of large pathogenic GAA repeats. In conclusion, we present a reliable, simple, fast, and inexpensive method for the detection of FRDA (GAA)n repeat expansions.

P1039. Predictive Genetic Testing Of Some Common Multifactorial Diseases. Where We Are Now And What We Expect From It.

V. Baranov
Institute of Obstetrics & Gynecology; St.Petersburg, Russian Federation
baranov@vb2475.spb.edu

Overview of the present achievements , problems and obvious pitfalls in quickly expanded area of predictive medicine. On the ground of genetic nets of some common multifactorial disorders original data dealing with predictive testing of endometriosis (E), osteoporosis (O), bronchial asthma(BA) are presented. Genetic testing of the functionally inferior alleles of detoxification (environmental) genes such as glutathione -S-transferases M1 (GSTM1) and N-acethytransferase-2 (NAT 2) was found to be very informative for elaboration of efficient treatment strategy for E; genetic testing of GSTM1 and GSTT1 polymorphisms turned to be very useful in prediction of inherited predisposition to BA, while testing of three major genes, participating in osteogenesis (VDR-3, COL1A1 & CALCR) gives a chance to reveal individuals prone to O. Overepresantation of some detoxification system alleles in the patients with chronic bronchitis, among pregnant women with repeated miscarriages as well as in cystic fibrosis patients with sever lung complications is shown. Exponential growth of genetic tests, their wide and frequently non critical application opens highly risky avenue for their misuse and misinterpretation. Some already existing regulations in gene testing policy in Europe and its position in the present Russia are briefly outlined.

P1040. Analysis Of Environmental Genes Polymorphisms As Predictive Genetic Test For The Treatment Efficiency Response In Endometriosis Patients

***T. Ivaschenko**, N. Shwed, H. Kramerova, V. Baranov*
Institute of Obstetrics &Gynecology; St.Petersburg, Russian Federation
TIV@ti2629.spb.edu

The polymorphisms of five genes (CYP1A1, mEPHX1, CYP2E1, NAT2, GSTM1) responsible for xenobiotic conjugating enzymes of Phase I and Phase II detoxification system were studied by PCR-RFLP in the blood spots from 68 patients with endometriosis subjected to complex operative and conservative treatment. 23 patients showed clinical improvements after postoperative immunomodulator therapy (Group I) and 45 patients were resistant to the treatment (Group II). We haven t found significant difference for the polymorphism of CYP1A1, mEPHX1 genes in this two groups. The frequency of slow allele for CYP2E1gene was significant higher in Group I (14,3% versus 4,4% in Group II).The analysis of the polymorphism enzymes of Phase II detoxification system have shown that the frequency of GSTM1 gene deletion was significant higher for Group II (76,6% versus 17,3% in Group I; p=0.001). Significant difference between two groups of patients could be mainly attributed to increased frequency of S1 allele (47,8% versus 28,6%; p=0.05) and decreased proportion of F allele (25% versus 38,8%; p=0.05) for NAT 2 gene in Group II. Concordance of both GSTM1 0/0 and S/S homozygotes was recorded in 46,7% of genotypes in the Group II patients and wasn t found in Group I patients. All these results prove clearcut preponderance of GSTM1-0 homozygotes and slow alleles of NAT2 gene in endometriosis patients resistant to immunomodulator therapy. Molecular analysis of GSTM1 and NAT2 gene polymorphisms might be suggested as a feasible predictive test for the choice of adequate treatment strategy in E. patients.

P1041. Association Of Three Detoxificaton Genes Polymorphism With Atopic Asthma In Adult Patients From St.petersburg.

***O. G. Sideleva**[1], T. E. Ivaschenko[1], T. E. Gembitskaya[2], V. S. Baranov[1]*
[1]Ott s Inst Obstet. & Gynecol.; Saint-Petersburg, Russian Federation; [2]Inst. of Pulmonology; St. Petersburg, Russian Federation
olgasid@yahoo.com

The genetic polymorphisms of glutathione-S-transferase M1 (GSTM1), glutathione-S-transferase T1 (GSTT1) and cytochrome P4501A1 genes responsible for xenobiotic conjugating enzymes of Phase II and Phase I detoxification system were studied by PCR-RFLP in 49 adult patients with atopic asthma sensitive to the house dust. The control group consisted of 66 individuals of Russian origin, living in St. Petersburg. We haven t found significant difference for Ile—Val polymorphism of CYP1A1 gene. The analysis of the genes, coding Phase II detoxification system enzymes, have shown that the frequency of GSTM1 gene deletion was significantly higher for asthmatic patients (75,5% versus 41,0% in control; p=0.001). Individuals with absence of the GSTM1 gene were at approximately 4,5—fold higher risk of developing asthma (OR=4,45; 95%CI=2,01-9,83). Proportion of glutathione-S-transferase theta null genotypes (GSTT10/0) appeared to be significantly increased in the group of asthmatics (77,6%) compared to controls (23,5%), with an OR of 11,7 (95%CI=5,13-26,88). Concordance of both GSTM1 0/0 and GSTT10/0 homozygotes was recorded in 61,2% of genotypes in the group of asthmatic patients and only in 9,1% of the control group .The comparable OR in the presence of both null genotypes was 15,8 (95%CI=6,37-39,12). Thus, strong association of atopic asthma with null alleles of GSTM1 and GSTT1 genes was proved in the present study. Molecular analysis of GSTM1 and GSTT1 genes polymorphism might be useful for presymptomatic prediction of atopic asthma.

P1042. Evaluation of the predictive value of the diagnostic criteria for the Marfan syndrome (MFS) with respect to the outcome of fibrillin-1 mutation analysis.

***A. De Paepe**, B. Loeys, I. Delvaux, S. De Bie, L. Nuytinck*
University Hospital Ghent; Gent, Belgium
anne.depaepe@rug.ac.be

We have performed a clinical and molecular study in 200 consecutive patients referred with a diagnosis or suspicion of MFS. Careful clinical evaluation against the Gent Nosology showed that 108 patients fulfilled the diagnosis of MFS,whereas 92 patients were diagnosed with a Marfan-related condition. An FBN1 mutation was found in 69 patients with the clinical diagnosis of MFS (68 %). In several MFS families in which no mutation could be identified the disease segregated with the FBN1 locus. The only significant difference observed in MFS patients with and without a mutation was the incidence of ectopia lentis ($p < 0.04$). No correlation was found between the type and location of the FBN1 mutation and the severity of the phenotype. Ten mutations were found in the patients not fulfilling the diagnosis of MFS; 2 patients with predominant ectopia lentis, 1 with mitral valve prolapse syndrome, 1 with isolated aortic aneurysm and 6 in a group of young children with emerging MFS, which were too young to fulfill the diagnostic criteria. Our results show a significant difference in the number of FBN1 mutations in patients fulfilling versus not fulfilling the diagnostic criteria for MFS. The findings underscore the need for careful clinical follow-up over time, especially in young children at risk, before excluding the diagnosis of MFS. Further molecular studies by gene sequencing and linkage analysis in a selected group of MFS patients without an FBN1 mutation are currently ungoing in order to resolve the question about locus heterogeneity in MFS.

P1043. A New Method For Identification Of Osteoporotic Risk By VDR-Genotyping

***G. K hne**, A. A. Krehan, E. Schnakenberg, C. Tamak, S. Wasch tza, L. Wehmeier*
AdnaGen AG; Hannover-Langenhagen, Germany
lw@adnagen.com

Genetic factors have been identified to account for up to 80% of bone mineral density variance in young adults. It was reported that polymorphism in the vitamin-D receptor (VDR) gene is associated with the disposition for the development of osteoporosis. So far, several mutations have been identified within the VDR gene. One mutation detectable by restriction fragment polymorphism (Bsm I) in intron 8 have been described to be associated with bone mineral density. With the help of genotyping methods it is possible to identify the predisposition of osteoporotic risk factors rapidly. In this investigation we report about a rapid method for the detection of vitamin-D receptor mutation in intron 8 based on real-time PCR technology. We have developed a simple protocol specific for this mutation which make it possible to differentiate between bb, Bb or BB (homozygous mutation) genotypes. While the wild-type corresponds to high bone mineral density the mutated allele could be associated with low bone mineral density. This procedure enables us to identify osteoporotic risk persons at an early stage. In addition, this test system is a genetic diagnostic service of our company.

P1044. How much should research subjects charge for their DNA?

J. C. Bear
Memorial University of Newfoundland; St John s, NF Canada
bearre@mun.ca

The mapping of loci conferring liability to single gene disorders and common conditions of complex inheritance is now done primarily for commercial reasons. The participation of a research subject involves only provision of a blood sample and health and pedigree data; physical risk and inconvenience to subjects are negligible. For these reasons, conventional models of compensation for research participation are not appropriate. Participation cannot be expected to be altruistic, given the commercial basis of the research. Nor is it appropriate to limit compensation to reimbursement for the costs the subject incurs by participating (such as lost income and travel expenses), or to limit compensation to a (low) notional wage paid for the time and effort of participation. Genome research companies sometimes provide population-level inducements to relatively small and genetically isolated populations (for instance health care facilities or services), or offer future treatment at no cost to families in which single-gene disorders occur. These compensation approaches cannot readily be generalized to studies in large populations, or to studies of common disorders of complex inheritance. An appropriate alternative compensation model treats individuals and families DNA as a resource in their possession, which a genome research company may wish to extract. Compensation can be based on potential or actual profits from exploitation of the resource, that is, profits from the sale of therapies developed from the knowledge the genomic study provides. Compensation would appropriately take the form of a lump-sum payment for access to a participant s DNA, royalties, or some combination. These alternatives would remit an appropriate portion of profits to a research subject. Such a compensation regime would result in payment to research subjects considerably larger than those commonly made now. Sample calculations which allow for the probability of success of individual research studies, and an equal division of profit between the company and research subjects, suggest compensation for DNA access of at least $50,000 per research subject, royalties in the order of 0.005% of annual profit from sales of the product, or a combination of these options.

Also presented orally in Concurrent Session 15: Poster Discussion Genetic Testing and Screening

P1045. High-throughput mutation screening by capillary array electrophoresis; Application to molecular diagnosis of hypertrophic cardiomyopathy

***L. A. Larsen**[1], M. Christiansen[1], J. Vuust[1], M. Johnson[2], C. Brown[2], C. Wike[2], H. Moolman-Smook[3], P. Skytt Andersen[1]*
[1]Statens Serum Institut; Copenhagen, Denmark; [2]Applied Biosystems; Foster City, CA United States; [3]US/MRC Centre for Molecular and Cellular Biology; Tygerberg, South Africa
lal@ssi.dk

The human genome project has resulted in the isolation of several thousand genes involved in human disease. These results raise new possibilities for precise molecular diagnosis of genetic disorders and hence for genetic counselling and therapeutic intervention. Consequently, there is an increasing demand for high-throughput mutation detection methods. Here, we present a method for large-scale mutation screening based on automated single strand conformation polymorphism (SSCP) analysis by capillary array electrophoresis with multicolour fluorescence detection. The method was developed and tested using a commercially available 16-capillary array system (ABI3100). This setup allows for unattended screening of 576 samples at three temperatures in less than 24 hours. Each genotype results in a distinct, highly reproducible conformation pattern, which allows for automated scoring of known allelic variants using dedicated software. Re-analysis of known mutations in the MYH7 gene associated with hypertrophic cardiomyopathy showed that screening at three different temperatures (18¡C, 25¡C, and 35¡C) was necessary for 100% sensitivity. We used the method to screen 130 South African probands with hypertrophic cardiomyopathy for mutations in the TNNI3 gene and found one novel R186Q missense mutation and nine novel single nucleotide polymorphisms

P1046. Experiences of cystic fibrosis carrier couples identified before the birth of an affected child

***L. Henneman**[1], L. Kooij[2], L. P. Ten Kate[1]*
[1]Vrije Universiteit Medical Centre; Amsterdam, The Netherlands; [2],; Groningen, The Netherlands
l.henneman.humgen@med.vu.nl

This qualitative study describes the experiences of cystic fibrosis (CF) carrier couples prospectively identified in CF families, in order to explore their experiences with genetic testing and counselling and the impact on reproductive behaviour and family relations. Of 12 couples identified until 1997, 7 couples participated in semi-structured interviews and 2 couples filled out questionnaires, 2-6 years after receipt of test-results. After receiving the results, most couples reported that they were shocked, because they did not expect to be both carriers. More anxiety was expressed by those who were pregnant (n=4) at the time of testing. Some subjects reported difficulties in disclosing the results to family members. The reactions of family members were not always supportive. After testing, all viable pregnancies (16 in 8 couples) were monitored by prenatal diagnosis, and all those affected were terminated (6 in 4 couples). Some couples perceived loss of reproductive expectations. For couples having children after testing, concerns about the correctness of results of prenatal diagnosis during infancy and how to inform the children may emerge. Couples generally reported a positive counselling experience, although dissatisfaction was reported on the psychological guidance during pregnancy. Most couples did not regret testing. In general, couples supported the idea of screening, although various concerns were expressed. The results suggest a favour for testing outside pregnancy. The findings may be useful to explore possible dilemmas raised by the introduction of population screening. Observations reported here also might apply to other recessively inherited disorders.

P1047. Cftr Mutation Scanning By Denaturing Hplc

V. M. Howell, *A. Proos, F. Beach, C. Jensen, G. Watts, L. Burnett*
Royal North Shore Hospital; St Leonards, Australia
vhowell@med.usyd.edu.au

Testing for cystic fibrosis mutations is a cumbersome process. Over 900 mutations have been detected, but our testing is currently restricted to the 25 most common mutations in the European population. We have evaluated a reverse-phase ion-paired denaturing HPLC system for the detection of mutations in the CFTR gene. Following amplification, fragments are denatured and slowly re-annealed to ensure the formation of heteroduplex and homoduplex species if a mutation is present. The renatured products are injected into the HPLC in the presence of an ion-pairing reagent. This allows the DNA to bind to the polymeric stationary phase. The negatively charged DNA is eluted against an increasing gradient of acetonitrile and is detected by a change in the absorbance at 260nm. Under partially denaturing conditions (created by increasing the temperature of the column), the less stable heteroduplexes elute from the column earlier than the more stable homoduplexes. When only 1 species is present in a fragment, the chromatogram will show only 1 peak. A heterozygous sample will show up to 4 peaks, representing the 2 heteroduplex and 2 homoduplex populations present. We evaluated this system by screening 6 CFTR exons containing 17 known mutations. The mutations consisted of substitutions, 1, 2 and 3 bp deletions, and a 2 bp insertion. Following optimisation, all 17 mutations were detected. We found denaturing HPLC to be a rapid, economical and highly sensitive technique for mutation scanning and plan to extend our CFTR testing to include mutation scanning.

P1048. Mutational spectrum of LDLR gene mutations underlying familial hypercholesterolaemia in South African Jews

M. Callis[1], *F. J. Raal*[2], *S. Jansen*[1], *R. Thiart*[3], *H. Nissen*[4], *G. Pilcher*[5], *M. J. Kotze*[3]
[1]*University of the Free State; Bloemfontein, South Africa;* [2]*Department of Medicine, University of Witwatersrand; Johannesburg, South Africa;* [3]*University of Stellenbosch; Stellenbosch, South Africa;* [4]*Odense University Hospital; Odense, Denmark;* [5]*University of Witwatersrand; Johannesburg, South Africa*
gnmgmc@med.uovs.ac.za

Objective. A cohort of 21 unrelated index patients with familial hypercholesterolaemia (FH) were screened for mutations in the low density lipoprotein receptor (LDLR) gene, in order to define the spectrum of mutations underlying the FH phenotype in the South African Jewish population. Methods. Multiplex polymerase chain reaction (PCR) amplification for detection of the prevalent South African founder mutations was applied, followed by heteroduplex-single strand conformation polymorphism (HEX-SSCP) analysis and/or denaturing gradient gel electrophoresis (DGGE) in mutation negative samples to identify LDLR gene defects in South African Jews with clinical signs of FH. Results. The FH Lithuania mutation (652delGGT) reported in the majority of South African Jews with FH, was detected in 57% (12/21) of the index cases studied. Seven of the remaining nine index patients were subjected to extensive mutation analysis of the LDLR gene and mutations Q64X, Q104W, D206E, IVS9+1G?A, N407K, V408M and IVS14+5G?A were identified. In all samples extensively examined, mutations were detected. Mutation N407K is associated with a mild FH phenotype. Conclusions. Although the high frequency of 80% previously reported for mutation 652delGGT in the South African Jewish population may be an overestimation, predominance of this deletion-mutation (75%) was confirmed in FH patients of Lithuanian origin, and a high sensitivity of extensive combined mutation screening strategies demonstrated.

P1049. Evaluation of the angiotensin-converting enzyme gene as a modifier locus for familial hypercholesterolaemia in the genetically homogeneous Afrikaner population

M. J. Kotze[1], *M. Callis*[2], *S. Jansen*[2], *C. L. Scholtz*[1]
[1]*University of Stellenbosch; Stellenbosch, South Africa;* [2]*University of the Free State; Bloemfontein, South Africa*
gnmgmc@med.uovs.ac.za

Objective. Previous studies have demonstrated that the deletion/deletion (DD) genotype of the angiotensin-converting enzyme (ACE) gene may increase the risk of myocardial infarction and coronary heart disease (CHD) in patients with familial hypercholesterolaemia (FH). We determined the frequency of this polymorphism in South African Afrikaners with FH and population-matched controls, in an attempt to determine the appropriateness of multilocus genotyping in CHD risk assessment. Methods. The genotype distribution of the ACE polymorphism was compared in 205 FH heterozygotes, 45 FH homozygotes and 215 controls (including 95 elderly subjects) of the same population. All the FH patients were heterozygous or homozygous for one of the founder-related low-density lipoprotein receptor (LDLR) gene mutations, D154N, D206E or V408M. Results. The ACE DD genotype was detected at a significantly lower frequency in the FH heterozygotes (P<0.004) compared with younger controls. No significant differences were observed in FH homozygotes (P<0.175) (when compared with the younger control group) and between FH patients with and without CHD. Allelic distribution differed significantly between the two control groups (P<0.031), with the I-allele predominantly in the elderly. Conclusions. Comparative analysis of molecularly-characterised FH patients and population-matched controls may represent a useful study approach to reveal clinically relevant allelic differences. The potential for different degrees of linkage disequilibrium of the ACE insertion/deletion (I/D) polymorphism with other potentially significant sequence changes in the gene and the modest degree of increased risk for CHD associated with the ACE DD genotype, argues against the likelihood of this marker being a clinically useful indicator of increased risk for CHD in Afrikaner FH patients.

P1050. Effective and time saving detection of mutations in BRCA1 exon 11 using Restriction Endonuclease Fingerprint-Single-Strand Conformation Polymorphism (REF-SSCP) with automated capillary electrophoresis in an ABI 310.

P. Kringen[1], *H. Eiken*[2], *J. Pedersen*[1], *K. Berg*[1], *S. Egedal*[3], *K. Tveit*[4], *T. Ikdahl Andersen*[4]
[1]*Department of Medical Genetics, Ullev l University Hospital; Oslo, Norway;* [2]*Medinnova, National Hospital; Oslo, Norway;* [3]*Department of Medical Genetics,Ullev l University Hospital; Oslo, Norway;* [4]*Department of Oncology, Ullev l University Hospital; Oslo, Norway*
pedro.kringen@ioks.uio.no

A large proportion of women in in the industrial countries are affected by breast cancer. Aproximately 5-10% of these cases may be the result of inherited cancer predispositions by mutations in the highly penetrant dominant breast cancer susceptibility gene BRCA1. More than a thousand sequence alterations including mutations and polymorphisms are scattered throughout this large gene and makes screening for mutations a complicated task. In the present study 75 patients with hereditary breast/ovarian cancer was screened for mutations using restriction endonuclease fingerprint-single-strand confarmation polymorphism (REF-SSCP) with automated capillary electrophoresis in an ABI 310. The strategy is based on digesting four fluorescent labelled PCR-products covering BRCA1 exon 11 with suitable restrictions enzymes. Each digest was analysed subjected to nondenaturing conditions and all samples showing bandshifts were sequenced to pinpoint the nature of the mutation. A total of 13 different DNA variants were detected by capillary REF-SSCP, two of which gave frameshifts and one missense mutation never previously reported in the BIC database. We conclude that REF-SSCP by automated capillary electrophoresis is a rapid, efficient and reliable method to screen for BRCA1-mutations.

P1051. National genetic register for Huntington s disease in Bashkortostan Republic of Russia

I. A. Kutuyev[1], *R. V. Magzhanov*[1], *E. K. Khusnutdinova*[2]
[1]*Bashkir Medical University; Ufa, Russian Federation;* [2]*Institute of Biochemistry and Genetics; Ufa, Russian Federation*
ildus@ufacom.ru

For helping in the efficient genetic counselling to affected subjects and relatives, and to monitor changes in the population at risk a genetic register (GR) for Huntington s disease (HD) was created in 1998 for the Interregional Medical Genetic Center. GR is based on card index principles and includes identifying information of subjects, anamnesis, clinical and genealogical data, results of molecular genetic testing. Patient s data is refreshed on every visit to Genetic Center. GR consists of for main parts; patients data, reports, data export, and services. Patients data includes forms on patient s information, patients searching form, and family forms with genealogy tree. Reports contain 4 forms; 1) subjects selection by living/birthplace place, 2) molecular genetic testing results for all patients, 3) all epidemiological data for the selected regions and in a whole 4) separate patient s report. All reports can be viewed or printed. Services include letter printing to patients and relatives and tools for connecting with the same remote databases in other regions for data updating. GR allows rapid check on the risk status of family members, make up lists of needing visits subjects and provide assessment of trends in migrations, prevalence, birth rate, and other variables. In a whole GR can allow providing more effective control over the HD patients and family members in the region. At present register includes information about 164 patients (111 alive) and 172 risk group family members. 62 patients and 167 unaffected persons with high risk were tested by molecular genetics methods.

P1052. Incorporating biparietal diameter measurements into risk calculations for open neural tube defects - potential impact in publicly-funded care

C. Meier[1], *A. M. S. Summers*[2], *P. R. Wyatt*[2]
[1]*Ontario Maternal Serum Screening Database; Toronto, ON Canada;* [2]*North York General Hospital; Toronto, ON Canada*
cmeier@nygh.on.ca

Women in Ontario are routinely offered second trimester maternal serum screening (MSS) during their pregnancies. MSS provides pregnancy-specific, chemistry-adjusted risks for trisomy 21 (Down syndrome), trisomy 18 (Edward syndrome) and open neural tube defects (ONTDs). About 65,000 patients are seen per annum. The risk assigned for ONTDs is based on the background prevalence, gestational age and maternal serum alpha fetoprotein (MSAFP) MoM value. This risk is also adjusted for other factors (race, multiple gestation, insulin-dependent diabetes). Gestational age is based on LMP or ultrasound dating. The calculated (posterior) risk for ONTDs has historically been based on the combined prevalence of both anencephaly and spina bifida (in Ontario, both prevalences are approximately 0.5 per 1,000). By excluding the risk of anencephaly by the use of software modifications, a more accurate risk can be presented to patients. The purpose of this study was to model the impact of such a risk adjustment, using the Ontario Maternal Serum Screening Database. Of the 254,833 women screened in a five-year interval from November 1st 1995 to October 31st 2000, 114,760, 57% received some kind of dating ultrasound in their pregancy. 40% of these dating ultrasounds were based on BPD which was recorded on their initial MSS form. Up to 75% of all pregnancies dated by ultrasound were late enough (10w6d) to warrant using BPD. Of the 57,350 dated by BPD, 2.5% or 1,412 had a positive screen result. If the risk for NTDs for these women was modified to exclude anencephaly, only 756, or 1.3% would have screened positive. If ultrasonographers had been encouraged to use BPD wherever possible and had done so, 1,279 fewer screen positives would have been seen, and the overall positive rate for NTDs would have dropped from 2.5% to 1.6%, while affording pregnant women more accurate risk figures and a similar detection rate for NTDs. Given the expense of and anxiety generated by false positives, the adoption of BPD dating and additional markers and their incorporation into screening algorithms is recommended, particularly in systems supported by public taxation.

P1053. Integration of Genetic Testing Services into Practice in the United States

I. M. Lubin, *B. Chen, A. Faucett, R. S. Whalen, J. Boone, R. Martin*
Centers for Disease Control and Prevention; Atlanta, GA United States
ilubin@cdc.gov

The human genome project and technological advances in the biomedical field have resulted in the discovery of a multitude of genes and the means to identify mutations predictive and causative of disease. In the United States, these advances have led to concerns regarding the accuracy, availability, use of, and access to genetic testing by the Public. To address these issues, the Task Force on Genetic Testing, later superceded by the Secretary s Advisory Committee on Genetic Testing were established. These groups, working together with professional organizations and the private sector, formulated recommendations toward ensuring the safety and effectiveness of genetic testing. The recommendations advocate the availability of appropriate education for the Public and health care providers, assurances for appropriate informed consent, protection against genetic discrimination, additional safeguards to ensure quality in laboratory practices, and the continued involvement of the Public in the decision making process. Accordingly, the Centers for Disease Control and Prevention, the Health Care Financing Administration, and the Food and Drug Administration have taken on the responsibility to evaluate and formulate policies and regulations that address issues surrounding the use of genetic laboratory services. A process is being developed to ensure that genetic tests introduced into clinical and Public health practices are analytically and clinically valid and have clinical utility. Also, recommendations, policies, and regulations are being developed to ensure laboratories offering genetic tests are appropriately credentialed. These efforts are being undertaken to ensure public access to quality genetic tests and allow the continued development of new genetic tests.

P1054. Screening Of Hemochromatosis; Results Of A Large Survey On 8663 Persons In Brittany.

A. Jouanolle[1], *Y. Deugnier*[2], *CES St-Brieuc*[3], *CES Rennes*[4], *CES St-Nazaire*[5], *J. Y. Le Gall*[6], *V. David*[6]
[1]*CHU Pontchaillou; Rennes, France;* [2]*Clinique des Maladies du Foie et Inserm U522; Rennes, France;* [3]*CNAM; St-Brieuc, France;* [4]*CNAM; Rennes, France;* [5]*CNAM; St-Nazaire, France;* [6]*UMR 6061 CNRS; Rennes, France*
anne-marie.jouanolle@chu-rennes.fr

Hemochromatosis is a common autosomal recessive disorder of iron metabolism in Caucasiens and its systematic population screening may be advocated. In Northern Europe, most cases of hemochromatosis are related to homozygosity for the C282Y mutation of the HFE gene. Before establishing a policy for the screening of hemochromatosis it is mandatory (i) to assess the penetrance of the C282Y homozygosity (ii) to choose the strategy of screening ; phenotypic parameters (=iron indexes) or genetic testing (=C282Y mutation). Subjects and methods. 8663 persons attending 3 Health centres in Brittany were enrolled in a large survey; 3132 men (25 to 40y) and 5531 women (35 to 50y) were tested for the C282Y mutation. Then fasting serum tests (serum iron, transferrin saturation and ferritin) were performed in the C282Y homozygotes. Results. In total, 54 homozygotes (10 men, 44 women) were identified giving a prevalence of 0.62% higher than that predicted (0.52%). Observed prevalence was 0.32% in men and 0.8% in women. All men had a transferrin saturation greater than 50%, 7/10 a ferritin greater than 237 moles/l, and 8/10 were symptomatic (fatigue, arthralgias or increased ALT). Only 50% of the women had a transferrin saturation greater than 45%, 40% a ferritin greater than 104 moles/l and 34% were symptomatic (fatigue and/or arthralgias). Conclusion. In men younger than 40y, biochemical penetrance of C282Y homozygosity is complete and phenotypic identification of homozygous men could be based on a transferrin saturation threshold of 40%. In women, penetrance is incomplete and not relevant to age or genital status, but transferrin saturation is unable to predict C282Y homozygosity.

Also presented orally in Concurrent Session 15: Poster Discussion Genetic Testing and Screening

P1055. Mutation Screening of the PKD1 gene in 55 Families of Hellenic Origin

C. Deltas[1], *I. Bouba*[2], *M. Koptides*[3], *A. Pierides*[4], *K. Siamopoulos*[5]
[1]*The Cyprus Institute of Neurology and Genetics; Nicosia, Cyprus;* [2]*The Cyprus Ins. of Neurology and Genetics; Nicosia, Cyprus;* [3]*The Cyprus Inst. of Neurology and Genetics; Nicosia, Cyprus;* [4]*Nicosia General Hospital, Dept. of Nephrology; Nicosia, Cyprus;* [5]*Univ. of Ioannina; Ioannina, Greece*
Deltasco@mdrtc.cing.ac.cy

Mutations in the PKD1 gene on chromosome 16p13.3 cause the most common form of autosomal dominant polycystic kidney disease (ADPKD). Patients from 55 Hellenic families from Greece and Cyprus were investigated in our laboratory. The unique part and part of the reiterated region of the PKD1 gene were screened for mutations by SSCP analysis. The part of the duplicated region under investigation included exons 16-22 and 23-33. These were obtained by Long Range-PCR utilizing unique PKD1 primers. Three nonsense mutations were identified in exons 40, 44 and 45 (W3794X, R4020X, C4986X). Nonsense mutations were not found in the PKD1 duplicated region, however, deletions and missense changes were identified. In three families deletions of 3bp in exons 20 and 24 were observed, which resulted in an aminoacid deletion without influencing the reading frame (7946del3, 8047del3, and 9142 or 9143del3). A 14bp deletion was observed in exon 16 (7177del14) and a 8bp deletion was observed in exon 21 (8183del8). The 7946del3 and 8183del8 mutations occurred on the same chromosome. Five missense mutations were observed (P2471L, Q2519L, T2649I, H2921P, V3375M) in exons 18, 19, 21, 23 and 31 respectively. However, we only have clear evidence that H2921P is pathogenic. The other missense mutations occurred in single ADPKD families and were co-segragating with the disease phenotype. Also, 18 polymorphisms were observed, either in exons or introns. The majority of them did not affect the aminoacid sequence, but six of them resulted in a substitution by biochemically similar aminoacids and they were present in multiple families. Regarding the missense mutations and polymorhisms, the results should be interpreted with caution, since no functional assay is yet available and small changes in aminoacid sequence may or may not influence the structure of the protein, thus affecting its function.

P1056. Mutation screening and prenatal diagnosis in Turkish 21-hydroxylase deficiency families, and development of a novel semi-quantitative PCR / enzyme digestion based approach for detection of large scale deletions/conversions of the gene.

T. Tukel[1], *M. Yuksel-Apak*[2], *J. Q. Wei*[3], *O. Uyguner*[2], *F. Bas*[4], *D. X. Song*[3], *H. Kayserili*[2], *N. Saka*[5], *H. Gunoz*[4], *R. C. Wilson*[3], *M. I. New*[3], *B. Wollnik*[2]
[1]*Division of Medical Genetics, Department of Pediatrics, Istanbul Medical Faculty, Istanbul University; Istanbul, Turkey;* [2]*Division of Medical Genetics, Child Health Institute, Istanbul University; Istanbul, Turkey;* [3]*Department of Pediatrics, Weill Medical College, Cornell University; New York,*

NY United States; [4]Division of Pediatric Endocrinology, Department of Pediatrics, Istanbul Medical Faculty, Istanbul University; Istanbul, Turkey; [5]Division of Auxology, Child Health Institute, Istanbul University; Istanbul, Turkey
ttukel@istanbul.edu.tr

21-hydroxylase deficiency is a recessively inherited disorder resulting from mutations in the CYP21 gene which encodes an important enzyme in the steroidogenesis pathway. The worldwide frequency is 1/14000 live births. CYP21 gene is located along with the CYP21P pseudogene in the HLA major histocompatibility complex on chromosome 6. The 98 % similarity of two genes and the fact that almost all frequently reported disease causing mutations reside in the pseudogene and are transferred to the active gene by gene conversion makes the molecular diagnosis difficult. Molecular analysis based on allele-spesific PCR using mutation site-spesific primers was performed in 30 Turkish patients and their families. The patients were screened for the 8 most frequently reported mutations and the frequencies were determined; PRO30LEU (0 %), IVS2AS,A/C-G,-13 (25.9 %), EXON 3 — 8 bp DEL (3.7 %), ILE172ASN (9.2 %), EXON 6 Cluster (ILE237ASN, VAL238GLU, MET240LYS) (3.7 %), VAL281LEU (0 %), GLN318TER (9.2 %) and ARG356TRP (11.1 %). Large scale deletions and 5 end conversions were idenfied with Southern blot analysis using DIG-labeled probes. The frequencies were found to be 9.2 % and 18.5 %, respectively. In 9.5 % of disease causing alleles none of these mutations were found. The sequence analysis of the gene in these patients still continues. In 4 families with known mutations, prenatal diagnosis were performed upon families requests. Additionally, a semi-quantitative PCR / enzyme digestion based approach for detection of large scale deletions/conversions of the gene was developed for routine diagnostic purposes and its accuracy was shown by comparison with the results of Southern blot analysis.

P1057. European Directory of DNA Laboratories - A registry which endeavours to spread information among European labs involved in the diagnosis of genetic disorders

E. Ngalula Kabanga[1], *C. Ramirez-Albott*[1], *D. Barton*[2], *H. Boman*[3], *M. Cardoso*[4], *B. Dallapiccola*[5], *M. Delpech*[6], *R. Elles*[7], *X. Estivill*[8], *C. Fonatsch*[9], *E. Kavanakis*[10], *C. Muller*[11], *M. Nordenskjold*[12], *A. Orpana*[13], *D. F. Schorderet*[14], *M. Schwartz*[15], *H. Smeets*[16], *A. Verloes*[17], *L. Van Maldergem*[1]
[1]*Centre de Genetique Humaine, Institut de Pathologie et de G n tique; Loverval, Belgium;* [2]*National Centre for Medical Genetics; Dublin, Ireland;* [3]*Haukeland Hospital; Bergen, Norway;* [4]*Instituto de Genetica Medica Jacinto de Magalhaes; Porto, Portugal;* [5]*University La Sapienza; Roma, Italy;* [6]*Universit Ren Descartes; Paris, France;* [7]*St Mary s Hospital; Manchester, United Kingdom;* [8]*Hospital Duran i Reynals; Barcelona, Spain;* [9]*Institut fur Medizinische Biologie; Wien, Austria;* [10]*St Sophia s Children s Hospital; Athens, Greece;* [11]*University of Wuerzburg; Wuerzburg, Germany;* [12]*Karolinska Hospital; Stockholm, Sweden;* [13]*University of Helsinki; Helsinki, Finland;* [14]*Centre hospitalier Universitaire Vaudois; Lausane, Switzerland;* [15]*Dept of Clinical Genetics, Rigshospitalet; Copenhagen, Denmark;* [16]*Stichting Klinische Genetica Zuid-Oost Nederland; Maastricht, The Netherlands;* [17]*Centre Universitaire Wallon de G n tique; Liege, Belgium*
eddnal@skypro.be

In 1995, the need for a comprehensive directory of genetic laboratories at a European level, as well as a listing of the services they could offer, led to the establishment of the European Directory of DNA Laboratories (EDDNAL). An on-line version of this directory (www.eddnal.com) has been available on the net since January 1997. The on-line directory is designed to promote information exchange between European centres and to improve awareness of the availability of services for rare genetic conditions. EDDNAL currently provides information for 317 laboratories and lists services for 580 genetic conditions. Through constant updating of information, EDDNAL endeavours to improve completeness of the registry thereby facilitating professional usage. Furthermore, EDDNAL aims to improve the quality of the services provided by professionals through the promotion of laboratory participation in a quality assessment scheme. EDDNAL also intends to promote the diagnosis of very rare genetic diseases with a consequent improvement in both the handling of such conditions and in the genetic counselling provided. The development of the EDDNAL database and web-site should result in an overall decrease in the total cost of DNA diagnoses of rare genetic disorders due to a European concerted approach based on the prevalence of such disorders.

P1058. Risk perception of participants in a family based genetic screening programme on familial hypercholesterolemia

M. C. van Maarle*, M. E. A. Stouthard, N. S. Klazinga, G. J. Bonsel*
Academic Medical Center; Amsterdam, The Netherlands
m.c.vanmaarle@amc.uva.nl

Introduction Familial Hypercholesterolemia (FH) is a hereditary metabolic disease and a major risk factor for cardiovascular disease. In the Netherlands, relatives of genetically confirmed FH patients are screened by genetic testing. Of these screenees, the risk perception in terms of the perceived probability of getting a heart attack later in life was studied. We studied both the influence of test result and lapse in time on risk perception, and the consequential influence of risk perception on cholesterol lowering medication use and stated attitude towards gene therapy. Methods The risk perception of 647 screenees was measured on 3 occasions (after approach, 3 days and 7 months after the test result). Presentation of the risk was pre-categorised and given both as numerical (1 in x) and verbal probability. Medication use was asked 7 months and consideration of gene therapy 18 months after screening. Results Screen positives perceived a higher risk than screen negatives, except in verbal risk perception 7 months after screening. Perceived risk was highest directly after approach and decreased over time. Both screen positives and negatives underestimated the actual risk. Of the screen positives, 68% would consider gene therapy. The higher the perceived risk, the more likely a person was to be on cholesterol lowering therapy or to opt for gene therapy. Conclusion The screening programme raised the awareness of the screenees for their risk of cardiovascular disease and encouraged engagement in preventive behaviours, including medication use and gene therapy.

P1059. An SSCP-based carrier test for spinal muscular atrophy (SMA)

A. Tacconelli[1], *S. Semprini*[1], *F. Capon*[1], *F. Brancati*[2,3], *B. Dallapiccola*[2,3], *G. Novelli*[1]
[1]*Tor Vergata University of Rome; Rome, Italy;* [2]*La Sapienza University of Rome; Rome, Italy;* [3]*Css Mendel Institute; Rome, Italy*
atacconelli@tiscalinet.it

Spinal muscular atrophy (SMA) is an autosomal recessive disorder with a newborn prevalence of 1 in 10,000, and a carrier frequency of 1 in 40-60 individuals. The SMA locus has been mapped to chromosome 5q11.2-13. The disease is caused by a deletion of the SMN gene, often encompassing other genes and microsatellite markers. The SMN gene is present in two highly homologous copies, SMN1 and SMN2, differing at five nucleotide positions. Only homozygous SMN1 mutations cause the disease. The sequence similarity between the SMN1 and SMN2 genes can make molecular diagnosis and carrier identification difficult. We developed a sensitive and reliable molecular test for SMN1 carrier identification, by setting up a non-radioactive SSCP-based method, which allows for the quantification of the amount of the SMN1 gene product with respect to a control gene. The assay was validated in 56 obligate (ascertained) carriers and 20 (ascertained) non-carriers. The sensitivity of the test is 96.4%, and its specificity, 95%. In addition, six of seven SMA patients without homozygous deletions presented with a heterozygous deletion, suggesting a concomitant undetected point mutation on the non-deleted SMN1 allele. Therefore, the present test is effective for detecting compound/hemizygote patients, for testing carriers in SMA families, and for screening for SMA heterozygotes in the general population. Project founded by Italian CNR (P.F. Biotechnology).

P1060. Metachromatic Leukodystrohpy; Mutation screening

F. Joncourt[1], *U. Wiesmann*[2], *S. Gallati*[3]
[1]*Children s University Hospital; Berne, Switzerland;* [2]*Children s University Hospital, Department of metabolic disease; Berne, Switzerland;* [3]*Children s University Hospital, Human Molecular Genetics; Berne, Switzerland*
franziska.joncourt@dkf2.unibe.ch

Metachromatic leukodystrophy (MLD) is an autosomal recessive disease caused by deficiency of the lysosomal enzyme Arylsulfatase A (ASA). Depending on whether the mutations in the ASA-gene cause complete or only partial loss of enzyme-activity age of onset and the course of the disease vary. MLD is lethal and no specific treatment can be offered to patients. Genetic counseling and the offer of prenatal diagnosis are therefore a need. They are complicated by the great variety of different mutations occuring in the ASA gene. The aim of this study is to identify disease causing ASA-mutations and to study their nature and distribution in our patient population. Mutation screening was started in a group of 50 MLD patients. Fifty healthy individuals serve as controls. Preliminary results; a partial screening of the ASA gene using SSCP and sequencing lead to the identification of 24 mutant alleles carrying 20 different mutations, 9 of which were novel. 85% of mutations found were nucleotide substitutions (missense/nonsense), 10% splice site mutations and 5% small deletions. This corresponds well to the distribution found among known ASA-mutations. An unexpected result was that 13 of 29 patients tested so far were found to be heterozygous for the so-called pseudodeficiency-allele Upon

completion of the mutation screening the question will be answered, whether the high pd-allele frequency in our patients is due to an association of this allele with a specific ASA-mutation. Our method efficiently detects known as well as unknown mutations and thus represents a useful tool for carrier detection and prenatal diagnosis of MLD.

P1061. Complete scanning of the hereditary hemochromatosis gene (HFE) using Denaturing High Performance Liquid Chromatography

***G. Le Gac**, C. Mura, O. Ragu n s, C. F rec*
INSERM, EFS, CHU, UBO; Brest, France
gerald.legac@univ-brest.fr

Hereditary hemochromatosis (HC) is a common autosomal recessive disorder of iron metabolism. Genetic testing for the C282Y and H63D HFE gene mutations now plays an important role both in diagnosis of the disease and detection of asymptomatic patients. However, between 4% and 35% of HC probands are C282Y or H63D heterozygotes or non-carrier of C282Y or H63D. The description of fifteen novel mutations now emphasizes the importance of a complete HFE coding region scanning in these HC probands. The aim of our study was to evaluate the Denaturing High Performance Liquid Chromatography (DHPLC) to set up a rapid and cost-effective pre-screening assay of the complete HFE coding region. The melting characteristics of the HFE gene were predicted using the wavemakerTM software. PCR primers were designed in order to amplified DNA fragments covering each translated exons with flanking intronic splice site sequences and to obtain the smallest difference as possible between melting domains. Then, analysis conditions for each HFE coding fragment were established separately according to its experimental melting curve. To achieve the highest accuracy we tested all the known HFE mutations and three HFE nucleotide polymorphisms, localized in exons or melting domains without mutation. The collection of the HFE mutations and polymorphisms was created using a site directed mutagenesis approach. All mutations and polymorphisms were detected and, we thus proposed, that the PCR-DHPLC method can be used to search known and unknown mutations in the HFE coding region of HC probands with at least one chromosome without an assigned mutation.

P1062. Clinical, Biochemical, FRAXA, FRAXE and 15q11.2-q12 Parental Methylation Pattern Investigation in 110 Institutionalized Individuals with Severe Mental Retardation.

***M. V. Mulatinho**[1,2], N. H. C. Aquino[3], M. J. Braga[4], E. J. C. Silva[1], P. S. Correia[1], A. A. P. Santa-Rosa[1], M. L. Oliveira[4], M. M. G. Pimentel[2], J. C. Cabral de Almeida[1,3], J. C. Llerena Jr[1,3]*
[1]Instituto Fernandes Figueira (IFF/FIOCRUZ); Rio de Janeiro, Brazil; [2]Departamento de Biologia Celular e Gen tica (IBRAG/UERJ); Rio de Janeiro, Brazil; [3]Unidade de Citogen tica Humana (IBCCF…/UFRJ); Rio de Janeiro, Brazil; [4]Departamento de Bioqu mica (IQ/UFRJ); Rio de Janeiro, Brazil
viannam@gbl.com.br

This study report a genetic investigation in 110 institutionalized individuals with severe mental retardation (MR) ranging from 1 to 68 years of age (median 27,64; sex ratio 1.2F;1M) using clinical examination, conventional cytogenetic, molecular methodologies for FRAXA, FRAXE and 15q11.2-q12 methylation pattern and also screening for inborn errors of the metabolism. We excluded 25 individuals (22,7%) with an acquired cause of MR and in the remaining 85 subjects, 8,2% had a chromosomal abnormality [four trisomy 21, including two mosaics, two mos45,X/46,XX, including a 68 year old woman, and one case of del(3)(q24~25)]. One atypical case of Angelman syndrome was diagnosed, however no FRAXA and FRAXE amplification were found. Among the metabolic disorder,20% of all individuals had an increased rate of aminoacids in urine. No cases of homocystinuria, phenylketonuria, or methylmalonicaciduria were detected. Classical genetic syndromes were present, including one neural tube defect spectrum, two true microcephalic syndrome, one Norrie syndrome, one Pelizaeus-Merzbacher syndrome, one panhypopituitarism dwarfism and one possible velo-cardio-facial syndrome. Two cases had similarly affected sibs and in eight cases a familial history of MR was reported. Few rarer syndromes such as W syndrome and a syndrome associated to cutis giratum were also clinically diagnosed. In 42% of cases no etiological classification was possible.Our results points to the great heterogeneity of the etiology in MR, such as previous reports, and justify the application of other refined research methodologies in the screening of genetic causes.The mean age of the population investigated, mostly adults, may impose some obstacles to establish a clinical diagnosis; in the other hand, it may illustrate the natural clinical history of rare genetic diseases associated to severe MR. (Finantial supports; FAPERJ, CNPq, CEPUERJ)

P1063. The Hungarian Cystic Fibrosis Database; Statistical Analysis and Genetic Data

***M. Garami**[1], K. N meth[1], G. Borgulya[1], K. Holics[2], R. jhelyi[2], A. V radi[3], G. Fekete[1]*
[1]2nd Department of Paediatrics, Semmelweis University; Budapest, Hungary; [2]Heim P l Hospital for Sick Children; Budapest, Hungary; [3]Hungarian Academy of Sciences; Budapest, Hungary
miklos@gyer2.sote.hu

Cystic fibrosis is a human genetic disorder caused by mutations in the CF-transmembrane conductance regulator. Mutations in the gene encoding this protein disrupt electrolyte secretion, leading to hyperosmolar viscous mucus. The Hungarian Cystic Fibrosis Database Project was started in 1998. We established a unified score system for all the Hungarian CF patients, including their clinical status and their genetic background, too. The Hungarian CF Registry contained 345 patients in 1998. Using these data allows us to perform comprehensive scientific analysis of CF patients distribution in Hungary, their current age, manifestation; gender ratio; pulmonary and gastrointestinal performance status; genotype vs. phenotype correlation; Kaplan — Meier analysis. The systematic mutation analysis for deltaF508 mutation was started in 1990 in our laboratory. In the first year 80 patients had been screened. Out of them 42 patients proved to be homozygous and 11 heterozygous for this mutation. In order to determine mutations in the CFTR gene, 1826 Hungarian persons have been tested. We isolated DNA from whole blood, followed PCR amplification, restriction enzyme digestion and fragment analysis. From 1992 we extended our capacity up to the most common five European mutations (deltaF508, G542X, G551D, R553X and N1303K) and occasionally (since 1998) we identify further 26 mutations, too. In 152 cases (34%) deltaF508 were presented in homozygous and in 116 cases (26%) in heterozygous forms. Detection rate was 4,5% for mutation G524X; 2% for R553X and 2,2% for N1303K mutation. Mutation G551D was not present in our cases. In 54 cases prenatal diagnosis was successfully done. Thirteen samples DNA carried the disease mutations. Intragenic microsatellite analysis for indirect study of CFTR gene has been performed since 1989, too. In Hungary at the present all suspected CF patients get the possibilities of mutation analysis, indicated by specialists and appropriate criteria.

P1064. Ethical and Social Consequences of Genetic Screening for Hemochromatosis

***D. Avard**[1], B. M. Knoppers[1], P. C. Adams[2]*
[1]University of Montreal; Montreal, PQ Canada; [2]University of Western Ontario; London, ON Canada
denise.avard@umontreal.ca

Hemochromatosis, the most common known genetic disorder among Caucasians, is an autosomal recessive condition associated with excessive absorption of iron which may result in liver cirrhosis, heart failure, diabetes, impotence, and arthritis and cause early death. Considering the relative large number of people affected (about 0.5%) of the population, the potential severity of the disease and the relative ease of prevention and treatment of the disease, population screening for hemochromatosis has been advocated. However, genetic screening, possible since the discovery of the HFE gene, is controversial. The relationship between genotype (presence of mutants of the HFE gene), iron overload and clinical hemochromatosis is complex, and not fully understood and the possibility remains that a large proportion of asymptomatic homozygotes may never develop iron overload. Possible negative consequences for the individual include increased anxiety, and possible discrimination in obtaining insurance or employment. There are also implications for the family, who have a high risk of having the affected gene and may or may not want to know. In addition, the social costs such as stigmatization as well as the resource costs associated with limited health care resources will be considered. This paper will review key ethical and social consequences for both homozygotes (high risk for the disease) and heterozygotes (carriers) by examining the professional debate, by comparing guidelines and policies from different countries and by evaluating lessons learned from screening programs for Cystic Fibrosis(CF), Sickle Cell or Tay-Sachs diseases.

Also presented orally in Concurrent Session 15: Poster Discussion Genetic Testing and Screening

P1065. Use of DNA-based mutation detection to evaluate the Glucose-6-phosphate Dehydrogenase (G6PD) enzyme assay in screening heterozygotes

***V. M. S. Lam**[1], J. L. C. Chan[1], P. Hung[2], M. D. Y. Au[1]*
[1]Department of Biochemistry, Faculty of Medicine, The University of Hong Kong; Hong Kong, Hong Kong Special Administrative Region of China; [2]Neonatal Screening Laboratory, Kwong Wah Hospital, Department of Health; Hong Kong, Hong Kong Special Administrative Region of China

hrmbvel@hkucc.hku.hk

In many parts of the world including Hong Kong and South China where G6PD deficiency is prevalent and with the wide spread use of some drugs e.g., Chinese herbal medicine, G6PD deficiency is a health problem. In Hong Kong, new-borns in government hospitals are screened by the enzyme assay. The at risk identified are counselled to avoid the offending agents, thus alleviating much of the clinical consequences. Since G6PD is X-linked, it is often difficult to classify the genotypes, particularly the heterozygous females, based on enzyme activity measurements alone. We have used two DNA-based mutation detection methods, the denaturing gradient gel electrophoresis (DGGE) and single stranded conformation polymorphism (SSCP) to evaluate the validity of the enzyme activity cut-off of 9.0 IU/gHb currently adopted to distinguish the heterozygotes from the normal females. From the results of 195 female samples with G6PD activity 1 IU above the cut-off , about 7% of G6PD deficient heterozygotes were missed. For an enzyme activity of 9.0 to10.4 IU/gHb, about 9% female heterozygotes had been identified by the DNA-based detection in these samples. The frequency of each of the common Chinese mutations from samples with enzyme activity 9.0 to 12.4 IU/gHb correlates well with the frequency of each of these mutations found in the local population. The implications of these findings and the use of enzyme assay alone for screening will be discussed.

P1066. Clinical genetics in the developing countries; The Brazilian situation

A. P. Marques-de-Faria[1], *A. X. Acosta*[2], *V. E. F. Ferraz*[2,3], *D. Brunoni*[4]
[1]*Departamento de Gen tica M dica, Faculdade de Ci ncias M dicas, Universidade Estadual de Campinas; Campinas, Brazil;* [2]*Departamento de Gen tica, Faculdade de Medicina, Universidade de S o Paulo; Ribeir o Preto, Brazil;* [3]*UNAERP; Ribeir o Preto, Brazil;* [4]*Centro de Gen tica M dica, Universidade Federal de S o Paulo; S o Paulo, Brazil*
faria@bestway.com.br

In the developing countries, where more than 80% of the world s population lives and the majority of children will be born, there are important obstacles for the progress in clinical and medical genetics. Among medical professionals and public health officials, issues as poverty, illiteracy, high infant mortality and other urgent matters concerning basic health care usually have precedence over genetic diseases and birth defects. However, the general prevalence of these conditions seems higher than in developed nations and the limited resources enhance the burden on individuals, families and populations. In addition, the requirement for genetic services is increasing, as a consequence of the advances in medical genetics, and this reinforces the need for programs in management and prevention especially at a primary health care level. Brazil has a total population estimated around 167 millions inhabitants with a wide range of regional inequalities. Total infant mortality rate, e.g., is 37.5 per 1000, but it ranges from 60.4 in the less developed Northeast region to 25.8 in the more industrialised region of Southeast, while the poverty rate is 52.9% in the former and 16% in the latter. These contrasts are reflected in the implementation of genetic services, the majority located in urban centres as tertiary care university hospitals in the South and Southeast regions, with an emphasis still on dysmorphology, routine cytogenetics and counselling. There are few biochemical laboratories and DNA-based diagnosis is rising, particularly with research purposes. A current overview concerning these issues will be presented. Strategies including governmental and community participation, improvement of epidemiological data, development of rational and ethical genetic health programs are among the proposals for providing appropriate genetic services for the Brazilian population.

P1067. Lay Advocacy Organizations as Collaborative Partners in Genetic Research

S. F. Terry[1,2], *P. F. Terry*[3,2], *V. H. Whittemore*[1,4], *J. Cody*[5,1], *M. E. Davidson*[1]
[1]*Genetic Alliance; Washington, DC United States;* [2]*PXE International; Sharon, MA United States;* [3]*Genomic Health; Palo Alto, CA United States;* [4]*Tuberous Sclerosis Alliance; Silver Spring, MD United States;* [5]*Chromosome 18 Registry; San Antonio, TX United States*
sterry@geneticalliance.org

Based on decades of partnering with lay advocacy groups involved in research, the Genetic Alliance defines research as a shared enterprise collaboration between participants and researchers. The cultures of scientific investigators and lay advocacy often differ, resulting in dynamic and creative tensions between shared and divergent interests. Lay advocacy groups are empowered to assume their role as equal partners in research. Lay advocacy groups represent research participants interests — both those directly involved in the study and those who stand to be impacted by study results. They are able to initiate research with a clear focus and make decisions about research that will prove most effective for the condition. Lay advocacy groups are optimally positioned to collect epidemiological and phenotypic data. By living in a virtual community , which promotes the sharing of stories, they have their hands on essential information that is usually inaccessible to clinicians or researchers who employ more traditional participant and tissue solicitation methods. A number of models will be presented. In addition, as proteomics takes center stage, research will require collaborations in well-developed networks of researchers, participants and their respective organizations. These partnerships will be both the catalyst and fuel for acceleration of research that is essential to advancing new understandings. In the past academia and industry have often worked on parallel tracks, apart from the lay advocacy and public sectors. Increasing numbers of research collaborations give rise to effective and efficient interactive models that can sustain the essential resources for genetics research.

P1068. Lay Advocacy Group Facilitates International Research Collaborative

P. F. Terry[1,2], *S. F. Terry*[2,3], *A. S. Marais*[2,4], *I. P. Ronchetti*[2,5], *T. Le Roux*[2,6], *C. D. Boyd*[7], *L. G. Bercovitch*[2,8]
[1]*Genomic Health; Palo Alto, CA United States;* [2]*PXE International; Sharon, MA United States;* [3]*Genetic Alliance; Washington, DC United States;* [4]*University of Cape Town; Cape Town, South Africa;* [5]*University of Modena; Modena, Italy;* [6]*Lasell College; Newton, MA United States;* [7]*University of Hawaii; Honolulu, HI United States;* [8]*Brown University; Providence, RI United States*
pat@genomichealth.com

Lack of data about manifestations, natural history and epidemiology of rare genetic disorders inhibit research. Often difficulty identifying affected individuals limits the funding possibilities and makes the acquisition of patient samples and data difficult. Further, the agenda of researchers and participants can diverge in ways that ultimately delay progress. Pseudoxanthoma elasticum (PXE) is a rare genetic disorder. PXE International, a lay advocacy organization founded in 1995, initiates, supports and funds research. It also provides support for affected individuals, their families and their healthcare providers. Establishment of various programs in 21 countries world-wide is critical to providing genetic services and supporting research. Empowering the individuals in numerous countries and cultures requires an investment of resources in these communities. As a result, the PXE International Blood and Tissue Bank has branches in Italy and South Africa. The results of efforts made by PXE International are the PXE International Research Consortium comprised of15 laboratories world-wide, a multidisciplinary approach forging collaborations amongst many specialists, a focused agenda which accelerates research in the service of consumer interests, and increased congressional awareness for real outcomes of medical research funding. The organization is also uniquely able to safeguard confidentiality by acting as a firewall between researchers and participants. Genetic lay advocacy using this model can benefit from the added advantage of international collaboration, furthering genetic research for rare disorders.

P1069. Molecular Genetics viewed from the Health Technology Assessment Perspective

I. R. Blancquaert[1], *L. Bouchard*[2], *Y. Chikhaoui*[1], *G. Cleret de Langavant*[1]
[1]*Agence d valuation des technologies et des modes d intervention en sant ; Montr al, PQ Canada;* [2]*Universit d Ottawa; Ottawa, ON Canada*
ingeborg.blancquaert@aetmis.gouv.qc.ca

Health Technology Assessment (HTA) provides legitimacy to health services planning. Given the drive towards evidence-based medicine and policy, the introduction of new technologies is closely scrutinised and expected to produce measurable benefits. In genetics, the technology transfer from research to clinical services has not traditionally been supported by a formal evaluation process (in contrast to therapeutics or medical devices). The Qu bec Agency for Health Services and Technology Assessment evaluated the relevance of implementing several molecular tests into clinical services and assessed the corresponding diagnostic/screening strategies. The purpose of this communication is to discuss the evaluative approach used as well as the challenges and potential contribution of HTA. Our experience shows that the usual criteria for assessing diagnostic/screening tests do not constitute a satisfactory framework for molecular tests. Evaluating test validity is complicated by the constant evolution of techniques, the frequent absence of standardisation and the need to distinguish between clinical and analytical validity. The utility of diagnostic/screening strategies may be difficult to measure, because of the psycho-social and collective nature of benefits and risks, and is often poorly documented. Besides the scientific literature, HTA needs to consider the regional and organisational context, which affects the utility, feasibility and

acceptability of alternative strategies. HTA provides legitimacy to the decision-making process by formulating recommendations that take into account the available data and their limits. Another contribution of HTA is to define the conditions required to ensure the provision of appropriate and co-ordinated services, which are relevant to planning and policy making.

Also presented orally in Concurrent Session 15: Poster Discussion Genetic Testing and Screening

P1070. Gene Expression Profiling - a new service from the Resource Center in Berlin and Heidelberg

C. Maercker[1], *D. Lutz*[1], *G. Mall*[2], *K. Amann*[3]
[1]*Resource Center (RZPD) Heidelberg; D-69120 Heidelberg, Germany;* [2]*Dept. of Pathology; 64283 Darmstadt, Germany;* [3]*Dept. of Pathology, University of Erlangen; 91054 Erlangen, Germany*
c.maercker@dkfz.de

The gene expression profiling service of the Resource Center (RZPD) is open for all groups working on functional genomics of human, mouse and rat. We isolate poly A+ RNA, synthe-size P33 labeled cDNA and hybridize it with the RZPD Unigene filter sets of human (set I; about 33.800 gene and EST clones, set II; about 76.000 clones), mouse (about 25.000 clones) or rat (about 26.000 clones). Phosphorimaging and evaluation of the data by different soft-ware tools make it possible to find out with genes are over- or underexpressed, for example in a certain tissue in comparison to a control sample. In the moment a public database is set up to make the data available for all people working with our filters or other microarrays. In the work shown here we address the question, why renal failure in rats causes cardiac dis-eases. Cardiovascular complications in patients with renal failure are an extremely important clinical problem. The characterisitc changes of cardiovascular disease in renal failure can be nicely reproduced in the experimental model of the subtotally nephrectomized rat (SNX). In order to identify genes which are differentially regulated during the course of the disease the gene expression profiles of hearts of sham-operated rats (controls) and SNX with renal failure of 12 weeks duration where compared. The hybridizations were repeated several times and also performed with different Unigene sets (rat, mouse, human) under stringent condi-tions. We were able to confirm results of previous experiments, showing an activation of car-diac interstitial cells in SNX rats, for example by specific growth factors. The behavior of a number of other genes could help us to explain certain cardiovascular changes in uremic rats. Due to the homology of many rat cDNAs to human and/or mouse genes, it was possible to interpret the results from the different filter sets by computer analysis. In addition, we could clearly document that we are able to produce reliable data with our experiments.

P1071. Molecular basis of hypoxanthine-guanine phosphoribosyltransferase deficiency in Italian Lesch-Nyhan patients; identification of five new mutations.

C. Lievore[1], *L. De Gregorio*[2], *W. L. Nyhan*[2], *M. Carella*[1]
[1]*Department of Medical Genetics, Baschirotto Institute for Rare Diseases (BIRD EUROPE onlus); Costozza di Longare (VI), Italy;* [2]*Department of Pediatrics and Institute for Molecular Genetics, University of California San Diego; La Jolla, CA United States*
laboratorio@birdfoundation.org

Lesch-Nyhan syndrome is an X-linked recessive disorder involving the purine metabolism, with resultant hyperuricemia, choreoathetosis, self-mutilation, and profound neurologic dysfunction. A deficiency of the enzyme hypoxanthine guanine phosphoribosyl-transferase is responsible for the disease. The human HPRT gene is located at Xq26-27 and consists of nine exons spanning approximately 45 Kb. We have recruited twelve Italian unrelated Lesch-Nyhan patients diagnosed with LN syndrome according to the clinical and laboratory findings. Studies by PCR and direct sequencing on genomic DNA from patients revealed two missense mutations, two nonsense mutation, two mutations in the splicing acceptor sites, three large deletions leading to a loss of more exons, two insertion of one base, and one compound mutation consisting in a four base deletion with a three base insertion. Five of these twelve mutations have not been previously described; 74C>G (Pro25Arg) in exon 2, 329-332delCAAC insTCT in exon 4, 506insC in exon 7, IVS6-1G>A in the acceptor site of intron 6, and IVS7-1G>C in the acceptor site of intron 7. Four mutations have been previously described in unrelated families, and three mutations have been already published. Characterization of the HPRT mutations allowed us to detect the carrier status in eight females from seven families. In one family the mutation was de novo. Identification of mutations provides a valuable tool for confirmation of the diagnosis, for identification of relatives who might be potential carriers, for pleimplantation testing, and for prenatal diagnosis.

P1072. Screening of pancreatic secretory trypsin inhibitor (PSTI) mutations in chronic pancreatitis by D-HPLC

C. Le Marechal[1], *O. Raguenes*[2], *I. Quere*[2], *M. Audrezet*[2], *B. Mercier*[2], *J. Chen*[1], *C. Ferec*[2]
[1]*Etablissement Fran ais du Sang-Bretagne; Brest, France;* [2]*Universite de Bretagne Occidentale and Centre Hospitalier Universitaire; Brest, France*
cedric.lemarechal@univ-brest.fr

Over the past five years, gain-of-function missense mutations in the cationic trypsinogen gene have been increasingly identified as being associated with hereditary pancreatitis or/and sporadic pancreatitis. Based upon a candidate approach, presumably loss-of-function mutations in the pancreatic secretory trypsin inhibitor (PSTI) gene, particularly a p.N34S missense mutation, was also identified as being strongly associated with chronic pancreatitis (CP) (Chen et al, J Med Genet 2000,37;67-9; Witt et al. Nat Genet 2000,25;213-6; Pfutzer et al. Gastroenterology 2000,119;615-23). We extended to evaluate the PSTI gene in a large cohort of unrelated French subjects with CP by Denaturing High Performance Liquid Chromatography (DHPLC). The major new findings are as follows. Firstly, the p.N34S carrier frequency in the <20 years group is significantly higher than the >20~65 years group, suggesting an increased environmental contribution to the disease in the later group. Secondly, the p.N34S is in complete linkage disequilibrum with another two variants, IVS1-37T>C and IVS3-66_-65insTTTT. Thus, the [IVS1-37T>C + N34S + IVS3-66_-65insTTTT] haplotype suggests a unique origin. Thirdly, in addition to other known variants (eg., c.-53C>T and c.-41G>A), novel variants (eg., c.-2C>A, p.L12F and p.L14R) were also detected, albeit with a much lower frequency compared with the p.N34S mutation. These results help further resolve the elusive etiology of sporadic pancreatitis.

P1073. Analysis of dystrophin mRNA in patients with DMD, BMD and XLDC

L. Fajkusova[1], *M. Tvrdikova*[1], *Z. Lukas*[2], *J. Fajkus*[3]
[1]*Research Institute of Child Health; Brno, Czech Republic;* [2]*Faculty Hospital Brno; Brno, Czech Republic;* [3]*Institute of Biophysics, Academy of Sciences of the Czech Republic; Brno, Czech Republic*
lenkafajkusova@volny.cz

Complete dystrophin mRNA sequence has been analyzed in 36 Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) patients. Deletions and duplications in mRNA were detected using reverse transcription-polymerase chain reaction and point mutations were found using the protein truncation test. Individual mutations were characterized by sequence analysis and their correlation with diagnosis of DMD or BMD was checked. One patient with BMD phenotype was associated with nonsense mutation E1110X. In the case of the E1110X mutation an alternative splicing of dystrophin mRNA (3486-3640del) was detected in this patient, which included the E1110X mutation site (nucleotide 3536) and did not change the translation reading frame. Expression of the major dystrophin mRNA isoform (from the muscle-, brain- and Purkinje-promotors) has been analyzed in 15 patients with suspicion on X-linked dilated cardiomyopathy (XLDC). Analysis of the expression of three dystrophin mRNA isoform was complemented by DNA analysis of the muscle promotor, the first exon and first exon-intron boundary. The work was supported by Ministry of Health of the Czech Republic, project no. NA/5227-3

P1074. Cystic Fibrosis Newborn Screening; Rare Cftr Mutations In Hypertrypsinaemic Neonates

D. A. Coviello[1], *R. Padoan*[2], *A. Bassotti*[2], *M. Seia*[1], *A. Ambrosioni*[1], *C. Corbetta*[3]
[1]*Molecular Genetic Laboratory, Istituti Clinici di Perfezionamento; Milan, Italy;* [2]*CF Centre, Istituti Clinici di Perfezionamento; Milan, Italy;* [3]*Neonatal Screening Centre , Istituti Clinici di Perfezionamento ; Milan, Italy*
coviello@unige.it

Recently neonatal hypertrypsinaemia has been associated to the presence of increased frequency of rare CFTR mutations and IVS8-5T polymorphism. In the period October 1998-October 2000, neonatal screening program performed in out laboratory on the population of Lombardia region, identified 25 hypertrypsinaemic infants presenting borderline sweat test (chloride value between 30 and 60mmol/L). The aim of our study was to assess the incidence of rare CFTR mutations or polymorphisms in infants with neonatal hypertrypsinaemia and with a borderline sweat test in the first months of life. Among the 25 hypertrypsinaemic infants 22 were heterozygous for one CFTR mutation identified within the neonatal screening program (IRT/IRT+DNA by PCR/OLA assay for up to 31 mutations). An extended analysis of CFTR gene was performed on the 25 hypertrypsinaemic infants, using DGGE technique on 11 out 27 exons, sequencing analysis of splice site polymorphism in intron 8 (TG)mTn and sequencing analysis of DNAs that showed an abnormal DGGE electrophoretic pattern.

The results identified a total of 17 different CFTR alleles; eight F508del, four R117H, two G542X, two N1303K, two D1152H, R347P, 2183AA->G, 621+1G->A, 2789+5G->A, R553X, R347H, R117L, V1153E, Y1032C, 711+3G->A. In addition we identified a novel mutation D110E (2 cases). Furthermore IVS8-(TG)mTn polymorphisms were tested, and the haplotype TG12T5 was identified in seven infants. In total we identified 14 compound heterozygotes (seven with two CFTR mutations, seven with one mutation/TG12T5), 10 subjects with only one mutation identified, whereas in only one patient no gene alterations were found. In conclusion we suggest that a high frequency of CFTR gene mutations is present among hypertrypsinaemic neonates presenting a borderline sweat test in the first months of life. Moreover we suggests that some immunoreactive trypsin positive neonates are actually affected by a very mild form of CF, an extended genetic analysis is recommended and furthermore clinical follow up in specialised Centres is needed to diagnose mild or atypical forms of CF.

Also presented orally in Concurrent Session 15: Poster Discussion Genetic Testing and Screening

P1075. Sequence alterations in the gene for LDL receptor synthesis in Czech hypercholesterolemic patients

***V. Kuhrova**, H. Francova, P. Zapletalova, E. Hrabincova, R. Slovackova, T. Freiberger*

Research Institute of Child Health; Brno, Czech Republic

vera.kuhrova@volny.cz

Objective; Mutations in the gene for low density lipoprotein receptor (LDLR) has been associated with increased total and LDL cholesterol levels, leading to accelerated atherosclerosis. The aim of our study was to define sequence variations causing familial hypercholesterolemia (FH) phenotype in Czech patients. Methods; 625 patients with high total and LDL cholesterol, normal serum triglyceride and with family history of premature CHD were selected for study. Screening of the mutant exons of LDLR gene was performed using heteroduplex analysis, SSCP, DGGE, DNA sequencing and PCR/RFLP analysis. Results; Molecular searching for mutations in coding sequence of above mentioned gene resulted in the identification of 29 probably pathogenic sequence variations and 8 silent mutations, many of which are new and were not described previously. Conclusion; Knowledge of mutations causing autosomal dominant lipoprotein disorder - FH is an aid for unambiguous diagnosis, facilitates genetic consulting at early age together with preventing disease manifestation. This study was supported by the Internal Grant Agency of the Ministry of Health of the Czech Republic, project no. NE/5554-3.

P1076. Molecular genetics of Familial Mediterranean Fever in Cyprus

***R. Mean**[1], C. Costi[2], E. Rossou[2], V. Hadjiroussos[3], P. Petrou[4], C. Deltas[2]*

[1]The Cyprus Institute of Neurology and Genetics; Nicosia, Cyprus; [2]The Cyprus Inst. of Neurology and Genetics; Nicosia, Cyprus; [3]Clinician in Private Practise; Limassol, Cyprus; [4]Apollonion Private Hospital; Nicosia, Cyprus

Deltasco@mdrtc.cing.ac.cy

Familial Mediterranean fever (FMF) is an autosomal recessive disease of high prevalence around the Mediterranean countries with four ethnic groups being particularly affected, namely Non-Ashkenazi Jews, Armenians, Arabs and Turks. Typically it presents as acute episodes of periodic fever accompanied by complaints of abdominal pain, chest pain, or joint pain. The actual attack usually lasts from 12 to 72 hours, with arthralgia or arthritis often lasting longer. The most dangerous potential complication of FMF is amyloidosis than can lead to end-stage renal failure. Atypical phenotypic presentation makes diagnosis difficult, and in many occasions diagnosis is established by exclusion. The administration of Colchicine constitutes an effective treatment. The responsible gene, MEFV, is placed on chromosome 16p13.3 and it encodes a 781 aminoacid protein, Pyrin /Marenostrin. About 20 mutations have been identified so far, some of them being very frequent, accounting for a high percentage of patients. Founder effects have been postulated to be responsible for the high frequency of certain mutations in selected populations, rather than recurrence. Molecular epidemiological investigation of the Greek-Cypriot population reveals that about 1/25 is a carrier of one of three mutations, V726 A being the most frequent. Among 44 MEFV chromosomes analysed, the results are; V726A 36.4%; F479L 20.4%; M694V 18.2%; E148Q 4.5 %; M680I 2.3 %; Unknown 18.2%. Mutation F479L is rather rare in other populations. These results suggest that contrary to previous believes FMF is not a rare condition in Cyprus, and molecular testing is expected to assist further in identifying ambiguous cases.

P1077. The European Molecular Genetics Quality Network (EMQN)

***S. Patton**[1], R. Elles[1], D. Barton[2], C. Mueller[3], M. Losekoot[4], S. Ramsden[5], S. Stenhouse[6], B. Rautenstrauss[7], M. Simoni[8], P. Vogt[9]*

[1]EMQN, c/o Regional DNA Laboratory, St Mary s Hospital; Manchester, United Kingdom; [2]National Centre for Medical Genetics, Our Lady s Hospital for Sick Children; Dublin, Ireland; [3]University of Wuerzburg; Wuerzburg, Germany; [4]Leiden University Medical Centre; Leiden, The Netherlands; [5]Regional DNA Laboratory, St Mary s Hospital; Manchester, United Kingdom; [6]Northern Regional Genetics Service; Newcastle-upon-Tyne, United Kingdom; [7]Univesitat Erlangen-Nurnberg; Erlangen, Germany; [8]Institute for Reproductive Medicine, University of Munster; Munster, Germany; [9]Institute of Human Genetics, University of Heidelberg; Heidelberg, Germany

spatton@smh5.cmht.nwest.nhs.uk

Molecular Genetics testing forms an increasingly important part of the diagnostic process in all branches of medicine. Studies of the reliability of such testing have indicated a significant level of inaccuracy in laboratory reports, arising from errors in sample identification, genotyping or interpretation. The European Molecular Genetics Quality Network (EMQN) aims to raise and maintain the quality of Diagnostic Clinical Molecular Genetics Testing. One way of doing this is to provide standard external quality assessment (EQA) schemes to allow diagnostic laboratories to test and improve their proficiency. Since its inception in 1997, EMQN has provided fifteen EQA schemes in six disease-specific areas. Separate posters describing each disease-specific scheme can be found alongside this poster. EQA schemes are designed to test the ability of laboratories to interpret data in the light of clinical information supplied with a referral, and to produce a clear and accurate report. Laboratories from all EU countries and several others have participated in EMQN EQA exercises. In 2000, the schemes evaluated 207 returns from laboratories, a 65% increase on 1999. The standards of accuracy were high but significant error rates were found and methods of reporting and interpreting data were varied. The error rate indicates a clear need for EQA to measure current standards of proficiency and encourage laboratories to raise their technical performance. Differences in interpretation of data and reporting standards can be addressed by bringing centres together to discuss a consensus and agree guidelines for best practice in diagnostic clinical molecular genetics.

Also presented orally in Concurrent Session 15: Poster Discussion Genetic Testing and Screening

P1078. An External Quality Assessment scheme for genetic testing of familial Breast/Ovarian Cancer

***C. Mueller**[1], D. Stoppa-Lyonnet[2], U. Kristoffersson[3]*

[1]University of Wuerzburg; Wuerzburg, Germany; [2]Institut Curie; Parris, France; [3]Lund University Hospital; Lund, Sweden

crm@biozentrum.uni-wuerzburg.de

Breast and ovarian cancers (Br/Ov) are the most frequent cancers in women with a life-time risk of about 10 % in Western countries. About 5 % of all cases are thought to be familial due to mutations in the BRCA1 and BRCA2 genes. Given the prevalence of Br/Ov cancers, mutation screening of the BRCA genes is now being offered to at risk women. The main benefit of mutation detection is the predictive testing of relatives of identified mutation carriers. In 1999 a pilot External Quality Assessment (EQA) scheme was offered by the European Molecular Genetics Quality Network (EMQN) for the genetic testing of the BRCA1 gene. Following the successful trial, the scope of the scheme was widened in 2000 to include a case of a predictive test for a known family mutation and testing for the BRCA2 gene. The scheme was offered to a wider audience with 24 laboratories from 14 countries participating. Error rates were calculated from the total number of alleles analysed by all laboratories. On this basis, the overall error rate (% of incorrect alleles) was 2,9 % (1.25% in 1999). Most errors were the result of failure to identify a given mutation. Variation in the interpretation and reporting was noted. Reasons for the increased error rate and variation in interpretation and reporting will be given in context with other EMQN disease-specific EQA schemes. The results emphasise the need for additional efforts in raising standards through the provision of EQA schemes and best practice guidelines.

P1079. An External Quality Assessment scheme for genetic testing of Huntington Disease

***M. Losekoot**[1], F. Laccone[2], S. Stenhouse[3], V. Biancalana[4], B. Bakker[1], S. Patton[5], R. Elles[5]*

[1]Leiden University Medical Centre; Leiden, The Netherlands; [2]Institute of Human Genetics, Universtaet Gottingen; Goettingen, Germany; [3]Northern Regional Genetics Service; Newcastle-upon-Tyne, United Kingdom; [4]Institute of Biological Chemistry, University Louis Pasteur; Strasbourg, France;

[5]EMQN, C/o Regional DNA Laboratory, St Mary s Hospital; Manchester, United Kingdom
m.losekoot@kgc.azl.nl

Huntington Disease (HD), a progressive fatal neurodegenerative disorder, is the most common CAG repeat disease with an incidence of 1 in 10,000 people in Europe. HD is caused by an expansion of a highly polymorphic CAG repeat in exon 1 of the Huntingtin gene. Genetic testing for this expansion is routinely offered in diagnostic molecular genetics laboratories. In 1997, the European Molecular Genetics Quality Network (EMQN) organised a pilot External Quality Assessment (EQA) scheme for genetic testing of the HD gene with 14 participants from 13 different European Union countries. The HD scheme is now the longest running EMQN scheme with 39 participants (18 countries) in 2000. As the number of participants has increased, there has been a consequent rise in the error rate (% of incorrect alleles) to 2.6% (2000) from 1.3% (1997). The error rates were calculated from the number of alleles analysed by all laboratories. In contrast, the precision of genotyping has increased with 74% (2000) of all alleles typed within the error limits (+/- 1 repeat unit for alleles up to 40 repeats and +/- 4 repeats for alleles >40). Reporting styles varied but interpretation was more consistent with the majority of laboratories covering most of the interpretative criteria adequately. The results emphasise the importance of EQA schemes in measuring and raising the standards of diagnostic genetic testing.

P1080. An External Quality Assessment scheme for genetic testing of Duchenne and Becker Muscular Dystrophy

S. Stenhouse[1], *S. Ramsden*[2], *G. Mattius*[3], *M. Jean-Pierre*[4]
[1]*Northern regional genetics Service; Newcastle-upon-Tyne, United Kingdom;* [2]*Regional Genetics Service, St Mary s Hospital; Manchester, United Kingdom;* [3]*Katholieke Universiteit Leuven; Leuven, Belgium;* [4]*Groupe Hospitalier Cochin; Paris, France*
s.a.r.stenhouse@ncl.ac.uk

Duchenne Muscular Dystrophy (DMD) is the most common muscle wasting disease in children with an incidence of 1 in 4,000 males. A milder variant of the disease, Becker Muscular Dystrophy (BMD) is less common with an incidence of 1 in 18,000 males. Both diseases are due to mutations in the Dystrophin gene on the X-chromosome. Genetic testing for DMD and BMD by mutation screening of the dystrophin gene is routinely offered in diagnostic molecular genetics laboratories. The main benefit of this testing is carrier detection in relatives of affected individuals. In 1998, a pilot External Quality Assessment (EQA) scheme for DMD and BMD was offered by the European Molecular Genetics Quality Network (EMQN); 15 laboratories participated. Following the successful trial, the scheme was offered to a wider audience and the number of participating laboratories has increased to 33 (16 countries) in 2000. Error rates from the scheme were calculated from the total number of alleles analysed by all labs. The overall error rate (% incorrect alleles) for the 2000 scheme was 2.02% (4.44%, 1998; 0.98%, 1999). The structure of the scheme has evolved in line with problems identified in each round of EQA. In 1998, 3 deletion cases were provided. In 1999, a pedigree was included and participants asked to perform linkage analysis. In light of problems identified with the interpretation of linkage analysis, a linkage question was also included in the 2000 scheme. The scheme will be described in full and the results for both genotyping and interpretation will be discussed and lessons drawn from the development of the scheme.

P1081. An External Quality Assessment Scheme for genetic testing of Friedreich Ataxia

D. Barton[1], *E. Sistermans*[2], *M. Claustres*[3]
[1]*National Centre for Medical Genetics; Dublin, Ireland;* [2]*University Hospital Nijmegen; Nijmegen, The Netherlands;* [3]*Institut de Biologie; Montpellier, France*
david.barton@olhsc.ie

Friedreich ataxia (FRDA), an autosomal recessive neurodegenerative disorder, is the most common hereditary ataxia with an estimated prevalence of 1 in 50,000. The most frequent genetic abnormality seen in FRDA is the homozygous expansion of a GAA repeat in the FRDA gene. The genetic test for FRDA is particularly useful because different forms of ataxia may be difficult or impossible to distinguish by clinical examination alone. In 1998, the European Molecular Genetics Quality Network (EMQN) organised a pilot External Quality Assessment (EQA) scheme for genetic testing of the FRDA gene with 10 participants from 10 different European Union countries. No sample identification or genotyping errors were recorded, but a small number of errors or omissions of interpretation were identified. Accordingly, a best practice workshop was organised to develop best practice guidelines for FRDA testing. These guidelines are available on the EMQN web site (www.emqn.org). Following the successful pilot, a second scheme was offered in 2000. 34 laboratories including 3 from the USA participated. Results will be reported, and any required amendments to the guidelines identified by this process.

P1082. An External Quality Assessment scheme for genetic testing of Charcot-Marie-Tooth disease

B. Rautenstrauss[1], *J. Lupski*[2], *V. Timmerman*[3]
[1]*Universitat Erlangen-Nurnberg; Erlangen, Germany;* [2]*Baylor College of Medicine, Texas Medical Centre; Houston, United States;* [3]*Universitaire Instelling Antwerpen; Wilrijk — Antwerpen, Belgium*
berndwr@humgenet.uni-erlangen.de

Charcot-Marie-Tooth disease type 1 (CMT1) is a clinically and genetically heterogeneous disorder of the peripheral nervous system. Patients show severely reduced nerve conduction velocities (NCV<38 m/s). CMT type 1A is the most frequent autosomal dominantly inherited form caused by a 1.5-Mb tandem duplication in chromosome 17p11.2-12 comprising the peripheral myelin protein 22 (PMP22) gene. Genetic testing for CMT1A is routinely offered in diagnostic molecular genetics laboratories and performed using a wide variety of methods. The main benefit for patients is avoidance of a sural nerve biopsy for diagnostic purposes. In 1999, a pilot External Quality Assessment (EQA) scheme for CMT was offered by the European Molecular Genetics Quality Network (EMQN). From 14 registered laboratories a total of 11 participated. Following this successful trial, the scheme was offered to a wider audience in 2000 with 20 laboratories participating (23 registered). No genotyping errors were detected in either scheme. The scheme will be described in full and the results for both genotyping and interpretation will be discussed. Lessons drawn from the development of the scheme should help in harmonising laboratory standards for the genetic diagnosis of CMT.

P1083. An External Quality Assessment scheme for genetic testing of Y-Chromosomal Microdeletions

P. Vogt[1], *M. Simoni*[2]
[1]*Institute of Human Genetics; Heidelberg, United States;* [2]*Institute of Reproductive Medice, University of Munster; Munster, Germany*
peter_vogt@med.uni-heidelberg.de

The PCR screening of microdeletions of the Y chromosome has become an important diagnostic step in the workup of male infertility. However, there is still uncertainty about how this diagnosis should be performed. There are suggestions that the large variation in deletion frequency reported in the literature (varying between 1 and 55%) could be due to the various selection criteria of the patients analysed, but methodological aspects could also play a role. Like other genetic diseases, the overall quality of molecular diagnosis of Y-chromosomal microdeletions should be controlled by adopting strict internal quality control measures and by participating in external quality assessment (EQA) schemes. Such an external quality assessment project was started on the initiative of the European Academy of Andrology (EAA). Three preliminary trials involving 29 European laboratories have given a state-of-the-art picture of the overall diagnostic performance, showing an overall rate of misdiagnosis of about 5% for both the AZFb and AZFc regions. In collaboration with the European Molecular Genetics Quality Network (EMQN) the data from the preliminary trials were used to generate the first guidelines for molecular diagnosis of Y-chromosomal microdeletions and were presented at a best practice workshop held in the spring of 2000. An official EQA programme for Y-chromosomal microdeletions was then initiated as a joint initiative of the EAA and the EMQN. The first EAA/EMQN trial EQA scheme started in October 2000 and includes 57 participants from 20 countries. The results will be presented.

P1084. A rare homozygous rhodopsin splice site mutation ; To test or not to test, is there a question?

L. J. Greenberg, *L. Roberts, R. Ramesar*
University of Cape Town; Cape Town, South Africa
JG@ANAT.UCT.AC.ZA

Mutations in the rhodopsin gene can cause both dominant and recessive retinitis pigmentosa (RP), a major cause of genetic blindness. Most changes are in the coding regions of the gene, although some splice site mutations have been described. A homozygous IVS4+1G-T mutation in the rhodopsin gene has been detected in a South African (SA) patient with RP. A low incidence of RP amongst heterozygous carriers of this mutation has been reported but it is unclear whether the mutation results in a dominant or recessive allele. To our knowledge, this mutation has never been reported in the homozygous state. Analysis of the extended family indicate no obligate heterozygous carriers of this mutation with any obvious loss of vision. These findings were made during a screening programme of SA patients with inherited retinal degeneration. The question is now whether

to inform the family and should all the potential heterozygous carriers be investigated. Knowledge itself without the ability to act on it, may be comforting to some, but may simply aggravate the situation for others. An awareness of the predisposition to genetic diseases may have negative consequences for asymptomatic individuals. This could also result in discrimination if this information is available to employers and insurance providers. Information diffuses rapidly and its consequences are felt beyond the presenting patient. Children specifically are passively affected. It may be decades before the absolute value of genetic testing can be proposed with any degree of certainty. To test or not to test, even when and who to test therefore remains an unresolved question.

P1085. Multiehnic Thalassemias And Haemoglobin Hereditary Disorders In Italy

P. Primignani, *M. Travi, C. Curcio, A. Biasi, D. A. Coviello, A. Cant Rajnoldi*

Molecular Genetic Laboratory, Istituti Clinici di Perfezionamento; Milan, Italy

coviello@unige.it

The world distribution of beta and alpha thalassemias is well documented but adequate data on gene frequency defects are available only for a small number of populations. Beta disease is common throughout the Mediterranean regions, Middle East, India, Southern Asia starting in Southern China, stretching through Thailand, Cambodia, Laos and Malaysia peninsula. Alpha disease is extremely common in part of Africa, Mediterranean region, Middle East, Southern Asia and Pacific islands. Multiethnic Italian reality in the last five years required new molecular approach to cover different mutation patterns and to achieve full DNA information in couples at risk. In 1997 foreign people with regular permit were more than 2.000.000 but the number does not include children and clandestines (the last more than 1.000.000). Moreover, in comparison with France and Germany where immigration is homogeneous, the phenomenon in Italy is characterized by a great heterogeneity, infact a part of immigration in Italy during the last years arrived from North Africa, Senegal, Albania, ex Yugoslavia, Romania, Poland, Bulgaria, Philippines and China. In North Italy actually lives more than 5% of immigrants, many of them are at risk for thalassemias or hereditary haemoglobinopathies such as Hb S and a great increase of this phenomenon is expected in the near future. Screening programs for prevention of these diseases, and prenatal diagnosis have determined in a marked reduction in the birth rate of affected children. The practical problems associated with genetic screening of beta thalassemia are now a minor problem thanks to the new molecular technologies and, on the other hand, alpha thalassemia gene defects are partially but more easily detectable by PCR protocols. In our 15 years activity we performed 1765 DNA prenatal diagnoses of Thalassemias and hereditary Haemoglobinopathies. Since 1995 foreign people began to ask counseling for haemoglobin disorders to our Centre (estimate frequency = 6%). The geographic origin is the following; Africa (3,2%), Asia (0.7%), East Europe (1.3%) Middle East (0.3%) and South America (0.5%). Since the phenomenon is increasing in relation to the establishment of permanent family groups we need to adapt the screening program to the new ethnic Italian distribution and we look forward to organize a more appropiate genetic counselling that include; Easy and wide educational programs on the territory. Easy and multiethnic PCR protocols to achieve molecular information in couple at risk for different thalassemia syndromes. Easy and convenient access to the hospital or genetic departments thank to the use of translators and guide lines on original language.

P1086. Mutation analysis of the MECP2 gene in Italian Rett Patients using denaturing high performance liquid chromatography.

M. Carella[1], *R. Cattin*[1], *B. Giometto*[2], *B. Tavolato*[2], *M. L. Giovannucci-Uzielli*[3], *C. Lievore*[1], *A. Marin*[1], *P. Gasparini*[4], *P. Nicolao*[2,1]

[1]*Department of Medical Genetics, Baschirotto Institute for Rare Diseases (BIRD EUROPE onlus); Costozza di Longare (VI), Italy;* [2]*Department of Neurological and Psychiatric Sciences, Second Neurological Clinic, Padua University; Padua, Italy;* [3]*Genetics and Molecular Medicine Unit, Department of Pediatrics, University of Florence, Azienda Meyer; Firenze, Italy;* [4]*Medical Genetics Service, IRCCS - CSS Hospital; San Giovanni Rotondo (FG), Italy*

carella@tigem.it

Rett Syndrome is an X-linked dominant neurodevelopmental disorder characterized by several clinical abnormalities, such as progressive encephalopathy, autistic behavior and stereotyped hand movements. Classical RS features seem to occur almost exclusively in females, with an estimated prevalence of one in 10,000-15,000 female birth. The vast majorities of Rett cases (99%) are sporadic in origin, and are due to de novo mutations. Mutations in the Methyl CpG Binding protein 2 gene have been identified in roughly 75 % of classical Rett girls. We collected DNA samples from 42 Italian classical Rett girls, and screened the MECP2 coding region for mutations by Denaturing High-Performance Liquid Chromatography (DHPLC), and subsequent direct sequencing. DHPLC is a recently developed method for mutation screening combining high sensitivity, reduced cost per run and high throughput. In our study, 16 different de novo MECP2 mutations, seven of which were previously unreported, were found in 32/42 Rett girls (76%). Seven recurrent mutations were characterized in a total of 22 unrelated cases. Initial DHPLC screening allowed the identification of 14/16 different mutations (87%); after optimal conditions were established, this figure increased to 100%, with all recurrent MECP2 mutations generating a characteristic chromatographic profile. Standardization of DHPLC reagents and methods seems to be critical to the reliability and accuracy of mutation prediction. Detailed analysis of signature shape, retention time, and retention time differences between peaks will allow signature-based sequence identification, at least for the most common MECP2 mutations and SNPs.

P1087. The mutation rates of ten human autossomal microsatellites

A. M. L. Vercesi[1], *S. D. J. Pena*[1,2]

[1]*Depto. Bioqu mica e Imunologia - Universidade Federal de Minas Gerais; Belo Horizonte, Brazil;* [2]*N cleo de Gen tica M dica de Minas Gerais - GENE/MG; Belo Horizonte, Brazil*

averc@mcafeemail.com

There is consensus that microsatellites evolve by stepwise mutations and that the mutation rate is positively correlated with repeat size. However, there is disagreement about the influence on the mutation rate of the size and nature of the repeat motif, GC content of flanking regions, influence of heterozigosity and direction biases. Molecular studies of germinative mutations are needed to elucidate their mechanisms Here we present mutation data on ten new human microsatellite loci that have been chosen because they have average heterozigosity > 0.6, different amplicon sizes that permit multiplexing (all ten loci can be amplified in a single PCR reaction and analyzed in the same electrophoretic run using a single fluorophore). We analyzed 27,086 parent/child allelic transfers selected from paternity cases. We observed 26 instances of father-child mismatches in cases where fatherhood had been otherwise proven. Cases due to null alleles were verified and removed from the study group. The average mutation rate was 10-3 per locus per meiosis but there was considerable heterogeneity according to sex and locus. The average paternal mutation rate was 1.3 X 10-3 and the average maternal mutation rate was 0.4 X 10-3. No mutations were observed in three loci, while ten different mutations were seen in one single locus (D10S1237). All mutations were sequenced. The vast majority (92%) were single step mutations. In general our results are concordant with previous family studies of microsatellite studies in humans and increase considerably the database of well-studied human mutational events on which to test mechanistic hypotheses.

P1088. Molecular detection MTM1 gene mutations in Myotubular Myopathy Using DHPLC

M. R. D Apice[1], *A. De Luca*[1,2], *E. Flex*[1,2], *B. Dallapiccola*[2,3], *G. Novelli*[1]

[1]*Dip of Biopathology and Diagnostic Imaging, Tor Vergata University; Rome, Italy;* [2]*CSS Mendel Institute; Rome, Italy;* [3]*Dip of Sperimental Medicine and Pathology , La Sapienza University; Rome, Italy*

dapice2@yahoo.it

X-linked myotubular myopathy (XLMTM; OMIM# 310400) is a severe congenital muscle disease caused by mutations in the myotubularin gene. This gene encodes for a dual specific phosphatase belonging to a large gene family highly conserved through evolution. To date, 133 mutations distributed in all exons of the MTM1 gene have been identified in a large number of families. Since the majority of MTM1 mutations are private and rare, their detection is difficult and labor-extensive using traditional screening approaches. We set-up and formatted a denaturing high performance liquid chromatography (DHPLC) method to detect MTM1 mutations in all coding exons of the gene. Our protocol has revealed the pathogenic mutation in 8 XLMTM patients screened for the entire coding sequence of the gene. DHPLC identified 7 point mutations previously described [R37X, T(191-11)A, 646-647insA, T197I, R253X, G378R, G402R]. In addition, we identified two novel mutations, one in exon 8 [P199S] and one in the donor splice site of exon 14 (1644+2)insG. In conclusion, we provided that DHPLC analysis is a sensitive and specific method to detect mutations in MTM1 gene.

P1089. Towards an efficient and sensitive molecular genetic test for Neurofibromatosis type 1 (NF1)

L. M. Messiaen, *T. Callens, G. Mortier, N. Van Roy, F. Speleman, A. De Paepe*
Center Medical Genetics, University Hospital; Gent, Belgium
Ludwine.Messiaen@rug.ac.be

Although NF1 is one of the most common autosomal dominant disorders with 50% of patients presenting as de novo cases, efficient and sensitive mutation analysis of the NF1 gene is still not available for clinical use. We developed a combined approach starting from short-term cultured T-lymphocytes leading to detection of NF1 mutations in 95% of typical NF1 patients, fulfilling the N.I.H. diagnostic criteria. The cascade of analyses includes; 1) protein truncation test (PTT) followed by cDNA and gDNA sequencing of the region of interest; 2) intragenic microsatellite analysis followed by FISH to detect total gene deletions and/or by sequencing of the complete cDNA to detect missense mutations; 3) Southern blot and cytogenetic analysis. So far 112 bona fide mutations were identified in 121 unrelated patients. For 9 patients, steps 2 and 3 of the cascade are still ongoing. All mutations were characterized at the genomic and RNA level. Using the PTT only, the mutation was identified in 100 patients and the mutational spectrum consisted of 26 frameshift, 40 nonsense and 34 splicing mutations, 25 of the latter caused by alterations outside the canonical AG/GT splice sites. Furthermore, 8 missense or small in frame deletions, 3 total gene deletions and 1 translocation t(14;17)(q32;q11.2) were found by steps 2 and 3 of the cascade. Our approach may be helpful in establishing the diagnosis of sporadic patients at an early age or not totally fulfilling the N.I.H. diagnostic criteria and in providing the means to prenatal or preimplantation diagnosis, especially in sporadic patients.

P1090. Genotype-phenotype-diversity in familial cylindromatosis; A mutation in the tumor suppressor gene CYLD underlies multiple tumors of skin appendages.

J. Frank
University Clinic of the RWTH Aachen; Aachen, Germany
jorgefrank@yahoo.com

Familial cylindromatosis (Brooke-Spiegler-syndrome) (OMIM numbers 123850; 313100) is a rare skin disorder, inherited in an autosomal dominant fashion and usually presenting in the second or third decade of life. With female preponderance, dermal tumors predominantly arise in hairy areas of the body with approximately 90% on the head and neck, rarely on the face or trunk. Transformation to malignancy seems to be rare. Recently, the susceptibility gene CYLD which reveals the genetic attributes of a tumor-suppressor gene (recessive oncogene) has been localized to chromosome 16q and mutations have been identified in affected families. Here, we studied the molecular basis of familial cylindromatosis in a multigeneration family of German origin. Using PCR, microsatellite analysis, heteroduplex analysis, and automated sequencing, we identified a frameshift mutation in the CYLD gene in several individuals. Mutation analysis further allowed exclusion of the tumor predisposition in a 6-year old boy. Interestingly, in several family members with identical genotype we observed a distinct phenotype. While some individuals revealed discrete small skin colored tumors localized in the nasolabial region, one family member showed expansion of multiple big tumors on the trunk and in a turban like fashion on the head. The reasons for different clinical expression patterns of the same genetic defect in this disease remain elusive. Identification of the CYLD gene and modern genetic techniques enable us to rapidly confirm putative diagnoses on the genetic level, to give a prognosis for family members at risk for developing multiple tumors, and to offer genetic counseling for affected families.

P1091. National Genetic Service In Bulgaria

I. Kremensky, *S. Andonova, A. Ashikov, V. Chorbov, B. Georgieva, M. Ivanova, N. Ivanova, S. Jankova, A. Jordanova, R. Kaneva, E. Michaylova, R. Petkova, T. Petrova, A. Savov, A. Todorova, B. Zaharova*
Laboratory of Molecular Pathology; Sofia, Bulgaria
kremensk@ns.medfac.acad.bg

Since 2000 year in Bulgaria started a National Programme for Prevention and Prophylaxis of Inherited Disorders. It is organized in five regional centers. In four of them only genetic counseling and postnatal cytogenetic diagnostic is performed. All other activities are concentrated in the genetic center of Sofia. Here, based on functional criteria, several laboratories are involved; Laboratory for Biochemical Genetics (performing mass screening for PKU - 70 000 newborns, selective serum screening for Down syndrome and neural tube defects in first and second trimester, selective screening for galactokinase deficiency- 15 000 newborns); DNA laboratory for pre- and postnatal diagnostic of the most common monogenic disorders (CF, b-thalassaemia, DMD/BMD, SMA, CMT, PKU, LGMD2C, MCAD, Wilson disease, Haemophilia A and B, Down syndrome); Laboratory for Molecular Cytogenetic analyses (performing about 1 500 postnatal and 500 prenatal tests annually) and a team of 6 ultrasound specialists dealing with diagnostics of chorion villi sampling, amniocenteses, cordocenteses and prenatal diagnostics of malformation syndromes. For a small country with limited resources the centralized model of genetic services offers a series of advantages. The same structure, communications and genetic register are used for the different screening programs with the same analytic technologies for most of the cases.

P1092. Primary Health Care Approaches for the Control of Congenital Disorders.

A. L. Christianson[1], *B. Modell*[2], *V. Boulyjenkov*[3], *A. Alwan*[3]
[1]*Dept of Human Genetics, University of Pretoria; PRETORIA, South Africa;* [2]*Dept of Primary Care and Population Sciences, RF and UC Medical School; London, United Kingdom;* [3]*WHO; Geneva, Switzerland*
christal@medic.up.ac.za

Global decline in infant mortality and birth rate, especially in developing nations, is shifting the focus of health policy from acute conditions of environmental origin towards the management of chronic diseases and disability. This change is notable in the areas of reproductive and child health. An increasing proportion of infants born with congenital disabilities are now being diagnosed, surviving and thus constituting an increasing health burden. This health burden could be greatly reduced by deploying approaches outlined in the 1985 WHO definition of a control programme for a congenital disorder. These approaches also offer people with genetic disadvantage choices that enable them to live and reproduce as normally as possible in their circumstances. However, no comprehensive model of these interventions is available. Consequent on a previous WHO Eastern Mediterranean Regional Office (EMRO) initiative a WHO Advisory Group was convened in Cairo in December 1999 to make recommendations on Public Health Approaches for the Control of Genetically Determined Disorders and Birth Defects in Primary Health Care . The global epidemiology and health burden of congenital disorders was reviewed. It was recognized that medical management of these conditions entailed costs that escalated exponentially as patient survival increased and this required to be counterbalanced by effective prevention programmes. These included basic primary health care reproductive health approaches, including accessible family planning, adequate diets and micronutrient supplementation, prevention and management of maternal infections and avoidance of teratogens. Implementation of such programmes can reduce the birth prevalence of congenital disorders to a baseline level, with further reduction then depending on population screening programmes to identify individuals at increased risk of having children with specific disorders. As no standard set of interventions is universally applicable, the Advisory Group developed a package of recommendations that a country could contemplate implementing according to its needs and circumstances. It further proposed that countries could form a national task force to develop a national plan utilizing baseline epidemiological data. A draft protocol and operating manual, developed from a second EMRO initiative, were provided. Such a plan would require systems for education and audit, and could be implemented following a pilot project. The need for technical support from selected experts and relevant WHO programmes in a long-term working relationship was foreseen.

P1093. Use of short tandem repeats (STRs) for diagnosis of the Charcot-Marie-Tooth 1A duplication

A. Vandenberghe[1], *P. Latour*[2], *N. Levy*[3], *R. Bernard*[3], *A. Boyer*[3], *L. Boutrand*[4], *F. Claustrat*[2], *G. Chazot*[2], *M. Boucherat*[2]
[1]*Faculty of Pharmacy; Lyon, France;* [2]*Neurog n tique Mol culaire, Laboratoire de Biochimie, H pital de l Antiquaille; Lyon, France;* [3]*D pt. G n tique M dicale, H pital d Enfants de la Timone; Marseille, France;* [4]*D pt. G n tique Mol culaire et Biochimie Clinique, Facult de Pharmacie; Lyon, France*
vandenbe@rockefeller.univ-lyon1.fr

We tested a number of repeat sequences identified from sequenced BAC clones covering 100% of the PMP22 duplicated region (MIT Center for Genome Research, Cambridge MA, USA) for polymorphic behavior. We selected sequences with (i) five or more tandem repeats and (ii) possessing neighboring sequences suitable to identify unique primers for specific amplification. Ten such repeat sequences (4 tri-, 4 tetra- and 2 pentanucleotide repeats) were amplified by PCR using standard conditions. Three of this short tandem repeats (STRs) were polymorphic and found suitable for genotyping. The heterozygosity for normal controls is between 0.69 and 0.80. Less than 1% of healthy controls are non informative for the 3 STRs. The PCR products show clean amplification without stuttering bands. The separation of the different alleles is accurate on non denatur-

ing polyacrylamide gels, and allows economical silver staining detection. When duplicated, dosage differences between alleles are observed. The 3 STRs cover about 0.5 Mb of the central part of the CMT1A duplication and enclose the PMP22 gene. Until now, we found 9 CMT1A families which showed normal CMT-REP profiles. Very interestingly the most distal of the STRs revealed the duplication in all 9 families. The frequency of CMT1A duplication with normal CMT-REP profiles is 2.0% in a series of 480 duplicated CMT 1A patients. Genotyping with these new STRs now definitely replaced other polymorphic systems (RFLPs and poly(AC) repeats) in our routine diagnosis. An automated genotyping format using 2 different fluorescent labels was successfully tested.

P1094. Frequency of Fragile X syndrome in South Indian Mental retarded patients of un known etiology

G. N. Mallikarjuna Rao[1], *A. Jyothy*[1], *M. Swarna*[2], *M. Sujatha*[2], *M. P. J. S.Ananda Raj*[2]
[1]*Institute of Genetics and Hospital for Genetic Diseases; Hyderabad, India;* [2]*Institute of Genetics and Hospital for Genetic Diseases; Hyderabad, India*
MALLIKGENE@REDIFFMAIL.COM

Fragile X syndrome is the most common form of inherited mental retardation (MR) with frequency of 1 in 4000 males and 1 in 8000 females. The frequency of fragile X syndrome in mentally retarded males ranges from 0 to 11 %. Our study is based on 101 patients of Mental retardation of unknown etiology referred from various hospitals in Andhra pradesh and neighboring states. Cytogenetic analysis was done to identify fragile site at Xq27.3 using folate deficient medium(TC199). Molecular diagnosis was done using PCR and southern blot analysis.The full mutation of the FMR1 gene i.e >200 CGG repeat expansion at the 1st exon of this gene cannot be amplified by PCR method. The un amplified samples were subjected to southern blot analysis using Ecor1/Eag1 double digest and probing with Stb12.3 probe. 7 unrelated patients out of 101 patients of MR were of full mutation (>200 repeat) thus the frequency of fragile x syndrome was 7%. this study helped identify the Fragile X patients among non specific MR patients and also in developing strategies for definite diagnosis of this syndrome

P1095. Genetic Evaluation Of Male Infertility In Venezuelan Patients

T. Aguirre, *M. M. Piras, R. Sotelo, L. Lozano, F. R squez, M. E. P o, A. Kovac«s, A. Sosa, I. Reyes, A. Tr as, R. Piras*
Centro M dico Docente La Trinidad; Caracas, Venezuela
taguirre@reacciun.ve

The availability of Assisted Reproduction Techniques, like ICSI, allows procreation by males with severe infertility problems. The knowledge that genetic abnormalities account for many cases of idiophatic infertility, led us to evaluate a group of Venezuelan infertile patients. In 1998, we began a genetic evaluation program which includes Y chromosome microdeletions by PCR, mutational analysis of the CFTR gene (cystic fibrosis) and, in selected cases, karyotype. To date, we have evaluated a group of 190 consecutive patients with a range of indications for ICSI, this group included azoospermic/oligozoospermic men. Y chromosome microdeletions were found in 4 patients (one in AZFb and three in AZFc). Abnormal karyotypes were identified in 4/65 (6%) of the patients (two 45, XY t(14;21), one 47, XXY and one 47,XYY). Mutation analysis of the CFTR gene resulted in one patient compound heterozygote for Delta F508/R117H and five patients heterozygote for one mutation in the CFTR gene (two R117H/-, two DeltaF508/- and 621+ G to T/-). These results demonstrate that genetic factors are involved in some cases of infertility in Venezuelan males, with a frequency (7 %) comparable to that observed worldwide. Therefore, in our population, a complete genetic evaluation is highly recommended before the ICSI procedure.

P1096. Sensitivity of DHPLC for the detection of low level mosaicism in tuberous sclerosis

K. Mayer, *S. Schirdewahn, H. D. Rott*
Institute of Human Genetics; Erlangen, Germany
kmayer@humgenet.uni-erlangen.de

In tuberous sclerosis complex (TSC) parental mosaicism is often observed in a family with more than one affected child from parents without clinical signs of TSC. Parental germ-line mosaicism can be shown by haplotype analyses whereas parental somatic mosaicism is usually detected by a reduced ratio of the mutant-to-normal allele. Direct detection of somatic mosaicism is even more difficult. During mutation screening in TSC we directly discovered two monosymptomatic cases of somatic mosaicism in a total of 30 sporadic patients with a TSC1 or TSC2 mutation. A further case of parental somatic mosaicism was uncovered by family analysis. The two directly discovered mosaics were initially detected through the sensitivity of the protein truncation test (PTT) and promoted by a predominance of the mutated allele in lymphoblastoid cell lines compared to peripheral blood cells. Suspected low level mosaicism in lymphocyte DNA could be proven by cloning of the PCR product and confirmation of a mutation by sequencing individual clones in one case and selective amplification of the mutated allele after enzymatic digestion of the wildtype allele in the two other patients. Direct sequence analysis of peripheral blood DNA failed to confirm the mutations In all three cases no matter what type of labeling (primer or dideoxynucleotides) and what kind of automatic sequencer (LICOR or ABI310) was used. In contrast, the underrepresented mutated alleles could be directly detected by DHPLC analysis through heteroduplex formation. Titration experiments of cloned PCR products could proof a minimal proportion of 5% of the mutated allele. Our report illustrate the importance of considering somatic mosaicism in sporadic TSC cases with clinical symptoms restricted to single organs and the necessity of sensitive methods for their detection.

P1097. Alpha Thalassemia Frequency in S o Jos do Rio Preto, State of S o Paulo, Brazil.

E. C. Schiaveto, *C. R. Bonini Domingos*
Instituto de Bioci ncias Letras e Ci ncias Exatas/ UNESP; S o Jos do Rio Preto, Brazil
manuschiaveto@bol.com.br

The alpha thalassemia characterizes itself for the presence of a fast haemoglobin, with unstable characteristics, forming inclusion corpuscules in the erythrocytes, denominted H haemoglobin. The carrieers present anemia with variable grades, hypochromia, microcytosis and poikilocytosis. The phenotype seriousness is directly related wiht the loose of alpha genes synthesis, being classified according his defect. The goal of the present study was verify the presence of alpha thalassemia in blood samples conducted to the Haemoglobin Labooratory, of the Biology Departement in the Instituto de Bioci ncias Letras e Ci ncias Exatas/ UNESP, in S o Jos do Rio Preto, state of S o Paulo, Brazil, of two groups; newborns and anemia carriers to explain. 712 blood samples of adult individuals and 813 navel cord samples were analysed, in the period f 1998-200, picked with EDTA on 5%, that were submited to a cytological and biochemical tests and a electrophoretic procedures, confirming the presence of Hb H or Hb Bart«s. Among the total analysed, 322 adult individual samples and 139 navel cord samples presented alpha thalassemia. The answer for a «prevention program fr this genetic alteration type must be wisely elaborated, considering the age group that will be studied and the edicative material. A prevention program for this alteration type has a huge importance, since the alpha thalassemias are frequent in the population, because makes possible an improvement in the carrier life quality.

P1098. Diagnoses of Childhood Onset Neuronal Ceroid Lipofuscinoses; Clinical, Pathological, Biochemical, and Molecular Aspects.

W. Ju, *D. Moroziewicz, W. T. Brown, N. Zhong*
New York State Institute for Basic Research; Staten Island, NY United States
omrddzhong@aol.com

Three childhood onset neuronal ceroid lipofuscinoses (NCLs), the infantile (INCL), classical late-infantile (LINCL), and juvenile (JNCL), are underlied by gene CLN1, CLN2, and CLN3, respectively. They are among the most common pediatric neurodegenerative disorders in the United States. Clinically, they all share similar symptoms. Pathologically, lysosomal storage of lipofuscins can be detected with electron microscope. Emzymatically, deficiency of palmitoyl-protein thioesterase 1 (PPT1) and (tripeptidyl peptidase 1) TPP1 can be assayed for INCL and LINCL. Molecularly, more than 100 mutations have been identified, which include 39 from INCL, 39 from LINCL, and 32 from JNCL. Testing five mutations, the c. 223A-G (Y109D) and c. 451 C-T (R151X) in CLN1, c. 622C-T (R280X) and IVS5-1G-C in CLN2, and a deletion of 1.02-kb genomic fragment involving exon 7-8 of CLN3 may detect ~75% of clinically referred chilhood NCLs. Applying enzyme assay and molecular testing to NCL family members, we are able to identify carriers and presymptomatic affected individuals. As well, prenatal diagnostic testing enables to distinguish affected, carrier, and normal pregnancies. Combining clinical information such as the age at onset and initial signs, ultrastructural examination for characterizing lipofuscin profile(s), enzymatic analyses for PPT1 and TPP1, and molecular testing, we are able to distinguish over 90% childhood NCL patients and reevaluate and confirm clinical diagnoses.

P1099. Quality of life in children with trisomy 21 - is an induced abortion inevitable

M. Meyer, *J. Kunze, J. Pelz*
Institut fuer Humangenetik; Berlin, Germany
joerg.pelz@charite.de

In clinical practice it is the obligation of a clinician to treat a patient according to evidence based principles. A quite different situation exists in screening, where healthy individuals are investigated to identify risks of disorders, which justify costs and hazards of further investigation. The situation becomes even more complex, in prenatal diagnosis, where in most cases physicians take action not to treat or to avoid disease but to prevent the birth of a diseased child. This is the ultimate action, which should not be taken by a physician, unless the disease is serious and no effective treatment is available. The antenatal screening for Down syndrome, in case of a positive test, results in the overwhelming majority of case in an induced abortion, a consequence broadly accepted in both the medical and the social community. In a case-control study we investigated different aspects of quality of life in 51 children with trisomy 21 and in 52 control children of their peer group. Parents were personally interviewed with a structured questionnaire. Quality of life in children with trisomy 21 can primarily be defined by good health, an intact and caring family environment, by integration and autonomy. Second-rate are those areas where the disability shows strongest manifestation; somatic, intellectual and communicational development. Many health problems of children with Down s syndrome are treatable with modern medical methods, integration can be facilitated by an appropriate, affectionate and empathic social environment. It seems to be wrong to assume that children with trisomy 21 have a low quality of life. An induced abortion after prenatal diagnosis of trisomy 21 is not justifiable, at least if one takes/respects the viewpoint of the Down syndrome child.

P1100. The integration of human genetics in the realm of medicine

B. Press, *J. Pelz*
Institut fuer Humangenetik; Berlin, Germany
bjoern.press@web.de

Within the different specialities of medicine human genetics is one of the youngest. The introduction of its concepts and its molecular methods has changed the understanding of disease and the possibilities of diagnostics. This development is often seen as a paradigm shift and compared with the introduction of the cellular pathology by Virchow. It has an influence on medical thinking and on the use of basic medical concepts. Nevertheless the position of human genetics in the realm of medicine and its role in the clinical setting is far from being clear and established. The present work analyses the understanding of doctors of tomorrow (1) of human genetics function in the realm of medicine and (2) of terms like prevention , disease and patient in clinical settings related to human genetics. Three groups of students of medicine differing in the level of clinical and theoretical experience (first, third and sixth year of medical education) were interviewed by a structured questionnaire focusing on the above mentioned topics. Human genetics is not seen as a medical discipline in the traditional sense by the interviewees. The role of human genetics within medicine is seen in diagnostics and in science. It lacks the traditional role of many medical specialities; caring and healing, both taking place in the different specialities per se. The clinical application leads to dissension s in the concepts of (1) prevention, which in human genetics is mainly understood as prevention of the ill instead of prevention of illness; (2) the role of patient and physician, which in the setting of prenatal diagnosis is far from clear. This may be one of the problems of human genetics to establish itself as a medical discipline.

P1101. Provision of genetic services ; what do the professional guidelines tell us?

B. Godard[1], *M. Bobrow*[2], *J. Cassiman*[3], *G. Evers-Kiebooms*[3], *D. Coviello*[4], *P. Farndon*[5], *H. K ri inen*[6], *U. Kristoffersson*[7], *M. Pembrey*[8], *S. Raeburn*[9], *J. Schmidtke*[10], *L. ten Kate*[11], *L. Tranebjaerg*[12], *S. Aym* [13]
[1]*University of Montreal; Montreal, PQ Canada;* [2]*University of Cambridge; Cambridge, United Kingdom;* [3]*University of Leuven; Leuven, Belgium;* [4]*Istituti Clinici di Perfezionamento; Milan, Italy;* [5]*Birmingham Maternity Hospital; Birmingham, United Kingdom;* [6]*Family Federation of Finland; Helsinki, Finland;* [7]*University Hospital; Lund, Sweden;* [8]*University College London; London, United Kingdom;* [9]*City Hospital NHS Trust; Nottingham, United Kingdom;* [10]*Medizinische Hochschule; Hannover, Germany;* [11]*Academisch Ziekenhuis Vrije Universit t; Amsterdam, The Netherlands;* [12]*University of Tromsoe; Tromsoe, Norway;* [13]*INSERM; Villejuif, France*
godardb@courrier.umontreal.ca

Our understanding of molecular genetics has created new possibilities to diagnose and to treat genetic disorders. This has increased public awareness of hereditary diseases, and there are an increasing number of families requesting genetic services, and more physicians prescribing genetic tests. Simultaneously, there are demands in European countries to cut down the costs of public health care. All this has created a situation where professional guidelines for the provision of genetic services are needed. In September 2000, an international workshop was organized by the ESHG-PPPC in Helsinki, to formulate guidelines for the provision of genetic services in Europe. Participants were concerned about equal access and effectiveness of genetic services, quality assessment of services, professional education, multidisciplinarity and division of tasks, as well as networking. Participants identified a number of policy areas requiring (international) coordination and the establishment of coherent (international) policies. The purpose of this presentation is to systematically examine the existing professional guidelines, legal frameworks and other documents related to the organization of genetic services, mainly from Europe but also from other international organizations. Since professional guidelines can improve the quality, appropriateness, and cost-effectiveness of genetic services, and can serve as valuable educational tools, it is important to determine to what degree they document the best practice standards, in which areas they may be deficient, and whether there were changes over time. This review is an essential step to lead to the efficiency in use of health care resources and the establishment of common guidelines for all European countries.

P1102. Primary Health Care approache for prevention of Genetic Disorders

A. Samavat
Disease Control Dpartment; Tehran, Islamic Republic of Iran
samavat@orang.net

Iran is a vast country with a iand area about 1.6 million square kilometers and a population size of more than 60 million.Iran also has different ethnic groups. The rate of consanguinous marriages in Iran is high and the IMR(infan mortality rate)is improving and reaching below 40(22).In this situation planning the prevention programme of Genetic Disorders is one of the necessary duty of the Health Autorities in Iran. The primary health care approaches for the prevention and management of Genetic Disorders provids aspecial possibility to plan the prevention programme,for Iran and other developing countries. ThePrenatal Diagnosis is one of the microstructures was set up in PHC system in Iran to prevent the thalassemia.The other microstructures which were set up in the PHC are Lab. M.,Counselling M.,Education M.These microstructuers not only accelerat the prevention programme of thalassemia but also provide paved path for other prevention prgramme of the Genetic Disorders such as Haemophilia,down,.....

P1103. Services for the management and prevention of genetic disorders, birth defects and disabilities in South Africa - successes and challenges

N. Madolo
Department of Health; Pretoria, South Africa
madolN@health.gov.za

Medical genetic services are integrated into maternal, child and women s health services at national and provincial level in South Africa. The national office, in collaboration with a task team nominated by each of the nine provinces, and funded by the World Health Organisation, has developed policy guidelines for the management and prevention of genetic disorders, birth defects and disabilities. These guidelines outline services which should be offered prior to conception, during pregnancy, at birth, in infancy and childhood, as well as in adolescence and adulthood. Services to be offered at the community, district, regional, provincial, and national levels are outlined as well as the training needs of personnel at each of these levels. Capacity for the implementation of these guidelines varies greatly within the provinces and support has to be provided by the national office and the academic centres. There are presently only four clinical geneticists, ten genetic counsellors, and approximately eighty nurses with training in genetics for a population of 40 million people. This clearly impacts on appropriate diagnosis and the quality of care that can be offered to individuals with genetic disorders and birth defects. Effective monitoring of birth defects is dependent on the accurate diagnosis and reporting, again highlighting the need for advanced training in genetics. Public awareness of genetic conditions is done in collaboration with non-governmental organisations, which also assist in the implementation of genetic services at the local level. These policy guidelines are a beginning from which appropriate accessible and equitable medical genetic services can be developed in a country with many pressing health priorities.

P1104. 1987 — 2000; Thirteen Years Of DMD/BMD Prevention In Italian Families

S. Tedeschi[1], *M. Castagni*[1], *P. De Leonardis*[1], *F. Lalatta*[2], *V. Briscioli*[2], *D. A. Coviello*[1], *A. Cant Rajnoldi*[1]
[1]*Molecular Genetic Laboratory, Istituti Clinici di Perfezionamento; Milan, Italy;* [2]*Medical Genetic Unit, Istituti Clinici di Perfezionamento; Milan, Italy*
coviello@unige.it

Duchenne Muscular Dystrophy (DMD) and its allelic milder form Becker Muscular Dystrophy (BMD) account for 50% of all muscular dystrophies and are caused by mutations on Dystrophin gene. Deletions and duplications are responsible for two third of cases of affected males and the remaining 30% is caused by point mutations. Disease prevention is performed by carrier detection and prenatal diagnosis (PND). Risk evaluation needs to be defined before performing PND but often women at risk are already pregnant when decide to ask per PND. If deletions or duplications are not detected, either on index case or on at risk women, point mutation analysis should be performed, but since Dystrophin gene is the largest discovered to date in humans (79 exons), point mutation analysis is too time consuming to be performed in PND. Our centre is performing familial studies and prenatal diagnoses of DMD/BMD on Italian population since 1987. Our registry includes more than 1000 family groups, and we performed 586 prenatal diagnoses over a period of 13 years. Our experience combined to technological progress allowed us to set up an easy and fast strategy for accurate carrier detection and PND. Deletion and duplication detection for males and females by Quantitative Multiplex PCR Etidium Bromide stained on 32 exons and PCR-based linkage analysis using very informative STR and RFLP can actually be performed with low costs and good accuracy in 5 days. Of 317 PND carried out on male foetuses, 182 diagnoses of healthy males were confirmed at birth by follow up. Our experience both at laboratory level and at counselling level have improved the quality of the service offered. We observed an interesting evolution of the risk modification during the years with the reduction of pregnancies with foetuses with intermediate risk.

P1105. Gastrochisis and Omphalocele in the Genetics Clinic

P. Hwang, *B. G. Kousseff*
University Of South Florida; Tampa, FL United States
bkoussef@hsc.usf.edu

Since 1960s improved definition, diagnosis, and reporting of gastroschisis (G) and omphalocele (O) have shown that the frequency of gastroschisis is on the increase. While most large studies document this increase, the textbooks in English still state that O (incidence of 1;4,000) is more common that G (incidence 1;6,000). Retrospective analysis of all 36,665 probands/families evaluated at the USF genetics clinic between January 2, 1982 and December 31, 1999 showed 121 patients with G and 98 with O. The higher number of G reversed the customary G; O incidence ratio of 1/6,000;1/4,000. Of the G patients 37/121 had karyotypes, all were normal. In the O cohort 75/98 had karyotypes and 18 were abnormal (24%); 12/18 had trisomy 18, 2/18-trisomy 21, 1/18-trisomy 13, 1/18-del (18p)/i (18q), 1/18-inv (2)(p11q12) mat and 1/18-inv (3)(p13q11) mat. In the G cohort 43/121 (36%) had associated anomalies compared to the O cohort with 67/98 (68%). 5/121 pregnancies with G were interrupted; 2/121-miscarried and there were 2 stillbirths. 33 pregnancies remained with unknown to us outcome (lost to reevaluations). Among the 79 liveborn the prematurity rate was 57%. 11/98 O pregnancies were electively interrupted; additional 6/98 miscarried and 23 remained with unknown outcome. Among the 58 liveborn patients the prematurity rate was 36%. The mortality rate in the G cohort was 9% (7/79) and in the O cohort it was 15% (9/58). This study contributed to several issues in these abdominal defects; G is more common that O, has lower mortality rate, and higher rate of prematurity compared to O.

P1106. The Yq Microdeletions in Iranian patients with Idiopathic Azoospermia

A. Hatami[1], *F. Sahebjam*[1], *K. Kahrizi*[2], *R. A. Nadji*[1], *F. Pourfarzad*[2], *H. Najmabadi*[2,1]
[1]*Karimi-Nejad Pathology and Genetics Center; Tehran, Islamic Republic of Iran;* [2]*University of Welfare and Rehabilitation; Tehran, Islamic Republic of Iran*
maryamneishabury@yahoo.co.uk

Male factor is the cause of infertility in about 50 per cent of infertile couples. Five to twently per cent of infertile men with severe oligozoospermia or azoospermia have Yq microdeletion. Recent studies has shown the presence of 4 regions on the internal 6 of Y chromosome associated with male infertility. These are AZFa , AZFb, AZFc and AZFd which are involved in the process of spermatogenesis. DAZ and RBM are two multicopy gene families which are expressed only in testes and have an important role in spermatogenesis. They are located at AZFc and AZFb respectively. Microdeletions in theses regions cause severe oligozoospermia or azoospermia. In this study 30 STSs were selcted to screen for Yq microdeletions in patients with severe oligozoospermia or azoospermia . Cytogenetic, urologic , hormonal and other organic causes leading to azoospermia and oligozoospermia were ruled out in these patients. Yq microdeletion was detected in 4 out of 16 patients.

P1107. Alpha thalassemia deletion analysis in Iran

M. Neishabury[1], *H. Najmabadi*[1,2], *A. A. Poorfathollah*[3], *N. Amirizadeh*[1], *W. Krugluger*[4]
[1]*University of Welfare and Rehabilitation; Tehran, Islamic Republic of Iran;* [2]*Karimi-Nejad Pathology and Genetics Center; Tehran, Islamic Republic of Iran;* [3]*Iran Blood Transfusion Services; Tehran, Islamic Republic of Iran;* [4]*Institute of Clinical Chemistry, Rudolfstiftung Hospital,; Vienna, Austria*
maryamneishabury@yahoo.co.uk

a-thalassemia is one of the most prevalent haemoglobin disorders in the world. The molecular basis of a-thalassemia are deletions of variable lenght involving one or both a-genes at the a-globin gene cluster. Functional point mutations leading to inactivation of the a-genes are less frequent. So far, no comprehensive population screening for a-thalassemia has been performed in Iran and no molecular diagnostic services are available for this disease. As a result, a considerable number of patients with microcytic, hypochromic anemia and normal Hb A2 levels might be misdiagnosed as silent b-thalassemia or the molecular basis of disease remains unidentified. In this study we have screened 25 Iranian patients randomly chosen from a pool of patients with microcytic, hypochromic anemia and negative results in b-thalassemia genotyping for the 2 most frequent a-thalassemia deletions (-a 3.7, -a 4.2). Analysis was performed using deletion-specific PCR amplification followed by agarose gel electrophoresis of the resulting PCR-fragments. No -a 4.2 deletion was detected. However, 40 % of analyzed cases demonstrated the -a 3.7 deletion, either in the homozygous or heterozygous state. This study suggests, that the -a 3.7 deletion is common cause of microcytic, hypochromic anemia in Iran. The results are in accordance with previous studies, reporting a remarkable high frequency of -a 3.7 in the Middle East. Routine screening for this mutation will improve the molecular diagnosis of anemia in Iran.

P1108. Application of the INNO-LiPA CFTR12 and INNO-LiPA CFTR17+Tn tests for neonatal screening of cystic fibrosis using blood spot samples

C. Van Loon, *I. De Canck, P. Hendrix*
Innogenetics N.V.; Gent, Belgium
connyvlo@innogenetics.be

As large-scale screening of newborns for cystic fibrosis based on the immunoreactive trypsinogen (IRT)/DNA protocol is being considered, there is a need for a DNA method which can simultaneously detect many mutations and is suitable for blood spots. We evaluated the INNO-LiPA CFTR12 and INNO-LiPA CFTR17+Tn tests which detect 29 mutations (and the Tn alleles) and their wild type sequences in the CFTR gene to assess their suitability for routine analysis of blood spot samples. Both tests are line probe assays (LiPAs) based on the reverse hybridization principle. Using these tests, and depending on the country, up to 94% of the mutant alleles can be detected. We defined an extraction method that is easy and rapid to perform. This method was tested with twenty random samples. Results showed that a small adaptation of the multiplex amplification profile of the INNO-LiPA CFTR tests (increase in the number of cycles from 30 to 35) was necessary. After increasing the number of amplification cycles, all DNA samples were successfully amplified. The hybridization results were comparable to those obtained with samples extracted from whole blood. Test results were concordant with data obtained using other mutation detection methods. These results indicate that the INNO-LiPA CFTR12 and INNO-LiPA CFTR17+Tn tests are suitable for the detection and identification of an important number of CF mutations in the CFTR gene using blood spots.

P1109. Steinert s myotonic dystrophy in Romania

D. Coprean[1], *M. Popescu*[2], *A. Bosca*[1]
[1] *Iuliu Hatieganu University of Medicine and Pharmacy; Cluj-Napoca, Romania;* [2] *Horia Radu Center of Neuromuscular Pathology; Valcele, Romania*
dmarineanu@personal.ro

Steinert s myotonic dystrophy (SMD), the most frequent myopathy of the adult, is a multisystemic disease. SMD is characterized by; myotonia, muscular dystrophy, cataract, hypogonadism, frontal alopecia, and ECG changes. SMD is genetically and clinically heterogeneous. SMD, an autosomal dominant disease, is caused by mutations of the myotonin gene

located in 19q13.3. The genetic defect consists in the amplification of the CTG triplet from the region 3 of the myotonin gene. The severity of the symptoms depends on the number of CTG triplet repeats; 3-35 repeats in healthy persons, 50-100 repeats in premutation carriers, 100-2000 repeats in affected patients (2000 or more repeats in severely affected patients). According to the onset age and the severity of symptoms, the following SMD forms are known; adult (classical, onset at 25-35 years, slow evolution), congenital (neonatal, dramatic evolution), infantile (onset at 5-10 years, slow evolution), and minimal (onset after 50 years). This study was performed on the 188 SMD cases admitted to the Horia Radu Center of Neuromuscular Pathology, Valcele, Covasna, in the period 1986-1999. The study methods used were the familial inquiry, completion of the genetic record, reconstruction of pedigrees, and the biostatistical method. The analysis of pedigrees confirms the autosomal dominant transmission of SMD. The SMD cases represent 11% of all muscular dystrophy cases admitted in the same period. We found an increased SMD incidence in the districts Brasov, Sibiu, Timis, and Bihor. This can be explained by the founder couple effect and the different pressure of mutagenic and selection factors.

P1110. Evaluation of a molecular diagnostic assay for the detection of 29 mutations and polymorphisms in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene.

A. Myers[1], *R. Nersesian*[1], *K. E. Colvin*[2], *L. Wang*[2], *M. Macek Jr.*[3], *R. K. Saiki*[1]
[1]*Roche Molecular Systems Inc.; Alameda, CA United States;* [2]*Roche Molecular Biochemicals; Indianapolis, IN United States;* [3]*University Hospital Motol; Prague, Czech Republic*
angela.myers@roche.com

Genetic testing for molecular defects in the CFTR gene has become an important area in laboratory testing. However, because of the large number of documented molecular defects in the CFTR gene, only a small number of laboratories have implemented the techniques necessary to exhaustively analyze a patient s CF locus. Nevertheless, only a relatively small handful of mutations recur at significant frequency and we have developed an assay to test for 25 of these CFTR mutations and polymorphisms recommended by the American College of Medical Genetics (ACMG). The assay is capable of detecting 80-90% of the defective alleles in Caucasian Americans and has a significant detection level in other ethnic groups. This may be sufficiently sensitive for routine carrier screening and for the majority of diagnostic requests. The ability to test for CFTR mutations at the molecular level has already improved the diagnosis of symptomatic patients and expanded the reproductive options for family members of CF patients. The same technology also holds promise of identifying asymptomatic carriers and at-risk couples without family history. Here we report the analysis of 400 clinical specimens that were previously genotyped by other methods (restriction enzyme digestion, PCR mediated site directed mutagenesis, DGGE, or DNA sequencing) using a striped array of 55 immobilized, sequence-specific oligonucleotides designed to detect genetic defects responsible for CF and other CFTR-associated pathologies. The assay includes 24 mutations with frequencies >= 0.1% in Caucasian populations, achieving 80-90% sensitivity and an additional mutation of African origin providing 60-70% coverage among African-Americans. Genomic DNA is amplified in a single 15-plex polymerase chain reaction amplification system followed by detection of the 25 mutations and 4 polymorphisms (intron 8 Tn locus, I506V, I507V, and F508C) using a low-density oligonucleotide probe array. The format of the assay permits the simultaneous screening of many samples for multiple mutations and can be readily modified to combine the detection of the most frequent mutations with other allelic variants common to specific ethnic groups. Expanding the panel of screened mutations only marginally increases the sensitivity of the assay. This molecular diagnostic assay is simple and accurate. The assay identifies most of the carriers and affected persons (high sensitivity) with few false positives (high specificity) and may be used in carrier screening and in conjunction with conventional tests for newborn screening.

P1111. Genetic Testing and Human Resource Management

I. Eckerstorfer
University of Z rich; Z rich, Germany
ingeck@hotmail.com

Abstract Genetic Testing and Human Resource Management Corporations are faced with the fact that employees with unstable health conditions are less resourceful due to their absence from the workplace caused by sickness. Genetic testing seems to be a method in human resource management to find out about genetically caused health problems of employees. As a result, corporations start to use genetic testing as a guiding tool throughout their recruitment procedures for potential candidates. The aim of this primarily investigation is to examine the opinions and expectations of personnel managers of the Migros Genossenschafts-Bund (MGB) in Switzerland regarding the use of genetic testing in human resource management. To carry out this task, questionnaires have been answered by all MGB personnel managers. Secondly, group discussions with these managers, representing 80.000 employees in industrial, service and association sectors, have been conducted. MGB s code of conduct supports reliability, confidence and ethical aspects. The author would like to thank MGB as the first corporation in Switzerland for its visionary permission to this inquiry.

P1112. Quality Assessment Study of Molecular Biological Markers resulting in Recommendations for Quality Guidelines, Technical Standards and New Definitions

I. M. Ambros[1], *J. Benard*[2], *M. Boavida*[3], *N. Bown*[4], *H. Caren*[5], *V. Combaret*[2], *B. De Bernardi*[6], *O. Delattre*[2], *M. C. Favrot*[2], *N. Gross*[7], *C. Hattinger*[1], *P. Kogner*[8], *R. Ladenstein*[1], *A. Luegmayr*[1], *J. Lunec*[4], *T. Martinsson*[8], *K. Mazzocco*[6], *J. Michon*[2], *S. Navarro*[9], *R. Noguera*[9], *A. D. J. Pearson*[4], *U. P tschger*[1], *S. Rumpler*[1], *F. Speleman*[10], *G. P. Tonini*[6], *N. Van Roy*[10], *P. F. Ambros*[1]
[1]*CCRI, St. Anna Kinderspital; Vienna, Austria;* [2]*France;* [3]*Portugal;* [4] *United Kingdom;* [5]*The Netherlands;* [6]*Italy;* [7]*Switzerland;* [8]*Sweden;* [9]*Spain;* [10]*Belgium*
ambros@ccri.univie.ac.at

Background; Genetic criteria become increasingly important in the treatment stratification of pediatric tumors. Amplification of the *MYCN* oncogene is one of the most powerful markers indicating an aggressive tumor behavior and is consequently used for therapy stratification. A quality control study was initiated by the ENQUA Group to guarantee reliable and uniform quality of *MYCN* data and of other genetic features, the prognostic impact of which is evaluated prospectively.
Methods; A panel of twelve coded tumor specimens was analyzed in eleven laboratories from nine European countries. Southern blot (SB), PCR and FISH were used for *MYCN* and chromosome 1p36.3 evaluation and flow FCM/ICM for tumor cell ploidy assessment.
Results; Altogether, 350 investigations were performed leading to 23 divergent findings (8/160 MYCN, 9/114 1p36.3 and 7/42 ploidy results). 34 results were judged as not evaluable. No or incorrect determination of the tumor cell content turned out to be one of the biggest problems leading to results based on the investigation of normal cells. After discussions and reviews of the tumor samples, 17 of 23 differing findings were judged as false results. The diagnostic errors were lowest for MYCN analyses determined by SB (3.7%) and FISH (4.1%), and highest for ICM (17.3%).
Conclusions; This study demonstrated the difficulties and limitations for each technique and the problems in the evaluation and interpretation of the results. Moreover, it led to technique standardization and to the development of a common language for the interpretation of the molecular-genetic results which is based on uniform and unequivocal definitions.
The project has been funded by the DGV, No. 98 36220

P1113. Non-paternity and Social Policy

P. Millar
University of Calgary; Calgary, AB Canada
pmillar@cadvision.com

The issue of non-paternity, where the presumed father is not the biological father, has become more prominent in recent years because of the reliability and reduced costs of DNA-based testing and because of the increased emphasis that governments have been placing on child support and its enforcement. This paper reviews the dimensions of the issue or non-paternity, its impact on child outcomes and rights, and the implications for medical, legal and social policy.

P1114. Computational systems for Comparative Genomics

C. Suter-Crazzolara, *G. Kurapkat*
LION bioscience; Heidelberg, Germany
suter@lionbioscience.com

Current world wide genome projects confront scientists with a wealth of sequence information. Both the sizes and the number of biological databases grow exponentially. Intelligent systems are needed to bridge the widening chasm between data collection and interpretation. To interpret data from newly sequenced pro- and eukaryotic genomes, comparative genomics is rapidly gaining importance. Even distant organisms encode proteins with high sequence similarity. Gene order in different genomes is also well conserved. We have employed both these observations to create a versatile computational genome comparison system (genomeSCOUT“). This application can be used for projects spanning

from the identification of drug targets or the optimization of organisms for biotechnology, to in depth characterization and encyclopedic annotation of completed or partial genomes. This application is based on the well established data integration system SRS [Etzold, T. et al., 1996. Methods Enzymol. 266; 114-128]. In a first step, information about gene order and protein homology (e.g. homologs, orthologs and clusters of orthologous groups, COGs) is efficiently collected and automatically stored in five separate databases. Next, these databases are queried interactively to carry out the actual genome comparisons. Key benefits; 1. High speed handling of large genomic data sets. The most complex queries give results instantly. 2. User friendly graphical representations of search results, 3. Unique, SRS-based linking functions between all databases result in the accumulation of a wealth of biological information, 4. Reliable addition of genomes is straightforward, and 5. High flexibility allows effortless integration of additional public or proprietary databanks and applications. (http;//www.lionbioscience.com/)

P1115. Genomes and Gene Names

***M. W. Wright**, E.A. Bruford, H.A. Wain, M.J. Lush, E. Stupka, R. Lovering, M.S. Povey.*
HUGO Gene Nomenclature Committee, UCL; London, United Kingdom
nome@galton.ucl.ac.uk

We have recently increased our resources to enable us to tackle the task of assigning symbols for the whole of the human genome. Despite calls to give each gene a unique reference number, there is still a need for symbols for genes, especially when they are discussed in the literature. Standardised gene nomenclature is an essential resource and the HUGO Gene Nomenclature Committee (HGNC) is committed to supplying unique gene symbols and names for the ever growing number of human genes. Designations describing structure, function or homology are preferred where possible. We work closely with authors and researchers in each field, online databases such as LocusLink and EnsEMBL, and other species specific databases, in particular the Mouse Genome Informatics Nomenclature Committee. We have already assigned symbols for the finished sequences for chromosomes 22 and 21 (Dunham et al. 1999; Hattori et al. 2000),providing a total of nearly 600 approved symbols, and have recently been working on new data sent to us by various sequencing centres. Indeed, for the chromosome 21 publication we were able to ensure that 88% of the genes (including pseudogenes) had approved symbols. With the ever-increasing use of electronic databases this will greatly aid future information retrieval for all interested in the human genome. The HUGO Gene Nomenclature Committee webpage can be found at URL http;//www.gene.ucl.ac.uk/nomenclature/ and can be contacted via email; nome@galton.ucl.ac.uk

P1116. The Mouse Genome Database (MGD); A Comprehensive Biological Information System for the 21st Century http;//www.informatics.jax.org/)

***L. J. Maltais**, J. A. Blake, J. E. Richardson, J. A. Kadin, C. J. Bult, J. T. Eppig, M. G. D. Staff*
The Jackson Laboratory; Bar Harbor, ME United States
ljm@informatics.jax.org

The laboratory mouse is the premier model for understanding human biology and disease. Much contemporary research focuses on comparative analysis of mouse and human sequence data with emerging function of biology implications. Thus, annotating the mouse genomic sequence and integrating biological knowledge are of vital importance to the scientific community. The Mouse Genome Database (MGD) is the community database for comprehensive information on the mouse. MGD provides a full range of information including; genomic, structural, and phenotypic data on gene definition, identification, classifications and nomenclature; genetic, cytogenetic, physical, and (species) comparative maps and data supporting those maps; clones/ESTs/probes/PCR characterizations and sequence links for DNA and proteins; allelic polymorphism data; mammalian homologies for model organisms and domestic species; and descriptive phenotypic information for genes, mutations, and mouse strains. MGD is interconnected with other online resources such as SWISS-PROT, LocusLink, GenBank, , PubMed, OMIM, and databases for other species, including GDB, RatMap, SheepBase, PiGbase, BovMap, FlyBase, etc. Two new features have recently been added to MGD. The first is the use of controlled vocabularies for the description of the molecular function, biological process and cellular component of gene products as part of the Gene Ontology (GO) project. These terms can be used as attributes of gene products across species contributing to the development of comprehensive comparative maps and facilitating queries across multiple databases. The second recent feature of MGD is allele description enhancements, developed, in part, to support the explosion in new mutant allele discovery from mutagenesis projects and gene targeting efforts. Allele records now include information on inheritance and the molecular mutation involved, and are being annotated with phenotypic data and human disease model information. All allele data are fully integrated with sequence, ortholog, gene expression, and strain polymorphism data. MGD is supported by NIH grant HG00330

P1117. Turbogene; An access tool to genetic web resources.

***J. Granados Riveron**, L. Machuca Tzili*
Hospital General de Mexico, Facultad de Medicina UNAM; Mexico City, Mexico
tadeo61@yahoo.com

Genetics has become one of he most rapidly expanding sciences. It is very difficult keeping up to date using conventional methods such as reviewing printed journals, catalogs, or scientific textbooks. The use of electronic publications and databases have therefore became essential for geneticists. Information obtained owing to Human Genome Project and research efforts around the world is stored and classified in a growing number of databases that are accessed through the Internet. In addition, many of the scientific journals focused in genetics have generated their own Web sites, from where it is possible to have access in variable degree to its content. However, recent rapid proliferation of these and other kinds of web resources for geneticist may confuse new potential users. We are presenting a new web site, called Turbogene. The purpose of our site is to provide a powerful access tool to genetic web resources. The current content of the site is divided in several main categories; Journals, Databases, Software and Societies. The Journals option displays a listing of links for over 120 scientific journals whose main interest is focused to genetics and molecular biology. The Databases option includes over 50 links, subdivided in the following categories; Inherited Disorders, Dysmorphology, Cytogenetics, Genomics, Mutations, Proteins, Enzymes, Mitochondrials, Laboratory resources and Support groups. The Software option is a catalog of programs used to pedigree analysis, primers design, sequence analysis and linkage analysis. The content of the site is updated every week. The URL of Turbogene is; http;//members.fortunecity.com/turbogene.

P1118. The BioKnowledge“ Library — A Multispecies Knowledge System for Protein Annotation

K. E. ONeill
Proteome Inc.; Beverly, MA United States
keo@proteome.com

With a growing number of genomes close to completion, Proteome s BioKnowledge Library “ provides a unique resource built by comprehensive curation of the research literature for model organisms, S.cerevisiae (YPD “), S.pombe (PombePD“), C.elegans (WormPD “), C.albicans (PathoPD “), and for human, mouse, and rat proteins (HumanPSD“). Using the sequenced genomes as frameworks, our curators collect protein knowledge currently scattered throughout the literature, and put it into a concise format. The information is available in both a highly navigable format, and a relational format which allows for convenient data mining. Applications for the BioKnowledge Library“ include genome annotation, comparative genomics, interpretation of functional genomics, proteomic datasets and disease and pathway studies. The Human Proteome Survey Database (HumanPSD“), a component of the BioKnowledge Library “, contains up-to-date information for over 10,000 known human proteins, 6,000 mouse proteins, and 3,000 rat proteins. An additional component is GPCR-PD “, which represents in depth annotation of the literature for the G-protein coupled receptors, an important class of drug target proteins, their ligands and their associated signaling pathways. As the sequence of the human genome is completed, annotations for predicted human proteins are being added with the intention of creating the complete Annotated Human Proteome. The model organism volumes are freely available to academic researchers, while subscriptions are available to all others (www.proteome.com).

P1119. Annotation of the Human Genome; Use of the Genome as an Organizing Principal for Data Integration

N. Tsinoremas
DoubleTwist Inc; Oakland, CA United States
nickt@doubletwist.com

A rough draft of approximately 90% of the human genomic sequences is now available in the public domain. Interpretation and extraction of knowledge from these sequences represent a challenge for scientific community. In this presentation we will discuss our current efforts to utilize these sequences in order to create the best view of the human genome. In addition, we will present a computational environment that can be used for efficient integration of diverse but relevant biological information. DoubleTwist

provides a system that allows easy and comprehensive analysis against all publicly available human genome sequence data, whether finished or framework sequences. Gene models from the majority of the human genes have been derived. SNP information has been integrated in the genome view, as well as in each gene model. Using a sophisticated JAVA-based viewer, scientists are able to visualize similarities to protein and protein domain signatures, alternative splicing information, SNPs, Mapping information, as well as a comparative view between human and mouse genes. In our view, the human genome serves as a scaffold to integrate different data types including expression, genotyping and pharmacogenomics information. Our system is based on an open architecture paradigm, which allows such easy integration of all the diverse types of information on top of the genomic information.

P1120. Navigation through the space of gene interactions

S. Heymann
Kelman GmbH; Berlin, Germany
sheymann@kelman.de

Even now that the sequencing of the human genome is nearly complete, scientists are still only at the threshold of understanding the functions of individual genes. Kelman offers a high-end solution of bioinfomatics and functional genome research which ensures new levels of data consistency and exploitation. By means of the SPRAB technology we can predict the genetically-determined functional relationships between proteins. Resulting from the in silico protein-protein interaction mapping we are able to construct a GeneNetwork, which provides an indepth understanding of gene interplay, because it involves gene products in all their known molecular versions. So it becomes possible to embed every DNA sequence in a network of other genes to find functional connections, which otherwise wouldn t have attracted attention. This unique approach is now completed by a novel tool for visualization of data and for navigation within the network of physical protein-protein interactions; the NetNavigator. Network and navigation tool are provided with an easy to handle user interface, so offering the opportunity for efficient analyses of the growing amount of functional gene data according to different criteria. The application of Kelman s tools is exemplified here by means of a case study in the field of neuro-degenerative diseases. The computational protein-protein interaction mapping approach uncovered stringent relations between genes involved in neuro-degenerative pathologies with hereditary background.

P1121. Proteomics Analysis using Web resource

G. Kadambi
DoubleTwist.com; Oakland, CA United States
geetak@doubletwist.com

Proteomics seeks to provide structural and functional information for all proteins, specifically referring to individual genomes. The true value of the genome sequence information will be realized when a function is assigned to all encoded proteins. In our effort to provide a complete solution for bioinformatics tools we provide online agents and behind the firewall products. DoubleTwist has partnered with Molecular Simulations Inc (MSI) to provide on-line bioinformatics tools to help in assigning molecular function to protein sequences. FoldSearch is the first available Agent. This Agent is built on MSI s SeqFold technology. And provides an ability to search for homologous sequences when sequence identity is too low for standard sequence search engines. FoldSearch searches a submitted protein sequence with a protein folds library curated from the PDB. The FoldSearch Agent identifies similar protein sequences bases upon protein primary and secondary structure considerations. In my talk I will discuss the software and the scientific details of FoldSearch and its use via the Internet.

P1122. A New Paradigm for Biomarker Identification

M. Xiong
University of Texas-Houston; Houston, TX United States
mxiong@utsph.sph.uth.tmc.edu

The identification of specific targets for diagnostic or therapeutic use has been a principle goal for both clinicians and biomedical researches. So far, in biomedical research, major methods for identification of disease genes is based on positional cloning, and linkage and linkage disequilibrium analyses. In this report, a new paradigm, which incorporate feature selection into gene expression recognition, will be proposed for the discovery of disease genes and biomarkers for diagnosis, drug efficacy and toxicity. The feature selection is defined as selecting a subset of features (genes) out of original candidate features that performs the best under some data mining systems. Since criterion for feature selection is fundamental to the identification of biomarkers, several criteria based on separability and information measures will be proposed. The wrapper, which involves linear discriminant analysis, logistic regression and support vector machines, and filter methods will be studied. Tabu serach algorithms will be utilized to select best subsets of genes according to criterion values. The proposed methods for gene identification will be applied to colon and breast gene expression data sets. The impact of the criteria, various learning algorithms and searching techniques on the classification capacities will be evaluated and the biological implication of identified genes by the proposed methods will be explored using these two data sets

P1123. Data Mining a SQL Database Using Multidimensional On-Line Analytical Processing (MOLAP) Cubes

R. W. Finnegan, *D. Goldman*
NIH; Rockville, MD United States
rwf@mail.nih.gov

We developed a SQL (structured query language) database with phenotype information to support analysis of our single nucleotide polymorphism (SNP) genotyping data as part of our goal to characterize gene variants related to complex diseases. The SQL database was designed containing genotypes, diagnoses and responses to questions requiring the subject to give a discrete rating. A multidimensional on-line analytical processing (MOLAP) database was constructed using the data contained in the original SQL database. The MOLAP database (example here called; Finn) uses a cube structure in place of the two-dimensional tables seen in typical SQL databases. The data in these cubes can be viewed in the form of a pivot table using an Internet browser or a spreadsheet application. The pivot table produced here represents data from the cube in the MOLAP database containing dimensions for genotyping results of the serotonin receptor HTR2a 1438 G > A SNP, subject diagnosis (based on a SCID (Structured Clinical Interview for DSM-IV Axis I Disorders) results)) of alcohol dependence (Dx code = 12) and their rated response to the question; How often do you drink alone? (depicted as alone, 50 = 50 percent of the time). Implementing MOLAP cubes containing the dimensions necessary to support complex trait analysis enables us to review the data prior to performing a statistical analysis.

P1124. RAMEDIS - Patient Database for Genetic Metabolic Diseases on the Internet

U. Mischke[1], *G. Frauendienst-Egger*[1], *D. Scheible*[1], *T. T pel*[2], *R. Hofest dt*[2], *F. K. Trefz*[1]
[1]*Klinik f r Kinder- und Jugendmedizin Reutlingen, Steinenbergstr. 31; 72764 Reutlingen, Germany;* [2]*Institut f r Technische und Betriebliche Informationssysteme, Otto-von-Guericke-Universit t Magdeburg, Universit tsplatz 2; 39106 Magdeburg, Germany*
grn.kkh-rt@t-online.de

Compiling molecular genetic and clinical data of patients with inborn errors of metabolism in a database has become a very efficient tool for genotype-phenotype correlation. In addition, epidemiological information can be drawn. This has been realized for patients with PKU (http;//www.mcgill.ca/pahdb/) and atypical forms with tetrahydrobiopterin deficiency (http;//www.bh4.org./). We developed a database which is not limited to data input of only one disease. Using the terminology of our knowledgebase METAGENE[1] standardized data input of any genetic metabolic disease is possible. Clinical, biochemical, ethnic origin and molecular genetic data are included. RAMEDIS is based on an ORACLE database management system. So far, the data input from three different centers in Germany with 20 genetic metabolic diseases and 150 patients are completed. The database will be opened to the scientific community for data input as an electronic publishing tool. Questions of keeping a high quality data input and patient s confidentiality will be discussed. [1] Wissenschaftliche Verlagsgesellschaft mbH, Birkenwaldstr.44, D-70191 Stuttgart

P1125. Relation Database as an useful tool in genetic study for the Multiple Sclerosis.

M. Belotti, *G. Motta, I. Prokopenko, C. Montomoli, L. Bernardinelli*
Dep. Applied Health Sciences, University of Pavia; Pavia, Italy
belotti@borromeo.unipv.it

Data collection, management and control are key stages in the genetic study. The relation Database (DB) was set up for the case register of patients with Multiple Sclerosis (MS) resident in Nuoro province, Sardinia, Italy, a sampling frame for epidemiological and genetic study. Clinical, demographical and genetic information obtained for MS patients and their families were managed under MS-Access 7.0 software. As to high prevalence of MS and relatively low population density in Sardinia it was expected to find many common relatives among patients family members. DB structure was developed with the purpose to analyse extended pedigrees. Special tools were developed to export pedigree data into programmes for

genetic analysis. During data insertion DB automatically searches for identical demographic and personal data, and establishes simple links of the type mother - child or father - child between individuals. This tool allows to construct many-generations pedigree for any individual of the DB. At the moment DB contains family data for 320 MS patients among 470 in the case register. 69 multiplex cases of 1st, 2nd, 3rd, 4th and higher degree of relationship were found among them. Some of the relationships between individuals were found automatically without knowing them a priori. We found this relation DB to be the useful tool for data control and management in a genetic study carried out in an archaic, isolated, genetically homogeneous, inbred population. It allows us to manage a very complex data set that will also include in the future genotyping data.

P1126. Congenital Heart Diseases; Gene Expression Database

S. Sperling[1], *D. Seelow*[1], *S. Mebus*[2], *P. E. Lange*[2], *H. Lehrach*[1]
[1]*Max-Planck-Institut f r Molekulare Genetik; Berlin, Germany;* [2]*Deutsches Herzzentrum Berlin; Berlin, Germany*
sperling@molgen.mpg.de

To decipher genes responsible for heart development and malfunction in human, we set up a database linking gene expression profiles of myocardial samples from patients suffering from congenital heart diseases to the anatomical state and clinical data. The samples are processed by complex RNA hybridisation on cDNA filter containing PCR products of 72.000 clones (Human Unigene Set). The filters are exposed in a phosporimager and automatically assessed. For phenotyping a dedicated diagnostic scheme allows us to distinguish between separate cardiac morphological and hemodynamical features rather than to rely exclusively on the diagnosis. Expression profiles and diagnostic criteria are managed by a non-commercial database system (MySQL) with a web-based interface. The system permits the search for differentially expressed genes correlated to selected cardiac anatomical features, lists all informations available for a gene or clone and integrates our internal database. Furthermore, it will provide links to Internet databases for the sequence of interest. The integration of different species (e.g. mouse, rat and zebrafish) is planned for the future. The obtained data should enable us to identify genes involved in heart development as well as genes responsible for adaptation mechanism influenced by hemodynamical changes correlated to the heart malformation. With these data, new targets for cardiac adaptation to common diseases like hypertension or heart failure should be identified.

P1127. Protein structures as indicators of phylogeny; Analysis of the short-chain alcohol dehydrogenase superfamily

R. Breitling, *J. Adamski*
GSF-National Research Center for Environment and Health; Neuherberg, Germany
breitling@gsf.de

As part of our attempt to use phylogenetic data for the functional characterization of novel proteins, we have developed a 3D-structure-based approach to establish very ancient divergences within a complex protein superfamily. In implementing this approach, pairwise superpositions of X-ray structures are used to calculate similarity scores as input for a neighbour-joining tree-building algorithm. The resulting phylogeny is validated by comparison with the results of sequence-based algorithms and biochemical data. It is also possible to use the 3D-data as a template for the reliable determination of the phylogenetic position of novel proteins as a first step towards functional predictions. As an examplary application we have analysed the short-chain alcohol dehydrogenases (SCAD), a large and diverse superfamily of ancient origin. Several of its members play a role in human physiology and disease, especially in the metabolism of steroid substrates (e.g. progestins, estrogens, androgens, and corticosteroids). Their involvement in common human disorders such as cancer, osteoporosis, and Alzheimer disease makes them important candidate drug targets. Using the 3D-structure-based approach we were able to discern new patterns in the phylogenetic relationships of the SCAD superfamily, including a basal dichotomy of the 17beta-hydroxysteroid dehydrogenases. Using those results as background, we were able to assign unexpected functions to some recently described SCAD members.

P1128. AIDBase ; G6PD, an integrated database for Glucose-6-Phosphate Dehydrogenase (G6PD) Mutations

C. J. Kwok[1], *A. C. R. Martin*[2], *S. Au*[3], *V. Lam*[1]
[1]*Department of Biochemistry, The University of Hong Kong; Hong Kong, Hong Kong Special Administrative Region of China;* [2]*School of Animal and Microbial Sciences, Whiteknights, University of Reading; Reading, United Kingdom;* [3]*Department of Biochemistry, The University of Hong Kong; Hong Kong Special Administrative Region of China; and Section of Structural Biology, Institute of Cancer Research, Chest Beatty Laboratory; London, United Kingdom*
ckwok@hkusua.hku.hk

Hereditary deficiency in human Glucose-6-phosphate dehydrogenase (G6PD) is estimated to affect about 400 million people world-wide. The highest prevalence rates are found in tropical Africa, the Middle East and sub-tropical Asia, some parts of the Mediterranean and in Papua New Guinea. The most common clinical manifestations of G6PD deficiency are neonatal jaundice and acute haemolytic anaemia. AIDBase ; G6PD (http;//www.bioinf.org.uk/g6pd/) is a newly created web-accessible relational database of human Glucose-6-phosphate dehydrogenase (G6PD) mutations. It integrates mutations at the DNA and protein levels with clinical manifestations, references to the biochemical variants initially documented and structural consequences of the mutations. The database provides a form for submitting additional mutation data and can perform an automatic analysis of mutations likely to have a significant impact on the structure of the protein. This procedure identifies mutations which distort secondary structure, destroy hydrogen bonding and electrostatic interactions or simply cause bad clashes. Residues involved in the active site and the dimer interface and residues conserved among the G6PD of different species have also been identified. The database is also linked to other major bioinformatics, mutation, disease and health care databases e.g., OMIM, HGMD, HGBASE and PDB, relevant to understanding G6PD deficiency and its management. The database can provide both science researchers and clinicians insights into the molecular aspects and clinical significance of G6PD deficiency. It also facilitates the understanding of the structure and function relationships of the enzyme.

P1129. Identification of candidate genes predisposing to age-related macular degeneration by systematic EST based expression profiling

H. B. St hr, *H. L. Schulz, N. Mah, B. H. F. Weber*
Human Genetics; Wuerzburg, Germany
huge014@rzbox.uni-wuerzburg.de

Age-related macular degeneration (AMD) is an increasingly common, multifactorial disorder causing severe vision impairment among the elderly population. With no effective therapy available at present, the identification of predisposing genetic factors and the determination of their contribution to AMD is urgently needed. Towards this goal, we are pursuing a strategy that is based on in silico EST expression profiling and which is aimed at the generation of a comprehensive catalogue of genes preferentially active in the human retina. Subsequently, these genes will be assessed in SNP-based association studies in large AMD cohorts. As a first step retina EST sampling was performed in the UniGene database and has identified a total of 1241 EST clusters with at least 30% of the ESTs in each cluster originating from retina cDNA libraries. In a pilot study 180 clusters with varying retina and non-retina EST contents were analyzed for their in vitro expression by RT-PCR. This approach identified 69 clusters representing genes with putative retina- or retina/neural- specific transcription. Thus far, six retinal genes have been fully characterized and include four genes with unknown function as well as two genes belonging to well characterized gene families. Another three clusters correspond to transcripts previously identified by the Kazusa cDNA sequencing project. At least ten of the 69 clusters are located in non-coding regions of known human or rodent genes and thus may represent partially spliced transcripts. Extrapolation of our preliminary results suggests that at least 130 genes within the selected 1241 EST clusters may have a specific function in the neuronal tissues including the retina.

P1130. A Primary Transcript Map for the Familial Juvenile Hyperuricemic Nephropathy (FJHN) Critical Region on Chromosome 16

P11.2.

B. Stiburkova[1], *K. Hodanov* [1], *L. Ondrov* [1], *M. Zik nov* [1], *M. Jer bkov* [1], *M. Nov* [1], *C. Vlcek*[1], *J. Paces*[1], *J. Majewski*[2], *J. Ott*[2], *S. Kmoch*[1]
[1]*Center for Integrated Genomics; Prague, Czech Republic;* [2]*Rockefeller University; New York, NY United States*
bstib@lf1.cuni.cz

Familial juvenile hyperuricaemic nephropathy (FJHN) is an autosomal dominant renal disease, characterized by juvenile onset of hyperuricemia, gouty arthritis and progressive renal failure at an early age. Recently we have identified a locus for FJHN on chromosome 16p11.2 and found evidence for genetic heterogeneity and reduced penetrance of the disease (Stiburkov et al, (2000) Am J Hum Genet 66;1989-1994). Our results and a recent publication on localization of the FJHN gene in another family (Kamatani et al. (2000) Arthritis Rheum 43; 925-9) have narrowed the 1.7 cM candidate region, between markers D16S403 and D16S3113. BAC

clones spanning approximately 2 Mb of critical region were isolated and a physical map is constructed using a combination of STS content analysis and BAC ends sequencing. Known genomic sequences of the clones were BLASTed against various EST and protein databases. So far, these analyses revealed presence of 10 known genes and 69 putative transcripts. Corresponding EST clones were isolated and sequenced. Full length cDNAs are currently being PCR-RACEd, sequenced and its expression profiles are studied on a multiple tissue cDNA panel. Genes expressed in the kidney are prioritized for mutation analysis in affected pedigrees.

P1131. A Large Proportion of Coding Region Mutations in Medium-Chain Acyl-COA Dehydrogenase (MCAD) Lead to Low Levels of mRNA

B. S. Andresen[1], *K. B. Nielsen*[2], *N. Gregersen*[3]
[1]*Aarhus University; Aarhus, Denmark;* [2]*Institute of Human Genetics and Research Unit for Molecular Medicine (MMF); Aarhus, Denmark;* [3]*Research Unit for Molecular Medicine, Aarhus University; Aarhus, Denmark*
brage@biobase.dk

In eukaryotic cells there exist systems that recognize and degrade mRNA s with premature termination codons (PTC). This process is named nonsense mediated decay (NMD). We have studied the possible involvement of the NMD system on MCAD mutations. We used analysis of cDNA from >30 unrelated patients to evaluate the effect on mRNA levels of >20 different (missense, silent, splice, PTC) MCAD mutations. Most patients are compound heterozygotes with the prevalent mutation 985G in one allele. Therefore, we could monitor the amounts of mRNA from alleles with 20 different mutations using allele specific quantitation in a LightCycler based 985G assay. Patients having two PTC mutations were studied by PCR of cDNA and northern blotting. All PTC mutations located upstream of the last exon resulted in low levels of mRNA, whereas a PTC in the last exon or mutation of the translation initiation codon did not affect mRNA amounts. By adding translation blockers to patient cell cultures we could demonstrate that NMD in MCAD is translation dependent. To our surprise, also some of the missense mutations had drastic effects on MCAD mRNA amounts. One of the missense mutations, that did not create a pseudosplice site, resulted in complete exon skipping, and we speculate that it disrupts an exonic splice enhancer. Studies employing mini-gene constructs is in progress in our laboratory. We speculate that aberrant mRNA processing caused by coding region mutations may be more frequent than previously thought.

P1132. Construction of a Physical Map of the CHRNA7 Gene/Duplication Region on 15q13-q14

A. J. Makoff, *B. P. Riley*
Institute of Psychiatry; London, United Kingdom
a.makoff@iop.kcl.ac.uk

Several linkage studies have implicated markers in the vicinity of the alpha-7 nicotinic cholinergic receptor subunit gene (CHRNA7) on chromosome 15q13-q14 in both schizophrenia and epilepsy. However, this region is complicated by the presence of a partial duplication of exons 5-10 of CHRNA7. We have made a detailed study of genomic clones in the human databases to construct a physical map of this complex region. This has identified clones defining three types of gene; CHRNA7, several copies of a novel putative gene and a hybrid between the two. The upstream sequences of the novel and hybrid genes contain four previously identified exons plus a novel exon. The downstream sequence of the novel gene contains three novel exons. The central six exons of this eight exon cassette are part of a 23 exon putative kinase gene on chromosome 3, while the 5 - and 3 -most exons are found only on chromosome 15. A fourth, less conserved copy of most of this cassette has also been identified on a different chromosome 15 segment. We have constructed a >2.8 Mb physical map of the region from BAC and PAC sequences deposited in databases. The map places the partial duplication in opposite orientation to CHRNA7. Together, the data support an evolutionary model involving a large deletion followed by a further rearrangement, giving rise to the partial duplication of CHRNA7. This physical map shows the complexity of the CHRNA7 region and the highly duplicated and repetitive nature of its large-scale structure.

P1133. Identification and Characterization of mutations in families affected with Corneal dystrophy.

N. S. Udar, *S. Sheikhavandi, S. Yelchits, R. Silva-Garcia, M. Chalukya, V. Yellore, K. Small*
UCLA School of Medicine; Los Angeles, CA United States
Nitin@ucla.edu

A group of four corneal dystrophies inherited in an autosomal dominant pattern are characterized by bilaterally symmetrical disorder with progressive accumulation of corneal deposits that begin to appear during the first two decade of life. Progressively these opacities cause serious visual handicaps, often requiring phototherapeutic keratectomy or corneal transplantation. A single gene BIGH3 was identified accounting for all four disorders - Granular, Lattice type I, Adeline and Reis Buckler and localized on human chromosome 5. Experiments have been done with different ethnic population to study the hot spot nature of these mutations. We have studied a total of 4 families affected with Granular corneal dystrophy (CDGG1), Reis Buckler (CDRB) and Lattice type I (CDL1). SSCP analysis was carried out on 47 members of a Reis Buckler family with 10 affected and 21 affected, 80 unaffected for a Granular corneal dystrophy family and . Sequence analysis revealed a R555Q mutation for the CDRB family, R555W mutation for the CDGG1 families and R124C for the CDL1 family. We carried out haplotype analysis on our individuals which represent Caucasian and on family from Sri Lanka. Our results support the hot spot theory instead of the common ancestor hypothesis to account for the mutations.

P1134. Evidence for a putative cancer associated gene specific to Barrett s Oesophageal Cancer at 17

P13.1

J. R. Dunn[1], *B. C. Oates*[2], *J. Garde*[1], *J. R. Gosney*[3], *A. J. M. Watson*[2], *J. K. Field*[1]
[1]*University of Liverpool; Liverpool, United Kingdom;* [2]*Department of Medicine, University of Liverpool; Liverpool, United Kingdom;* [3]*Department of Pathology, Royal Liverpool University Hospital; Liverpool, United Kingdom*
jrdunn@liv.ac.uk

Allelic imbalance (loss of heterozygosity) studies of 41 markers on chromosome 17 in nineteen Barrett s Oesophageal Cancer (BOC) specimens have identified thirteen common minimally deleted regions (MRs I-XIII). MRs II, VII and VIII are located at the sites of the Tumour Suppressor Genes p53, NF1 and BRCA1 respectively; MRs III, IV, V (C17p), and VI, IX, XII, XIII (C17q) are novel regions. The highest frequency of loss of heterozygosity (LOH) was observed at MRIII (91%), which is centromeric to but distinct from p53. Twenty-three premalignant and BOC tissue samples taken throughout the length of five oesophagectomy specimens were also analysed for C17 LOH. We found evidence for a clonal pathway of LOH in four specimens that began as one or more small deletions in histologically early tissue, spreading to larger deletions in BOC. LOH at MRIII was seen in the histologically earliest tissue in all five specimens. The minimal region at MRIII is 300-400 kb in size, is contained on three BAC clones, two of which have been completely sequenced, while sequencing of the third is underway. Analysis of this sequence data has shown that MRIII contains at least four characterised genes and a possible further eleven previously undescribed genes from the EST data. We are currently characterising these putative cancer associated genes for BOC and investigating mutation patterns in affected tissue.

P1135. Candidate gene analysis in the Tylosis Oesophageal Cancer (TOC) region on chromosome 17q25

J. Jones[1], *J. Langan*[1], *K. Evans*[1], *A. Ellis*[2], *J. Field*[1], *J. Risk*[1]
[1]*Liverpool University; Liverpool, United Kingdom;* [2]*Royal Liverpool University Hospital; Liverpool, United Kingdom*
j.jones01@liv.ac.uk

The association between the autosomal dominant skin disorder, tylosis, and oesophageal carcinoma has been identified in 2 large families in the UK and USA and a smaller German family. The disease locus was mapped by haplotype analysis to chromosome 17q25. Physical mapping shows that the 0.5Mb minimal region is covered contiguously by 3 BAC clones. The sequence data for 2/3 of this region is available in the public domain and analysis has been carried out using the NIX suite of programs available from the UK Human Genome Mapping Project (HGMP). NIX analysis identified at least 10 characterised candidate genes in the TOC minimal region and at least 40 other, previously uncharacterised, gene fragments. GalNAcI alpha 2-6 sialyl transferase and GalNAcII alpha 2-6 sialyl transferase (SThM) map to the proximal end of the TOC minimal region and may be involved in the development of cancer. The intron/exon structure of the genes was confirmed by EST alignment using Sequencher. Both genes were analysed for polymorphisms and mutations in the coding regions using family members. Two polymorphisms were identified in SThM in the 5 UTR and intron 7 but these were shown not to be tylosis specific. Expression data for both genes in a variety of normal and tumour tissues will also be presented. The criteria used to select further candidate genes for analysis will be discussed.

P1136. Exclusion of six candidate genes in distal hereditary motor neuropathy type II linked to chromosome 12q24.

J. I. Irobi, E. Nelis, J. Meuleman, K. Venken, E. De Vriendt, P. De Jonghe, C. Van Broeckhoven, V. Timmerman
Molecular Genetics Laboratory, Flanders Interuniversity Institute for Biotechnology (VIB), Department of Neurology, University Hospital Antwerp (UZA), University of Antwerp (UIA); Antwerpen, Belgium
jirobi@uia.ua.ac.be

Distal hereditary motor neuropathies (distal HMN) are clinically and genetically heterogeneous and are divided in seven subtypes according to mode of inheritance, age at onset and clinical evolution. We performed a genome-wide search in a multigenerational Belgian pedigree with autosomal dominant distal HMN type II. Significant linkage was obtained between markers D12S86 and D12S340, suggesting that a gene causing distal HMN II is located on chromosome 12q24.3. In a previous mutation analysis we were able to exclude the human phospholipase A2 gene (PLA2A) as the responsible gene for distal HMN II. Here we report the exclusion of six other known genes, all located within the distal HMN II region; Two cytoskeletal proteins; (paxillin and restin), acidic ribosomal phosphoprotein (RPLP0) and nucleoside diphosphate kinase (NM23-NDP Kinase), and two ESTs; one homologous to a human G- protein-coupled receptor (HM74) gene and the other to a human voltage-gated calcium channel beta subunit (CACN§3). The coding region of these positional as well as functional candidate genes for distal HMN II, were analysed in patients and healthy individuals for possible mutations. DNA sequence analysis was performed but no disease causing mutations in any of the six genes could be identified, excluding them as disease-causing gene for distal HMN II

P1137. A complete gene catalogue of human Xp11.4 harboring disease loci for diabetes mellitus type I, mental retardation and retinal disturbances

J. Ramser[1], G. Wen[2], Y. Demirci[3], H. Achatz[1], S. Engert[1], C. Pusch[4], I. Martinez Garay[5], A. Hardcastle[6], K. Badenhoop[7], M. Gorin[3], M. Platzer[2], A. Meindl[1]
[1]Ludwig Maximilians University; Munich, Germany; [2]Institute for Molecular Biotechnology; Jena, Germany; [3]Department of Ophthalmology and Human Genetics; Pittsburgh, PA United States; [4]Universitaets-Augenklinik; Tuebingen, Germany; [5]Universidad de Valencia; Valenica, Spain; [6]University of London; London, United Kingdom; [7]University of Frankfurt; Frankfurt, Germany
juliane@pedgen.med.uni-muenchen.de

Different disease entities like two syndromic forms of mental retardation, diabetes mellitus type I and two eye diseases have been mapped to the short arm of the X-chromosome including the Xp11.4 region. In order to isolate genes, involved in these conditions, we have constructed a complete BAC/PAC-contig for the Xp11.4 region between markers DXS8025 and DXS228 encompassing about 3.0 Mb. For the proximal part of this region, between DXS993 and DXS228, a complete transcription map was established. Six distinct genes were found in this 1.5 Mb large interval, four of them were screened for mutations in patients with the complete form of congenital stationary night blindness (CSNB1). A novel gene termed NYX (Nyctalopin on X chromosome) was found to be mutated in CSNB1 families (Pusch et al., Nature Genetics 2000). NYX is widely and low expressed and codes for a 481 amino acid protein (nyctalopin). In order to identify candidate genes for X-linked cone dystrophy (COD1), we are now analysing the 1.5 Mb large distal part of the contig (DXS8025-DXS993). Sequence analysis resulted in the identification of three genes so far. They escape X-inactivation and are currently screened for mutations in COD1 families. Genes isolated from this region will also, together with genes from the adjacent fully characterized Xp21.1 region, be evaluated for variants in patients suffering from diabetes mellitus type I. Finally, all genes from the Xp11.4 region and from the proximally located regions Xp11.3 and Xp11.23, can be screened for mutations in Prieto- and Renpennning syndrome, respectively.

P1138. Application of fuzzy logic to haplotype pattern mining in case-control study

B. A. Skierczynski[1], C. Nguyen[1], M. Blumenfeld[2], M. Torre[1], I. Chumakov[2], J. Link[1], D. Cohen[2]
[1]Genset Corp.; La Jolla, CA United States; [2]Genset SA; Paris, France
skierb@genxy.com

Most human cancers (and other complex diseases) are characterized by genomic instability. The genetic events underlying its development are poorly understood. In a majority of cases, cancer does not occur as a simple mongenic disease with clear Mendelian inheritance. Results throughout literature support the view of multigenic etiology for complex diseases. While the methodology for the mapping of Mendelian disorders is relatively well developed, the successful identification of genes in a complex trait is still difficult to predict. Direct application of the LD (linkage and association analysis) have proven difficult for complex traits due to combined action of multiple genes influenced by environmental factors. The single-marker LD methods cannot fully describe possible association(s) in an entire chromosomal region. To increase the statisticical power to detect the presence of a disease locus (loci) the LD analysis can be extended from single marker to multiple-marker haplotypes. In general, we do not have a precise chromosomal location of the disease-susceptibility locus (loci) forcing us to search through all possible haplotypes surrounding multiple locations as well as spanning them. To understand and estimate structure and parameters within the genetic architecture of quantitative traits we have developed a method based on fuzzy logic, discovery of recurrent patters and multilocus haplotypes, and applied to the case-control association study for family and sporadic occurrence of the prostate cancer. Results suggest that combining the application of the fuzzy logic, pattern recognition and multi-markers LD methods with large numbers of (SNP) markers should enable identification of patterns of genetics instability with potential prognostic, diagnostic and disease-susceptibility loci utility.

P1139. Genopure - A novel magnetic bead DNA purification system for MALDI-TOF MS analysis

U. Rapp[1], M. Kostrzewa[1], J. Bimmler[1], I. Thomas[1], T. Wenzel[1], E. Nordhoff[2], H. Rauth[2], T. Fr hlich[1]
[1]Bruker Saxonia Analytik GmbH, Bioanalytics; Leipzig, Germany; [2]Max-Planck-Institute of Molecular Genetics; Berlin, Germany
AKR@bdal.de

MALDI-TOF mass spectrometry has a high potential for high throughput DNA analyses because of its accuracy, speed, automation capabilities, and cost-effectiveness. Unfortunately, molecular biological reactions are performed in the presence of high amounts of salts and detergents which form adducts with DNA or interfere with matrix crystallization, respectively. Therefore, effective DNA purification is indispensable for successful and valid MALDI-TOF MS analysis. We present a novel magnetic DNA purification system which gives excellent results in subsequent mass spectrometric measurement. Small double stranded PCR products were precipitated on paramagnetic beads using an appropriate binding solution. The binding of DNA to the particles did not require any labeling of primers or dNTPs. After several washing steps with buffers containing either ethanol or isopropanol, DNA was eluted and measured with 3-HPA matrix in a MALDI-TOF mass spectrometer. Residual PCR primers were removed while PCR products down to about 50 bp in size were recovered with high yield. Quality of the acquired spectra was superior to that of alternative purification protocols. Alternatively, eluted PCR-Products were used for subsequent reactions like primer extension or restriction enzyme digest. Primer extension products or very small restriction enzyme digest products (about 20 bp) were again purified by the magnetic bead system to MALDI quality with high recovery using a dedicated binding buffer. The combination of both purification procedures enabled the genotyping of SNPs by performing PCR, purification of the PCR product, primer extension or digest, and clean-up of the allele specific products in one reaction tube. High quality spectra could be acquired for several model systems allowing the unambiguous determination of genotypes. The bead handling can easily be automated on standard pipetting robots thereby enabling the processing of thousands of samples per day.

P1140. High-throughput Mutation Detection and Screening by Constant Denaturant Capillary Array Electrophoresis

M. Minarik[1,1], J. Bj¿rheim[2], K. Dains[1], M. Mahtani[1], P. Ekstr¿m[2]
[1]Molecular Dynamics; Sunnyvale, CA United States; [2]The Norwegian Radiumhospital; Oslo, Norway
marek.minarik@am.apbiotech.com

Project Description;
The discovery of low frequency DNA point mutations and single-nucleotide polymorphisms (SNPs) presents one of the major challenges in genetic analysis in the post-genome era. Among various techniques, constant-denaturant capillary electrophoresis (CDCE, introduced in 1994 by Khrapko et al.) is a viable tool for mutation detection. CDCE offers a high degree of sensitivity as well as ability to detect low level mutations. The CDCE instrument reported in the literature did not have any automatisation with regard to change of polymer and loading of sample. Consequently running large number of samples was laborious and time consuming.
The present work describes a transfer of established CDCE technology to the multicapillary array format enabling an increase in sample throughput that is required for large scale mutation detection and screening. In this initial work, the main emphasis was towards the optimization of the separation of wildtype homoduplex and mutant homoduplex and heteroduplexes

by determining the optimal temperature in a step-wise fashion. The separating conditions for the fragment were achieved with a combination of denaturing capacity of LPA matrix and elevated capillary temperature.
Here we present data using high-throughput CDCE technology to identify point mutations in exon 1 of the *k-ras* gene. The samples analyzed were obtained from colorectal carcinoma patients previously described and analyzed by manual CDCE. The main objective of this study was to elucidate the feasibility of transferring the CDCE technique from a single to multi-capillary platform. In addition, the detection sensitivity was measured by identifying a number of mutants included in a wildtype population.

P1141. FP-TDI; A high-throughput, homogeneous assay for SNP genotyping coupled with effective data management.

S. F. A. Grant, *R. Kern, K. Kumpf, B. M ller-Myhsok, R. Tolle*
LION Bioscience; Heidelberg, Germany
struan.grant@lionbioscience.com

With the increasing demand for higher throughput SNP genotyping, many technologies have become available or are currently in the development pipeline. However, with such large volumes of information, robust data management is crucial for subsequent statistical analysis.
We have adopted the Perkin Elmer Wallac Victor^2 V multilabel counter for a template-directed dye-terminator incorporation assay with fluorescence polarization (FP- TDI) detection. As there are no instrument-specific application notes for this SNP genotyping methodology, filters and instrument settings had to be experimentally validated. Consequently, by using a specific combination of filters, we have observed consistent discrimination through a 2-dye approach with a 1 second read per well.
To aid high-throughput screening, we have maximized the homogeneity of the assay by employing a standard touchdown protocol for all PCRs. This approach has been designed to ensure specific and maximum PCR yield in a limited primer and ddNTP concentration environment. In addition, following the optimization with 96 well plate assays, the system has been successfully scaled up to a 384 well plate assay format.
From the resulting data, a SNP genotype can be efficiently assigned to an individual through a graphical visualization tool that clusters the genotypes. This tool is part of a single platform with multiple views designed to handle both SNP and microsatellite data. The data is then subjected to a set of rigorous tests including control value checks, pedigree checking and an analysis of individual/marker history to determine if conflicting genotypes exist. Based on these checks plus samples that did not yield a clear genotype, a repeat plate is proposed by the system to allow minimum effort in assembly for the laboratory. Finally, the data can be exported in linkage format for haplotype and statistical analysis.
Also presented orally in Concurrent Session 5: Poster Discussion Methods to Detect Genomic Variation

P1142. SNPstream(TM) 5K, a Microsphere-based System for Determination of Single Nucleotide Polymorphisms by Primer Extension

M. A. Kunkel, *R. Liberchuk, C. A. Gelfand, M. Boyce-Jacino*
Orchid BioSciences, Inc.; Princeton, NJ United States
mkunkel@orchid.com

The accelerating interest in single nucleotide polymorphism (SNP) genotyping and the sweeping pharmacogenetic implications of correlating SNPs to phenotypes have resulted in the need for simple, highly accurate SNP genotyping methods. Orchid BioSciences has developed SNPstream(TM) 5K, an adaptation of Orchid s SNP-IT(TM) primer extension genotyping assay to Luminex s novel microsphere-based assay platform. A significant benefit of the system is its ability to rapidly analyze multiplexed solution-phase SNP-IT(TM) assays. The result is highly accurate SNP genotyping system that maintains the flexibility to adapt to virtually any SNP loci of interest. The advantages of this microsphere-based system will be described, along with multiplex genotyping, reproducibility, and accuracy results achieved with the system.
Also presented orally in Concurrent Session 5: Poster Discussion Methods to Detect Genomic Variation

P1143. The SNPcode(TM) Genotyping System; A Generic System for SNP Genotyping on High Density Arrays

M. A. Donaldson, *J. A. Lathrop, C. A. Gelfand, M. T. Boyce-Jacino*
Orchid BioSciences, Inc.; Princeton, NJ United States
mdonaldson@orcbid.com

With the completion of the Human Genome Project, a substantial need to obtain rapid, low cost information on genetic variations has emerged. Single nucleotide polymorphisms (SNPs) are being shown in ever increasing numbers to correlate to medically relevant conditions, such as adverse drug response or predisposition to disease. Understanding the correlations between SNPs and phenotypes requires high-throughput, high-density SNP detection systems enabling hundreds or thousands of SNPs per sample to be genotyped in a low cost, robust fashion. Orchid BioSciences has developed the SNPcode(TM) system, combining Orchid s SNP-IT(TM) primer extension genotyping assay with the GenFlex(TM) Tag Array chip from Affymetrix. The result is a versatile genotyping system capable of scoring from hundreds to thousands genotypes on a single chip. The inherent generic design of the chip allows the user to adapt any SNP loci of interest to this platform. The SNPcode system utilizes a bifunctional SNP-IT primer with the 3 sequence complementary to the SNP-containing PCR amplicon, and the 5 sequence complementary to one of the 2000 unique tag sequences on the GenFlex chip. The assay protocol begins with multiplex PCR amplification, followed by multiplexed solution-phase SNP-IT primer extension. The SNP-IT products are hybridized to the chip, sorting the multiplex reaction by hybridization of the specific tags on each SNP-IT primer to its unique complement on the chip. More than 1000 SNPs have been analyzed on the system to date. We will present use of this new genotyping system in comparison to results collected on other genotyping platforms.

P1144. Genome Wide SNP Detection Using Subtraction Remainder Quantification Analysis and DNA Sorting on Microbeads.

C. Dando, *K. Locke, R. Fu, E. Beilharz, S. Brenner, G. Albrecht*
Lynx; Hayward, CA United States
cdando@lynxgen.com

Single nucleotide polymorphisms (SNPs) represent powerful markers for the mapping of genes and mutations related to important traits, diseases and for the study of pharmacogenetics. We describe our Megatype“ technology that permits the identification of SNPs that are differentially represented in two populations without the need for prior knowledge of the SNP sequences. Genomic DNA fragments that exhibit a specific class of SNPs are selected using our proprietary method and loaded on to microbeads using our Megaclone“ technology as described in Brenner et al, PNAS. 97;1665-1670 (2000). DNA probes which reflect the allelic frequencies of the different SNP bearing fragments in each population are then prepared from the two different sample sources using the same proprietary method and tagged with two different, but complementary adapter sequences. In order to reduce the complexity of human probe libraries we first fractionate the probe by using a similar but less complex library to capture relevant sequences. To increase the sensitivity of subsequent analysis competitive hybridization of the tagged probes to the target DNA on microbeads results in a 3 bp, 3 -end overhang. Ligase is added to the beads such that the two complementary 3 overhang sequences undergo intra-bead ligation. Molecules remaining following intra-bead ligation represent the molar excess of probe from the sample source. The allelic frequency of these sequences can then be measured by the addition of a pair of fluorescently labeled decoder molecules, followed by analysis and sorting by flow cytometry.
Also presented orally in Concurrent Session 5: Poster Discussion Methods to Detect Genomic Variation

P1145. High-throughput, automated methods for targeted Single Nucleotide Polymorphism (SNP) discovery

J. V. Platko, *R. J. Prill, T. Lavery, A. Rachupka, R. Onofrio, D. Richter, J. Ireland, M. Cargill, E. S. Lander*
Whitehead Institute, Center for Genome Research; Cambridge, MA United States
platko@genome.wi.mit.edu

Single Nucleotide Polymorphisms (SNPs) are the most common form of variation in the human genome and are amenable to high-throughput genotyping methods. Powerful approaches are being used to discover hundreds of thousands of SNPs randomly distributed across the genome. Our group has taken a targeted approach in order to discover SNPs in specific genes and genomic regions from selected individuals. The polymorphisms we discover are used as genetic markers to investigate human history, evolution, disease, and population structure. Designing an efficient, high-throughput system for targeted SNP discovery has held many challenges. These include retrieving sequences for genes of interest, designing primers for PCR amplification, designing and managing a laboratory workflow, and analyzing and interpreting the resulting data. We screened over 2600 genes by designing primers from mRNA sequence and sequencing cDNA templates. Now that the human genome sequence is nearing completion, we initiated the use of genomic DNA as the template. We adapted software to separate an mRNA sequence into exons for primer design. We found that genomic DNA based PCR has a product 92% of the time compared to 66% for the cDNA based assay. The success rates for sequencing these PCR products also differ between the two methods

(89% for DNA, 70% for cDNA). Once sequencing is complete, we analyze the results using the automated program, Polyphred. We currently cover at least 48Kb per person per week. Information on all aspects of our system from genomic sequence through polymorphisms is entered into databases and can be retrieved by both laboratory personnel and researcher. As of 12/15/00, our lab discovered and validated over 4400 polymorphisms that have been applied to studies on human populations and disease.

Also presented orally in Concurrent Session 5: Poster Discussion Methods to Detect Genomic Variation

P1146. Multiplex SNP scoring of cardiovascular candidate genes by a novel labeling strategy and microcapillary electrophoresis.

***C. Virgos**, M. Pho, A. Sassi, T. Matray, J. Wei, J. Wu, D. Dutta, S. Leon, S. Muley, S. Williams, S. Singh, E. S. Mansfield, M. T. Cronin*
ACLARA Biosciences Inc; Mountain View, CA United States
cvirgos@aclara.com

An increasing number of SNP scoring methods to support association studies and specific genotyping panels used for phenotype prediction are being developed. These methods will have to meet stringent requirements of high-throughput, accuracy, flexibility and reduced cost. By combining a proprietary new probe labeling strategy (eTag[TM] reporters) with existing, robust genotyping biochemistry such as TaqMan[R], ACLARA Biosciences is developing a flexible, multiplex assay format for multiplex SNP scoring. In the eTag label strategy, each probe of a multiplexed set is labeled with a fluorogenic eTag, which has a unique and characterized electrophoretic mobility. Following the reaction, the cleaved eTags are separated by electrophoresis and genotyping information is obtained by interpretation of the obtained electropherograms. Specific genotyping panels can be constructed by selection from a library of characterized eTags. Additional eTags sets can be used as internal control markers. We are currently optimizing the SNP assay using the eTag approach for analysis on both commercial capillary electrophoresis instrumentation (ABI 310 and ABI 3100) and our own plastic, multichannel LabCard[TM] devices. Multiplex SNP analysis of synthetic target and genomic DNA for the cardiovascular targets LPA, ApoA4, ApoB, ApoE and CETP will be discussed.

P1147. Direct IBD Mapping; Gene Mapping Using Microarrays

***D. Smirnov**, S. Narasimhan, T. Weber, A. Bruzel, V. Cheung*
Childrens Hospital of Philadelphia; Philadelphia, PA United States
dsmirnov@mail.sas.upenn.edu

Genomic mismatch scanning (GMS) is a hybridization-based gene mapping method, which allows selective recovery of genomic DNA fragments that are identical-by-descent (IBD) between two individuals. During GMS procedure, restriction-digested genomic DNA from two subjects are denatured, mixed and allowed to reanneal to form heterohybrids. Homohybrids and mismatch-containing heterohybrids are then selectively removed by treatment with methylation-sensitive restriction enzymes, exonuclease, and Escherichia coli mismatch repair proteins. The isolated DNA fragments can be then mapped to their genomic regions by genotyping with microsatellite markers, however genomic microarrays can be used to map IBD DNA much more efficiently. The combination of GMS and IBD mapping using genomic microarrays is known as direct IBD mapping . To allow mapping any disease genes, one needs a set of well-characterized genomic clones. Currently, in our laboratory, a mapping project, which will produce a set of well-characterized BAC clones covering entire human genome at ~ 1 Mb resolution, enters its final phase. Mapped BAC clones will be placed in map order onto a DNA microarrays. This allows easy determination of the genomic address of DNA fragments isolated through the GMS procedure. Here we will discuss potential applications and experimental specifics of direct IBD mapping.

P1148. Use of Custom, in situ Synthesized, Oligonucleotide Arrays in Gene Expression Analysis

***A. B. Lucas**, S. Y. Fujimoto, P. D Andrade, Z. A. Keval, A. Franke, L. N. Liu, D. Q. Bi*
Protogene Laboratories, Inc.; Menlo Park, CA United States
alucas@protogene

Microarray expression analysis has become one of the most widely used functional genomics tools. We have developed the use of surface tension patterns on glass slides to allow the *in situ* synthesis of arrays of oligonucleotides directly on the glass surface using ink jet technology to dispense reagents. This versatile method offers the ability to customize the arrays at the time of synthesis, and allows precise feature placement and morphology. This control, and the ability to create arrays of T_m-matched oligonucleotides, gives excellent performance and quantification in hybridization reactions. Our group has optimized aspects of target preparation and labeling, hybridization conditions, and data analysis. Examples of microarray performance will be shown for the analysis of gene expression.

P1149. Using Lab On A Chip Technology to Assess Sample Quality for Microarrays

***D. Vitale**, D. Ilsley, K. Hahnenberger*
Agilent Technologies; Palo Alto, CA United States
deborah_vitale@agilent.com

Microarray technology enables parallel measurement of thousands of expressed genes while minimizing sample consumption. One important step in the microarray process is sample preparation. Lab-on-a-Chip technology provides a complete solution to improving sample preparation for DNA microarrays by identifying potential problems that may result in poor array performance. For example, RNA preparations that are degraded or contain ribosomal RNA or genomic DNA may result in poor probe labeling and can easily be identified using the RNA6000 kit. Fluorescently labeled probe preparations that may result in poor array performance can also be determined. The Agilent 2100 Bioanalyzer rapidly analyzes RNA preparations for integrity, yield, and genomic DNA contamination. Information with regard to amount, size, and the degree of purification of fluorescently labeled cDNA and cRNA probes can also be obtained and strongly correlates to array performance.

P1150. Statistical Inference in Array Genomics

***R. Nadon**[1], P. Shi[1], E. Woody[2], H. Hubschle[1], N. Rghei[1], P. Ramm[1]*
[1]Imaging Research Inc.; St. Catharines, ON Canada; [2]University of Waterloo; Waterloo, ON Canada
robert.nadon@imaging.brocku.ca

Gene expression arrays present unique challenges for statistical inference. The typically small number of replicated expression values in array studies make the use of standard parametric statistical tests problematic. Such tests have low sensitivity and return potentially inaccurate probability values. We present novel alternative statistical inference procedures which circumvent these difficulties. The procedures stem from the concept that each gene represents an individual experiment. Accordingly, arrays present large numbers of small sample experiments tested under the same conditions. Given this, it should be possible to pool the random error estimates across all genes. We have developed two variants of the pooled error method, which we refer to as common error and fitted, to estimate random error associated with each transcript. Our pooled error methods estimate random error from the entire array and can function with as few as two replicates. Because our random error estimates are based on large samples, they provide more accurate and precise estimates than other methods. With these accurate error estimates in hand, classical statistical techniques can be applied to array analyses, providing statistical tests that are sensitive to small differences in expression. The methods will be illustrated with experimental data.

P1151. Making DNA microarrays — optimisation and comparison of various DNA immobilisation strategies

***L. Ondrov** [1,2], M. Nova[1], M. Jerabkova[1,2], M. Hrebicek[1,2,3], S. Kmoch[1,2,3]*
[1]Institute for Inherited Metabolic Disorders; 1st Faculty of Medicine; Prague, Czech Republic; [2]Center for Integrated Genomics; Prague, Czech Republic; [3]GeneAge Technologies; Prague, Czech Republic
londr@lf1.cuni.cz

Efficient and uniform immobilisation of target DNA molecules onto a glass surface is one of the critical steps in making DNA microarrays and is central to the quality of the resulting data. We have adopted and compared immobilisation of amino-modified DNA onto a poly-L-lysine and 3-glycidoxypropyltrimethoxysilane-modified glass surfaces, disulphide-modified DNA onto a 3-mercaptopropyltrimethoxysilane-modified glass surface and a disulphide-modified DNA covalently cross-linked to 3-mercaptopropyltrimethoxysilane onto unmodified glass surface. Individual chemistries were tested with Cy5 and Cy3 labelled PCR products or Cy5 labelled oligonucleotides. Target DNA was spotted by GeneSurfer arrayer (GeneAge Technologies) and individual reaction steps were followed by IVL laser scanner (Genomic Solution). Our experience showed that published immobilisation protocols were usable only after careful optimisation. Poly-L-lysine chemistry is useful for binding of amino-modified and even of non-modified DNA. However, blocking procedure limits its application only for hybridisation experiment. Disulphide chemistry is applicable for both hybridisation and primer extension experiments. Procedure is fast, effective and cheap what makes it preferable for high-throughput array production.

P1152. Improved background correction for spotted DNA microarrays

C. Kooperberg
Fred Hutchinson Cancer Research Center; Seattle, WA United States
clk@fhcrc.org

Most microarray scanning software for glass spotted arrays provides estimates for the intensity for the foreground and background of two channels for every spot. The common approach in further analyzing such data is to first subtract the background from the foreground for each channel, and to use the ratio of these two results as the estimate of the expression level. The resulting ratios are, after possible averaging over replicates, the usual inputs for further data analysis, such as clustering. If with this background correction procedure the foreground intensity was smaller than the background intensity for a channel that spot (on that array) yields no usable data. In this paper it is argued that this preprocessing leads to estimates of the expression that have a much larger variance than needed when the expression levels are low.

P1153. Serial Analysis of Gene Expression using the Applied Biosystems Capillary Electrophoresis Platforms

K. M. Gunning
Applied Biosystems; Foster City, CA United States
GunninKM@appliedbiosystems.com

In today s genomic age, the draft completion of the human genome has allowed researchers access to a wealth of information with respect to gene identity and gene expression profiles. Scientists have developed novel ways in which to elucidate this information and SAGE is deemed one of the more comprehensive methods available for rapid, detailed analysis of large numbers of cellular transcripts. To further enhance the rapidity at which one can determine this global gene expression profile, one needs more efficient and precise methods for analyzing these transcripts and for assessing their abundance. Automated capillary electrophoresis provides a platform whereby the sequence of these many transcripts can be determined with speed and precision. The ABI PRISM 3700 and the ABI PRISM 3100, the most recent addition to the CE family of platforms, provide the SAGE researcher with a means by which they can determine quickly and reliably, the transcript information necessary to build these gene expression profiles. These platforms partnered with the uniformity of signal strength and length of read of BigDye“ terminators, are the efficient solution for large scale transcript analysis using the SAGE method. We will demonstrate the ease of use and flexibility of these platforms as a preferred method for the sequence analysis of ditags in the SAGE process.

P1154. Hazardous Waste-Associated Acquired Genetic Disorders

G. McKinstry
Gene Amplification; Dunwoody, GA United States
gmckinst@bellsouth.net

Gene Amplification is an environmental consulting agency located in a northern suburb of Atlanta. The company is 14 years old. Recently, the company has focused on genomics and bioinformatics. Of great interest has been acquired genetic disorders due to exposure of humans living around uncontrolled hazardous waste sites. There has been much attention given to the exposure problem in the United states [(Agency for Toxic Substances and Disease Registries (ATSDR), Environmental Protection Agency (EPA), National Research Council (NRC)] and to serious environmental cleanup efforts (Superfund Sites). Popular Superfund sites have included Love Canal, New York, Woburn, Massachusetts, Hinckley, California, and Toms River, New Jersey. Other cancer-related hotspots have been recently highlighted (USA TODAY, October 5, 2000). There is serious concern for those individuals exposed to toxic wastes and their genetic-related health problems. Increased occurrences of childhood leukemias, brain cancers, liver cancers, breast and ovarian cancers, gastrointestinal cancers, miscarrages, birth defects and low-birth-weight babies all have been found associated with uncontrolled hazardous waste sites. Gene Therapy, Genetic Testing and Prenatal Diagnosis are new medical procedures that can be applied to deal with hazardous waste associated acquired genetic disorders (HWAAGD). A database (HWAAGD) is being compiled and HWAAGD models formulated showing how these new genetic procedures can be used to deal with cases reported for completed exposure pathway sites. Weekly environmental updates are also provided at www.geneamplificationintl.com highlighting these and other environmental -related issues. These updates can be easily downloaded to most computer printers.

P1155. Association studies in pharmacogenomics-how valid are they?

L. J. Sheffield[1,2], *S. Forrest*[1], *P. Ioannou*[1], *N. Cranswick*[1,3]
[1]*Murdoch Childrens Research Institute; Parkville, Australia;* [2]*University of Melbourne; Parkville, Australia;* [3]*Royal Childrens Hospital; Melbourne, Australia*
sheffld@cryptic.rch.unimelb.edu.au

An exciting promise of pharmacogenomics research is to develop gene tests that will predict which individuals are at increased risk of side effects and which individuals will benefit most from each specific drug. Association studies are often used. A target group is selected (eg those with abnormal drug clearance and/ or side effects) and they are compared with a control group with no side effects or normal clearance. We discuss the issues relating to selecting these groups and what sample size is required. It is important to know the population distribution of the polymorphisms being studied and if possible select the control group from this population so the design becomes a nested case-control study. This allows true estimates of the risks for target group for each allele type. The presentation will discuss various methods for adjusting for population admixture such as matching case and control or alternatively using family based studies such as the TDT test. Once a suspected polymorphism is identified there needs to be collaborative studies to verify the findings. An alternative is to examine if the polymorphism found to be important actually affects gene function. We have developed an in vitro method, of inserting a particular single base change into a gene and studying the change in function using an attached reporter molecule

P1156. Measuring the affects of aneuploidy; gene expression differences between Down syndrome and normal human fetal brain

R. Lyle[1], *P. Chanson*[1], *C. Gehrig*[1], *H. S. Scott*[2], *S. E. Antonarakis*[1]
[1]*University of Geneva Medical School; Geneva, Switzerland;* [2]*The Walter and Eliza Hall Institute of Medical Research; Victoria, Australia*
Robert.Lyle@medecine.unige.ch

Down syndrome (DS) or Trisomy 21 is the most common genetic cause of mental retardation, occuring in approximately 1/700 live births. In addition, there are other neurological phenotypes associated with DS, including brachycephaly, microcephaly and early onset Alzheimer s like desease. The presence of an extra copy of certain but not all of the approximately 250 genes on HC21 is likely to contribute to the DS phenotype. However, the effect of each of these gene products to the specific phenotypes could be either direct of indirect through processes that affect the expression of other genes or gene products. This hypothesis leads to the need for the exploration of global gene expression studies in cells and tissues from individuals with DS and from model organisms (see also Chrast et al. this meeting). Our aim is to use techniques available for measuring global gene expression (SAGE, macroarrays and microarrays) to analyse the differences in gene expression in aneuploid versus normal mammalian brain cells to identify genes and pathways with altered expression levels in DS phenotypes. Here we describe results using large nylon macroarrays that contain over 30 000 human genes to analyse gene expression differences between DS versus normal human fetal brain at 20 weeks of gestation. We have identified a number of genes which show consistent changes within different DS and normal samples and are confirming these changes by Northern blot and RNase protection. Comparison of data from human and mouse models of DS, as well as data from different techniques (SAGE), will enable us to identify the most important genes and pathways involved in the DS neurological phenotype.

P1157. Performance Evaluation Of A Novel Application For Comparative Dna Sequencing Analysis And Mutation Detection.

C. Kosman
Applied Biosystems; Foster City, CA United States
kosmanca@appliedbiosystems.com

We have developed a new practical application for comparative DNA sequencing analysis. The focus of this new application is the discovery or identification of sequence variants in a data set of sequence derived from a single locus in various individuals. This tool allows for the rapid and accurate analysis, and alignment of multiple sequence comparisons containing mixed-base positions against a reference sequence. This application uses procedures and algorithms better suited to the unique nature of comparative sequencing data. Specifically, the prior knowledge of a reference sequence available in many comparative sequencing projects is used to streamline and automate portions of the analysis. In addition, new algorithms were developed that leverage the high degree of sequence similarity found in comparative sequencing applications to aid in the analysis. The

software performs sequence analysis and aligns the analyzed sequence to a reference sequence. It also aligns imported sequences that contain variants. It will then analyze the compared sequences, provide protein translation and report the analysis in a convenient format. Here, we present the results from the performance evaluation of this new software tool on several types of comparative sequencing data sets. For these analyses, this new application produced results concordant with those previously obtained using standard DNA analysis tools while providing substantial performance advantages over these tools. In all cases the analyses were completed in significantly less time and required less manual manipulation of the data. The innovative approaches employed in this tool result in less user intervention needed to achieve improved mutation detection.

P1158. Automatic method to construct complete genome high-resolution physical maps integrating data from different sources

J. Sainz, *S. Gudjonsson, G. Sigurdsson, K. Agnarsson, O. Atladottir, U. Benitez, S. Einarsson, H. Magnusson, K. Mylonas, G. Thorisson, D. Oskarsson, K. Stefansson, J. Gulcher*
Decode Genetics; Reykjavik, Iceland
sainz@decode.is

We constructed a high resolution physical map of the human genome including markers and genes using a method that integrates data from different sources. We store the data in relational databases (Oracle and Access) and use queries that process and analyze the data to construct physical maps integrating all available information. We integrated data generated in our group with data from the public domain. We used the fingerprinting contig database from Washington University and the Sanger Center, and the Human Genome Project Working Draft sequence from the University of California at Santa Cruz. We generated hybridization data with 8.040 microsatellite markers versus BACs from the RPCI-11 library. Electronic PCR versus the human genome draft sequence was performed with approximately 1,000.000 markers designed from BAC end sequences, known STSs, gene Reference Sequences, UniGene clusters and sequences contained in one Affymetrix expression chip. Positional discrepancies from different sources were analyzed and a final location was provided to the markers. The integrated physical map includes physical locations for 9.446 microsatellite markers, 9.127 gene Reference Sequences, 9.147 Affymetryx expression chip sequences and 17.380 markers from UniGene EST clusters. The high degree of automation of the process allow us to rapidly update and construct a human genome physical map with new information on a monthly basis.

P1159. Towards a complete transcript map of the human X chromosome

R. Sudbrak[1], *S. Hennig*[1], *G. Teltow*[1], *K. Rak*[1], *P. Sterk*[2], *H. Lehrach*[1]
[1]*Max-Planck-Institute for Molecular Genetics; Berlin, Germany;* [2]*EMBL-EBI; Hinxton, United Kingdom*
sudbrak@molgen.mpg.de

The X chromosome is one of the best characterized human chromosomes. Most genes on the X chromosome display a haploid status in males and as a consequence, recessive mutations in X-linked disease genes result in hemizygous affected males and heterozygous (usually asymptomatic) carrier females. The easily recognizable inheritance pattern of X-linked diseases allows instant assignment of the causative genes to the X chromosome. For these reasons, genetic analysis and mapping of genes on the X chromosome have been greatly facilitated. It is estimated that the X chromosome contains 1.500-4,000 genes, but only 446 UniGene cluster (December 2000 version) have been assigned to the chromosome so far. By the analysis of the working draft sequence of the human X chromosome we will assemble a close to complete X chromosome gene collection. So far, we identified 1500 X-linked UniGene cluster. The majority of clusters are aligned to the sequence of the goldenpath (http;//genome.ucsc.edu/). A set of two IMAGE clones per cluster were identified and re-arrayed. This array will greatly accelerate physical mapping and candidate gene approaches aiming in identifying disease-causing mutations. In order to identify transcripts not covered by UniGene clusters we used gene prediction methods for generation of hypothetical cDNA and peptide sequences. These in-silico gene targets are evaluated in a second step by doing sequence comparisons against all available mammalian Unigene sets, Protein databases and model organisms, including non-public EST databases generated at MPIMG. Potential novel gene targets are classified then by low or no detectable homology to any of the human databases. Out of these only the predictions with several exons and a minimum cDNA length of more than 500 bp were initially considered for further analysis. The analysis of the genomic sequence of the human X chromosome resulted in a set of 700 novel gene candidates, where no significant sequence homology to the human Unigene set could be detected, and which fulfilled the arguments mentioned above. These candidates are presently under investigation.
Results are stored in The Integrated X chromosome Database (IXDB, http;//ixdb.molgen.mpg.de/). In addition to our own mapping and in-silico data IXDB integrates data from other public databases as well as data from the EC Transcript Map Consortium. Nine chromosome spanning and eleven contig maps can be viewed.

P1160. A Comprehensive BAC Resource

S. Zhao
The Institute for Genomic Research (TIGR); Rockville, MD United States
szhao@tigr.org

Bacterial Artificial Chromosome (BAC) clones carry large insert (80-300 kb) and are used by large genome centers and small laboratories. Human Genome Project and other research projects have generated extensive maps, sequences, annotation and function information for many BACs. The data are extremely valuable. However, currently, different pieces of information are scattered over different places and an integration of the existing data for each BAC is lacking. Researchers have to spend significant amount of time searching for information of the BACs that they are interested in, and often are unable to obtain a complete picture that sometimes results in duplication of efforts. Although a few sites start to offer a more complete set of data for human BACs, these resources are aimed to facilitate the collaboration among genome centers to finish the human genome and therefore are not easy to use by the general research community. In order to utilize the massive data efficiently and to realize the ultimate goals of the genome projects, a centralized place that gathers, integrates and makes the data easily accessible is greatly needed. Towards this end, we build a comprehensive BAC resource for species including human, mouse, rat, and zebrafish. The current database contains over 630,000 human BACs and 265,000 mouse BACs that are mapped, with 23,000 human clones and 1,000 mouse clones having sequences and annotation. The database is freely accessible via the web and supports sequence or clone searches and anonymous FTP. The relevant sites and resources are described at
http;//www.tigr.org/tdb/BacResource/BAC_resource_intro.html.

P1161. A 1 Mb Resolution BAC Map Spanning the Human Genome

S. L. Narasimhan, *T. Weber, M. Arcaro, M. Morley, A. Bruzel, V. G. Cheung*
University of Pennsylvania, Children s Hospital of Philadelphia; Philadelphia, PA United States
sandya2@mail.med.upenn.edu

We are generating a repository of mapped bacterial artificial chromosome (BAC) clones that covers the human genome at a resolution of ~ 1 Mb. These clones are designed as reagents for microarray-based gene mapping and Direct Identical-by-descent Mapping . They can also be used to identify and characterize chromosomal abnormalities. The BAC clones from the Roswell Park Cancer Institute human male BAC library (RP- II) have been mapped to radiation-hybrid (RH) markers by filter hybridization and PCR. The clones are characterized by HindIII fingerprinting, end sequencing, and fluorescent in situ hybridization . These characterized clones allow us to integrate the RH map with cytogenetic map and the human genome sequence. Currently, we have over 3,000 mapped BAC clones, and characterization is near completion. A database, GenMapDB, http;//genomics.med.upenn.edu/genmapdb, has been developed to provide information on these mapped clones as well as to consolidate multiple resources related to this project. In this presentation, we will discuss the content of this clone collection and illustrate its use in genome analysis.

P1162. Annotation of Human Chromosome 7

S. W. Scherer[1], *J. Cheung*[1], *K. Nakabayashi*[1], *J. Vincent*[1], *E. Petek*[1], *J. Herbrick*[1], *J. Grover*[1], *B. Kerem*[2], *M. Li*[1], *G. Boocock*[1], *J. Rommens*[1], *L. Osborne*[1], *A. Boright*[1], *L. Tsui*[1]
[1]*Hospital for Sick Children; Toronto, ON Canada;* [2]*Hebrew University; Jerusalem, Israel*
steve@genet.sickkids.on.ca

The aim of our project is to establish a fully annotated physical, DNA sequence, and gene map of human chromosome 7. As part of this annotation we are also collecting and characterizing all clinical, functional, and biological information relevant to chromosome 7. Through analysis of DNA sequence data in the public and Celera databases we could detect corresponding genomic fragments for over 98% (6,500 of 6,579) of the STSs known to map to chromosome 7. Initially, 70 large (>500 kb) DNA

sequence contigs could be established with the average length being 1.6 Mb and the largest being 11.5 Mb. To complete the map of chromosome 7 we have determined the end-sequence of 200 cosmid or BAC clones and generated over 700 kb of genomic DNA sequence resulting in coverage or closure of 16 gaps. Our analysis indicate most of the euchromatic region is now covered but that the pericentromeric region is still under-represented requiring additional mapping and sequencing work. Moreover, a 100-200 kb duplication at 7p11.1 was identified to be present in 30 copies elsewhere in the genome and another 200 kb segment was found in 3-4 copies at 7q11.23, further complicating assemblies. With the current DNA sequence map we have, so far, identified over 718 full-length genes and 1382 additional transcriptional units (total of 2100) on chromosome 7. Also, 100 rearrangement breakpoints, 700 FISH mapped clones, 3 fragile sites, and 3 imprinted regions could be accurately placed on the DNA sequence-based map for future disease gene research.

P1163. Analysis of Gene Transcripts of Chromosome 21 by HPSF Oligonucleotide Microarrays

H. Donner[1], B. Drescher[2], J. Weber[2], S. Wegener[1], H. Paul[1], R. Kirchner[1], ***W. Mann[1]***
[1]Department of DNA microarrays, MWG-Biotech AG; Ebersberg, Germany; [2]Department of Bioinformatics, MWG-Biotech AG; Ebersberg, Germany
wmann@mwgdna.com

Chromosome 21 is the smallest human autosome and an additional copy causes Down syndrome, the most frequent genetic disorder of mental degradation in humans. The complete DNA sequence was published recently, leading to 127 known as well as 98 predicted genes. Based on the complete chromosomal DNA sequence we have computationally designed specific oligonucleotides for all known, predicted as well more than 275 possible gene transcripts, which had been annotated using the Annotation EngineTM module of our BioGISTTM software package. For every gene transcript three different oligonucleotides were robotically spotted (Affymetrix 417 arrayer) onto glass slides. Those microarrays were hybridized with fluorescent labeled cDNA derived from lymphoblastoid cell lines of two patients suffering from Down syndrome as well as healthy controls. Gene transcripts were regarded to be present if two of three oligonucleotides showed significant values above background. Also variations in the mRMA amount between patients and controls were monitored and data obtained from these experiments will be presented. Because of the recently published DNA sequence, cDNA clones of many known, predicted as well as possible genes are not available. HPSF oligonucleotide microarrays therefore provides a valuable tool for first phase expression analysis of genes not known to be expressed so far.

P1164. Pyrosequencing Technology For Signature Tag Determination.

K. Hjelm*, P. Blomgren, F. Lysholm, G. Magnusson, R. Kroon, A. Tallsj*
Pyrosequencing AB; Uppsala, Sweden
katarina.hjelm@pyrosequencing.com

Pyrosequencing is a non-electrophoretic DNA sequencing method based on the detection of pyrophosphate released as a result of nucleotide incorporation by DNA polymerase. An enzyme cascade converts the pyrophosphate to light which is detected by the camera in a PSQ“96 System (Pyrosequencing AB). The system includes a PSQ“96 instrument with dedicated software and reagent kits for the application. Each reagent kit contains the enzyme and substrate mixtures and the four nucleotide solutions, ready for use. Sequence stretches of some 20 bases can be used to identify specific genes or genomes. Such stretches are known as Signature Tags . Pyrosequencing“ technology has now been adapted to enable the reading of these signature tag sequences. The components of a new reagent kit allow parallel processing of up to 96 samples within approximately one hour. Samples are easily prepared from PCR products. As the four nucleotides are added sequentially to the reaction mixture, the sequence of an unknown sample is easily determined from the developing pyrogram“ in which peaks are generated as nucleotides are incorporated. A dedicated algorithm for automatic base calling and quality assessment from Pyrosequencing“ data has been developed. The results are stored in a local database, fully traceable and easily editable, if desired. On average, over 20 bases were correctly sequenced on a number of templates, differing in origin, GC content and length. The suitability of Pyrosequencing“ technology for Signature Tag sequencing was clearly demonstrated.

P1165. Recombination, interference and sequence; comparison of chromosomes 21 and 22.

A. Collins*, W. Tapper, X. Ke*
University of Southampton; Southampton, United Kingdom
arc@soton.ac.uk

The euchromatic sequences of chromosomes 21 and 22 are almost complete and have similar lengths (33.7 - 34.6 Mb). This similarity largely controls for the influence of sequence length making comparison of patterns of recombination and chiasma interference interesting. There are marked differences between the two chromosomes in both of these aspects. Overall chromosome 21 shows less recombination, particularly in males, than chromosome 22 (56 versus 71 cM). The level of chiasma interference is rather close to the genome average of p=0.35 (in the Rao et al., mapping function) for both sexes for chromosome 22 and for female chromosome 21. However, the paternal chromosome 21 level is p=0.05. This indicates very strong chiasma interference. We suggest that these differences depend on the very different sequence composition of the two chromosomes, particularly in the distribution of GT/CA repeats and/or tetranucleotides. We observe that recombination in males is significantly associated with these repeat tracts. For chromosome 21 the longer repeat tracts are localised to the sub-telomeric region only but they are more widespread on 22. This suggests there may be fewer opportunities for double recombination events to occur, within a critical distance, for chromosome 21, consistent with higher levels of interference. The sex-difference may be related to the greater condensation of chromosomes in paternal meiosis, which restricts recombination to areas with the longer repeat tracts.

P1166. Mapping of DNA loop organization of a region within chromosome 16q22.1 containing the LCAT gene cluster.

S. A. Shaposhnikov[1,2]*, E. Frengen[2], V. V. Zverev[1], H. Prydz[2]*
[1]Research Institute for Viral Preparations; Moscow, Russian Federation; [2]Biotechnology Centre of Oslo; Oslo, Norway
sergeys@biotek.uio.no

The present work is focused on the exploration of domain organization of a specific region within human chromosome 16q22.1 containing a tight cluster of genes, the LCAT gene cluster. We suggest that the organization of the gene cluster has biological significance. We have recently generated a high-resolution integrated map covering 2.8 Mb surrounding the LCAT gene cluster (Frengen, E., Rocca-Serra, P., Shaposhnikov, S. et al., Genomics 70(3), in press, 2000). This map includes a candidate region for containing a putative tumor supressor gene. The approach used in the present work is based on the ability of cellular topoisomerase II to cleave DNA in the presence of several anticancer agents. The cleavage sites are associated with regions of DNA attachment to the nuclear skeleton, and indicate DNA loop anchorage sites. Thus, verifying the topoisomerase II cleavage sites within the region allow mapping of the domain organization. Human cells have been exposed to topoisomerase II inhibitor, the DNA from the cells was cleaved with restriction enzymes, separated by pulsed field gel electrophoresis, blotted, and hybridized with probes from the LCAT gene cluster region. The domain maps allow relation of the structural architecture of the region to the functional organization of the DNA.

P1167. Latest Advances in SNP Genotyping by Dynamic Allele Specific Hybridization (DASH)

W. M. Howell*, M. Jobs, L. Feuk, T. Emahazion, J. A. Prince, A. J. Brookes*
Center for Genomics Research, Karolinska Institute; Stockholm, Sweden
Anthony.Brookes@cgr.ki.se

DASH is a method for high-throughput genotyping of SNPs, insertion/deletions (indels), and other sequence variations (Genome Res, 2001, 11;152-162; Nature Biotech, 1999, 17;87-88). Allele discrimination is based upon real time detection of hybridisation stability differences in duplex DNAs involving fully matched or allelic-mismatched structures. The published procedure involves PCR amplification of a target sequence (with one primer biotinylated), streptavidin affinity capture of one product strand, hybridisation of an unlabelled oligonucleotide probe (matching one allele) at low temperature in the presence of a double-strand specific fluorescent dye, and steady heating through a temperature range while monitoring fluorescence. Changes in the amount of duplexed probe-target material are thereby tracked as a melting-curve profile, indicating whether the probe-target duplexes were perfectly matched, subtly mismatched, or a heterozygous mixture of the two. A validation study was performed, involving application of DASH to 89 randomly selected proven SNPs. Non-optimized assay designs (worst-case scenario) resulted in 79 functional assays (89%). Secondary structures in target sequences were found to be critically harmful to DASH performance. Identifying and altering key PCR primer bases involved in secondary structure formation can overcome this

problem. This led to an improved design success rate of essentially 100% (6/6 initially non-functioning DASH assays were restored via this optimized design strategy). A software tool for automated DASH assay design that incorporates the new design system has now been developed. Subsequent DASH studies demonstrated that the method provides genotyping accuracy >99.9%, and a reproducibility of 100%. With the above DASH system an individual worker can easily genotype several thousand samples/week.

To move towards genotyping hundreds of thousands of samples, we are exploring many new DASH modifications. Reaction principles are now in place for scaling up via i) micro-volume (<2?L) PCR in 1536-well plates, ii) employing liquid-handling-free spun-arrays for DASH execution on flat surfaces, and iii) incorporating multiplexed potential by using FRET-based signal production. Final imaging of DASH melting-curves will employ a CCD camera. We anticipate that a fully validated system along these lines will be produced this year.

Also presented orally in Concurrent Session 5: Poster Discussion Methods to Detect Genomic Variation

P1168. Gene Dosage using Real Time PCR ; Application for Deletion Carrier Diagnosis in Galactosaemia.

C. Costa[1], *M. Brivet*[1], *M. Abramowicz*[2], *E. Vamos*[2], *A. Legrand*[1], *J. Costa*[3]
[1]*H pital Bic tre AP-HP; Kremlin Bic tre, France;* [2]*Universite Libre Bruxelles H pital Erasme; Bruxelles, Belgium;* [3]*H pital Am ricain de Paris; Neuilly, France*
catherine.costa@bct.ap-hop-paris.fr

In two related galactosaemic patients from an Ashkenazi jewish family, no PCR amplification of the 11 exons of the uridyltransferase gene (galt) was possible. A homozygote Galt gene deletion was then suspected. Successful co-amplification by duplex PCR of another gene eliminated the hypothesis of PCR inhibitors. Sequencing of the 11 exons in the parents did not reveal any heterozygote mutation or polymorphism and suggested a heterozygote deletion in both parents. To confirm the possible deletion, real time quantitative PCR on a LightCycler system was used. The Galt gene and as reference internal control the superoxyde dismutase (SOD) gene were co-amplified in the same tube by duplex PCR in DNA from patients, parents and controls; they were compared to a range of serial DNA dilutions for quantitation. The two genes were submitted to the same PCR fluctuations since they were amplified in the same reaction. The copy number of Galt gene was about twofold lower in each parent s DNA (ratio Galt/SOD=0.43 and 0.47 for mother and father respectively) than in control DNA (ratio=1.2+/-0.2) thus confirming the heterozygous status for the deletion. The quantitative real time PCR by duplex PCR in a single tube on genomic DNA represents an interesting alternative to time-consuming and labor-intensive Southern blotting or pulsed field gel electrophoresis, for the diagnosis of deletions, especially in heterozygous patients. Complete GALT gene deletion is rare and was only described previously in two other unrelated Ashkenazi jewish families.

P1169. A Novel Mutation In The PHKG2 Gene In A Case Of Phosphorylase Kinase Deficient Liver Glycogenosis Associated With Cirrhosis

C. Bruno[1], *F. Menni*[2], *K. E. Niezen-Koning*[3], *C. Minetti*[1], *R. Parini*[2]
[1]*Dept. of Pediatrics, Istituto Giannina Gaslini; Genova, Italy;* [2]*Dept. of Pediatrics, Istituti Clinici di Perfezionamento; Milano, Italy;* [3]*Dept. of Pediatrics, Beatrix Children s Hospital; Groningen, The Netherlands*
cb2246@yahoo.com

We describe a novel homozygous PHKG2 mutation in a boy with liver Phk deficiency who developed cirrhosis early in infancy. A 9-year-old boy, the second child of healthy consanguineous Pakistani parents, presented at 16 months with hepatomegaly and jaundice. Blood tests showed liver cell necrosis, cholestasis, severe hypocalcaemia, and microcytic hypocromic anaemia. Ultrasonography showed an enlarged and homogeneously hyperecogenic liver. A liver biopsy revealed severe fibrosis; hepatic debranching and phosphorylase enzyme activities were normal while phosphorylase kinase activity was undetectable The clinical follow-up of the child showed persistent alteration of function liver tests with a normal glucose control and a correct diet treatment. A second liver biopsy performed at the age of 8 years showed liver cirrhosis. Direct sequencing of the PHKG2 gene revealed a single homozygous T-to-C mutation at nucleotide position 1122 in exon 10, replacing an encoded leucine with proline at codon 357 (L357P). This mutation segregates with the biochemical phenotype, and it was not found by us in 50 normal controls. These data enlarge the spectrum of mutations associated with PhKG2 gene and further confirms that PhK deficient liver glycogenosis associated with cirrhosys is caused by PHKG2 mutations.

P1170. Glycogen Storage Disease type Ia; molecular study in Brazilian patients

E. L. Sartorato[1], *F. C. Reis*[1], *H. C. Caldas*[1], *I. V. D. Schwartz*[2], *R. Giugliani*[2], *M. G. Burin*[2], *D. Y. J. Norato*[1]
[1]*Universidade Estadual de Campinas; Campinas - S o Paulo, Brazil;* [2]*Universidade Federal do Rio Grande do Sul; Porto Alegre - Rio Grande do Sul, Brazil*
sartor@unicamp.br

Mutations in the glucose-6-phosphatase (G6Pase) gene are responsible for glycogen storage disease type Ia (GSDIa). It is characterized by growth retardation, hepatomegaly, hypoglicemia, hyperlipidemia and lactic acidosis. In this study we are reporting G6PC gene mutations in eight of the twenty-five Brazilian patients with clinical symptoms of GSDIa . Five previously described mutations (R83C, Q347X, V338F, D38V and G68R) were detected. The two most common mutations identified were R83C and Q347X, accounting for 8 out of 14 (57,14%) mutant alleles. It was also analyzed the 1176 polymorphism and two intronic mutations (IVS3-58T>A and IVS4+10G>A). We used the minigenes strategy in order to verify the effect of this intronic mutations in the splice mechanism. This study underscores that molecular genetic analysis is a reliable and convenient alternative to the enzyme assay in a fresh liver biopsy specimen to diagnose GSDIa.

P1171. A novel splicing mutation (IVS4—3C>G) in the G6Pase gene of five Portuguese GSD Ia patients

H. Rocha[1], *L. Diogo*[2], *E. Martins*[3], *A. Cabral*[4], *C. Sousa*[1], *I. Maire*[5], *L. Vilarinho*[1]
[1]*Instituto de Gen tica M dica Jacinto de Magalh es; Porto, Portugal;* [2]*Hospital Pedi trico de Coimbra; Coimbra, Portugal;* [3]*Hospital Maria P a; Porto, Portugal;* [4]*Unidade de Doen as Metab licas, Servi o de Pediatria de Hospital Santa Maria; Lisboa, Portugal;* [5]*Service de Biochimie P diatrique, H pital Debrousse; Lyon, France*
hugo.rocha@igm.min-saude.pt

Glycogen Storage Disease Type Ia (von Gierke disease, GSD Ia, MIM# 232200) is an autossomal recessive metabolic disorder caused by a deficient activity of the catalytic unit of the microssomal glucose-6-phosphatase (G6Pase) system. Since the characterisation of the G6Pase catalytic unit gene, a steadily growing number of mutations, responsible for GSD Ia, have been reported. The identification of mutations allows a non-invasive DNA-based method for the diagnosis of patients suspected of having GSD Ia, but has also important implications with respect to carrier detection and prenatal diagnosis. Here we describe a new mutation in intron four of the G6Pase gene, that cause a change in the acceptor site for splicing. This mutation, IVS4-3C>G, was found in five Portuguese patients with a previous enzymatic diagnosis of GSD Ia (three homozygous and two heterozygous), and was absent from the 228 control alleles tested. Due to the unavailability of liver biopsy from any of these patients, it was not possible to confirm the presence of the aberrant mRNA. In spite of this limitation, there are some reasons that strongly suggest that this mutation cause an aberrant splicing of the G6Pase gene, leading to an impaired activity of the G6Pase; (i) the nature of the substitution (only 1% of the acceptor splicing sites have a guanine at that position); (ii) the frequency of this mutation among Portuguese patients (17% of the alleles); (iii) the absence of any other mutation in the G6Pase gene of the homozygous patients and (iiii) the absence from the control alleles.

P1172. Cytochrome oxidase deficiency presenting with microcephaly and agenesis of the corpus callosum

A. M. Innes[1,2], *D. Applegarth*[3], *M. Collison*[2], *S. S. Seshia*[2], *L. E. Seargeant*[2], *M. Del Bigio*[2], *B. Robinson*[4], *C. R. Greenberg*[2]
[1]*University of Calgary; Calgary, AB Canada;* [2]*University of Manitoba; Winnipeg, MB Canada;* [3]*University of British Columbia; Vancouver, BC Canada;* [4]*University of Toronto; Toronto, ON Canada*
Micheil.Innes@CRHA-Health.Ab.Ca

A term baby weighing 3.2 kg (50%) and measuring 50 cm (50%) was born to an apparently non-consanguineous couple from a remote aboriginal Cree community in Manitoba. Her head circumference measured 31.5 cm (<5%). She developed a severe lactic acidosis (5-10uM) with an elevated lactate/pyruvate ratio. Positive investigations included severe microcephaly with agenesis of the corpus callosum on MRI, an abnormal muscle biopsy with prominent mitochondria and on histochemical analysis many fibres lacked or had diminished cytochrome oxidase (COX) activity. COX activity was also low (20% of control) in isolated muscle mitochondria, with very mildly decreased complex I activity, normal complex II and citrate synthase activities. COX activity, PDH activity, Succinate Cytochrome c Reductase and the lactate/pyruvate ratio in cultured skin fibroblasts were normal. Mitochondrial DNA deletion studies were normal and the NARP mutation was absent. The lactic acidosis resolved in 2-3

weeks but the patient developed severe apnea , intractable seizures, and showed no neurodevelopmental progress. She died at age 15 months with a head circumference of 36 cm. The COX complex (complex IV of the mitochondrial respiratory chain) is responsible for the final event of electron transport. This is a 13 subunit enzyme, the largest 3 subunits are encoded by the mitochondrial genome, and the 10 other subunits are encoded by the nuclear genome. A recent review (Robinson, 2000) summarizes the 5 well-delineated phenotypes associated with COX deficiency. These include a tubulopathy/ataxia phenotype (COX 10), Leigh disease (SURF1 and other genes), a fatal infantile phenotype with severe acidosis and brain sparing , a cardiomyopathy phenotype (SCO2) and a phenotype with mild pyschomotor retardation and eventual fulminant acidosis unique to regions of Quebec (2p16). To our knowledge, our patient s presentation with evidence of prenatal brain effects manifesting as severe microcephaly and agenesis of the corpus callosum is unique. Given the uneven COX staining in different muscle fibres suggesting heteroplasmy, an underlying mtDNA abnormality affecting COX activity and oxidative phosphorylation appears most likely.

P1173. A novel SURF-1 mutation in Leigh syndrome caused by cytochrome c oxidase deficiency

M. DiRocco[1], *M. Bado*[2], *L. Arata*[3], *P. Broda*[2], *U. Caruso*[2], *V. Capra*[3], *P. Tortori-Donati*[4], *C. Minetti*[2], *C. Bruno*[2]
[1]*II Div. of Pediatrics, Istituto Giannina Gaslini; Genova, Italy;* [2]*Neuromuscular Diseases Unit, Univ. of Genova, Istituto G. Gaslini; Genova, Italy;* [3]*Div. of Neurosurgery, Istituto G. Gaslini; Genova, Italy;* [4]*Div. of Neuroradiology, Istituto G. Gaslini; Genova, Italy*
majadirocco@ospedale-gaslini.ge.it

We have identified a novel mutation in the SURF-1 gene in a child with clinical and neuroradiological evidence of Leigh syndrome and cytohrome c oxidase deficiency (COX). A 15-month-old girl, first child of non related Italian parents, presented at 1 year of age with failure to thrive, neurodevelopmental regression and frequent episodes of vomiting. Physical examination showed facial dysmorphism, hirsutism, and muscle hypotonia. Venous blood lactate was elevated (32mg/dl, normal < 22). GS-MS of urinary organic acids showed a massive excrection of lactate and related metabolites. Plasma and urinary amino acids were normal. Brain MRI showed bilateral increase signal of thalami on T2-weighted scans. Histochemistry of skeletal muscle showed virtually absent cytochrome c oxidase (COX) activity and no ragged-red fibers. Biochemical analysis of a muscle homogenate confirmed an isolated reduction of COX. Genomic DNA was isolated by peripheral limphocytes and SURF-1 gene was amplified by PCR protocols. Sequencing analysis of the SURF-1 gene revealed a novel heterozygous single base-pair deletion at nucleotide position 566 (566delG) in exon 6, which predicts a truncated surf1 protein. A mutation in intron sequence or in other regulatory sequence of SURF-1 is predicted to account for the missing second mutation.

P1174. Tissue-specific distribution of mtDNA mutations in 26 MERRF and MELAS patients

M. Tesarova, *M. Capkova, V. Kaplanova, H. Hansikova, J. Zeman*
Department of Paediatrics, First Faculty of Medicine, Charles University; Prague, Czech Republic
marketa.tesarova@seznam.cz

Clinical symptoms in patients with mitochondrial encephalomyopathies are very heterogeneous. Oxidative phosphorylation system, as the only exception in mammalian cell, is encoded by both the nuclear DNA (nDNA) and the mitochondrial DNA (mtDNA). The presence of homogeneous population of mtDNA molecules in tissue is called homoplasmy, the presence of wild-type and mutated mtDNA molecules is known as heteroplasmy. Clinical manifestation of mtDNA mutations occurs when the percentage of mutated mtDNA copies reaches a threshold value, when the cell is no more able to tolerate the mutation and decrease in activity of several complexes of respiratory chain cause insufficient energy provision. In this study we analysed tissue-specific distribution of mtDNA mutation A3243G and mtDNA mutation A8344G in blood, muscles, hair roots and skin fibroblasts from 26 patients in 10 families with MELAS and MERRF syndrome. In patients with syndrome MERRF and MELAS, the level of heteroplasmy varied between 6% and 95%. The range of heteroplasmy of mtDNA mutations in various tissues in one patient was quite broad (from 48% to 95%). The highest heteroplasmy was found in muscles, followed on with hair roots. Low levels of mutated mtDNA molecules were found in blood and fibroblasts. In most of investigated families the portion of mutated mtDNA molecules increase in descendant generations, the increase wasn«t the same in all studied tissues. Supported by GACR 302/99/0648.

P1175. MEHMO (Mental retardation, Epileptic seizures, Hypogonadism and -genitalism, Microcephaly, Obesity); A new X-linked mitochondrial depletion syndrome.

D. Lev[1], *E. Leshinsky-Silver*[1], *A. Singer*[2], *R. Navieux*[3], *N. Bibi*[4], *V. Barash*[5], *M. Sadeh*[1], *T. Lerman-Sagie*[1]
[1]*Wolfson Medical Center; Holon, Israel;* [2]*Kaplan Medical Center; Rechovot, Israel;* [3]*The Mitochondrial and metabolic disease Center, UCSD; San Diego, CA United States;* [4]*Barzilai Medical Center; Ashkelon, Israel;* [5]*Hadassa Medical Center; Jerusalem, Israel*
dorlev@post.tau.ac.il

MEHMO is a new X-linked disorder characterized by mental retardation, epileptic seizures, hypogonadism and genitalism, microcephaly, obesity and hypotonia. It was recently assigned to the locus Xp21.1-p22.13 that is flanked by CYBB and DXS365. We describe a child with MEHMO and lactic acidosis .A muscle biopsy revealed markedly reduced activities of complexes 1,3 and 4 of the electron transport chain Histological staining showed mitochondrial proliferation and lipid storage. Elecron microscopy revealed abnormal huge mitochondria with concentric cristae and electron dense bodies. Southern blot analysis of the child s DNA extracted from muscle biopsy using both mitochondrial and nuclear probes revealed a normal mtDNA size but a profound depletion (30%) of the mtDNA.
These findings suggest a deficiency in one or a fewfactors involved in the processing or control of mitochondial DNA synthesis. This is the first report of identification of MEHMO as a mitochondrial disease and the first X-linked mitochondrial depletion syndrome. MEHMO should be added to the growing list of neurologic disorders presented with mitochondrial DNA depletion. The applications of these findings to prenatal diagnosis and to the understanding of the processes leading to mtDNA synthesis are discussed.

P1176. Construction of transgenic mice carrying human mitochondria

V. Sokolova, *N. Arbuzova*
Institute for Experimental Medicine; St.-Petersburg, Russian Federation
molgen@molgen.iem.ras.spb.ru

A human mitochondrial suspension isolated from HepG2 culture was microinjected into fertilized eggs of CBA«C57BL mice. In the first experimental series the microinjected zygotes were cultured in vitro to the blastocyst stage. All embryos survived and developed normally. Previously, species-specific primers were designed for the human mtDNA and the endogenous mt-DNA in the embryos. An Alu1 cleavage site was found in the human mtDNA sequence which is absent from the murine mtDNA. This allowed us to discriminate the amplified human mtDNA from that of the mouse. A presence of human mtDNA was shown by PCR with species-specific primers at every stage of development of the preimplantation embryos. Then 2- and 4-cell embryos derived from microinjection zygotes were separated to blastomeres. More than once human mtDNA was revealed only in one blastomere after the first cleavage of a zygote. In some cases one blastomere of the 4-cell embryo carried no human material and another showed the presence of somewhat lower amount of human mtDNA. In all cases when microinjected zygotes reached the blastocyst stage human mtDNA was detected. In the second experiments the microinjected zygotes were transplanted into the uteri of recipient mice. DNA of foreign mitochondria was found by PCR with species-specific primers in the embryos at 8th - 13th days of embryogenesis. The per cent of transgenic embryos was 13%. The several 13th-days embryos were divided to different tissues and organs which further were analyzed separately for presence of human mtDNA. Heteroplasmic mice produced by this technique will be useful in study of mitochondrial dynamics and may hasten the creation of animal models of human mt-DNA-based diseases.

P1177. Molecular characterization of phosphofructokinase deficiency (Tarui s Disease) in a Chinese male

P. S. Lai[1], *H. H. Khng*[1], *P. Kuperan*[2], *S. Miwa*[3], *H. Kanno*[4], *W. C. Yee*[5]
[1]*National University of Singapore; Singapore, Singapore;* [2]*Tan Tock Seng Hospital; Singapore, Singapore;* [3]*Okinaka Memorial Institute for Medical Research; Tokyo, Japan;* [4]*Okinaka Memorial Institute of Medical Research Institute; Tokyo, Japan;* [5]*National Neuroscience Institute; Singapore, Singapore*
paelaips@nus.edu.sg

Hereditary phosphofructokinase deficiency or Tarui s disease is an autosomal recessive disorder caused by a deficiency of phosphofructokinase (PFK) enzyme which catalyzes the ATP-dependent conversion of fructose 6-phosphate to fructose 1,6-biphosphate. Three isoenzymes of PFK have been identified, namely muscle (M), liver (L) and platelet (P). The PFK-M gene responsible for this disorder spans 30 kb and consists of 24 exons encoding three types of tissue-specific mRNA variants. We report the

molecular characterization of a 20 year-old Chinese male with PFK-M deficiency. The proband, an offspring from a consanguineous marriage, presented with exercise intolerance and compensated hemolysis. The genetic defect was identified through RT-PCR and sequencing analysis of mRNA transcripts. A single nucleotide substitution involving a C to T transition was identified in exon 6 of the PFK-M gene. The proband was homozygous for this mutation while the parents were observed to be heterozygous carriers. This mutation generated a stop codon predicted to result in premature termination of translation of the PFK enzyme. This is the first PFK-M mutation known to us to be reported in an ethnic Chinese patient.

P1178. Prosaposin Deficiency Due To 1 Bp Deletion In Sap B Domaine; The Evidence For Functional Sap A Deficiency Due To Nonsence-mediated Decay.

M. Jer bkov *[1,2], M. Hreb cek[1,2], H. Hulkov [1], J. Ledvinov [1], H. Poupetov [1], B. Asfaw[1], L. Bern [1], M. Elleder[1]*
[1]Institute for Inherited Metabolic Disorders; 1st Faculty of Medicine; Praque, Czech Republic; [2]Center for Integrated Genomics; Prague, Czech Republic
mcerv@lf1.cuni.cz

We describe a case of a fatal infantile storage disease with hepatosplenomegaly and severe neurological disease caused by a deficiency of prosaposin, precursor protein giving rise to saposins A,B,C, and D. Saposins are essential for in vivo activation of lysosomal hydrolases taking part in the sphingolipid degradation cascade. The patient was homozygous for 1bp deletion in saposin B domain (c803delG), leading to a frameshift and premature stop codon. In obligatory heterozygotes the mutant mRNA was detectable only in nuclear RNA fraction of skin fibroblast, while in cytosolic RNA fraction it was undetectable presumably due to nonsense-mediated mRNA decay. Degradation of majority of the mutant mRNA may lead to functional deficit of saposin A, in spite of normal sap A domain coding sequence. The absence of immunodetectable saposins (saposins A-D) in patient«s tissues was accomompanied by a broad spectrum of stored sphingolipids encompassing ceramide mono-,di-, tri-, tetrahexosides and sulphatide ceramides. In heterozygous parents direct sequencing of RT-PCR products revealed only wild-type cDNA sequence, suggesting that mutant mRNA was significantly less abundant. Mutant cDNA was detected by ARMS only in RNA isolated from nuclear fraction of parents fibroblasts, it was not detectable in cytoplasmic fraction. This confirms that mutant mRNA is in cytoplasm essentially absent or at least significantly less abundant. These findings together with the lack of immunoreactive saposins A-D in the patient s tissues allow to postulate that the c803delG in addition to deficit of saposins B, C and D leads to functional deficit of saposin A due to nonsense-mediated decay.

P1179. Towards the understanding of the lysosomal transport disorders; proteomic maps of lysosomal membrane proteins.

A. V. Pshezhetsky*, V. Seyrantepe, J. Ausseil, K. Landry, Y. Chang*
Sainte-Justine Hospital; Montreal, Canada
alex@justine.umontreal.ca

Lysosomal membrane transport proteins mediate the traffic of protons, cysteine or activated acetyl residues into the lysosome and the catabolic products such as amino acids, monosaccharides and vitamins out of the lysosome. Lack or malfunction of lysosomal transporter proteins causes several severe genetic diseases of children including nephropathic cystinosis CTNS), Niemann-Pick disease type C, sialic acid storage disease (SIASD), cobalamin (cbl) F disease, mucolipidosis IV (ML IV) and Sanfilippo III type C (MPS IIIC). The goal of our proteomic-based research program is to identify the underlying cause of genetic diseases, as reflected by the protein patterns of affected cells and tissues. We have isolated lysosomal membranes from mouse and human liver, obtained their proteomic maps using two-dimensional gel electrophoresis with immobilized pH gradients and identified the major proteins by combining peptide mass fingerprinting, and amino acid sequencing. The obtained results have provided the basis to build a complete proteomic database of the lysosomal membrane. Then we have analyzed lysosomal membrane proteins from cultured skin fibroblasts of normal controls and patients suffering from cbl F and MPS IIIC and identified the protein spots that reproducibly differ between patients and control cells. Candidate disease-causing genes will be cloned, and studied by mutation analysis and/or by expression and functional characterization. This approach may be in the future extended to discovery of other disease-related membrane proteins in peroxisomes, microsomes, mitochondria and the plasma membranes.

P1180. Patients with Gaucher disease and Parkinsonian symptoms do not share a common genotype.

E. Sidransky*, N. Tayebi, M. Callahan, J. Park, B. K. Stubblefield*
National Institute of Mental Health; Bethesda, MD United States
sidranse@irp.nimh.nih.gov

There have been several reports of adult patients with Gaucher disease, the inherited deficiency of lysosomal glucocerebrosidase (GC), who have developed parkinsonian symptoms. We have observed that these patients generally have relatively mild visceral manifestations of Gaucher disease, but develop an early onset, treatment-refractory form of Parkinson s. We performed genotypic analyses of 9 adult patients with type 1 Gaucher disease and parkinsonian manifestations. The patients were from 8 different nations and included Ashkenazi and non-Jewish individuals. Direct sequencing of the 11 exonic regions of the GC gene demonstrated that no two patients shared the same genotype. The common N370S mutation, which was heretofore not associated with neuronopathic Gaucher disease, was encountered in 7 of the patients studied. One patient carried mutation L444P on the paternal allele and D409H on the maternal allele, but Southern blot analyses showed that the maternal allele had an additional 15 kb fragment resulting from a recombination between metaxin and its pseudogene. Metaxin, a convergently transcribed gene located adjacent to the GC pseudogene, encodes for a 317 aa protein which is part of a preprotein import complex in the outer membrane of the mammalian mitochondrion. Sequencing of the gene for metaxin along with the genes for parkin and alpha-synuclein is in progress for all of the 9 patients. Because Parkinson s disease is a relatively common disorder, thought to result from multiple etiologies, there also may be different genes contributing to this phenotype among our patients. Further analyses of the glucocerebrosidase locus in these patients are also being performed to explore the possible association of the parkinsonian symptoms and alterations in genes contiguous to glucocerebrosidase.

P1181. A study of the glucocerebrosidase gene mutations; Vaccinia expression system

K. Hodanova*[1,2], M. Hrebicek[1], Z. Melkova[3]*
[1]Institute of Inherited Metabolic Disorders, 1st Faculty of Medicine, Charles University; Prague, Czech Republic; [2]Centre for Integrated Genomics; Prague, Czech Republic; [3]Institute of Pathological Physiology, 1st Faculty of Medicine, Charles University; Prague, Czech Republic
khod@lf1.cuni.cz

Gaucher disease is the most frequent sphingolipidosis. It is caused by lysosomal b-glucocerebosidase (GCD) deficiency or rarely by the deficiency of sphingolipid activator protein, saposin C. More than 120 mutations were described in the human b-glucocerebrosidase gene in Gaucher patients. (1) The majority of GCD deficient Gaucher patients are compound heterozygotes. In non-Jewish populations rare or private mutations form about 25-30 % of mutant alleles. (2) Therefore, functional expression of mutant GCD is necessary to distinguish neutral mutations from deleterious mutations. We have used hybrid vaccinia expression system to study wild-type and mutant GCD. We have expressed novel and rare mutations found in Czech Gaucher patients. (3) The individual mutations were introduced by site-directed mutagenesis into expression vector pTM1-glu1, which contains human GCD cDNA under control of T7 promoter. (4, 5) We have constructed five recombinant vaccinia viruses carrying wild-type and mutant human GCDs. The tissue culture cell line was co-infected with VTF7-3 vaccinia virus, which expresses T7 RNA polymerase, and with each of the recombinant viruses. High levels of GCD expression were achieved. Plasmids carrying mutant 15 different GCD cDNAs were employed also for transient expression of GCD. In spite of the rather high background of tissue culture cell lines it was possible to distinguish polymorphisms and deleterious mutations in both cases. This work was supported by grant GA UK 131/97. 1 Beutler,E. and Gelbart, T. (1998) Blood cells, Molecules, and Diseases 24, 2-8 2 Horowitz M, Zimran A (1994) Hum Mutat 3; 1-11, 1994 3 Hodanova K, Hrebicek M, Cervenkova M, Mrazova L, Veprekova L, Zeman J. (1999) Blood Cells Molecules and Diseases 25; 287-289 4 Pasmanik-Chor, M. et al. (1996) Biochem J 317, 81-8 5 Pasmanik-Chor, M. et al. (1997) Hum Mol Genet 6, 887-95

P1182. Simultaneous Detection of Six Common Gaucher Disease Mutations by a Novel Reverse-Hybridization Assay

G. Kriegsh user*, A. Moritz, F. Kury, C. Oberkanins*
ViennaLab Labordiagnostika GmbH; Vienna, Austria
kriegshauser@viennalab.co.at

Gaucher disease is the most common glycolipid storage disorder known. The disease is characterized by glucocerebrosidase deficiency caused by mutations in the glucocerebrosidase (GBA) gene. Enzyme deficiency results in accumulation of glucocerebroside within the reticuloendothelial

system which may lead to hepatosplenomegaly, bone marrow suppression, and bone lesions. The disease is panethnic and has been divided into three clinical phenotypes (Types I-III) with Type I being most common. In the Ashkenazi Jewish population Type I disease frequency is estimated to 1 in 500 with a carrier rate to approximate 1 in 10. Enzyme replacement therapy is available for Type I Gaucher disease, resulting in clinical improvement and increased quality of live. Since the sequence of the GBA gene was first published in 1985, more than 100 mutations have been described. We have developed a reverse-hybridization assay for the rapid and simultaneous detection of six mutations predominantly found in the Jewish population; 84GG, IVS2(+1), 1226G, 1297T, 1448C and 1604A. The test is based on a single, multiplex DNA amplification reaction and ready-to-use test strips presenting a parallel array of oligonucleotide probes for each wild-type and mutated allele. The entire procedure from blood sampling to the identification of GBA mutations needs less than 6 hours and may be automated on existing equipment (e.g. TECAN profi-Blot). (kriegshauser@viennalab.co.at)

P1183. Genotype-phenotype Correlations In Romanian Gaucher Patients

C. Drugan[1], *L. Procopciuc*[1], *C. Caillaud*[2], *P. Grigorescu-Sido*[1], *T. Drugan*[1], *G. Jebeleanu*[1]
[1]*University of Medicine Cluj; Cluj, Romania;* [2]*Universite Paris V; Paris, France*
cdrugan@umfcluj.ro

Biochemical diagnosis of Gaucher disease in Romanian population became available since 1997. In this study we measured the enzymatic activities of acid beta-glucosidase in peripheral blood leukocytes and of serum chitotriosidase in 68 suspects selected according to clinical and histopathological criteria. 22 type 1 Gaucher disease patients were confirmed. Analysis of the frequent mutations in the acid beta-glucosidase gene; N370S, L444P, R463C, 84GG and of the recombinant alleles recNci I and recTL was performed by PCR-RFLP and by sequencing of the amplified fragments bearing the L444P substitution, respectively. Clinical severity was evaluated according to the Zimran severity score index. The results obtained indicate a high prevalence of the N370S allele (52.3%), followed by the L444P (22.7%), recNci I (6.8%) alleles and unknown mutations (18.2%). Genotype-phenotype correlations were similar to those reported for other Caucasian populations, with the N370S allele excluding the development of neurological symptoms and the L444P allele indicating a more severe course of the disease. This study indicates a high prevalence of type 1 Gaucher disease in Romanian population (22 confirmed patients in only 4 years of biochemical diagnosis) and points out to the necessity of introduction of the enzyme replacement therapy in Romania.

P1184. Human GM1-Gangliosidosis; Identification of Three Mutations in the Maltese Population.

A. E. Felice, *A. Alashtar, J. Vella, C. Al-Shinawi, S. Bezzina Wettinger, R. Blundell, C. Scerri, G. Grech*
Lab of Molecular Genetics, University of Malta; Msida, Malta
afel@biotech.um.edu.mt

The lysosomal storage disorder G_{M1}-gangliosisdosis is a genetic metabolic and neurological disorder caused by a complete or partial deficiency of the enzyme acid b-galactosidase. Infantile G_{M1}-gangliosisdosis is relatively common in the Maltese population , with a heterozygous frequency of 3.0%. The mutations responsible were studied in five unrelated Maltese families. Fragments containing all the exons and flanking regions, of the §-galactosidase gene where amplified using the polymerase chain reaction (PCR) and were either sequenced directly or scanned by single stranded conformational polymorphism (SSCP) analysis prior to sequencing. A double point mutation, CG?AT, within the IVS 7 of the §-galactosidase gene, at position 9 and 10 bp downstream of the 3 end of exon7 was identified in all the families studied and denoted as Spl7. Another mutation denoted as IVS14 nt —2G, an A?G transition located at the invariant AG dinucleotide of the 3 splice acceptor site of intron 14 has also been characterized in three of the families. The IVS14 nt —2G mutation was identified in patients who were also shown to be carriers of the Spl7 mutation. The R482H mutation, has been identified in two of the families. A single nucleotide polymorphism, IVS7 +10G>T (the G?T transversion in mutation Spl7), which has an allelic frequency of 1 in 16 in the Maltese population, has also been characterised. In conclusion, these data indicate that the Spl 7 and IVS14nt-2G mutations are probably linked, and show considerable molecular heterogeneity in the §- galactosidase gene of Maltese patients with GM1 gangliosidosis.

P1185. Type I Gm1 Gangliosidosis In South Brazil; Insights From The Haplotype Analysis In Families With The Two Common Mutations.

R. Giugliani[1,2], *C. Baiotto*[1,2], *C. Dornelles*[3], *R. Sano*[1], *J. Coelho*[1], *U. Matte*[1,2]
[1]*Medical Genetics Service - HCPA - UFRGS; Porto Alegre, Brazil;* [2]*Post Graduation Program in Genetics and Molecular Biology - UFRGS; Porto Alegre, Brazil;* [3]*FEPPS; Porto Alegre, Brazil*
rgiugliani@hcpa.ufrgs.br

Type I GM1 Gangliosidosis is a lysosomal storage disorder caused by the deficiency of beta-galactosidase. The accumulation of GM1 ganglioside causes neurological regression and death within the first two years of life. Its incidence (1;200,000 world-wide) is highly increased in South Brazil (1;17,000 live births), where only two mutations (R59H and 1627insG) are responsible for almost 60% of the alleles. We studied a group of 390 normal individuals in order to determine the frequency of carriers in the general population. In addition, to identify a possible common origin to these mutations, 26 patients and the controls were analysed for the presence of two polymorphisms (R521C and S532G). All patients were diagnosed by enzyme assay on plasma or leukocytes. Parents were also analysed to establish linkage phase. Mutation analysis in 24 new patients confirmed the frequencies of R59H (21%) and 1627insG (37%) assigned in 20 cases previously analysed. Mutation frequency in the controls was 0,13% and 0,38%, respectively. Therefore carrier proportion is 1;65, similar to that found in PKU. The frequency of the polymorphisms R521C and S532G was 0% and 18% in the patients and 3% and 7% in the controls. Haplotype analysis indicated a possible common origin for R59H, found to be linked to the G allele for S532G in two thirds of the patients. The other mutation, 1627insG, was found associated to the wild type haplotype in more than 90% of the cases. The wild type haplotype was also the most common haplotype, found in 86% of the alleles in the control group. R59H was first described in an Italian patient, and probably spread in Brazil with the Italian migratory waves. On the other hand, the link of 1627insG to wild haplotype in most cases suggests a more recent origin, and possibly recurrent, for this event. The collection of ethnic information and the analysis of 2 further markers close to the gene, in progress, should bring valuable information to clarify this issue (CNPq, FAPERGS, PRONEX/MCT).

P1186. A New Non-invasive Surrogate Marker For Assessment Of Large Arteries Involvement In Patients Affected With Fabry Disease

P. Boutouyrie, *S. Laurent, D. P. Germain*
H pital Europ en Georges Pompidou; Paris, France
dominique.germain@hop.egp.ap-hop-paris.fr

Background ; Fabry disease (FD) is an X-linked inborn error of glycosphingolipid metabolism due to deficient activity of the lysosomal enzyme alpha-galactosidase A. The enzymatic defect results in the deposition of uncleaved glycosphingolipids in tissular lysosomes and plasma. Whereas FD is primarily considered as a progressive small vessel disease, little is known about peripheral large arteries involvement. Recent studies have demonstrated that genetic engineering has removed many obstacles to the clinical use of enzyme replacement, and that the infusions of purified alpha-galactosidase A are safe and biochemically active, emphasizing the need for clinical and surrogate markers to monitor efficacy of enzyme replacement therapy. Methods ; We investigated common carotid and radial artery diameter, intima-media thickness (IMT) and distensibility in 21 hemizygous FD patients and 21 age-matched male controls, using high-definition echotracking systems and aplanation tonometry. Results ; Patients with FD had a significant two-fold increase in radial artery IMT and distensibility, independently of body surface area, age, and mean BP. With aging, the increase in radial artery IMT was 2.3-fold higher in FD patients than in controls. Carotid IMT was mildly but significantly increased in FD patients (+18 %), whereas distensibility was unchanged. Conclusion ; This is the first evidence of a major increase in arterial wall thickness, measurable at the site of a medium-sized artery in a cohort of patients with FD. The assessment of this newly recognized phenotype of FD, through non invasive procedures, may prove useful for future monitoring of enzyme replacement therapy.

P1187. Fabry Disease; Nine New Mutations in alpha-Galactosidase A Gene and Molecular Carrier Detection

R. Dobrovolny, *L. Dvorakova, M. Hrebicek, J. Ledvinova*
Institute of Inherited Metabolic Diseases; Prague, Czech Republic
rdobr@lf1.cuni.cz

Fabry disease, an X-linked inborn error of glycosphingolipid catabolism, results from the deficient activity of the lysosomal alpha-galactosidase A (E.C. 3.2.1.22). Affected individuals accumulate neutral glycosphingolipids

with terminal alpha-linked galactosyl moieties particularly in the lysosomes of blood vessels, heart, and kidney. Clinically affected are hemizygote males but heterozygote females may have an attenuated form of the disorder. This could be, besides other factors, due to X-inactivation pattern of female carriers.
We diagnosed biochemically 35 hemizygotes and 51 heterozygotes from nineteen Czech and Slovak families with Fabry disease. Mutations were found by direct sequencing of purified RT-PCR products from affected males or carrier females from 13 unrelated families. Nine new mutations were identified, each in a single family; Q280K, L294X, D155H, A153V, G43D, IVS 1^{+2}, IVS 5^{+3}, ˇEx2, and c. 734del59. In the remaining four families, four previously reported mutations were found; R301X, R342Q, Q340X, and N215S.
ARMS or PCR-RFLP methods were designed for specific detection of each of the mutations. To unravel the impact of X-inactivation status on clinical manifestation of Fabry disease in heterozygote females we optimised method for determination of X-inactivation pattern in tissues.
These studies permit precise heterozygote detection, prenatal diagnosis and further define the heterogeneity of mutations in alpha-galactosidase A gene causing Fabry disease. It also helps to delineate phenotype-genotype correlation. Determination of X-inactivation pattern can be used in further studies of Fabry disease clinical manifestations in heterozygotes.
Supported by grant projectsIGA MZ ?R NE 5770-3-99 and VS 96127

P1188. Identification of a novel alpha-galactosidase A mutation in a Japanese case of Fabry disease with systemic inflammatory findings

M. Iga
Miyazaki Medical College; Miyazaki, Japan
myiga@fc.miyazaki-med.ac.jp

A novel single point mutation, G43D in exon 1 of alpha-galactosidase A (alpha-Gal) was identified in a Japanese of Fabry disease. The patient complained high fever and arthralgia. Inflammatory findings were seen on laboratory analysis in addition to typical presentation of Fabry disease. The administration of prednisolone was effective to improve them. The alpha-Gal cDNA from the patient and his parents was synthesized and amplified by RT-PCR. DNA sequence analysis of 10 clones derived from each family member was performed. A single amino acid substitution G43D was found in 10 clones of the patient, 4 clones of his mother. No substitution was found in his father. The wild type alpha-Gal cDNA and the alpha-Gal cDNA carrying G43D mutation derived from the patient were used for transient expression in COS-7 cells. Northern blot assay showed that there was no difference between the wild type alpha-Gal and the mutant alpha-Gal G43D in the level of mRNA of alpha-Gal. However, the calculated alpha-Gal activity for G43D was 1% of that for the wild type alpha-Gal. These findings indicate that G43D is a specific mutation which causes deficiency of alpha-Gal activity without reduction of mRNA and results in Fabry disease. Because this is a novel mutation, G43D might be relevant to the atypical phenotype of this patient.

P1189. Results of a screening for most common mutations in metachromatic leukodystrophy patients with different types of the disorder.

A. Lugowska[1], *A. Tylki-Szymanska*[2], *B. Loeschl*[3], *B. Czartoryska*[1], *J. Berger*[4], *B. Molzer*[3]
[1]*Institute of Psychiatry and Neurology; Warsaw, Poland;* [2]*Child Health Centre; Warsaw, Poland;* [3]*Institute of Neurology, University of Vienna; Vienna, Austria;* [4]*Brain Research Institute, University of Vienna; Vienna, Austria*
lugowska@ipin.edu.pl

Metachromatic leukodystrophy (MLD; Mc Kusick 250100) - a genetically based metabolic disorder leads to demyelination of central and peripheral nervous system. It is caused by deficient activity of arylsulphatase A (ARSA; EC 3.1.6.1) which initiates the degradation of sulfatide - an essential component of the myelin. Three types of MLD are distinguished; late infantile, juvenile, and adult. Mutations 459+1G>A and P426L were most frequently found in MLD-patients from Western Europe. Each of these mutations accounted for 25% of all investigated MLD alleles. Mutation I179S was previously described to be the most common one among Polish late juvenile MLD-patients. A screening for these three mutations was performed in 33 unrelated MLD-patients from Poland; 12 individuals with late infantile, 15 - with juvenile, and 6 - with adult types of MLD. Genomic DNA was isolated from peripheral blood leukocytes and PCR-RFLP methods were used. Prevalence of 459+1G>A in the whole collective of MLD-patients was 21%. 459+1G>A accounted for 42% of investigated alleles in late infantile, 10% in juvenile, and 8% in adult MLD-patients. Prevalence of P426L in all MLD-patients was 18%. P426L accounted for 67% of examined alleles in adult and 13% - in juvenile MLD-patients. Prevalence of I179S in the whole group of MLD-patients was 11%. I179S accounted for 17% of examined alleles in adult as well as juvenile MLD-patients. P426L and I179S were not found in late infantile MLD-patients, what confirms previous observation that these two mutations cause MLD of late-onset types. Interestingly, I179S always occured in a heterozygous state.

P1190. Delineation of genes and mutations involved in the various clinical forms of ceroid lipofuscinoses in France.

C. Caillaud, *J. P. Puech, J. Manicom, L. Poenaru*
Laboratoire de G n tique, INSERM U129; Paris, France
caillaud@cochin.inserm.fr

Neuronal ceroid lipofuscinoses are inherited neurodegenerative disorders characterized by the accumulation of autofluorescent lipopigments in various tissues. Four NCL forms are distinguished according to clinical and morphological features ; infantile (INCL), late infantile (LINCL), juvenile (JNCL) and adult (ANCL). Eight loci have been described as responsible for these different forms, among which two encode soluble enzymes ; palmitoyl-protein thioesterase (CLN1), tripeptidyl-peptidase I (CLN2). In LINCL, the most common clinical form in France, the CLN2 locus was frequently involved, as demonstrated by the presence of a tripeptidyl-peptidase I (TPP-I) deficiency in a majority of patients. Complete sequencing of the CLN2 exons showed the predominance of two previously reported mutations (3556 G->C and Arg208Stop), accounting each for 30 % of the alleles. Various novel mutations such as deletions, splice or nonsense mutations were characterized on the other alleles. Some LINCL patients had no TPP-I deficiency and are likely candidate for the CLN6 or CLN7 loci. One patient with a juvenile form exhibited a TPP-I deficiency and carried the 3556 G->C mutation in association with a novel point mutation. A palmitoyl-protein thioesterase (PPT) deficiency was found in most of the INCL patients tested. The first CLN1 mutations characterized in these patients were previously reported mutations, except one novel point mutation. The study of the CLN3, CLN6, CLN7 and CLN8 loci is in progress among the uncharacterized patients. Delineation of the various loci involved in NCL will allow a reliable genetic counselling and prenatal diagnosis to couples at-risk for these diseases.

P1191. Molecular Genetics of Neuronal Ceroid Lipofuscinosis in Portugal

C. A. F. Teixeira[1], *A. Guimar es*[2], *M. J. Ferreira*[2], *L. Lopes*[3], *E. Pinto*[3], *S. Rocha*[3], *C. Caseiro*[3], *M. C. S. Miranda*[3], *M. G. R. Ribeiro*[3]
[1]*Genetic Neurobiology Department, Instituto de Biologia Molecular e Celular; Porto, Portugal;* [2]*Department of Pathology and Molecular Immunology, ICBAS; Neuropathology Department, HGSA; Porto, Portugal;* [3]*Genetic Neurobiology Department, Instituto de Biologia Molecular e Celular; Enzimology Department, Instituto de Gen tica M dica Jacinto Magalh es; Porto, Portugal*
gribeiro@ibmc.up.pt

The neuronal ceroid lipofuscinoses (NCL) are recessively inherited neurodegenerative disorders characterised by the intralysosomal accumulation of autofluorescent lipopigments. Four variants are classically distinguished according to clinical and ultrastructural features; infantile (INCL), late-infantile (LINCL), juvenile (JNCL) and adult (ANCL). Currently there are at least 8 genetically different variants and an accurate differentiation requires a multidisciplinary approach. Eighteen unrelated Portuguese patients presenting a clinical course and histopathological alterations of NCL have been studied. Based on clinical and morphological data 4 main groups were established; INCL/GROD (n=1), LINCL/CVP (n=3), JNCL/FPP (n=7) and vLINCL/CVP+FPP (n=7). The enzymatic determination of PPT and TPPI enzymatic activities in patient cell extracts, using synthetic fluorogenic substrates, showed that INCL was caused by a deficient PPT activity whereas the LINCL was due to a defective TPPI activity, thus establishing the diagnosis of CLN1 and CLN2, respectively. Mutation analysis by screening the 1.02kb deletion within CLN3 gene showed that JNCL patients were all homozygous for the same gene defect. The vLINCL patients presented a normal TPPI activity. The data suggest that 2 clinical forms of NCL, the JNCL and the vLINCL, associated respectively to CLN3 gene and to an yet cloned gene, might be prevalent in Portugal. The work was supported in part by the Portuguese Health Ministery.

P1192. Unusual clinical presentation in two cases of multiple sulfatase deficiency

S. Cuevas[1], *R. Rivera*[1], *E. Blanco*[1], *S. Kofman*[1], *C. Medina*[2], *M. Valdes*[3], *W. B. Rizzo*[4]
[1]*Hospital General de Mexico; Mexico D.F., Mexico;* [2]*Centro Medico de Occidente; Mexico DF, Mexico;* [3]*Instituto Nacional de Ortopedia; Mexico DF, Mexico;* [4]*Medical College of Virginia; Virginia, United States*

sergioa@servidor.unam.mx
Multiple sulfatase deficiency (MSD) is an inborn error of metabolism that combines clinical features of late infantile metachromatic leukodystrophy and mucopolysaccharidosis. The characteristic biochemical abnormality is a reduction in the activities of several sulfatases with consequent tissue accumulation of sulfatides, sulfated glycosaminoglycans, sphingolipids and steroid sulfates. In this study, we present two unusual cases of MSD with variable enzymatic deficiency of arylsulfatases A, B and C. Both patients lacked hepatosplenomegaly, had broad thumbs and index fingers, and exhibited an unusually slow progression of clinical symptoms. Olivopontocerebellar atrophy was present and one patient had a large retrocerebellum cyst. Mucopolysaccharides were not detectable in the urine from either subject. Leukocyte arylsulfatase A activity in RJ was 0.46 nmoles/mg protein/h and in RW was 0.0 nmoles/mg protein/h (normal 0.7-5.0 nmoles/mg protein/h). Leukocyte arylsulfatase B activity in RJ was 24 nmoles/mg protein/h and in RW 22 nmoles/mg protein/h (normal 115-226 nmoles/mg protein/h). Leukocyte arylsulfatase C was in RJ 0.30 pmoles/mg protein/h and in RW 0.28 pmoles/mg protein/h (normal 0.84 pmoles/mg protein/h). In conclusion, we presented two cases of MDS with mild clinical presentations not previously reported and variable enzymatic deficiency of arylsulfatases A, B and C.

P1193. Mutational analysis of 24 Niemann-Pick type A and B patients - 20 novel mutations

J. Sikora[1], *H. Pereira*[1], *M. Elleder*[1], *R. Wevers*[2]
[1]*Institute for Inherited Metabolic Diseases, 1st Medical Faculty of Charles University; Prague, Czech Republic;* [2]*University Hospital Nijmegen, Laboratory of Neurology 319; Nijmegen, The Netherlands*
jakub.sikora@lf1.cuni.cz
We have analyzed acid sphingomyelinase (ASM) gene mutations in 24 patients with Niemann-Pick disease (NPD) type A and B by direct sequencing of gDNA. Eighteen patients originated from former Czechoslovakia, six were from Netherlands. Niemann-Pick disease type A and B is an autosomal recessive disorder caused by deficiency of lysosomal ASM (E.C. 3.1.4.12) and the resultant lysosomal sphingomyelin accumulation. There are 21 ASM gene mutations published up to date. Among the patients from former Czechoslovakia (type A - 7 cases, atypical type A variant - 6 cases, type B - 5 cases,) eighteen mutations were described, fifteen of them novel. The novel mutations (type A associated - fsP189, S248R, D278A, Q292K, W533R; type B associated - G166R, P184L, R228H, A241V, R289H, Q292K, R376H, R474W a fsP595) were all in heterozygous state. Three novel mutations were identified (Q292K, D251E and L341P) among atypical type A patients. Mutation Q292K is prevalent among NPD patients from former Czechoslovakia (described on 38% of mutated chromosomes). Six cases (type A - 3 patients, type B - 3 patients) from Netherlands represent one quarter of Dutch NPD patients. Number of mutations in this group was six, five of them novel (type A associated - H319Y, F463S, P475L, Y537H, type B associated - P371S). All of the identified mutations were either small deletions or of missense type. We confirmed all the novel mutations by PCR\RFLP.

P1194. Clinical And Laboratorial Study Of 21 Cases Of Mucopolysaccharidoses

P. S. Gerritsen Plaggert[1], *L. M. J. Albano*[1], *S. M. M. Sugayama*[1], *L. Toma*[2], *J. Coelho*[3], *R. Giugliani*[3], *C. A. Kim*[1]
[1]*Genetics, Instituto da Crian a; S o Paulo, Brazil;* [2]*Molecular Biology, UNIFESP; S o Paulo, Brazil;* [3]*Medical Genetics, Hospital de Cl nicas; Porto Alegre, Brazil*
plaggert@aol.com
The mucopolysaccharidoses(MPS)are a heterogeneous group of inborn errors of lysosomal glycosaminoglycan metabolism. The importance of this group of disorders among the inborn errors of metabolism lead us to report 21 cases. We performed clinical, radiological and biochemical evaluations of the suspected patients, wich allowed us to establish a definitive diagnosis in 21 cases. The diagnosis was made in average at the age of 51 mo(ranging from 7 mo to 19 y). The patients were classified as follows; Hurler-MPS I(2 cases); Hunter-MPS II(2 cases); Sanfilippo-MPS III(3 cases); Morquio-MPS IV(4 cases); Maroteaux-Lamy-MPS VI(9 cases); and Sly-MPS VII(1 case). The high relative frequency of Maroteaux-Lamy disease contrasts with most reports in the literature and could express a populational variability. Not all patients showed increased GAG levels in urine, therefore, normal urinary GAG pattern does not rule out the possibility of a MPS. Diagnosis is not easy and confirmation is based with specific enzimatic investigation.

P1195. Creation of registration systems of patients with hereditary Gaucher disease in Russia

G. V. Smirnova, *I. N. Lunga, S. V. Stukalov, T. M. Bukina*
Medical Genetic Scientific Center RAMN; Moscow, Russian Federation
maria@co.ru
In Russia registration system of patients with Gaucher disease (GD) belonging to class of hereditary storage diseases has been worked up. With the help of complex of genetic-epidemiological and clinical genealogical methods informational arrays about families with patients suffering from GD have been collected. Upon this foundation the computer database about Gaucher patients has been created including information on 30 various sections; general information, methods of diagnostics, clinical status of proband, state of health of relatives, treatment of proband. Computer base contains information on 122 cases of GD with various clinical types. General analysis of computer database on GD showed such serious problems as revealing contingents of Gaucher patients and exact diagnostics. We ve executed registration of Gaucher patients and constant renewal of data upon patients addressing to doctor and active revealing patients in medical institutions. The complication of GD diagnostics is related with exclusive clinical polymorphism of this pathology and availability of hardly distinguishable geno- and phenocopies. Due to this and also to new prospect for Gaucher patients it was greatly important to receive biochemical verification of diagnosis. Diagnosis was confirmed in 66 patients by method of enzyme diagnostics (detection of glucocerebrosidase activity) and in 43 patients analysis of genome DNA was made and mutations N370S ? L444P were revealed. The created computer database of Gaucher patients is used for; revelation of groups of patients for making additional researches for verification of diagnosis made at clinical level; collection of population-statistical material for further development of medical-genetic researches of GD.

P1196. Evolution of Gaucher Disease during ERT and its Interruption

M. Ivanova, *B. Radeva*
Clinical Genetics, High Medical University-Sofia; Sofia, Bulgaria
pediatr@internet-bg.net
The enzyme replacement with Ceredase and Cerezyme is high effective for Gaucher s patients type 1. The establishing of this treatment is connected with a lot of ?conomical difficulties. The complications after stopping the ERT are unknown and the literature data are limited. The aim of our work was to evaluate the consequence of ERT interruption.Tree patients with Gaucher type 1 were investigated. Their diagnosis was confirmed with enzyme assays and DNA analysis. The ERT was proceed in 1994/1995,1997/1998 and 2000 years. During the ERT there were improvement of clinical, hematological and biochemical parameters. The 2 years interruptions because finantinal cause took to appear of epistaxis, post operative bleeding, bone pains, often infections. The splenohepatomegaly increased faster. There were registrated spleen infarctions. The growth development delay significantly. The hematological parameter worsed. The acid phosphatase and chitotriosidase elevated singnificantly.The results of retreatment during 2000 shown that the children need more high doses Cerezyme to rich the primary effect. Our analysis shown that the interruption of ERT cost more expensive as the regularly continued treatment with Ceredase and Cerezyme and has negative psychosocial effects.In conclusion we not recommende interruption of ERT for Gaucher s patients because is dangerous and lifethreatening.

P1197. Investigating roles for two human proteins with very long-chain acyl-CoA synthetase activity in the biochemical abnormality of X-linked adrenoleukodystrophy

S. J. Steinberg, *A. K. Heinzer, K. D. Smith, P. A. Watkins*
Kennedy Krieger Institute and Johns Hopkins University School of Medicine; Baltimore, MD United States
steinbergs@kennedykrieger.org
Mutations in the ALD gene are the molecular basis of X-linked adrenoleukodystrophy (ALD). Greater than 60% of these mutations result in an absence of ALD protein (hALDP) in the peroxisomal membrane of cultured fibroblasts. Peroxisomes isolated from human ALD fibroblasts demonstrate a reduced capacity to activate very long chain fatty acids (VLCFAs) to their coenzyme A derivatives and, thus, presumably have diminished very long-chain acyl-CoA synthetase (VLCS) activity. However, hALDP is not a VLCS and its function remains elusive. We have cloned two human genes that have VLCS activity; hVLCS and bubblegum (hBG). In addition to their capacity to activate VLCFAs, both hVLCS and hBG are active towards a broad range of substrates, including long chain, mono- and polyunsaturated and branched-chain fatty acids. hVLCS is expressed primarily in liver and kidney, although its transcript is detectable in brain; in

contrast, hBG is expressed principally in brain. Northern blots have revealed decreased bubblegum gene expression in ALD fibroblasts. A similar result was obtained using reverse transcription-PCR. Endogenous hVLCS is localized to both the peroxisome and endoplasmic reticulum, whereas hBG has a plasma membrane and cytosolic localization. Transient overexpression of hBG or hVLCS in ALDP-deficient fibroblasts does not correct the defect in peroxisomal §-oxidation that results from the faulty activation step. However, co-overexpression of hVLCS and hALDP in ALDP-deficient cells has a synergistic effect and restores §-oxidation beyond that obtained with hALDP alone. Further studies to elucidate a putative interplay between hALDP and hVLCS and/or hBG are in progress.

P1198. Gestational Loss Is A Complex Trait In The Long-chain Acyl-CoA Dehydrogenase Deficient Mouse Model

P. A. Wood, *P. S. Berger, D. A. Hamm, C. A. Pinkert*
University of Alabama at Birmingham; Birmingham, AL United States
paw@uab.edu

We previously found a significant reduction from the expected number of mice born that were either heterozygous (45% of expected) or homozygous (60% of expected) for the null allele for long-chain acyl-CoA dehydrogenase (LCAD). We hypothesized that this may be due to either an energy deficiency in the embryo or a toxic effect on the embryo in the heterozygous or homozygous mother. Since development from morula to blastocyst is a major energy consuming process, we hypothesized that LCAD deficient embryos would exhibit reduced developmental competence as demonstrated by a failure to proceed from compaction through blastocoel formation. To investigate the development of early embryos of all three genotypes (LCAD+/+, +/-, -/-), we cultured early embryos from day 1 post-copulation to day 8. We found a significant decrease in LCAD-/- embryos starting at days 3-4 (morula to blastocyst stages) resulting in a 8.7% survival rate as compared to 56% survival rate in LCAD+/+, and 32% survival rate in LCAD+/- embryos (p=0.012, ANOVA). We also have observed that we have been unable to develop congenic LCAD+/- mouse lines on the C57BL/6 background due to the severe rate of loss of offspring in G2 when backcrossing mice with the B6,129 starting genetic background, whereas the backcrosses done using 129/SvEv mice yielded the expected ratio of offspring with the mutant allele (~50%). We speculate that there is a profound modifier gene(s) in the B6 background that promotes for gestational loss that is independent of the energy deficiency that appears to be correlated with a simple gene dosage effect in the cultured embryos. These studies are particularly important to help understand the fact that no human LCAD deficient patient has been documented.

P1199. Clinical manifestation of muscular carnitine palmitoyltransferase II deficiency in infancy - detection by a new and non-invasive strategy using tandem mass spectrometry

K. E. Gempel[1], *S. Hofmann*[1], *B. F. Pontz*[2], *K. Gerbitz*[1], *M. F. Bauer*[1]
[1]*Institute for Clinical Chemistry, KH Munich-Schwabing; Munich, Germany;* [2]*Department of Paediatrics of the Technical University Munich; Munich, Germany*
Klaus.Gempel@lrz.uni-muenchen.de

Carnitine palmitoyltransferase II (CPT-II), an enzyme associated with the inner mitochondrial membrane, plays a crucial role in the transport of long-chain fatty acids into the mitochondrial matrix for b-oxidation. CPT-II deficiency is an autosomal recessive disorder which presents itself as two phenotypes; a severe infantile form and a milder muscular form which typically manifests around puberty. Patients with the adult form suffer from muscular pain, rhabdomyolysis and myoglobinuria triggered by extensive exercise. Currently, the diagnosis is based on the determination of CPT enzyme activity in muscle biopsies or fibroblasts. Here we report a non-invasive diagnostic strategy based on the tandem mass spectrometric (TMS) determination of acylcarnitines, which allows the detection of CPT-II deficiency in serum samples. In CPT-II deficiency, long chain acylcarnitines accumulate in the serum thereby generating a highly characteristic TMS spectrum. These spectra are characterized by distinct elevations of palmitoyl-, oleoyl-, stearoyl-carnitine. They are consistently obtained in all CPT-II patients, are absent in patients with unspecific muscle symptoms and controls, and differ markedly from that of other inborn errors of lipid metabolism. We propose that TMS of serum acylcarnitines allows us to easily screen suspect cases, thereby facilitating a rapid diagnosis and lowering the risk of serious complications. In some cases, TMS together with molecular genetic testing even renders it possible to establish the diagnosis without undertaking a muscle biopsy. This view is supported by the recent identification of a child presenting the typical symptoms of the adult form of CPT-II deficiency.

P1200. Molecular Analysis of Carnitine Acylcarnitine Translocase Deficiency in Twelve Patients ; Application for Prenatal Diagnosis.

M. Brivet[1], *J. Costa*[2], *A. Slama*[1], *A. Boutron*[1], *A. Legrand*[1], *C. Costa*[1]
[1]*H pital Bic tre AP-HP; Le Kremlin Bic tre, France;* [2]*H pital Americain de Paris; Neuilly, France*
catherine.costa@bct.ap-hop-paris.fr

CACT deficiency is a rare severe autosomal recessive disorder of long chain fatty acid oxidation and has a bad prognosis. A multiplex PCR followed by cycle-sequencing was developed to detect mutations in the CACT gene. The entire coding regions, intron/exon splice junctions were amplified in a single tube by multiplex PCR using 9 pairs of primers and the PCR products were submitted to cycle sequencing. Genomic DNA analysis was performed in 12 unrelated patients and was confirmed in parents in 9 families. On the 24 alleles, 11 molecular abnormalities were identified, two of them at a higher frequency. Among these two, a homozygote splicing mutation (261-10T->G) already described in 2 eurasian patients, was found in 3 unrelated asiatic patients. Two point mutations at the same codon (R178X, R178Q) were found in 3 other unrelated north-african families. Prenatal diagnosis carried out by our group were previously performed by enzymatic methods on cultured cells (trophoblasts or amniocytes). However results were obtained after several weeks of cell growth. Mutation identification has allowed a prenatal diagnosis in 4 families. In 2 families mutation analysis was started only after amniotic fluid sampling and prenatal diagnosis conducted on cultured amniocytes. In the two other families genomic studies were performed on crude chorionic villi and result was obtained in 3 days. Two carriers, an affected foetus and an unaffected foetus were identified. Long chain fatty acid oxidation assays and CACT activity measurements, performed on cultured trophoblasts and/or amniocytes confirmed the results one month later.

P1202. The modification of the tridimensional conformation of HMGCoA Lyase caused by novel mutations explains the 3-OH-3-metilglutaric aciduria in two patients

R. Aledo[1], *N. Casals*[1], *C. Mir*[1], *P. Gomez*[2], *R. Roca*[3], *D. Serra*[2], *G. Asins*[2], *J. Pi* [4], *J. Lloberas*[5], *J. Crespo*[6], *J. Abdenur*[6], *G. Besley*[7], *A. Valencia*[8], *F. Hedgardt*[2]
[1]*Unit of Biochemistry and Molecular Biology, Universitat Internacional de Catalunya; Sant Cugat, Spain;* [2]*Department of Biochemistry and Molecular Biology, School of Pharmacy, University of Barcelona; Barcelona, Spain;* [3]*Protein Design Group (CNB-CSCIC), Cantoblanco; Madrid, Spain;* [4]*Unit of Human Physiology, College of Huesca, University of Zaragoza; Zaragoza, Spain;* [5]*Department of Physiology (Immunology, Macrophage Biology), School of Biology, University of Barcelona; Barcelona, Spain;* [6]*Fundaci n para el Estudio de las Enfermedades Neurometab licas; Buenos Aires, Argentina;* [7]*Willink Biochemical Genetic Unit; Manchester, United Kingdom;* [8]*Protein Design Group (CNB-CSIC), Cantoblanco; Madrid, Spain*
raledo@csc.unica.edu

3-hydroxy-3-methyl-glutaric aciduria (HA) is an autosomal recessive inborn error of metabolism produced by mutation in 3-hydroxy-3-methylglutaryl-CoA lyase (HL) gene. HL is a mitochondrial enzyme, which catalyses the last step of both ketogenesis and leucine catabolism. Two novel point mutations in HL gene were found in patients from Argentina and United Kingdom with biochemically diagnosed 3-hydroxy-3-methylglutaric aciduria. Mutations Ser201Tyr and Asp204Asn, one in each patient, were detected by sequencing the PCR amplified exons. The Ser201Tyr mutation interferes with the proper assembly of the substrate as demostrated in a tridimensional model of HMGCoA lyase, and the Asp204Asn mutation breaks the interaction Asp204-His233 responsible for the nucleophilic attack to the substrate. The mutational changes in tridimensional models of HMGCoA lyase and their implications in the patients phenotypes are discussed.

P1203. Inborn Error of Metabolic Diseases in Malaysia

Y. Choy[1], *Z. Mohd. Yunus*[2], *D. Pertiwi*[2], *Z. Ismail*[2]
[1]*Pediatric Institute, Kuala Lumpur Hospital; Kuala Lumpur, Malaysia;* [2]*Institute of Medical Research; Kuala Lumpur, Malaysia*
choyo2tm.net.my

The exact incidence of many inborn error of metabolic diseases (IEMD) are unknown in many parts of the world especially the developing countries due to lack of diagnostic facilities and lack of awareness among the physicians in these countries. In fact, certain IEMD may be more common in these countries due to higher incidence of consanguinity. In Malaysia, laboratory diagnosis of IEMD was not available till recently. Under the Health Ministry New Policy we were able to set up a number of specialized diagnostic tests. Using High Performance Liquid chromatography for amino

acid analysis and Gas Chromatography-Mass Spectrometry for organic analysis we were able to confirm some of the amino acidopathies and organic acidurias. Out of the 470 samples received from all over the country from June 1999 till June 2000, 28 cases of IEMD have been confirmed using these tests. Out of these 28 cases, 7 cases of maple syrup urine disease (MSUD), 4 cases of tyrosinemia type I, 8 cases of urea cycle defects (UCD), 3 cases of methylmalonic acidemia (MMA), 3 cases of 3-hydroxyisobutyric acidemia, 1 case each of phenylketonuria, non-ketotic hyperglycinemia (NKH) and propionic acidemia (PA) were diagnosed. Three out of the eight cases of UCD were citrulinemia. Consanguinity of the parents was found in 8 of these cases (30%). History of neonatal death in siblings occurred in 6 cases (21%) and there was history of similar illness in the other living siblings in 3 cases (13%). These cases were definitely the tip of an iceberg and phenylketonuria was probably under-represented as most of the tests were done for sick children admitted to hospitals. But his data provides us a good argument for carrying out newborn screening for IEMD in the country as part of our effort to prevent and reduce genetic causes of mental and physical handicap.

P1204. Values of Urine Amino Acid in Pre-term Infants; Comparison of Urine Amino Acid Levels Before and After Pre-term Formula or Human Milk Fed

C. Chuang, *S. Lin*
MacKay Memorial Hospital; Taipei, Taiwan Republic of China
cck@ms1.mmh.org.tw

The change of amino acid (AA) concentrations in body fluids is critical for the diagnosis of aminoacidopathy and malnutrition. Because of the lower physiological maturity of organs in pre-term infants, AA metabolism is affected that results in inconsistency of AA concentrations in the body fluids, especially in urine. In order to accurately interpret urine AA results, well-documented urine AA control values for pre-term infants are absolutely required. In our study, we report the control range of urine AA concentrations in pre-term infants before and after formula or human milk fed, in the mean time, we also evaluate the difference of individual AA concentration among these 5 groups by proceeding statistical analysis. Urine amino acid levels (in nmol/mg cretinine) were measured by ion-exchange chromatography method. Urine samples were collected from 34 pre-term (28 - 35 Wks) infants before the first milk feeding, 1 and 4 weeks after milk fed, in which 12 infants fed with human milk and the rest 22 fed with pre-term formula milk with regular amounts. When comparing the urine AA values before and after milk fed by pair-t test, a significant mean difference was found between the first milk feeding group and the 1st week after milk fed group ($p < 0.05$), however, an insignificant mean difference was obtained between the first milk feeding group and the 4th week after milk fed group. For individual AA, all the infants either fed with formula or human milk showed significant decrease in concentrations of cystine (approximately 51.4 to 57.4% ł) and tryptophan (approximately 21.5 to 27.3%). Essential amino acids, such as valine, leucine, isoleucine, and phenylalanine, showed significant decrease in concentrations in the formula fed groups, but not in the human milk fed groups. Conclusively, urine AA levels in pre-term infants is 2 - 4 folds higher than that of full-term infants, and this confirmed the lower physiological maturity of digestive function and AA metabolism in pre-term infants. In addition, human milk is much better than pre-term formula milk in AAs supply for growth and development. Finally, the new-established urine AA control values is valuable for clinical application, especially for the diagnosis of AA metabolic disorders.

P1205. Aminoacidopathies with Mental Retardation Diagnosed in the Laboratory of Human Genetics of Cluj County Hospital, Integrated in the Department of Cell and Molecular Biology of University of Medicine and Pharmacy Iuliu Hatieganu from Cluj-Napoca, Romania.

R. C. Vulturar[1], *I. Benga*[2], *G. Benga*[3]
[1]*Department of Cell and Molecular Biology ,University of Medicine and Pharmacy; Cluj-Napoca, Romania;* [2]*Neuro-Psychiatry Clinic of Cluj County Hospital, Department of Paediatric Neurology, University of Medicine and Pharmacy; Cluj-Napoca, Romania;* [3]*Department of Cell and Molecular Biology, University of Medicine and Pharmacy Iuliu Hatieganu , Laboratory of Human Genetics of Cluj County Hospital; Cluj-Napoca, Romania*
Vulturar.Romana@umfcluj.ro

Plasma and/or urinary aminoacid analyses were performed by a two-dimensional thin layer chromatography procedure (Wadman & al. 1981) in children suspected for having perturbances of aminoacid metabolism. A number of over 1700 samples from patients reffered by the Neuro-Psychiatry Clinic and other Paediatric services from Cluj-Napoca were analysed between 1980-2000. Out of these, 54 cases were diagnosed as hyperphenylalaninemias/phenylketonurias, one case of oculocerebrorenal Lowe syndrome. These are definite disturbances of aminoacid metabolism associated with mental retardation. The molecular defects with metabolic blocks and accumulations of some metabolites in the body fluids explain the clinical features. We also include a case of sarcosinemia, a case of histidinuria, rare and controversial genetic disorders concerning neurologic abnormalities determination.

P1206. Alkaptonuria in Slovakia; Thirty two years of research on phenotype and genotype

S. Srsen[1], *C. R. Mueller*[2], *A. Fregin*[2], *K. Srsnova*[1]
[1]*Jessenius Faculty of Medicine; Martin, Slovakia;* [2]*Department of Human Genetics; Wuerzburg, Germany*
crm@biozentrum.uni-wuerzburg.de

The research programme on alkaptonuria (AKU; OMIM # 203500) in Slovakia was started in 1968 by the Institute of Clinical Genetics at Martin and, in its first stage, was focussed on clinical, biochemical, genetic and epidemiological questions. Based on a screening programme of now 610.000 inhabitants (including 509.000 newborns) the world-wide highest incidence of AKU (1 in 19.000) was recorded and a total of 208 patients (including 110 children) were registered. Through the co-operation of the families, extensive genealogical studies (sometimes over 2 centuries) were made possible. Most AKU ancestors could be traced to their original geographic locations, predominantly in remote mountain regions. A high degree of inbreeding was noted in these areas. These epidemilogical data formed the basis for molecular studies which began in 1991 in collaboration with the Wuerzburg group. The AKU locus was mapped to human chromosome 3q2 by orthology to the mouse locus aku, the homogentisate 1,2 dioxygenase genes (HGD) were cloned from mouse and man and nine different mutations were identified in AKU patients; These include 4 missense, 2 splice-site, 2 insertion and one novel 1 bp deletion mutation. The most frequent mutations in Slovakia are G161R and c.1278insC (P370fs) which were found on 16 (= 40 %) and 7 (= 17.5 %), respectively, of the 40 AKU chromosomes from the 20 index cases. To date, the genotypes of 28 patients and 68 relatives from 20 families have been established and served as the basis for genetic counselling. The highest incidence and allelic heterogeneity was observed in the Kysuce district (125.500 inhabitants) with 5 different mutations on 11 AKU chromosomes. An analysis of the association between HGD intragenic markers and mutations and of the geographical origins of the AKU chromosomes suggests that several founders have contributed to the HGD gene pool. While there is no straightforward explanation for this clustering of independent mutations, subsequent genetic isolation is likely to be responsible for the high prevalence of AKU in Slovakia.

P1207. Biotin deficiency effects on HCS and carboxylases genetic expression

A. Velazquez, *R. Rodriguez-Melendez, S. Cano-Colin, S. Mendez-Cruz*
National Autonomous University of Mexico; Mexico, D.F., Mexico;
velare@servidor.unam.mx

Biotin deficiency is a nutritional phenocopy of Multiple Carboxylase Deficiency. Regulation of genetic expression of carboxylases and holocarboxylase synthetase (HCS) is poorly understood, and may have important consequences for their genetic defects. We investigated the regulation of HCS mRNA and of pyruvate (PC), propionyl CoA (PCC) and 3-methyl crotonyl CoA (MCC) carboxylases mRNAs and their protein mass, in liver, kidney, muscle and brain. HCS mRNA levels were significantly reduced. The decrease in HCS mRNA was reversed when biotin was injected into deficient animals, the recovery being a delayed effect, apparent over 24 hours after vitamin administration. These changes seem to be the first known instance in mammals, of an effect of a water-soluble vitamin on mRNA functionally related to it. On the other hand, the expected carboxylases activity decrement was associated with a reduction in the amount of their enzyme proteins, but not in their mRNA levels. Only in the brain, PC and PCC amounts did not change significantly. In conclusion, this work provides evidence for biotin being a modulator of the genetic expression of the enzymes involved in its function as a cofactor. As such, it may be a useful model for probing a similar role for other water-soluble vitamins. (Sponsored by grants from Programa de Apoyo a Proyectos de Investigaci n e Innovaci n Tecnol gica UNAM, and Consejo Nacional de Ciencia y Tecnolog a)

P1208. Molecular and functional studies in patients with the cblE type of homocystinuria.

P. Zavadakova[1], *V. Kozich*[1], *T. Suormala*[2], *B. Fowler*[2]
[1]*Institute of Inherited Metabolic disorders; Praha, Czech Republic;* [2]*Univ. Child. Hosp.; Basel, Switzerland*
zavadakp@hotmail.com

Methionine synthase reductase (MTRR) is presumably required for reductive activation of methionine synthase which remethylates homocysteine. MTRR deficiency is a putative cause of the cblE type of homocystinuria, a rare autosomal recessive disorder. This disease manifests in childhood with megaloblastic anaemia, neurological disease and psychomotor retardation. MTRR gene mutations are reported in 9 patients with cblE type of homocystinuria but normal and mutant gene products have not yet been characterised.
Our ongoing studies are directed at the molecular characterisation of an additional 7 patients with clinical, biochemical and enzymological evidence of functional methionine synthase deficiency and cblE confirmed by complementation studies.
In one case we identified homozygosity for a 140 bp insertion (903ins140) caused by a T->C transition within intron 6 of the MTRR gene, presumably leading to exon splicing enhancer activation. First trimester prenatal diagnosis was possible in this family by analysis of this mutation. Another patient was heterozygous for a deletion of exon 2 at the mRNA level, the only nucleotide change in exon 2 and flanking intronic regions was an intronic mutation, IVS-201G->A. The mechanism by which this intronic mutation leads to exon 2 skipping is unknown. Transient transfection of two cblE fibroblast cell lines with a eukaryotic expression vector containing wild type MTRR cDNA, revealed 2.2 and 3 fold increases of methionine synthesis, compared with untransfected cells. Restoration of methionine synthase activity following transfection with wild type MTRR supports, with a functional assay, the idea that cblE is due to defects in the MTRR gene.

P1209. Protein aggregation - a common cause of cystathionine beta-synthase (CBS) deficiency.

M. Janosik[1], *J. Oliveriusova*[2], *J. P. Kraus*[2], *V. Kozich*[1]
[1]*Institute of Inherited Metabolic Disorders, Charles University; Prague, Czech Republic;* [2]*University of Colorado, Health Sciences Center; Denver, CO United States*
mjano@lf1.cuni.cz

Cystathionine beta-synthase (CBS) - the key enzyme in the transsulfuration pathway of homocysteine - is a homotetramer of 63-kDa subunits. Each subunit binds five ligands; serine and homocysteine as substrates, cofactor pyridoxal-5«-phosphate, allosteric activator S-adenosylmethionine and heme with unknown function. Deficiency of CBS is the most common cause of homocystinuria. The majority of mutations are missense mutations with unknown impact on protein function. We determined the degree of assembly of CBS subunits in cultured skin fibroblasts of 13 patients with CBS deficiency. The native western blot analysis showed only high molecular weight aggregates, and absence of the tetramer. Five selected mutants - A114V, A155T, E176K, I278T and del ex12 - were expressed in E. coli. All mutants formed aggregates lacking heme while only the A114V mutant yielded traces of active CBS heme-containing tetramer. An ongoing study will determine the effect of chemical chaperones on the folding of mutant CBS using both an E.coli and an eukaryotic expression systems. Our results show that the decreased affinity and/or inability to bind heme prevent correct folding and subsequent tetramer formation of mutant CBS. This can make the mutants less stable, prone to misfolding and aggregation. We postulate that, similarly to other genetic defects, mutant CBS misfolding and aggregation may be the primary defect in a significant proportion of homocystinuric patients.

P1210. Use of an integrated approach to diagnose and manage a symptomatic ornithine transcarbamylase deficiency female

F. Scaglia[1], *Q. Zheng*[1], *W. O Brien*[1], *J. Henry*[2], *J. Rosenberger*[2], *P. Reeds*[2], *B. Lee*[1]
[1]*Department of Molecular and Human Genetics, Baylor College of Medicine; Houston, TX United States;* [2]*Children s Nutrition Research Center and Department of Pediatrics, Baylor College of Medicine; Houston, TX United States*
fscaglia@bcm.tmc.edu

Females with ornithine transcarbamylase deficiency (OTCD) have a heterogeneous clinical expression. Diagnosing at risk OTCD females is important for determining reproductive risks and preventing potentially catastrophic hyperammonemia. Diagnosis of partial OTCD during asymptomatic periods is difficult. The allopurinol challenge used in the diagnosis of OTCD females may give rise to both false negatives and positives, and DNA diagnosis is unable to detect mutations in all cases, especially in OTCD females or unusual presentations in males. We have previously used the ratio of isotopic enrichments of ^{15}N urea/^{15}N glutamine (^{15}N-U/G) as a sensitive index of *in vivo* urea cycle activity. We have now applied this method in an integrated approach to aid in the diagnosis and prospective management of a symptomatic female with suspected partial OTCD in whom a mutation in the OTC gene was not found on clinical testing. The ^{15}N U/G ratio in this patient showed a severe reduction of *in vivo* urea cycle activity that correlated with deficient OTC activity in her liver, level of orotic aciduria, and severe clinical phenotype. Stable isotope studies may provide a sensitive tool in combination with other measures to aid in the diagnosis of at risk OTCD females. The decision to resort to orthotopic liver transplantation (OLT) in OTCD females is controversial requiring consideration of phenotypic severity, access to tertiary care centers, and social factors. In this patient, the use of this diagnostic approach in conjunction with a consideration of her clinical history and medical-social situation led to a decision for OLT.

P1211. Ornithine Transcarbamylase(otc)deficiency; Mutation Spectrum, Haplotypic Background And Diagnosis

L. C. C. Azevedo[1], *L. Vilarinho*[2], *A. Amorim*[3]
[1]*IPATIMUP; Porto, Portugal;* [2]*IGMJM; Porto, Portugal;* [3]*Faculdade de Ci ncias da UP/ IPATIMUP; Porto, Portugal*
luisa.azevedo@ipatimup.pt

Ornithine transcarbamylase (OTC; EC 2.1.3.3) deficiency is the most common inborn error of the urea cycle and shows an X-linked inheritance with frequent new mutations. Inherited deficiency of OTC in hemizygous affected males usually results in severe ammonia intoxication, lethargy, vomiting and early death. Mild forms of OTC deficiency may be present later, even during adulthood. Symptoms and signs may include coma, mental retardation, protein avoidance, headache, bizarre behaviour, or episodic hyperammonemia. The broad aim of this study is to provide biochemical and molecular characterisation of OTC deficiencies, and diagnosis of OTC asymptomatic carrier status in Portuguese affected families. Biochemical diagnosis is established by the finding of; increased plasma ammonia and glutamine; decreased plasma citrulline and increased urine orotate excretion. Tests of proteins and allopurinol loading are used in an attempt to detect carrier females when biochemical determinations are inconclusive. By PCR amplification followed by SSCP and HCSGE, we have detected two recurrent mutations (R141Q and G195R) and a novel mutation in the donor splice site of intron 2 (IVS2 +1 g?t). R141Q and G195R mutations were found in two unrelated females, and IVS2 +1 was found in a male with 10% of OTC activity determined by hepatic biopsy. Haplotypic information obtained with two flanking markers of OTC gene (DXS997 and DXS1068) and four intragenic markers (Ex4 46; IVS3-8nt; IVS4-7nt; Ex8 270) was performed in affected families in order to provide information about lineage haplotypes.

P1212. Evidence for Neuron Apoptosis Induced by Hyperphenylalanine

X. Gu[1], *X. Yang*[2], *R. Chen*[2]
[1]*Shanghai Institute for Pediatric Research; Shanghai, China;* [2]*Xin Hua Hospital, Shanghai Second Medical University; Shanghai, China*
xhkj@public.sta.net.cn

Objective This study is to investigate the neurotoxicity of hyperphenylalanine and to explore the possible mechanisms. Methods The primary cultured cortical and hippocampal neurons of embryonic rat were cultured in Neurobasal, exposed to hyperphenylalanine (Phe) and assayed the survival rate. The morphological changes were observed by special staining. bcl-2, fas and c-fos were tested by immunohistochemistry and RT-PCR. Results The survival rate of cortical neurons was significantly decreased compared to normal controls in present Phe at 300 mol/L, 600 mol/L, 1200 mol/L and shown a dose dependent. Positive apoptotic neurons increased with the concentration of Phe by TUNEL staining and observed under electronic microscope. The positive neurons of fas and c-fos increased than normal control by immunohistochemistry with the exception of bcl-2. The mRNA expression of c-fos increased accordingly while that of bcl-2 decreased. Conclusion Apoptotic neurons were induced by hyperphenylalanine. The abnormal expression of some genes may hasten the neuronal apoptosis, such as the upregulation of fas and c-fos genes and the downregulation of bcl-2.

P1213. X-linked ichthyosis; utilization of FISH and PCR in the analysis of the frequent occurrence of deletions of the STS gene in Mexico

A. Jimenez[1], *M. Valdes*[2], *L. M. Gonzalez*[1], *R. Rivera*[1], *S. Kofman*[1], *S. Cuevas*[1]
[1]*Hospital General de Mexico; Mexico DF, Mexico;* [2]*Instituto Nacional de Ortopedia; Mexico DF, Mexico*
sergioa@servidor.unam.mx

X-linked ichthyosis (XLI) is due to the steroid sulfatase (STS) deficiency. STS assay, PCR and FISH analyses are useful to establish diagnosis and to discard XLI-carriers. The present study analyzes the frequency and size

of molecular deletions of the steroid sulfatase (STS) encoding gene in a sample of 90 Mexican subjects with biochemical diagnosis of X-linked ichthyosis (XLI). XLI patients were analyzed through STS assay, PCR amplification of the 5 -3 ends and flanking markers of the STS gene and FISH analysis. No amplification of the 3 and 5 -ends of the STS gene by PCR was detected in the DNA of 86 patients, 2 samples presented a normal amplification, 1 amplified the 3 end and 1 amplified the 5 end of the gene. FISH analysis was positive for XLI in 87 patients, the 3 patients that showed a normal hibridization pattern were a point mutation and 2 intragenic deletions. 32 patients presented the rupture sites at flanking markers DXS996 and DXS278, 24 patients had the breakpoints at flanking markers DXS996 and DXS1134 and the rest (24 subjects) showed heterogeneous rupture sites. This report shows a very high frequency and heterogeneous pattern of deletions in the human STS encoding gene in a representative sample of the Mexican population, and it defines the characteristics of FISH analysis and PCR to diagnose XLI patients

P1214. Intragenic deletion of 3 bp of the STS gene causing XLI ichthyosis.

M. Valdes[1], *A. Jimenez*[2], *R. Rivera*[2], *S. Kofman*[2], *S. Cuevas*[2]
[1]*Instituto Nacional de Ortopedia; Mexico DF, Mexico;* [2]*Hospital General de Mexico; Mexico DF, Mexico*
sergioa@servidor.unam.mx

Steroid sulfatase (STS) deficiency is an inherited error of metabolism causing X-linked ichthyosis (XLI). Onset is at birth or early after birth and it is clinically characterized by dark, regular and adherent scales of skin. Approximately 85-90% of XLI patients have large deletions of the STS gene and flanking sequences. Only seven patients have been reported with partial deletions of the STS gene. These include; a deletion within intron 7 extending over exons 8-10 of the STS gene; a partial deletion that included exon 10; an intragenic deletion spanning exons 2-5; a partial deletion within intron 1 and between flanking sequences DXS1131 and DXS1133. It has also been reported two unrelated cases with partial deletions at the 5 end of the STS gene. In this study we describe an intragenic deletion of the STS gene in an XLI patient and his mother. The subject and his mother were classified through STS assay in leukocytes using 7-[3H]-dehydroepiandrosterone sulfate as a substrate. Exons 1-10 of the STS gene were analyzed by PCR and DNA sequence analysis. STS activity was undetectable in the XLI patient (0.0 pmol/mg protein/h) and very low in his mother (0.32 pmol/mg protein/h vs 0.84 pmol/mg protein/h of normal control). DNA sequence analysis showed a 3 bp deletion (AAGdel1252) within exon 7 of the STS gene in both the patient and his mother. At this moment, we have analyzed more than 100 XLI patients and found two partial deletions of the STS gene. This is the first 3 bp deletion of the STS gene causing XLI.

P1215. Homozygous form of porphyria variegata in childhood

E. Malonova[1], *J. Zeman*[1], *H. Puy*[2], *V. Da Silva*[2], *R. Rosipal*[1], *J. Deybach*[2], *P. Martasek*[1]
[1]*Department of Paediatrics, First Faculty of Medicine, Charles University; Prague, Czech Republic;* [2]*Centre Francais des Porphyries; Colombes, France*
emalonova@hotmail.com

Porphyrias are metabolic disorders caused by decreased activity of enzymes in hem biosynthesis. Their clinical manifestation in early childhood is rare and include congenital erythropoietic porphyria, erythropoietic protoporphyria and homozygous forms of acute hepatic porphyrias. We present the results of biochemical and molecular analyses in 3 children with homozygous form of porphyria variegata (PV). The clinical symptoms in two children were already described (1). The third patient is a boy with short stature, short fingers and slight mental retardation who developed severe photosensitivity in the age of two years. The plasma fluorescence emission maximum of 626 nm was pathognomonic for VP, and the increased protoporphyrin (PP-IX) levels in erythrocytes were in agreement with our previous description of increased PP-IX levels in homozygous cases with porphyria variegata (2). Genetic analysis was performed by denaturing gradient gel electrophoresis (DGGE) of protoporphyrinogen oxidase gene and by subsequent sequencing of atypical DGGE pattern. Two different mutations in the boy were found; 1072G>A resulting in Gly358Arg in exon 10 and IVS10+4T>G in intron 10. The Gly358Arg mutation is identical with the mutation we found in our 2 other patients with homozygous variegate porphyria (3). Expression of this mutation in E. coli showed 9.5% residual activity as compared to wt protoporphyrinogen oxidase. 1. Kordac V., Deybach J.-C., Mart sek P., et al., Lancet, I, 851, 1984. 2. Kordac V., Mart sek P., Zeman Z., Rubin A. Photodermatol. 2, 257, 1984. 3. Roberts A.G., Puy H., Dailey T., et al., Hum. Mol. Genet. 7, 1921, 1998. [Supported by grant GAUK 5/2000/C from Charles University, and by INSERM, France]

P1216. Investigation of inborn errors of purine and pyrimidine metabolism

R. Giguere, *D. Cyr, C. Auray-Blais, B. Lemieux*
Centre Hospitalier Universitaire de Sherbrooke; Sherbrooke, PQ Canada
rgiguere@courrier.usherb.ca

In the last decade, there have been many exciting developments in the field of inborn errors of purine and pyrimidine metabolism. These disorders should be suspected in any case of unexplained anaemia, failure to thrive, susceptibility to recurrent infection or neurological deficits with no current diagnosis, including autism, cerebral palsy, developmental delay, deafness, epilepsy, self-mutilation, muscle weakness, inability to walk or talk, gout and sometimes renal disease. The diagnosis of the majority of the known inherited defects of purine and pyrimidine metabolism can be achieved by the analysis of urinary excretion profiles by reversed phase high-performance liquid chromatography and multi-wavelengh UV detection. Once perfected, this technique has important clinical applications for high-risk screening and follow up. We have established control values for sixteen different metabolites, thus permitting the possibility of diagnosing thirteen different diseases. Since the beginning of our analyses (October 1998), we have found seven positive cases; one molybdenum cofactor deficiency, one dihydropyrimidine dehydrogenase deficiency and five adenine phosphoribosyltransferase deficiency. The analysis of purines and pyrimidines is a useful tool when investigating prenatal and neonatal diseases.

P1217. Clinical heterogeneity and molecular basis of MyoAdenilate Deaminase Deficiency (MADD). Aguennouz M., Rodolico C., Ciranni A., Migliorato A., Musumeci O., Vita G, and Toscano A.

M. Aguennouz, *C. Rodolico, A. Ciranni, A. Migliorato, O. Musumeci, G. Vita, A. Toscano*
Clinica Neurologica 2; Messina, Italy
aguenoz@www.unime.it

Background; MyoAdenilate Deaminase Deficiency (MADD) is responsible of a bening metabolic myopathy characterized by muscle fatiguability, cramps, myalgias, and more rarely, exertional myoglobinuria (primary form). The most known molecular defect of MADD is the nonsense mutation C34T transition at codon 12(Gln to Stop) in exon 2 in AMPD1 gene, predicting a severely truncated MAD peptide. MADD may also present in association with other neuromuscular disorders in the so called coincidental form. Furthermore, it is known that MAD isoform M is abundantly expressed in type 2b muscle fibers. We have recently observed a number of patients with myalgias, reduced percentage of type 2b fibers and low or absent MAD activity and no associated neuromuscular diseases. These features suggest a third form af MADD that can be defined as secondary form. Objective; To characterize the clinical, morphological, biochemical and molecular aspects of the three forms of MADD. Materials & Methods; We investigated 31 unrelated patients with MADD; 5 with the primary form, 18 with the coincidental form and 8 with the secondary form. Results; -13 patients are homozygous for the C34T mutation (5 primary and 8 coincidental form), presenting with a residual MAD activity <1% of control range. - 10 patients with coincidental form are heterozygous for the nonsense mutation and 5 of them had a very low biochemical activity <8%. - 8 patients with selective muscle type 2b fibers atrophy and a reduced MAD activity (4-30%) did not carry the MADD mutation. Conclusion; 1) patients with the primary form had the homozygous mutation and residual MAD activity <1%; 2) patients with the coincidental form may present with very low activity and homozygous or heterozygous trait for C34T mutation; 3)patients with the secondary form may have variable residual MAD activity (4 to 30%) but do not carry the most known MADD mutation.

P1218. G6PD Deficiency and Genetics in Turkey

H. Bagci
Pamukkale University; DENIZLI, Turkey
hbagci@pamukkale.edu.tr

Glucose-6-phosphate dehydrogenase(G6PD) deficiency was firstly identified from the results of research on primaquine, which is used in malaria therapy, and its effects on hemolysis during 1950 s in US. During the time-course of G6PD research, it is clearly observed that biochemical characteristics, molecular identification of the G6PD protein, variant analysis and the structure-function relationships has been intensively studied. The regional distribution of the G6PD deficiency is closely related with geographic regions which are endemic for malaria.
First enzyme dificiency was reported on 1940 in Turkey. According to the WHO criteria, there are about 20 biochemically identified variants in

Turkey. The variants named as Gd-Aksu, Gd-Serik, Gd-Korkuteli in Antalya region(western Mediterranean), on the other hand Gd-Mersin, Gd-Balcalı, Gd-Samanda?, Gd-Adana ve Gd-Antakya variants were identified in ukurova(eastern Mediterranean) region. The overall distribution of G6PD deficieny is about 5 % in Turkey. In ukurova region G6PD deficiency was reported by different research groups as 8.5 %(Ozsoylu etal, 1975), 5.8 % (Akoglu et al, 1981), 7.6 % (Kılınc et al, 1982), 8.3 % (Yuregir et al, 1984), 8.2 % (Aksoy et al, 1981), on the other hand in Antalya region, 9.2 % (Aksoy et al, 1990), in Eagean Region 2-9 % (Sipahioglu et al, 1979), in Eastern Turkey-Erzurum 1 % (Aksu et al, 1972).
The main molecular defect causing the G6PD deficieny in Turkey is the Mediterranean mutation identified as 563T. On the other hand, Med2 haplotype and 1311T mutation are observed together frequently.
(*)*G6PD Working Group;* Bagci H., Aksoy K., Tuli A., Acikbas I., Atalay E., Aksu A., Gumuslu S., Solak M., Turan Y., Demircan K.

P1219. Menkes disease in a girl with normal karyotype

L. Burglen[1], *C. Ioos*[2], *N. Horn*[3]
[1]*H pital Trousseau; Paris, France;* [2]*H pital Raymond Poincar ; Garches, France;* [3]*John F.Kennedy Institute; Glostrup, Denmark*
lydie.burglen@trs.ap-hop-paris.fr

We report on a girl affected with Menkes disease. She is the first child of young healthy parents. Two maternal uncles died in early infancy from Menkes disease. Her mother was shown to be carrier by DNA analysis with polymorphic markers. The girl was born prematurely at 35 weeks. First months were marked by chronic diarrhea and failure to thrive. Psychomotor development was delayed, with head control at 7 months. At 18 months, examination showed major hypotonia. She was unable to sit. Smiling, following with eyes and social contact were good. She had a prominent occiput, sparse scalp hair with pili torti and cutis laxa. Menkes disease was suspected on familial history. Serum copper and ceruloplasmin were slightly reduced. Menkes disease was confirmed by increased copper accumulation in cultured fibroblasts. Karyotype was normal. MRI showed cerebral and cerebellar atrophy. Abdominal ultrasound was normal, as were skeletal radiographs. Females affected with Menkes disease are extremely rare. Expression of the disease is explained in some cases by 45X syndrome or balanced translocations with breakpoints at the MNK locus. Non-random inactivation is supposed to be responsible for clinical expression females with normal karyotype. X inactivation study in lymphocytes is in progress.

P1220. Alpha-1-antitrypsin mutation spectrum; assessment of haplotype diversity of rare variants and identification of a novel Ser47Arg mutation.

S. Seixas[1], *G. Cordeiro-Ferreira*[2], *O. Garcia*[3], *J. Rocha*[1]
[1]*Instituto de Patologia e Imunologia Molecular da Universidade do Porto (IPATIMUP); Porto, Portugal;* [2]*Servi o de Pediadria, Hospital Fernando Fonseca; Lisboa, Portugal;* [3]*Area de Laboratorio, Ertzaintza, Seccion de Biologia; Bilbao, Spain*
sseixas@ipatimup.pt

We have assessed the levels of haplotype diversity associated with rare non-S and non-Z alpha-1-antitrypsin (PI) variants by analysing a SNP situated in intron Ic of the PI gene and three microsatellites located within or close to corticosteroid binding globulin (CBG), alpha-1-antitrypsin and protein C inhibitor (PCI) at chromosome 14q32.1; PCI-190kb-PI-60kb-CBG. Using protein isoelectric focusing followed by DNA sequence analysis a total of 35 unrelated variants belonging to 14 distinct allelic classes were identified in samples from Portugal, the Basque Country and S o Tom e Pr ncipe; I (Arg39Cys, n=7); a newly identified normal Ser47Arg mutation (Slisbon, n=2); Mmalton (Phe52del, n=2); T (Arg101His or Glu264Val; n=3); M4 (Arg101His or Asp376Glu; n=8); V (Gly148Arg; n=1); Plowell (Asp256Val; n=1); R281del (Arg281del; n=1); Pdonauwoerth (Asp341Asn; n=2); Zaugsburg (Glu342Lys; n=1); Q0mattawa (Leu353insT n=1); S o Tom (Pro362His; n=1); Mheerlen (Pro369Leu n=3); Mwurzburg (Pro369Ser; n=2). Haplotype and sequencing information suggest that some variants could have arisen either from recurrent mutation or intragenic recombination; the mutations defining the Mheerlen, and Q0mattawa variants were found in allelic backgrounds that are different from those originally reported; the I allele displays levels of haplotype heterogeneity compatible with at least one additional independent origin; M4 haplotypes suggest independent origins through recurrence of the His101Arg mutation and through recombination between M2 and M1Val213; the T allele is the probable result from recombination between S and M2 (or M4). These data confirm and extend previous observations that a limited repertoire of mutations is responsible for a significant fraction of PI sequence variation.

P1221. Triplet Duplication in ?1(I) collagen chain of proband with lethal osteogenesis imperfecta shifts register of alpha chains in helix and alters incorporation of mutant trimers into fibrils and ECM.

J. C. Marini[1], *W. A. Cabral*[1], *M. Mertts*[2], *S. Leikin*[2]
[1]*NIH/NICHD/HDB; Bethesda, MD United States;* [2]*NIH/NICHD/LPSB; Bethesda, MD United States*
oidoc@helix.nih.gov

We investigated the effect of shifting the register of the collagen helix by a single Gly-X-Y triplet on collagen assembly, stability and incorporation into fibrils and matrix. These studies utilized a collagen mutation occurring in lethal type II OI. The proband has a triplet duplication in COL1A1 exon 44 cDNA and gDNA subclones. The normal allele encodes 2 identical Gly-Ala-Hyp triplets at aa 868-874, while the mutant allele encodes three. The register shift delays helix formation, causing overmodification of all CNBr peptides. Pericellular processing of secreted procollagen demonstrated more rapid appearance of pC- and mature collagen than control. In matrix deposited by proband cultured fibroblasts, overmodified chains were abundant in the immaturely cross-linked fraction but constituted a minor fraction of maturely cross-linked collagen. Trimers purified from cell culture exhibited markedly different behavior depending on whether they had two (+/+), one (+/-) or no (-/-) mutant ?1 chains. Differential scanning calorimetry revealed that +/+ molecules denature in sequential steps, 6º, 5º and 4ºC below the Tm of wild type protein; +/- molecules denature in a single step, 2ºC below wild type. The three species appeared to be secreted in a 1;2;1 ratio consistent with random chain association. In vitro fibrillogenesis of this mixture produced fibers containing no +/+ molecules and smaller +/- to -/- ratio than the starting mixture. The rate and extent of fiber formation were comparable to wild type only at 4X higher protein concentration. The profound effects of shifting the collagen triplet register correlate with the severe clinical outcome.

P1222. Chitotriosidase activity in patients with beta-thalassemia

G. M. Altarescu, *B. Rudensky, A. Goldfarb, A. Abrahamov, D. Elstein, A. Zimran*
Shaare Zedek Medical Center; Jerusalem, Israel
altaresg@ninds.nih.gov

Objective; To measure plasma chitotriosidase levels in patients with b-thalassemia and to ascertain correlation with severity and/or other parameters. Introduction; Chitotriosidase, produced by macrophages, is a marker of severity and response to therapy in Gaucher disease. Since there is expansion of the reticuloendothelial system, chitotriosidase may be equally useful in b-thalassemia. Material and Methods; 39 adults (16-53 years; 30 with b-thalassemia major, 9 with intermedia), and 14 children (0.7-15 years; 12 with b-thalassemia major, 2 with intermedia) were tested for chitotriosidase and correlated with ferritin, hemoglobin, liver function tests, and genotype. Results; Plasma chitotriosidase levels were normal (<20 mU/ml) in all children. Only 16 adults had elevated levels and 12 of these, >80 mU/ml. There was no correlation with genotype, age, type, hemoglobin, transfusions/year, or chelating treatment. The patient with the highest chitotriosidase (1440 mU/ml) had the highest ferritin (5175 mg/l), required the most transfusions/year (40), and had abnormal liver tests. Ferritin and poor therapy compliance correlated with chitotriosidase levels. Discussion; Normal levels in the pediatric cohort and increased levels in only some adults may reflect status of iron overload in macrophages and may suggest a role for monitoring chitotriosidase. Our results are in contradistinction to Barone et al s in an Italian cohort. Conclusions; These results may implicate a role for plasma chitotriosidase levels in monitoring clinical parameters and response to therapy in patients with b-thalassemia.

P1223. Diagnosis of Congenital Metabolic Disorders by Gas Chromatography / Mass Spectrometry (GC/MS) in India - High-risk screening approach.

U. P. Dave[1], *J. Fernando*[1], *I. Matsumoto*[2], *C. Zhang*[2]
[1]*Centre for Research in Mental Retardation (CREMERE); Mumbai, India;* [2]*Matsumoto Institute of Life Science (MILS); Ishikawa, Japan*
cremere@bom5.vsnl.net.in

A rapid progress in human genetics, by the end of this millennium, is mainly due to advances in laboratory technology available at the fingertips of the clinicians for accurate, specific and reliable genetic diagnosis. However, genetics takes a back seat in the Indian scenario due to other national health priorities. The concept of Inborn Errors of Metabolism (IEM) being rare (4—6%), was based on the older conventional methods of TLC and GC. In view of this a powerful tool like GC / MS was used in the simultaneous detection of 101 congenital metabolic abnormalities of amino acids, organic acids, sugars, sugar acids, sugar alcohols, nucleic acids and

nucleic acid bases.
The present paper reports chemical diagnosis of 355 high—risk children with definite diagnosis of IEM in 58 cases (16.4%) using Mass Spectrometry. In critically—ill neonates, the metabolic abnormalities was 28.26% (13 out of 46 neonates). This emphasises the crucial role of GC/MS in preventing mortality and morbidity. The high—risk genetic factors were consanguinity, family history of mental retardation, still births and deaths. For transportation of air—dried urine filter paper , the referral system was established with the attending doctors in small towns and rural areas having limited laboratory infrastructure. The 22 disorders are considered as target diseases as some therapy and management is possible.
A few interesting cases will be presented where successful management, therapy and genetic counselling was done. Recognition of congenital metabolic disorders at the primary health care levels by creating awareness among medical professionals and developing resources like technical expertise and sophisticated equipments are the key factors in genetic healthcare. The Indian population with religious, racial and ethnic diversity indicates a great scope of GC / MS diagnostic technology in establishing the epidemiology of congenital metabolic disorders which is currently lacking.

P1224. Diagnosis, Handling And Evolution Of The Maple Syrup Urine Disease. presentation Of A Case.

G. Cossio
Hospital del Ni o de Panama; Panama, Panama
gcossio@sinfo.net

The Maple Syrup Urine Disease is an inborn error of metabolism produced by genetics mutation. In Panama, they have been identified few cases, all of them children from the indigenous group know as Tule. In these cases the fatality has been high in the absence of a precocious diagnostic that would let a better handling. We had the opportunity of handling a case with exanguine-transfusion and especial milk formula getting very good results clinical as well as those from laboratory. Clinical Case. It treats of a child of 59 days who begins his symptomatology since 6to day of life, with a clinical chart of irritability, strong and sharp crying, poor feeding, vomiting and hyporexia, he has treated in an ambulatory way. On the eleven day of the life he enters to the institution for being with greater irritability, without suction and with opisthotonos. After the handling in the Hospital and with stable clinical evolution the newborn goes out with a diagnosis of Syndrome of the rigid boy and a treatment with Mogadon at the 59 days. Hi is evaluated at genetic department, where is suspected the MSUD for clinical and urine s smell, it is realized 2-4 DNPH test which give positive. With the fact and the clinic syrup urine smell the patient is hospitalized with Maple Syrup Urine Disease diagnosis to be treated with peritoneal dialysis and special diet. The blood and urine aminoacid chromatography gives positive for leucine, valine and isoleucine giving no doubt in the diagnosis. The clinical characteristics that this patient shows are typical of the classic MSUD. We believe important to report this case because the signs and symptoms (including the characteristic odor of the urine) of a children who as been asymptomatic the first 4 days of life must orient us toward a metabolic disease, first in the group of the intermediates organic acidemias, which may be corroborated with laboratory test hat is available in our media and which can be made very fast.

P1225. Molecular genetic and gas chromatography - mass spectrometry studies in the Conradi-Huenermann-Happle syndrome; biochemical evidence for somatic and germ line mosaicism

C. Has[1], *U. Seedorf*[1], *F. Kannenberg*[1], *L. Bruckner-Tudermann*[1], *J. Kunze*[2], *E. Folkers*[3], *H. Traupe*[1]
[1]*University of Muenster; M nster, Germany;* [2]*University of Berlin; Berlin, Germany;* [3]*Regional Hospital; Zaandam, The Netherlands*
traupeh@uni-muenster.de

Recently , causative mutations in the gene for emopamil binding protein (EBP) have been identified in the Conradi-Huenermann-Happle syndrome (MIM # 302960). EBP also acts as a sterol isomerase and has an important function in the final steps of cholesterol biosynthesis. To better understand the functional genetics of the Conradi-Huenermann-Happle (CHH) syndrome we performed molecular genetic and sterol biochemical studies in 5 CHH syndrome families comprising 13 individuals. For appreciation of the functional consequences we studied serum samples using gas chromatography- mass spectrometry (GC-MS) analysis. Mutations in the EBP gene were found in all 5 families.Accumulation of cholesterol precursors such as 8- dehydro- cholesterol demonstrated that the mutations identified were functionally relevant.In one family the CHH syndrome concerned two affected daughters, although both parents were free of the disease. Surprisingly, we found a strong accumulation of cholesterol precursors also in the serum of the clinically unffected father and subsequently extended our molecular genetic analysis to hair bulbs. In DNA from hair bulbs, but not from lymphocytes of the father we could then identify the same mutation that was present in his clinically affected daughter (one other daughter had died due to the disease and could not be studied).We conclude that detection of cholesterol precursors by GC-MS analysis is a very appropiate method to confirm the clinical diagnosis of the CHH syndrome and can be helpful to detect somatic mosaicism.

P1226. The Haemochromatosis StripAssay; a reverse-hybridization assay for the molecular analysis of HFE and TFR2 gene mutations

A. Moritz, *F. Kury, C. Oberkanins*
ViennaLab Labordiagnostika GmbH; A-1110 Vienna, Austria
moritz@viennalab.co.at

Hereditary haemochromatosis (HH) is a very common autosomal recessive disorder of iron metabolism with a genetic incidence of about 1 in 200 individuals of Northern European descent. HH is characterized by progressive accumulation of iron in various organs (liver, heart, pancreas), ultimately leading to liver cirrhosis, diabetes, arthritis, cardiomyopathies and premature death. With early diagnosis and treatment by therapeutic phlebotomy survival of patients is normal. A number of point mutations within a novel MHC class I-like gene (HFE) have been identified and related to HH. While homozygozity for C282Y is observed in the majority of HH patients, the diagnostic value of other HFE mutations is still under investigation. More recently, mutations in the gene encoding transferrin receptor-2 (TFR2) have been found in individuals with non-HFE haemochromatosis. We have developed a reverse-hybridization assay (Haemochromatosis StripAssay) for the rapid and simultaneous detection of known mutations in the HFE and TFR2 genes. The test is based on a single, multiplex DNA amplification reaction and ready-to-use test strips containing oligonucleotide probes for each wild-type and mutated allele immobilized as parallel lines. The entire procedure from blood sampling to the identification of mutations requires less than 6 hours and may be automated on existing equipment (e.g. TECAN profiBlot). (oberkanins@viennalab.co.at)

P1227. Prader-Willi and Angelman syndromes; rapid molecular diagnosis with methylation-specific PCR for imprinting analysis

A. Gomez, *L. Sbaiz, A. Sedita, S. Bosso, E. Cocco, L. Leone, G. Restagno*
Molecular Genetics Laboratory, Children s Hospital Regina Margherita; Torino, Italy
grestagno@hotmail.com

Prader-Willi (PWS) and Angelman syndromes (AS) are caused by the loss of function of imprinted genes in proximal 15q. The PWS and AS imprinting mutations include the DMR (Regions of allele-specific Differential Methylation) at the CpG islands of the SNRPN locus which may lead to incorrect resetting of the imprint in the germ line. We are using M-PCR (Methylation-specific PCR) which can rapidly assess the methylation status of CpG sites within a CpG island, without using methylation-sensitive restriction enzymes. This assay entails initial modification of DNA by sodium bisulfite, converting only unmethylated cytosines to uracils and subsequent amplification with primers specifically designed for the methylated or unmethylated version of the CpG island of SNRPN. A retrospective study conducted on 42 DNA samples previously analyzed by Southern blotting showed 100% concordance on 15 PWS, 4 AS and 23 normal samples. A diagnostic test is now routinely performed on DNAs from various sources (blood, Guthrie cards, CVS). In the last year we analyzed 65 subjects with suspected PWS/AS; nine showed the methylation pattern of PWS, two the methylation pattern of AS and fifthy-four were normal. M-PCR is a reliable method in the differential diagnosis of neonatal hypotonias and of the various forms of obesity/hypotonia/mental retardation. The advantage of M-PCR, when compared with previous PCR-based techniques or Southern blot are the rapidity, sensitivity, the use of cheap reagents and the possibility of detecting the major classes of molecular defects causing PWS/AS (deletions, uniparental disomy and imprinting mutations) without the need of parental DNA.

P1228. High Throughput SNP Analysis on the ABI PRISM 7900HT Sequence Detection System

J. F. Stevens, *J. Ma, C. Lee, W. Bi, K. J. Livak*
Applied Biosystems; Foster City, CA United States
stevenjn@appliedbiosystems.com

The ABI PRISM¤ 7900 HT Sequence Detection System features a real-time PCR instrument with 384-well-plate compatibility and robotic loading. The 7900 HT Automation Accessory loads up to eighty-four 384-well plates

into the instrument without user intervention. Hand-held and integrated bar code readers simplify sample tracking, while continuous wavelength detection enables the use of multiple fluorophores in a single reaction. Sample tubes remain closed throughout the PCR and detection process to control contamination. The probe-based, fluorogentic 5 nuclease assay provides homogeneous detection of any SNP using universal reaction conditions. By using fluorogenic probes with a Minor Groove Binder (MGB) attached to the 3 end, robust discrimination is achieved even for SNP s in AT-rich DNA. These TaqMan¤ MGB probes also utilize a non-fluorescent quencher, which enables the detection of four reporter dyes, making it possible to multiplex TaqMan SNP analysis. These features coupled with developments to reduce reaction volume and automate probe manufacturing enable large scale and cost effective SNP analysis. Used as an endpoint reader, the ABI PRISM¤ 7900 HT Sequence Detection System can make up to 300,000 genotype determinations per 24-hr day.

Also presented orally in Concurrent Session 5: Poster Discussion Methods to Detect Genomic Variation

P1229. Determination of a Genome Wide SNP Allele Frequency Map in Three Defined Populations and CEPH Pedigrees for SNPs Identified by The SNP Consortium

***M. S. Phillips**, W. M. Ankener, M. G. Pohl, M. T. Boyce-Jacino*
Orchid BioSciences, Inc.; Princeton, NJ United States
mphillips@orchid.com

Now that the sequencing of the human genome is nearing completion, the initiation of the next phase or the annotation phase of the human genome project is starting to unfold. This next phase will focus on determining the number and types of genes present, identifying how these genes control and regulate biological processes and characterizing the variations that are observed between individuals. A key component of this analysis is the identification, localization and population frequency determination of single nucleotide polymorphisms (SNPs). In collaboration with The SNP Consortium (TSC), Orchid BioSciences is determining SNP allelic frequencies in samples from three different defined populations, and selected CEPH pedigrees. Using Orchid s high throughput single-base primer extension technology, SNP-IT, approximately forty individuals from each of the Caucasian, African American and Asian populations are being analyzed to estimate the frequencies of more than 60,000 SNPs distributed equally across the genome. The 60,000 TSC SNPs are also being analyzed on 10 CEPH pedigrees for the determination of both allelic frequency and phase of the SNP. The pedigrees are also useful as a tool for error checking of the SNP assays and the determination of haplotypes. Results from the analysis of the CEPH pedigrees will provide the initial components for a linkage disequalibrium (LD) map of the genome. New software tools are being developed and utilized to streamline this process. The results of these studies will form the basis for the first defined human SNP map and generate the resources necessary to conduct genome-wide association studies.

Also presented orally in Concurrent Session 5: Poster Discussion Methods to Detect Genomic Variation

P1230. Multiplex genotyping of SNPs using Pyrosequencing“

***A. Schiller**, J. Dunker, A. Alderborn*
Pyrosequencing AB; Uppsala, Sweden
annalotta.schiller@pyrosequencing.com

Pyrosequencing“, or real-time sequencing, is a fast and accurate method for SNP analysis. Pyrosequencing AB (Sweden) manufactures the PSQ96“ System, in which 96 different SNPs are analyzed in parallel in approximately 10 min. A dedicated SNP scoring software automatically delivers genotype and a quality assessment for each sample. In this study, the applicability of pyrosequencing for multiplex genotyping was investigated. SNPs from the Renin-Angiotensinogen-Aldosterone System (RAAS) were analyzed using pooled simplex PCR products followed by multiplex genotyping. To further enhance cost efficiency in SNP analysis, multiplex PCR followed by multiplex genotyping by pyrosequencing was tested using the Factor V Leiden and Prothrombin G20210A polymorphisms. The flexibility offered by pyrosequencing with respect to positioning of the sequencing primer relative to the SNP was exploited in experimental design of multiplex genotyping. Both strategies (pooled simplex PCR/multiplex pyrosequencing and multiplex PCR/multiplex pyrosequencing) resulted in highly reproducible and accurate SNP scores, which were in complete accordance with those obtained when genotyping each fragment independently. Design of sequencing primers in combination with a reaction specific dispensation order enabled separate typing of the SNP positions whilst keeping the genotyping quality identical to that of simplex genotyping. In addition, this approach allowed some unequal amplification efficiency to occur without negatively effecting the genotyping results. The sequences surrounding the SNPs confirmed the correct positioning of the primers on their respective gene sequences. Thus, pyrosequencing enables reliable and robust analysis of several SNPs in a single pyrosequencing reaction.

Also presented orally in Concurrent Session 5: Poster Discussion Methods to Detect Genomic Variation

P1231. Can the polymorphism rate of genes be correlated to the localization within the genome?

***K. Schiebel**, S. Beck, B. Orlicz-Welcz*
Institute for Biochemistry; Erlangen, Germany
katrin.schiebel@biochem.uni-erlangen.de

Inter-individual genomic variability is mainly due to single nucleotide polymorphisms (SNP) and variable numbers of tandem repeats (VNTR). Both kinds of variability are elevated in the genomic region of the protein phosphatase regulatory subunit gene PPP2R3L compared to the average polymorphism rate in the whole genome. The human PPP2R3L gene is located in the pseudoautosomal region 1 (PAR1) on human sex chromosomes Xp and Yp, 150 kbp from the telomer. The elevated variability of the PPP2R3L gene region is most likely caused by the exceptional features of PAR1. The increased recombination rate within the PAR1 and a GC content of 60 % both are supposed to increase the SNP rate whereas slippage and illegitimate recombination alter the number of tandem repeats. Correlations between genomic environment and polymorphism rate were investigated by comparing the variability of the PPP2R3L region with the genomic region of the closely related autosomal PPP2R3 gene.

P1232. Determining the approximate relatedness of individuals for Linkage Disequilibrium mapping

***M. Bahlo**, J. P. Rubio, H. Butzkueven, T. P. Speed, S. J. Foote*
The Walter and Eliza Hall Institute of Medical Research; Parkville, Australia
bahlo@wehi.edu.au

Kruglyak (1999) and others have cautioned against the use of LD (Linkage Disequilibrium) mapping in the general human population because of the prohibitive number of markers required to map genes. However, the density of markers required for LD mapping is much reduced in a population that is sufficiently young. Suppose we have a population of age approximately 6-8 generations. This type of population allows the use of microsatellite markers instead of SNPs for LD mapping, which are known to be more informative. However, in order to use such a young population it is imperative to have a representative sample of individuals. Otherwise individuals in the sample are more distantly related and LD is unlikely to be detected. Here we outline two strategies to identify people who are distantly related by 6-8 generations. One is a simple method based on the average IBD sharing of individuals and the other is a multipoint approach looking at the average length of segment shared by individuals. We try the methods on some simulated data as well as on some real data from Tasmania, Australia s island state.

P1233. A gene fine mapping measure based on Hardy-Weinberg disequilibrium among affected individuals

***R. Jiang**[1], J. Dong[1], F. Sun[2]*
[1]Michigan Technological University; Houghton, MI United States; [2]University of Southern California; Los Angeles, CA United States
rjiang@mtu.edu

Feder et al. (1996) used $p_{\rm excess}$ and F, which is based on Hardy-Weinberg disequilibrium, to study hereditary hemochromatosis (HH), and located the hereditary hemochromatosis gene to a genomic region $<$1-2 cM. $$F=\frac{H_0-H_e}{1-H_e}$$ where H_0 is the observed frequency of homozygosity and H_0 is the expected homozygosity assuming Hardy-Weinberg equilibrium among cases. We prove that F_M needs to be improved, and it is possible that the value of F_M at one marker locus is larger than that at another marker locus, and the latter is closer to the disease susceptibility locus. The reason of the inadequate performance of F_M is that F_M depends on the allele frequency of M. If the allele frequencies at different markers are different, which is usually the case, the maximum of F_M will not occur at the disease susceptibility locus. We propose a new measure of fine-scale mapping J, which is a modified version of $\hat F_M$. Our new measure does not depend on marker allele frequencies, and its maximum occurs at the disease locus. We do not need to assume a single ancestral haplotype. Assuming multiple ancestral haplotypes, we prove that $p_{\rm excess}$ is not a good measure of fine-scale mapping because it depends on marker allele frequencies. In theory, our new measure is better than both F_M and $p_{\rm excess}$. In simulations, our new measure outperforms F and $p_{\rm excess}$ for all disease models.

P1234. Accurate Estimation of Both Haplotype Frequencies and Phase of Each Subject Using Multilocus Genotypic Data from Unrelated Individuals Using EM Algorithm-based Program

Y. Kitamura[1], *M. Saito*[1], *A. Saito*[1], *H. Akama*[1], *H. Morisaki*[2], *T. Morisaki*[2], *N. Kamatani*[1]
[1]*Institute of Rheumatology, Tokyo Women s Medical University; Tokyo, Japan;* [2]*National Cardiovascular Center Research Institute; Osaka, Japan*
kitamura@ior.twmu.ac.jp

Haplotype analysis is becoming increasingly important for mapping disease genes. Usually, exact haplotypes are considered to be obtained only from pedigree data. Recently, however, methods for estimating the frequencies of the haplotypes from the genotypic data of unrelated individuals have been developed based on expectation-maximization (EM) algorithm. The previous study has suggested that the estimation of the sample frequencies of the haplotypes was accurate when the simulation data were analyzed. We examined whether the EM-based haplotype frequency estimation and the estimation of phases of unrelated subjects are accurate using the real data on smoothelin gene. Using the DHPLC and SSCP methods on PCR-amplified DNA, we were able to determine the haplotypes for 2 separate regions spanning 341-380 bp sequences in the smoothelin gene for 96 unrelated subjects (32 Japanese, 32 European American and 32 African American). After physically determining the haplotypes, we concealed the phases and applied the genotypic data to our EM-algorithm-based program. Our program estimates both haplotype frequencies and the probability distribution on the phases of the subjects, and can handle both biallelic and multiallelic loci. When the data from the 3 groups were applied separately, the program almost perfectly estimated the sample haplotype frequencies as well as the phases in the subjects. Mis-estimation rate was almost negligible. Although the present method should be tested for the cases where haplotypes span much wider regions of the chromosome, our data suggest that the results of the EM-based haplotype frequency and phase estimation from the data of unrelated individuals may be quite reliable for the data from various ethnic groups.

P1235. Simulation of Genotypes using Inheritance Vector

A. Saito*, M. Saito, Y. Kitamura, H. Akama, N. Kamatani*
Institute of Rheumatology, Tokyo Women s Medical University; Tokyo, Japan
asaito@ior.twmu.ac.jp

In a linkage study of a disease, it is important to know if the number of pedigrees that investigators have collected or plan to collect will be sufficient to detect linkage. Computer simulation of the genotypes in pedigrees is an effective method that can provide approximate answers to such questions. However, with the previous simulation programs based on the basic algorithm used in the LINKAGE package, it was difficult to simulate genotypes at more than a handful of loci. To investigate complex diseases, multipoint analysis with many markers will be crucial and it is necessary to simulate genotypes at large numbers of loci. In this study, we have developed a new computer program to simulate genotypes quickly at many loci with general Monte Carlo method and inheritance vector. Inheritance vector is a binary vector that describes the outcome of the paternal and maternal meioses giving rise to the nonfounders in the pedigree at every locus in the genome, which can indicate the precise inheritance pattern of the pedigree. Using inheritance vector, our program can simulate genotypes at many trait or marker loci quickly in a standalone linux environment and that supports efficient multipoint analysis of pedigree data.

P1236. Incorporating ages at onset in a generalized score statistic for linkage analysis

L. Hsu
Fred Hutchinson Cancer Research Center; Seattle, WA United States
lih@fhcrc.org

Data with varying age at disease onset arise frequently in studies of mapping disease genes for complex traits. In our earlier paper (Li and Hsu, 2000, Annals of Human Genetics, in press), we show that naively combining affected subjects with different ages at onset may result in a much reduced power in detecting linkage. Based on this observation, in this paper we propose a weighted generalized score test statistic, where the proposed weighting depends on a specified age-dependent penetrance function and on the observed age at onset in affected family members. The large sample theory shows that the proposed weighted test has a correct type I error rate even if the penetrance function is misspecified. It is shown analytically that under the Cox proportional hazards model for the major gene effects, the weighted test statistic gains more power than the one giving equal weights to all affected subjects, and that under- or over-misspecification of mode of inheritance and relative risk do not appear to affect the power greatly. As for illustration, we apply the proposed test to a simulated data set provided by the 12th Genetic Analysis Workshop organizers. It shows that the proposed test statistic yields strong evidence for the region where the underlying disease loci reside whereas the equal weight test statistic provides only modest evidence for the same region and yet indicates a strong signal to the location which is far away from the true locations.

P1237. Genotyping SNPs with exension of fluorescently labelled primers

G. Kirov
University of Wales College of Medicine; Cardiff, United Kingdom
kirov@cardiff.ac.uk

The genetic dissection of disorders of complex inheritance requires large-scale genotyping of SNPs. A number of methods have been developed for efficient and fast genotyping. Some of these methods, e.g. using polarisation and mass spectrometry, require expensive equipment and are not suited for small to medium-sized laboratories. Primer extension with fluorescently labelled ddNTPs can be performed on the more widely available automated sequencers. Here we report a variation of this method which we find more reliable and cheaper. We use extension primers labelled with 3 different dyes (FAM, HEX and TET). We designed our own size standards ranging from 13 to 43 bp, labelled with TAMRA. The 3 end of the primer extends not immediately up to the SNP, but one bp earlier. This reduces the interference of the first extension peak with the peak produced by the primer which has not incorporated any d/ddNTP. We use 3 dNTPs and 1 ddNTP for each reaction, so that the primer extends between 1 and 5 bp beyond the SNP, until it reaches the next ddNTP site. This produces 3 peaks which allow unambiguous calling of genotypes. The presence of a peak produced by the non-extended primer in each reaction allows to distinguish failed reactions and provides an internal size control. Out of 8 reactions, we produced reliable traces in 7 with our first attempt, and had to change the ddNTP in the last reaction to the alternative ddNTP. Using different sizes of primers, ranging from 15 to 31 bp, we were able to multiplex all 8 SNP for analysis on the same gel. The cost of an individual genotyping is $0.42, excluding the cost of primers. We also perfomed pooled DNA genotyping of these 8 SNPs on pools of 110 parent-offspring trios. One reaction did not produce reliable traces because of interference with the peak of the non-extended primer. One SNP had an allele with a very low frequency of 2% and could not be measured reliably. The remaining 6 SNPs showed highly reproducible patterns with a maximum variation between experiments of just 2.8%. Analysis of pooled and individual genotypings showed a maximum error of 1.4% for the true versus the predicted difference between two sets of pools. We conclude that the method is sufficiently accurate to be used as an initial screen in order to reduce the number of SNPs that justify individual genotyping.

Also presented orally in Concurrent Session 5: Poster Discussion Methods to Detect Genomic Variation

P1238. A multiplex solution for SNP interrogation using fifth-dye labeled short size standard and single-tube primer extension reaction

S. S. Kuo*, D. Wei, G. Ayanoglu, M. Johnson, C. Chang, P. Hanachi, A. B. Tomaney, P. Dong*
Applied Biosystems; Foster City, CA United States
kuoss@appliedbiosystems.com

A variety of methods may be used to characterize and to screen single nucleotide polymorphisms. Analysis platforms include microarray scanning, real-time PCR analysis (TaqMan), and MALDI-TOF. In addition, electrophoresis-based techniques include OLA analysis, dideoxy sequencing, and single-nucleotide primer extension. SNaPshot is a single-tube SNE reaction designed for elucidation of individual loci within known sequence contexts for the purpose of SNP screening and validation. We describe the development of a short size standard for analyzing small fragments on fluorescent detection systems. This standard contains 9 fragments ranging from 15 to 120 nucleotides, and is labeled with a fifth dye. During evaluation on various capillary electrophoresis platforms, we are able to achieve excellent precision and curve-fitting cross-platform. The fifth dye is spectrally well resolved from other dyes. This size standard is designed in particular to enable automated data analysis in methods for SNP detection, such as single-nucleotide extension assays. In our poster, we will demonstrate its utility in SNE assays. With the combination of different fragment lengths and four-color chemistry, the potential for multiplexing SNP loci exists for large-scale genotyping with minimal optimization.

P1239. Non-Mendelian single-nucleotide polymorphisms with frequent conversion to homozygosity

W. J. Broom[1], *M. J. Parton*[1], *P. M. Andersen*[2], *A. Al-Chalabi*[1], *N. Leigh*[1], *J. F. Powell*[1], *C. E. Shaw*[3]
[1]*Institute of Psychiatry; London, United Kingdom;* [2]*Umea University; Umea, Sweden;* [3]*Institute of Psychiatry and Guy s, King s & St Thomas s School of Medicine; London, United Kingdom*
spgtwjb@iop.kcl.ac.uk

As part of a SNP haplotype we sequenced a 7.5Kb region around the SOD-1 locus. We detected 11 novel SNPs and a 50 bp deletion. The two commonest SNPs were genotyped in 1100 individuals and we found that their allele frequencies were at great variance from Hardy-Weinberg equation (chi-squared values of 45 and 85). Six primer pairs were used to generate PCR products ranging from 150 to 1,200 bp and genotyping by restriction digest and direct sequencing gave identical results excluding an artefactual explanation. Transmission of the SNPs within CEPH pedigrees demonstrated Mendelian and non-Mendelian SNP inheritance patterns within the same PCR product. Conversion was bidirectional from T>C and C>T and dependant on the presence of at least one parental T allele. Transmission frequencies observed in this study for non-Mendelian SNPs are consistent with a population genetics model leading to a stable polymorphism. These events are not explicable by our current ideas of mutagenesis, such as methylation and deamination of cytosine residues, and suggest that SNP conversion occurs as a novel genomic editing event affecting both parental chromatids. Sites of SNP instability may significantly impact on research that assumes genomic stability and heritability of human SNPs.

P1240. Multiple, time-spaced injections onto the MegaBACE“ for high throughput SNP genotyping.

A. Shuster[1], *M. Minarik*[1], *D. Shen*[1], *K. Pirkola*[1], *K. Jones*[2], *R. Belcinski*[1], *A. Mamone*[2], *M. Mahtani*[1], *K. Dains*[1]
[1]*Amersham Pharmacia Biotech; Sunnyvale, CA United States;* [2]*Amersham Pharmacia Biotech; Piscataway, CA United States*
audrey.shuster@am.apbiotech.com

We have further developed and improved a method for high throughput SNP genotyping on the MegaBACE“ 1000. The system takes advantage of the instrument s flexibility to load multiple 96-well sample plates over a short period using a proprietary method of repeated, time-spaced injections. Samples are prepared in a single-tube SNuPE“ (Single Nucleotide Primer Extension) reaction that contains four different fluorescently-labeled dideoxynucleotides (terminators). After single base extension and cleanup, SNP products are loaded onto the MegaBACE by automated electrokinetic injection. Multiple, pulsed injections of up to 10-30 different SNP marker plates are then loaded into the same polymer matrix, spaced at two minute electrophoresis intervals. 1000 - 3000 SNP genotypes can be obtained with a time range of approximately 2-5 hours; further color and size multiplexing will greatly increase throughput. A characteristic injection marker is added to each sample prior to injection, acting both as internal control and also demarcating each injection from the next. A separate software package, SNP Profiler automatically processes the signal data and outputs the maximum likelihood SNP genotypes. The flexibility of the system is demonstrated by the ability of switching between three applications high throughput sequencing, SNP typing or STR genotyping in minutes.

P1241. Assessing Interference Levels in Mouse

N. J. Armstrong, *T. P. Speed*
University of California, Berkeley; Berkeley, CA United States
nicola@stat.berkeley.edu

Positive crossover interference has been documented in many organisms including both human and mouse (Zhao et al. 1995). The amount of interference appears to vary throughout the genome. QTL analysis and genetic map estimation are usually carried out under the assumption of no crossover interference. However, taking crossover interference into account creates more accurate genetic maps especially in the presence of missing or erroneous data. The genome averaged level of interference has previously been estimated for mice. We fit a chi-square model for interference to each individual chromosome for various sets of mouse data. The chi-square model has been found to fit data from experimental organisms well (Lin and Speed 1996). We estimate m, the level of interference for each chromosome. We also investigate if there is a significant difference in the level of interference between the sexes.

P1242. Evaluating the power for detecting complex traits using all of the nonparametric statistics implemented in SimWalk2 and GENEHUNTER2.

Y. Y. Shugart[1], *A. Collins*[2]
[1]*Johns Hopkins University; Baltimore, MD United States;* [2]*University of Southampton; Southampton, United Kingdom*
yyao@cidr.jhmi.edu

Several allele sharing based statistics have been developed as alternatives to parametric lod scores when the mode of inheritance of a genetic trait is unknown. It is important to evaluate the power of alternative statistics to detect linkage given a complex genetic background. Goldin and Weeks (1993) simulated data for five two-locus models and three heterogeneity models. The two-locus models include two dominant loci (DD), two recessive loci (RR), a dominant and a recessive locus (DR and RD). and a model with additive penetrance (AD). Heterogeneity models describe heterogeneity levels of 50 % (H50) and 25% (H25). Each replicate comprises 20 three generation pedigrees. The power to detect linkage using two nonparametric (NP) statistics implemented in GENEHUNTER2 and five NP statistics in SimWalk2 were compared using these simulated data sets. Our results demonstrate that Spairs performs well over all models although Spairs and Sall are comparable except for the RR model where Sall has lower power. Surprisingly, the E statistic in SimWalk2 appears to be less powerful than Sall under all models, particularly the RR model. Within SimWalk2 statistics A and C perform best for RR and statistics B,D and E are similarly powerful with DD and two heterogeneity models. The power to detect linkage for the AD data set is generally low although Sall performs the best amongst all of the statistics examined. We conclude that the choice of NP statistic greatly affects the power to detect linkage.

P1243. Genetic Polymorphism of the ACE and TNF-alpha in Childhood Asthma

M. Alikasifoglu, *G. Adalioglu, Y. Saraclar, A. Tuncer, E. Tuncbilek*
Hacettepe University; Ankara, Turkey
mehmet@gen.hun.edu.tr

An insertion-deletion polymorphism of the angiotensin-converting enzyme (ACE) gene has been shown to be associated with levels of ACE. Because ACE is heavily expressed in the lungs and plays a key role in the metabolism of angiotensin II and bradykinin, which are potentially involved in the pathogenesis of asthma. Tumour necrosis factor alpha (TNF-alpha) is a potent modulator of immune and inflammatory responses, and has been implicated in a variety of autoimmune diseases, including asthma. Interindividual variation in TNF alpha levels may be genetically determined and polymorphisms (G-A - 238 and - 308) within the TNF-alpha genes and nearby HLA Class II region have been associated with differences is TNF alpha production. In the present study, we examined whether asthma is linked with these ACE and TNF-alpha genes polymorphism. Patients with asthma (n=120, 74 males and 46 females; age 11.22 – 3.69) and 52 healthy controls were determined for their genotype by the polymerase chain reaction (PCR) method. Fourty-six asthmatics demonstrated the DD type (38.3%), 52 the DI type (43.4%), and 22 the II type (18.3%). The ACE gene polymorphism (D/I) did not show on association (odds= 0.65, % 95 CI, 0,67 —2,33, X2= 1,99, p>0,005). The allelic frequency of the TNF-alpha G-A - 238 among the asthma patients was not significantly different from among the control subjects. However, TNF-alpha G-A -308 allele was present at significantly higher frequency in cases than control (odds= 2,42, % 95 CI, 1-7-3, 42, p<0,005). No significant differences were evident in serum IgE levels, allergic and non-allergic asthma patients among the three genotypes. The ACE (D/I) polymorphism is not associated with asthma in this population. We conclude that the TNF-alpha G-A —308 polymorphism may form of the genetic predisposition to asthma.

P1244. Associations of multi-locus genotypes in the cytokine gene cluster on chromosome 5q31-35 in asthmatic patients in Iceland.

H. Hakonarson[1], *U. S. Bjornsdottir*[2], *A. Manolescu*[1], *T. Gislason*[2], *D. Gislason*[2], *J. Gulcher*[1], *K. Stefansson*[3]
[1]*deCODE genetics Inc.; Reykjavik, Iceland;* [2]*Vifilstadir University Hospital; Reykjavik, Iceland;* [3]*deCODE genetics Inc.; r, Iceland*
hakonh@decode.is

Asthma is a complex genetic disorder largely attributed to the interactions of the environment and multiple genes each potentially having relatively small effects. Several genes that map to chromosome 5q31-35 have been associated with asthma. While these genes are highly polymorphic no SNP is found to play a major role in asthma. This study addressed the hypothesis that specific series of SNPs within several genes at this locus may be associated with asthma. Thirteen SNPs were examined in 9 genes at the 5q31-35 locus in 94 asthmatic patients and 91 controls, by sequencing.

The patients phenotype was characterized by skin tests, PFT«s and methacholine challenge tests. The genotypes are denoted as MM, Mm, and mm, wherein M and m represent the major and minor allele frequencies, respectively. The analyses were based on the Hardy-Weinberg law and chi-square tests. No dominant series of genotypes was detected that extended to all nine genes. However, we observed significant correlation between the combinations of the MM and mm genotypes in 4 locations within the IL-13 gene (p<0.0001), but no differences were detected between patients and controls. Linkage disequilibrium was also observed between the SNP s in the IL-13 and IL-4 genes, and to a lesser degree between the IL-4 and IL-3 genes. Taken together, these observations demonstrate that specific genotypes in the IL-3, IL-4 and IL-13 genes are in marked linkage disequilibrium; however, their relevance to asthma remains uncertain. We are currently searching for possible associations of multi-locus genotypes with asthma in a larger cohort.

P1245. Informativity and results of LOH analysis for five APC gene polymorphic markers

T. Cacev, *S. Kapitanovic, K. Pavelic*
Rudjer Bockovic Institute, Division of Molecular Medicine; Zagreb, Croatia
tcacev@rudjer.irb.hr

We examined 46 cases of human sporadic colon cancer and corresponding normal tissue samples to evaluate the loss of heterozygosity (LOH) at the APC gene loci. DNAs were used for PCR, RFLP, VNTR and LOH analysis. To analyze LOH at the APC gene loci we used five polymorfic markers; three RFLP intragenic markers (exon 11 RsaI, exon 15 MspI, and exon 15 AspHI) and two VNTR flanking markers (D5S409 and D5S433). The informativity for all three intragenic RFLP markers was 50.0 % (23 of 46 assayed), and 21.7 % of tumors (5 of 23 informative) demonstrated LOH. The informativity for VNTR flanking markers D5S409 and D5S433 was 60.9 % (28 of 46 asseyed) and 87.0 % (40 of 46 asseyed) respectively. Eight of 28 informative tumors (28.6 %) demonstrated LOH for marker D5S409, and 14 of 40 informative tumors (35.0%) demonstrated LOH for marker D5S433. The informativity for all five APC loci was 100 % and 14 of 46 tumors (30.4 %) demonstrated LOH. Our study showed that it was necessary to use VNTR flanking markers D5S409 and D5S433 to increase the number of informative cases (from 50 % with intragenic markers) to 100 %. The highest informativity was observed for VNTR marker D5S433, 87 %.

P1246. Wet/Dry Cerumen Phenotype Maps to Chromosome 16 P11.2-16q12.1

H. Tomita[1], *K. Yamada*[1], *G. Mohsen*[1], *N. Niikawa*[1], *N. Kato*[2]
[1]*Department of Human Genetics, Nagasaki University School of Medicine; Nagasaki, Japan;* [2]*Department of Neuropsychiatry, Faculty of Medicine, University of Tokyo; Tokyo, Japan*
niikawa@net.nagasaki-u.ac.jp

Human cerumen (earwax) is a single-gene trait, consisting of two phenotypically distinct forms, wet and dry types (MIM 117800). The wet type is characterzied by light-/dark-brown, sticky and wet cerumen, while the dry cerumen is gray/tan, brittle and dry. The wet type (W) is dominant to the other dry type(w). Since the phenotype of homozygotes (WW) for the wet type is indistinguishable from that of heterozygotes, the wet phenotype is completely dominant. Frequencies of the wet and dry types vary in races. The former type predominates in most Europeans and Africans, whereas the latter is markedly prevalent in Mongoloids and native Americans, and intermediately in polulations in eastern Europe, Middle East and South Africa. Thus, it is most likely that the prototype in the human is the wet type. To localize this trait locus, we performed a linkage analysis on eight Japanese families segregating with dimorphism. Two-point linkage analysis provided a maximum LOD score of 11.19 (theta = 0.00; penetrance p = 1.0) at D16S3044. Haplotype analysis defined the locus within ~5.9 cM in the 16p11.2-16q12.1 region between D16S3093 and D16S3117. The localization will contribute to further isolation of the putative cerumen gene.

P1247. Linkage and association studies of Calcitonin Receptor gene with bone mineral density in Italian families

G. Malerba[1], *A. Sangalli*[1], *S. Mirandola*[1], *V. Braga*[2], *S. Adami*[2], *P. F. Pignatti*[1], *M. Mottes*[1]
[1]*Biology and Genetics, DMIBG; Verona, Italy;* [2]*Department of Rheumatology; Verona, Italy*
bobo@medgen.unvir.it

Osteoporosis is a disease associated to low bone mineral density (BMD) that has been described to be mostly under genetic control. Several candidate genes have been reported to be linked or associated to osteoporosis or related phenotypes. We collected 120 families (547 individuals, 180 sibships) through an osteoporotic subject. All families had at least 2 sibs, and parents when possible. All the individuals were phenotyped for BMD at 7 sites. We now report a linkage study of AluI polymorphism T1377C in the calcitonin receptor gene (CTR) with bone mineral density measured at 2 sites (femoral neck and Ward triangle) or with body mass index (BMI). Families were studied with non parametric linkage and transmission disequilibrium test methods. C allele frequency was 30%. Genotypes were in Hardy-Weinberg equilibrium. Sib-pair analysis showed linkage with femoral neck density (p=0.036), and BMI (p=0.038). Linkage was not observed at Ward triangle site even if the result was close to the significance threshold (p=0.06). TDT was performed by setting as affected the subjectes with a Z-score < -1. No preferential allele transmission to affected individuals was observed. Due to the low information content of the polymorphism (PIC=0.33), individuals will be further genotyped with DNA markers mapping close to CTR gene in order to increase the number of informative families. In conclusion, the calcitonin receptor gene or a closely located gene might be associated to bone mineral density in the Italian population.

P1248. Genetic Analysis of Two Pedigrees with Charcot-Marie-Tooth Neuropathy Type 1C

A. S. Golden[1], *V. A. Street*[1], *J. D. Goldy*[2], *B. L. Tempel*[1], *T. D. Bird*[3], *P. F. Chance*[2]
[1]*Otolaryngology-Head and Neck Surgery and V.M. Bloedel Hearing Research Center, University of Washington; Seattle, WA United States;* [2]*Pediatrics, Division of Genetics and Development, University of Washington School of Medicine; Seattle, WA United States;* [3]*Neurology, University of Washington School of Medicine and Veterans Administration Medical Center; Seattle, WA United States*
agolden@u.washington.edu

Charcot-Marie-Tooth (CMT) neuropathy is the most common inherited peripheral nervous system disorder affecting approximately 1 in 2000 individuals and characterized by degenerative changes in motor and sensory nerves. The hallmark of CMT Type I (CMT1) is reduced nerve conduction velocities (NCVs) (<40 meters/sec) and nerve biopsies that demonstrate hypertrophic demyelination. The present study includes two large five-generation CMT1C pedigrees (K1550 and K1551). Affected members have clinical findings and reduced NCVs consistent with CMT1. Male-to-male transmission is present, confirming autosomal dominant inheritance. Previous linkage analysis with markers from the CMT1A region on chromosome 17p11-12 and CMT1B region on chromosome 1p21 excluded linkage. Furthermore, the DNA duplication commonly associated with CMT1A is not present. Sequence analysis for five genes known to play critical roles in the development of demyelinating neuropathies, the peripheral myelin protein-22 (PMP-22), the myelin protein zero (MPZ), the early growth response 1 and 2 genes (EGR1 and 2), also known as Krox-24 and Krox-22, and PERP disclosed no abnormalities, confirming further genetic heterogeneity in CMT1 and indicating that the mutant gene in these CMT1C pedigrees represents a novel unmapped form of CMT1. To assign a chromosomal address, we are performing a 10 cM genome scan on both pedigrees. No linkage has been detected on chromosomes 5, 6, 14, and 19-22 for pedigree K1550, nor on chromosomes 19-22 for pedigree K1551.

P1249. Molecular genetic studies in autosomal recessive axonal Charcot-Marie-Tooth disease

D. M. Georgiou[1], *H. Jedrzejowska*[2], *B. Ryniewicz*[3], *I. Hausmanowa-Petrusewicz*[2], *E. Zamba*[1], *T. Kyriakides*[1], *L. T. Middleton*[4], *K. Christodoulou*[1]
[1]*The Cyprus Institute of Neurology and Genetics; Nicosia, Cyprus;* [2]*Neuromuscular Unit, Medical Research Centre, Polish Academy of Sciences; Warsaw, Poland;* [3]*Department of Neurology, Medical Academy; Warsaw, Poland;* [4]*Clinical Discovery Genetics, Glaxo Wellcome R&D; London, United Kingdom*
roula@mdrtc.cing.ac.cy

Charcot-Marie-Tooth (CMT) disease forms a group of heterogeneous disorders of the peripheral nervous system the main feature of which is distal weakness and wasting in the limbs, associated to other neurological manifestations, mainly affecting sensation, reflexes and muscle tone. CMT is the most common peripheral nerve disorder with an estimated frequency of 1 in 2,500. It may be inherited in an autosomal dominant, an autosomal recessive or an X-linked mode. There are two physiopathological types of CMT; the demyelinating type, characterised by decreased motor nerve conduction velocities (MNCV) and primarily affecting the myelin and, the axonal type, characterised by normal or slightly reduced MNCVs and primarily affects the axon. The two types may be distinguished based on neurophysiological and neuropathological criteria. One autosomal recessive axonal CMT locus has thus far been mapped, on chromosome 1q21.2-q21.3, but the corresponding gene has yet to be identified. We selected a

group of seven autosomal recessive axonal CMT families that were previously characterised at the clinical, neurophysiological and neuropathological levels for further molecular genetic studies. Forty-seven individuals, fifteen of them affected, were included in the study. These individuals were genotyped at marker loci spanning the known CMT loci. Families were analysed for linkage at these known CMT loci and slightly positive results were further evaluated by haplotype analysis. Three of the seven families (one Cypriot and two Polish families) linked to demyelinating autosomal recessive CMT loci.

P1250. A Genetic Study of a Large CMT Type 2 Family from Turkey; Exclusion of all Known CMT2 Loci

N. Bissar-Tadmouri[1], *Y. Parman*[2], *F. Deymeer*[2], *P. Serdaroglu*[2], *V. Timmermann*[3], *P. De Jonghe*[3], *C. Van Broeckhoven*[3], *E. Battaloglu*[1]
[1]*Bogazici University, Department of Molecular Biology & Genetics; Istanbul, Turkey;* [2]*Istanbul University Medical School, Department of Neurology; Istanbul, Turkey;* [3]*University of Antwerp, Department of Molecular Genetics; Antwerpen, Belgium*
tdmouri@boun.edu.tr

Charcot-Marie-Tooth neuropathy (CMT) is a heterogeneous group of disorders of the peripheral nervous system with a broad spectrum of clinical severity and different pattern of inheritance. Autosomal dominant CMT is classified into two forms based on neuropathological criteria. CMT1 is characterized by de- and remyelination, while CMT2 exhibits axonal degeneration. Genetic studies have shown that CMT2 is genetically heterogeneous. Several CMT2 loci have been identified on chromosome 1p35-p36 (CMT2A), chromosome 3q13-q22 (CMT2B), and chromosome 7p14 (CMT2D). A fourth CMT2 type (CMT2C) was not linked to any locus. Recently, the Neurofilament Light (NF-L) gene was found responsible for a new CMT2 type (CMT2E) localized on chromosome 8p21. Point mutations in the Myelin Protein Zero (MPZ) gene were found in some CMT2 families as well. In this study, we report a genetic study on a large CMT2 family from Turkey. Linkage analysis was performed by using a number of markers mapping to the CMT2A, CMT2B, and CMT2D loci, to the MPZ locus on chromosome 1q22-23, and within the NF-L gene. Lod scores have excluded the association of the disease in this family with any of the CMT2 loci, suggesting the presence of an additional CMT2 locus to be identified.

P1251. Genetic refinement of the locus for autosomal dominant colobomatous microphthalmia to a 10-cM interval on chromosome 15q12-q15

L. Morle[1], *L. Michon*[1], *M. Bozon*[1], *L. Duret*[2], *J. Zech*[3], *N. Alloisio*[1], *J. Godet*[1], *H. Plauchu*[4], *P. Edery*[4]
[1]*Centre de G n tique Mol culaire et Cellulaire, UMR CNRS 5534; 69622 Villeurbanne Cedex, France;* [2]*Biom trie et Biologie Evolutive, UMR CNRS 5538; 69622 Villeurbanne, France;* [3]*Service d Ophtalmologie, H pital Edouard Herriot; 69347 Lyon Cedex, France;* [4]*Service de G n tique, H pital H tel-Dieu; 69288 Lyon Cedex, France*
l-morle@univ-lyon1.fr

Congenital microphthalmia is a common disorder of embryonic eye development characterized by shortened total axial length. Isolated microphthalmia is genetically heterogeneous and may be inherited in an autosomal dominant, autosomal recessive, or X-linked manner. To our knowledge, only the CHX10 gene has been clearly involved in human microphthalmia. Here, we studied a five-generation family of Sephardic Jewish origin that included 38 members, of whom 7 have either unilateral or bilateral microphthalmia of variable severity inherited as an autosomal dominant trait with incomplete penetrance. After exclusion of several candidate loci, we performed a genome-scan study and recently demonstrated linkage to chromosome 15q12-q15. Positive LOD scores were obtained with a maximum at the D15S1007 locus (maximum LOD score 3.77, at recombination fraction 0.00). Haplotype analyses supported the location of the disease-causing gene in a 13.8-cM interval between loci D15S1002 and D15S1040. Further linkage analyses allowed us to refine the candidate interval to a 10-cM region. Candidate genes, namely Gremlin and Connexin36 (Cx36), are currently under study.

P1252. Molecular Analysis Of A Large Turkish Family With Autosomal Recessive Congenital Microphthalmia And Coloboma

F. Percin[1], *V. Seyrantepe*[2], *A. Yigit*[3], *S. Karslioglu*[3], *M. Sarfarazi*[1]
[1]*Molecular Ophthalmic Genetics Laboratory, University of Connecticut Health Center; Farmington, CT United States;* [2]*University of Montreal, Sainte-Justine Hospital Research Center; Montreal, PQ Canada;* [3]*Sisli Etfal Research and Training Hospital; Istanbul, Turkey*
Ferda@Neuron.uchc.edu

Congenital microphthalmia is a developmental ocular malformation characterized by shortened axial length of the globe. This condition is usually associated with coloboma. Non-syndromic microphthalmia is clinically and genetically heterogeneous and may be inherited in an autosomal recessive, dominant or X-linked manner. A number of chromosomal abnormalities have previously been reported with sporadic cases of congenital microphthalmia. So far only two genetic loci have been identified for familial cases. A locus was reported for a Pakistani family on 14q32. We mapped another locus to 14q24.3 in a large Turkish family and reported mutation in the CHX10 gene (Nat. Genet. 25; 397-401; 2000). Our linkage analysis of another 10 Turkish and Israeli families failed to show any linkage with DNA markers linked to MITF (3p14-p12), EYA2 (20q13), CYP1B1 (2p21) and other candidate regions (2q21-q22; 2q31 and 2q33) previously associated with this condition. Herewith, we report clinical and molecular study of a new large three-generation Turkish family with 44 members. Eight subjects presented with unilateral/ bilateral non-syndromic microphthalmia or anophthalmia with coloboma of iris and/or coroid. Two additional members presented with coloboma without any microphthalmia. Myopia and astigmatism are other associated anomalies in this family. DNA samples prepared from 26 members were used for linkage exclusion of 6 candidate genes including CHX10 (14q24.3), PAX6 (11p13), OTX2 and SIX1 (14q21-q22), RX (18q21-q22), VSX1 (20p11.2), as well as the published locus on 14q32. A genome-wide search of this family has recently been completed. Saturation mapping for an observed region of homozygosity is currently in progress.

P1253. Homozygosity mapping and analysis of a candidate gene in a family with an autosomal recessive high myopia and vitreoretinal dystrophy

M. Weigell-Weber[1], *G. Sarra*[2], *E. P. Messmer*[2], *M. Hergersberg*[3]
[1]*Institute of Medical Genetics; 8057 Z rich, Switzerland;* [2]*University Eye Hospital; Z rich, Switzerland;* [3]*Institute of Medical Genetics; Z rich, Switzerland*
weigell@medgen.unizh.ch

We describe a Swiss family, in which four of eight sibs show severe retinal changes, early-onset myopia maligna, excessive posterior staphyloma and presenile cataract. There was no evidence for myopia or another ophthalmological disease in the parents of the eight siblings or in one of the over thirty children. The posterior staphyloma clearly differentiates this vitreoretinal degeneration from other previous described ones. The mode of inheritance in this family with only one affected generation and both affected males and females is likely to be autosomal recessive. The hitherto unknown phenotype of the disease (implicating very rare occurence) and the origin of the parents from adjacent villages are compatible with a hidden consanguinity in this family. Therefore, homozygosity mapping seemed to be a promising approach to localize the causative gene in this family. In the case of a recessive disease in a consanguineous pedigree, the region surrounding the disease locus is assumed to be identical by descent in affected persons. In contrast, in unaffected members this region should not be identical by descent. More than 500 microsatellite markers distributed over the entire genome were investigated and have lead to the identification of a 12cM homozygous region. A candidate gene, encoding a protein of the extracellular matrix, was analysed by DHPLC analysis. Until now only polymorphisms and no mutations in this gene have been identified in several members of this family.

P1254. Association of IDDM and Single Nucleotide Polymorphisms (SNPs) in the AIRE gene

T. Nikopensius[1], *P. Peterson*[2], *J. Ilonen*[3], *M. Knip*[4], *K. Krohn*[2], *E. L hmussaar*[1], *A. Kurg*[1], *A. Metspalu*[1]
[1]*Institute of Molecular and Cell Biology, University of Tartu; Tartu, Estonia;* [2]*Institute of Medical Technology, University of Tampere; Tampere, Finland;* [3]*University of Turku; Turku, Finland;* [4]*University of Helsinki; Helsinki, Finland*
tiitn@ebc.ee

Type-1 diabetes (IDDM) is an autoimmune disorder resulting from destruction of the pancreatic islet cells, with the major susceptibility gene in HLA region on chromosome 6p21. IDDM is accompanying with Addison s disease in autoimmune polyendocrinopathy syndromes (APS), appearing as a common clinical phenotype in APS2 and a rare phenotype in APS1, which is only described autoimmune endocrine disease with Mendelian inheritance. The gene responsible for APS1 - AIRE (autoimmune regulator) - has been identified on chromosome 21q22.3 by positional cloning and characterised as a transcriptional activator. IDDM and APS1 are enriched in Finnish population. We have designed a DNA chip with AIRE sequence polymorphisms and mutations searching for allelic variants associated with IDDM and estimated the allele frequencies and heterozygosities for 11 SNP-s, nine out of them from AIRE coding region, in 120 patients with

IDDM and 135 controls from Finnish population. We used APEX technology for identification of different types of sequence alterations. APEX is a resequencing method based on two-dimensional array of oligonucleotides, immobilised via 5 end on a glass surface. The PCR-amplified and enzymatically digested DNA template is annealed to immobilised primers, which promote sites for template-dependent DNA polymerase extension reactions, using four fluorescently labeled dideoxynucleotides. The Genorama" imaging system and genotyping software is used for data analysis. We have found five SNPs to be polymorphic with heterozygosity index between 0.2 - 0.5. Three polymorphic SNPs could be considered as markers of preference for association/LD studies in IDDM patients. The most common APS1 mutations, R257X and 13-bp deletion in exon 8, showed no evidence for linkage to IDDM in the population studied.

P1255. A Single nucleotide polymorphism in the Delta1 gene mapped to IDDM8 is associated with Type I Diabetes Mellitus

O. S. Kokanovic[1], *G. E. Davies*[2], *T. Oda*[3], *S. Kazi*[2], *X. Wang*[2], *S. Guo*[2], *C. C. Settara*[3], *S. Ghosh*[1]

[1]*The Max McGee National Research Center for Juvenile Diabetes/Medical College of Wisconsin; Milwuakee, WI United States;* [2]*The Max McGee National Research Center for Juvenile Diabetes/Medical College of Wisconsin; Milwaukee, WI United States;* [3]*National Human Genome Research Institute/National Institute of Health; Bethesda, MD United States*

kokanovo@mcw.edu

Type 1 Diabetes Mellitus (T1DM) is an autoimmune disease that results in the destruction of the insulin-producing beta cells of the pancreas. Single nucleotide polymorphism (SNP-based candidate gene analysis in regions with evidence of linkage to T1DM is proving to be instrumental in discovering variants of genes involved in the pathogenesis and/or regulation of this disease. Delta 1 is a human transmembrane notch ligand that has recently been fully characterized and mapped to the IDDM8 region between microsatellite markers D6S446 and D6S281 (Oda et al., unpublished data) showing significant LD transmission in T1DM patients (T=58.2-68.1%, P<0.05; Owerbach, 2000). The Delta 1-Notch pathway may play a role in induction of peripheral tolerance. We identified 3 new SNPs within the Delta1 gene and carried out initial association analysis for 2 SNPs using 384 affected T1DM cases from HBDI repository and 192 matched controls. We found significant association for one intronic SNP in the T1DM population located 47bp away from the GT donor splice site of exon 4 (P<0.023). We will confirm this association with a TDT analysis of over 200 singleton families from Australia. An SNP located in the 3 UTR region showed no frequency differences in cases vs. controls. The third SNP located in the promoter has not been examined for association yet, but we have determined in silico that this polymorphism is present in a putative binding site for the transcription factor AP-2, thus potentially affecting the transcriptional regulation of Delta1.

Characterization of study populations and association results

			Intronic SNP #5		3'UTR SNP #29	
	Subjects	Age at Onset	Frequency of G allele	Frequency of A allele	Frequency of T allele	Frequency of G allele
Case Control (HBDI Repository)						
T1DM	384	<15	0.3833	0.6167	0.716	0.284
NGT	192	>30	0.4751	0.5249	0.724	0.276
P value				P<0.023		No significant difference

P1256. A Possible Locus for Setleis Syndrome, an Ectodermal Dysplasia Common in San Sebasti n, Puerto Rico

C. L. Cadilla[1], *L. Al-Gazali*[2], *N. Izquierdo*[3], *L. Nogueras*[1], *A. Santiago-Cornier*[4]

[1]*UPR School of Medicine, Dept. of Biochemistry; San Juan, PR United States;* [2]*United Arab Emirates University, Dept. of Paediatrics; Al Ain, United Arab Emirates;* [3]*UPR School of Medicine, Dept. of Ophthalmology; San Juan, PR United States;* [4]*Ponce School of Medicine, Dept. of Biochemistry; Ponce, PR United States*

c_cadilla@rcmaca.upr.clu.edu

Setleis Syndrome is a form of ectodermal dysplasia (Facial Dermal Dysplasia, MIM 227260) first described in 1963 in Puerto Rican patients that moved to New York from the town of San Sebasti n. This syndrome is characterized by bilateral temporal marks similar to forceps marks, an aged leonine appearance, puckered skin about the eyes, absent eyelashes on both lids or multiple rows on the upper lids and none on the lower lids, a nose and chin that felt rubbery, and eyebrows that slant sharply upward. Subsequent reports in the literature have described affected patients in Germany, Japan and Oman. We have identified several families with children affected with Setleis Syndrome in Puerto Rico as well as from another family in Oman. DNA analysis of candidate gene regions, using various loci implicated in other ectodermal dysplasias (1q32-44, 2q11-13, 3q27,11q23-q24 and 13q12), and a region in chromosome 1 recently proposed to be a possible locus for the Setleis Syndrome gene (1p36) was carried out by genotyping with STR markers (Marshfield). We observed markedly increased allele frequencies and allele sharing or homozygosity for marker GATA48B01 at locus D1S1660, when compared to normal Puerto Rican controls. This marker lies close to the plakophilin 1 gene, which has been associated with ectodermal dysplasia/skin fragility syndrome. Identification of the Setleis Syndrome gene may permit understanding the pathogenesis and permit prenatal diagnosis of this rare disorder in the Puerto Rican population. Support provided by NCRR RCMI G12RR-03051 and S06GM08224

P1257. Serotonin gene polymorphisms in chronic daily headache

D. Civitelli[1], *S. Carrideo*[1], *G. Nicoletti*[1], *F. Bono*[2], *A. Labate*[2], *D. Pirritano*[2], *A. Lucisano*[2], *E. V. De Marco*[1]

[1]*CNR-Istituto Medicina Sperimentale e Biotecnologie; Mangone (CS), Italy;* [2]*Universit Magna Graecia ; Catanzaro, Italy*

civite@imseb.cs.cnr.it

Chronic daily headache (CDH) is defined as headaches occurring more than 15 days per month and lasting more than four hours per day. It is a common cause of pain, affecting up to 5% of the population. Although it is often regarded as being caused predominantly by external factors, many patients report complaints of CDH in their family members. The neurotransmitter serotonin (5-HT) play a major role in the regulation of pain in the central nervous system and therefore may be involved in pathophysiology of CDH. In this study we performed a genetic association analysis to verify whether the 5-HT genes contribute to the risk of CDH. Polymorphisms in the genes coding for 5-HT2A, 5-HT2C, 5-HT6 receptors and 5-HT transporter (h-SERT) were investigated in a sample of 105 patients with CDH and 92 healthy control subjects all coming from the same geographical area. No significant differences in allele or genotype frequencies were found in the patients compared to the controls for any of the investigated polymorphisms. These data indicate that serotonin genes studied do not contribute to the genetic predisposition to CHD.

P1258. Confirmation of linkage of juvenile hemochromatosis on chromosome 1 in families from Saguenay Lac-Saint-Jean (Quebec, Canada).

S. R. Rivard[1], *C. Mura*[2], *L. Bechner*[3], *H. Simard*[4], *R. Simard*[4], *D. Grimard*[4], *C. Morin*[4], *J. Latreille*[1], *G. Le Gac*[2], *O. Raguenes*[2], *C. F rec*[2], *M. De Braekeleer*[5]

[1]*H pital Charles LeMoyne; Greenfield Park, PQ Canada;* [2]*EFS & Universit de Bretagne Occidentale; Brest, France;* [3]*INSERM; Paris, France;* [4]*Complexe Hospitalier de la Sagamie; Chicoutimi, PQ Canada;* [5]*Universit de Bretagne Occidentale; Brest, France*

marc.debraekeleer@univ-brest.fr

Juvenile hemochromatosis, also called hemochromatosis type 2, is a rare and severe form of hemochromatosis affecting sexes equally. Patients usually develop hypogonadotrophic hypogonadism in their 20s and heart failure and/or cardiac arrhythmias in their 30s. The locus of this autosomal recessive disorder was identified on the long arm of chromosome 1, in a 4 cM interval between markers D1S442 and D1S2347 (Roetto et al. 1999). Thirteen patients with criteria fulfilling those of juvenile hemochromatosis are followed on a regular basis for phlebotomies at the Complexe Hospitalier de la Sagamie in Chicoutimi (Quebec, Canada). Ten patients (76.9%) had hypogonadotrophic hypogonadism and 9 (69.2%) had cardiac arrhythmia and/or congestive heart failure. The remaining two patients were diagnosed at a very young age (9 and 10 years old). A sole patient was homozygous for the H63D mutation in the HFE gene associated with hemochromatosis type 1. The other patients were heterozygous for the C282Y or S65C mutations or did not carry a known mutation in the HFE gene. Furthermore, no novel mutation was found in the HFE gene (Rivard et al. 2000). All the patients are from Saguenay Lac-Saint-Jean, a region with a founder-like effect in which several autosomal recessive disorders have attained a high frequency. We obtained blood for DNA extraction from 11 families to perform linkage analysis with markers (D1S442, D1S2344, D1S498, and D1S2347) located on the long arm of chromosome 1. The maximum LOD score obtained was 4.12 with a recombination rate of 0.03. Although six different haplotypes were identified, the same haplotype (1-3-3-5) was found on 16 of the 26 hemochromotosis chromosomes (61.5%).

Three other haplotypes were found more than once; they are 1-3-1-5 and 1-2-3-5 present on three chromosomes each and 1-3-1-3 present on two chromosomes. Our results confirm that the locus for juvenile hemochromatosis in Saguenay Lac-Saint-Jean is located on chromosome 1q; they also suggest a founder effect in this population.

P1259. Association study between 5-HTT gene polymorphisms and Parkinson s disease

***E. V. De Marco**, D. Civitelli, F. Annesi, S. Carrideo, I. C. Cir Candiano, A. A. Pasqua, P. Spadafora, C. Tomaino, G. Nicoletti, D. Messina, G. Annesi*
CNR-Istituto Medicina Sperimentale e Biotecnologie; Mangone (CS), Italy
demarco@imseb.cs.cnr.it

The serotonin transporter (5-HTT) of the brain mediates sodium dependent presynaptic reuptake of serotonin and has great importance for modulating serotonine function. The SLC6A4 locus encoding this protein maps to chromosome 17q11.1-17q12. It spans 31 kb and consists of 14 exons. A functional polymorphism (5-HTTLPR) located in the proximal 5 regulatory region is a repetitive sequence with different allelic transcriptional efficiencies (transcriptional activity of the long variant is more than twice that of the short). A second polymorphism found in intron 2 (Stin2 system) and consisting of a VNTR of 17 bp segment, might have an indipendent role in influencing the stability of transcription complexes. We performed an association study to elucidate the role of the serotonin transporter gene as a susceptibility factor for Parkinson s disease (PD). A comparison of polymorphic regions of the serotonine transporter gene was carried out in 315 patients with PD. Diagnosis of PD was carried out according to the UK Brain Bank criteria among outpatients attending the Institute of Neurology at the University of Catanzaro. The control group included 185 healthy subjects from the same geographic area (Calabria — Southern Italy). No difference was found between allele or genotype frequency in the patients compared to the controls as regard the examined polymorphisms. This findings indicate that the investigated polymorphisms of the serotonine transporter gene are unlikely to play a role in genetic predisposition to Parkinson s disease.

P1260. Complete Genomic Screen in Familial Parkinson Disease

***W. K. Scott**[1], J. M. Stajich[1], B. L. Scott[1], M. A. Nance[2], R. L. Watts[3], J. P. Hubble[4], J. L. Haines[5], W. C. Koller[6], R. Pahwa[7], M. B. Stern[8], B. C. Hiner[9], J. Jankovic[10], F. H. Allen[11], C. G. Goetz[12], G. W. Small[13], N. G. Laing[14], L. T. Middleton[15], A. D. Roses[16], J. M. Vance[1], M. A. Pericak-Vance[1]*
[1]Duke University Medical Center; Durham, NC United States; [2]Struthers Parkinson s Center; Golden Valley, MN United States; [3]Emory University; Atlanta, GA United States; [4]Ohio State University; Columbus, OH United States; [5]Vanderbilt University; Nashville, TN United States; [6]University of Miami; Miami, FL United States; [7]University of Kansas; Kansas City, KS United States; [8]University of Pennsylvania; Philadelphia, PA United States; [9]Marshfield Clinic; Marshfield, WI United States; [10]Baylor College of Medicine; Houston, TX United States; [11]Carolina Neurological Clinic; Charlotte, NC United States; [12]Rush-Presbyterian-St. Luke s Hospital; Chicago, IL United States; [13]University of California, Los Angeles; Los Angeles, CA United States; [14]University of Western Australia; Perth, Australia; [15]GlaxoWellcome; Greenford, United Kingdom; [16]GlaxoWellcome; Research Triangle Park, NC United States
bscott@chg.mc.duke.edu

The objective of this study is to identify genetic loci linked to Parkinson disease (PD) in multiplex (more than one family member diagnosed with PD and available for study) families. Data from family, twin, and epidemiologic studies suggest that the etiology of PD is complex and comprised of genetic and environmental factors. Genes responsible for early-onset, autosomal dominant PD (alpha-synuclein) and juvenile-onset, autosomal recessive parkinsonism (parkin) have been recently identified. However, additional genes, particularly for the more common, late-onset familial form of PD, remain to be discovered. We studied 174 Caucasian multiplex (2 or more sampled PD patients) families [containing 870 sampled members (378 affected), 185 sampled affected sibling pairs and 70 other sampled affected relative pairs] ascertained as part of a multi-center genetic linkage study. Mean age at onset was 59.9–12.6 years. Diagnosis of PD was based on the presence of two of the following signs; tremor at rest, bradykinesia, and rigidity, as well as the absence of atypical features and other causes of parkinsonism. A clinical adjudication board reviewed all cases for consistency across sites. Marker genotypes were obtained on 344 microsatellite markers (average spacing 10 cM). Families were studied for linkage using a multi-analytical approach consisting of two-point parametric (MLOD) and multipoint non-parametric (LOD*) methods. To identify potential genetic heterogeneity by age at onset, 18 families having at least one family member with onset prior to age 40 were considered separately from the remaining late-onset families (n=156). Six regions generated interesting lod scores. The strongest results overall (MLOD or LOD* > 2) were obtained for markers on chromosomes 5q, 8p, 9q, and 17p-17q. Additional regions of interest (MLOD or LOD* > 1.5) were 14q and Xq. Results in the late-onset subset were similar to those obtained overall. In contrast, analysis of the 18 early-onset families detected significant evidence for linkage to chromosome 6q (MLOD and LOD* > 5), in the vicinity of the parkin gene. We conclude that several loci provide interesting linkage results in our family sample. Strong linkage of early-onset PD families to the region on 6q containing parkin supports its involvement in familial PD. Regions of interest on 5q, 8q, 9q, and 17 have been prioritized for follow-up and evaluation of positional and functional candidate genes in these regions is underway.

P1261. NACP/alpha-synuclein polymorphism in Parkinson s disease

***P. Spadafora**, A. A. Pasqua, F. Annesi, P. Serra, G. Nicoletti, E. V. De Marco, D. Civitelli, C. Tomaino, I. C. Cir Candiano, S. Carrideo, G. Annesi*
CNR-Istituto Medicina Sperimentale e Biotecnologie; Mangone (CS), Italy
spadafo@imseb.cs.cnr.it

NACP/alpha-Synuclein gene is located on human chromosome 4q21-23. Missense mutations are responsible for rare forms of autosomal dominant Parkinson (PD). The protein encoded by this gene has also been demonstrated in Lewy bodies of sporadic PD, suggesting that it may play a crucial role in a final common pathway. The exact mechanism by which NACP/alpha-Synuclein protein plays a role in neuronal degeneration is unclear. Variability in the NACP/alpha-Synuclein gene could lead to the aberration in the proteolytic pathway with following protein aggregation. We investigated a polymorphic dinucleotide repeat in the promoter region of the NACP/alpha-Synuclein gene in 197 PD patients from Southern Italy and 182 healthy control subjects matched for age, sex and geographical origin. Four different alleles ranging from 265 bp to 271 bp were identified. Allele frequencies were computed by counting genes from the observed genotypes. We did not find statistically significant differences in the NACP/alpha-Synuclein genotypes between the patients with PD and the control group (chi square; 3.272; df; 5; p; 0.658). In contrast to our findings, Kruger et al. 99 showed a significantly higher frequency of the 267 bp allele (1 allele) in PD patients than in controls. Our results thus provide further evidence that the variation within the NACP/alpha-Synuclein gene does not play an important role in the etiology of PD.

P1262. Systematic Search For Susceptibility Genes In Bipolar Affective Disorder

***J. Schumacher**[1], S. Cichon[1], D. J. M ller[1], M. H rter[1], M. Albus[2], M. Borrmann-Hassenbach[2], E. Franzek[3], J. Fritze[3], R. Kreiner[4], B. Weigelt[4], J. Minges[5], B. Lerer[6], C. Windemuth-Kieselbach[7], T. Wienker[7], M. P. Baur[7], D. Lichtermann[8], W. Maier[8], M. Rietschel[8], P. Propping[1], M. M. N then[1]*
[1]Institute of Human Genetics, University of Bonn; Bonn, Germany; [2]Department of Psychiatry, Mental State Hospital, Haar; M nchen, Germany; [3]Department of Psychiatry, University of W rzburg; W rzburg, Germany; [4]Department of Psychiatry, University of Dresden; Dresden, Germany; [5]Department of Psychiatry, University of Mainz; Mainz, Germany; [6]Department of Psychiatry, Hadassah University of Jerusalem; Jerusalem, Israel; [7]Institute of Medical Biometry, Informatics and Epidemiology, University of Bonn; Bonn, Germany; [8]Department of Psychiatry, University of Bonn; Bonn, Germany
johannes_schumacher@yahoo.de

Bipolar affective disorder is a severe psychiatric disorder with a high prevalence and substantial morbidity and mortality. Strong support for the role of genetic factors in the aetiology comes from twin, family, and adoption studies. We have been conducting a genome-wide search for susceptibility genes in a set of 75 families with bipolar affective disorder from Germany, Italy, and Israel, comprising 136 affected-sib-pairs and including a total of 444 individuals. Clinical assessment of subjects included a SADS-L interview by trained interviewers and review of all available clinical records and family history information. DSMIII-R was used as the main diagnostic system. Inclusion criteria for the systematically ascertained BP families were one proband with BPI, a secondary affected sib with either BPI, BPII, SA/BP, or UPR, and availability of both parents. If either parent was unavailable, unaffected sibs of the proband were collected. Genotyping was undertaken using fluorescent semiautomated technology using a map of 394 microsatellite markers at an average spacing of ca. 9.1 cM. Linkage analyses were performed using LINKAGE, GAS, and GENEHUNTER. We found significant evidence for a susceptibility locus in chromosomal region 8q24.Suggestive evidence for linkage was obtained at 1p33-p36, 2q21-q33, 3p14, 3q26-27, 4p16, 8p21, 10q25-26, 11p11-12, 13q11, 18p11.2, and 18q23.

P1263. Interleukin (IL)-1beta, IL-1alpha and IL-Receptor Antagonist Gene Polymorphism in Patients with Multiple Sclerosis.

V. Andreoli, *I. Manna, G. La Porta, A. La Russa, P. A. Serra, G. Nicoletti, M. Caracciolo, R. Cittadella*
Institute of Experimental Medicine and Biotechnology-National Research Council; Cosenza, Italy
andreoli@imseb.cs.cnr.it

Genetic susceptibility to Multiple Sclerosis (MS) is determined by many partially identified genes, each having a modest effect but together forming a major determinant of disease. The cytokine network, with its prominent role in immune and inflammatory responses, can be considered a potential factor of considerable importance. Interleukin (IL)-1alpha and 1beta are two proinflammatory cytokines involved in the formation of MS lesions. The IL1-receptor antagonist (IL-1RA) is a protein structurally related to IL-1beta, that effectively inhibits the proinflammatory effects of IL-1. To analyze the contribution of IL-alpha, IL-1beta and IL-1RA genes in the genetics predisposition to MS, we have examined four polymorphic genetic markers in 85 Italian patients with clinically definite MS and 130 healthy controls. No significant differences in genotypic and allelic frequencies were found between MS patients and healthy controls. Our results suggest that these polymorphisms do not contribute to the genetic susceptibility to MS .

P1264. Fine mapping of the multiple sclerosis susceptibility loci on chromosomes 5p14-p12 and 17q22-q24

H. M. F. Riise Stensland, *J. Saarela, M. Schoenberg Fejzo, D. Chen, M. Parkkonen, A. Jokiaho, A. Palotie, L. Peltonen*
UCLA School of Medicine; Los Angeles, CA United States
hriise@mednet.ucla.edu

Multiple sclerosis (MS) is a neurological disorder characterized by inflammatory demyelination of the CNS. Multiple studies have suggested a genetic component to MS, and genome wide scans have revealed several putative susceptibility loci. In the genetically isolated Finnish population, four main candidate regions have been identified; The HLA-locus on chromosome 6, the MBP-locus on chromosome 18 and two relatively wide regions on chromosomes 5p14-p12 and 17q22-q24, which are syntenic to the murine Eae loci 2 and 7, respectively. By using radiation hybrid mapping, FISH, and web-based approaches, we have further characterized the critical regions on chromosomes 5 and 17. We have analyzed a denser set of markers between D5S432 and D5S2061 (20 cM) on chromosome 5, and between D17S956 and D17S2182 (25 cM) on chromosome 17 in the Finnish multiplex families. Supportive evidence for linkage was obtained for both regions. Markers D5S1992 and D17S1825 gave the highest pairwise LODs (4.08 and 3.42, respectively), and both Genehunter and Simwalk2 multipoint analysis programs further restricted these putative MS loci. The subset of families originating from Southern-Bothnia, an internal isolate on the Western coast of Finland with a higher prevalence of MS, provided most information for the chromosome 17 locus. Forty singleton families originating from the Southern-Bothnia region, as well as additional 119 trios from other parts of Finland, have been genotyped for the best markers, but no significant association with MS could be detected. We are now analyzing 126 intragenic SNPs in positional candidate genes. By combining the information from the microsatellite markers and the SNPs we will monitor for allelic association and shared haplotypes in the Finnish MS alleles.

P1265. Exlusion of the ETM1 and ETM2 loci in a large Moroccan family with autosomal dominant essential tremor

L. Baala[1], *A. Sbiti*[2], *R. Nejjari*[2], *A. Munnich*[3], *S. Lyonnet*[3], *A. Sefiani*[2]
[1]*Department of Genetics INSERM U-393 Necker Hospital; Paris, France and Department of Genetics and Molecular Biology; Rabat, Morocco;* [2]*Department of Genetics and Molecular Biology INH; Rabat, Morocco;* [3]*Department of Genetics INSERM U-393 Necker Hospital; Paris, France*
baala@necker.fr

Essential tremor (ET) is a chronic neurological condition which may be regarded as the most common movement disorder in human. The main feature of ET is postural tremor of the arms, but the head, legs, trunk, voice, jaw, and facial muscles may be involved as well. ET can be either sporadic or familial (familial essential tremor, FET or ETM). In addition, the prevalence of Parkinson s disease and dystonia may be increased in families with ET. Autosomal dominant inheritance can be demonstrated in most families and two ET loci have been mapped on chromosomes 3q13 (ETM1, MIM 190300) and 2p25-p22 (ETM2, MIM 602134) respectively. Here we report on a large Moroccan family with isolated autosomal dominant ET. Interestingly, patients presented with a very early onset. They are mainly affected with fine rapid ET of both hands and arms. We tested microsatellites DNA markers at the ETM1 and ETM2 loci, and eventually excluded linkage to both regions suggesting further genetic heterogeneity of FET. Furthermore, the Parkinson s loci mapping to chromosomes 4 and 6 were also excluded. Ongoing gene mapping studies will hopefully shed ligh on the genetic variability of ET.

P1266. The Epithelial membrane protein 3 and Neurotrophin 4/5 genes are not mutated in autosomal recessive Charcot-Marie-Tooth with axonal degeneration linked to chromosome 19q13.3

A. Leal[1], *B. Morera*[2], *G. Del Valle*[3], *D. Heuss*[4], *C. Kayser*[4], *B. Neund rfer*[4], *R. Barrantes*[5], *A. Reis*[6], *B. Rautenstrauss*[7]
[1]*Institute of Human Genetics, University of Erlangen-Nuremberg; Erlangen, Germany; Gene Mapping Centre, Max-Delbrueck-Centre; Berlin, Germany; and Institute of Health Research, University of Costa Rica; San Jos , Costa Rica;* [2]*Unitat de Biologia Evolutiva, Universitat Pompeu Fabra; Barcelona, Spain;* [3]*Department of Neurology, San Juan de Dios Hospital; San Jos , Costa Rica;* [4]*Department of Neurology, University of Erlangen-Nuremberg; Erlangen, Germany;* [5]*Institute of Health Research, University of Costa Rica; San Jos , Costa Rica;* [6]*Institute of Human Genetics, University of Erlangen-Nuremberg; Erlangen, Germany; and Gene Mapping Centre, Max-Delbrueck-Centre; Berlin, Germany;* [7]*Institute of Human Genetics, University of Erlangen-Nuremberg; Erlangen, Germany*
aleal@humgenet.uni-erlangen.de

Charcot-Marie-Tooth disease (CMT), a group of hereditary motor-sensory neuropathies (HMSN), is a clinically and genetically heterogenous disorder of the peripheral nervous system. Clinically, CMT is divided into a demyelinating type 1 (CMT1), characterized by reduced nerve conduction velocities (NCV<38m/s) and an axonal type 2 with almost normal NCV (CMT2). We have studied a Costa Rican family with 18 affected members with autosomal recessive CMT2. Clinically, the age at onset of chronic symmetric sensory-motor polyneuropathy was 28 to 42 years (mean 33,8), and the electrophysiologic data clearly reflect an axonal degenerative process. After exclusion of linkage to known CMT loci a genomewide search was performed. Evidence for linkage to markers on chromosome 19q13.3 was found with a maximum two-point LOD score of 9.08 for marker D19S867 (recombination fraction of 0.0). Homozygosity mapping defines a region of 5.5 cM between markers D19S902 and D19S907 with a shared haplotype. We propose a new locus for CMT2, namely ARCMT2B. Candidate genes in this region were identified and analyzed, using denaturing high-performance liquid chromatography (dHPLC) and sequencing with patients, carriers and healthy controls. The epithelial membrane protein 3 (EMP3) gene enconding a PMP22 homologous protein and located on 19q13.3 was ruled out as being responsible for this form of CMT. Also in this region is located the gene that encodes the neurotrophin-4/5 (NT-4/5), a regulator of the development and maintenance of the nervous system, that possibly play a physiological role in the mature peripheral nervous system. However, no mutation in this gene was found in carriers and affected with ARCMT2B in the Costa Rican family.

P1267. Clinical and genetic study of a large Autosomal Dominant Spastic Paraplegia family from Southern Italy

M. Muglia[1], *A. Magariello*[1], *A. L. Gabriele*[1], *A. M. F. Patitucci*[1], *R. Mazzei*[1], *F. L. Conforti*[1], *M. Caracciolo*[1], *B. Ardito*[2], *G. Nicoletti*[1]
[1]*Institute of Experimental Medicine and Biotechnology, National Research Council; Mangone (CS), Italy;* [2] *Miulli Hospital; Acquaviva delle Fonti (BA), Italy*
muglia@imseb.cs.cnr.it

Hereditary Spastic Paraplegia (HPS) is a heterogeneous group of inherited disorders of the motor system characterized by progressive lower-extremity spasticity and weakness. Clinically HPS is classified as pure form if neurologic impairment solely affects the legs and complicated form if additional symptoms such as mental retardation, ataxia, epilepsy, optic atrophy and peripheral neuropathy are present. The pure form of HSP is most commonly inherited as autosomal dominant trait, although families with an autosomal recessive or with an X-linked mode of inheritance have also been described. Genetic loci for HSP are designed spastic gait (SPG) loci. To date seven SPG loci responsible for pure HSP have been described; SPG4 (2p22), SPG13 (2q24-q34), SPG8 (8q23-24), SPG3 (14q11-q21), SPG10 (12q13), SPG6 (15q11.1) and SPG12 (19q13). Only one gene at the SPG4 locus, designed spastin has been identified. In this study, we report a large pedigree with a pure form of HSP from Southern Italy. A total of 35 subjects were studied. Eleven members were found to be affected. The clinical picture was uniform in the affected members and characterized by spastic gait as prominent sign in all symptomatic patients with a variable degree of severity. The hypertonia of the lower limbs was associated with moderate spasticity at rest and normal or slight-

ly impaired muscle power. Using nine microsatellite markers located on chromosome 14, linkage to chromosome 14 was detected. The molecular data confirm, as previously reported, the mapping of the SPG3 locus in a region flanked by D14S75 and D14S1031.

P1268. Genetic Linkage Analysis In Two Autosomal Dominant Spastic Paraplegia Families.

A. Moerman[1], *S. Schraen*[2], *T. Stojkovic*[2], *A. Coeslier*[1], *O. Boute*[1], *S. Manouvrier*[1], *B. Sablonni re*[2]
[1]*Hopital Jeanne de Flandre; Lille, France;* [2]*H pital Roger Salengro; Lille, France*
a-moerman@chru-lille.fr

Familial spastic paraplegia (FSP) is a genetically heterogeneous group of neurodegenerative disorders of the central motor system, characterised by progressive weakness and spasticity of lower limbs. On the basis of additional neurologic or non-neurologic features, they may be divided into pure and complicated forms. Autosomal dominant, autosomal recessive, and X-linked recessive inheritance patterns have been described for each type. No definitive correlation between gene mutations and clinical phenotype has been described for autosomal dominant FSP, and genetic heterogeneity remain important. To date, seven distinct loci have been identified for the autosomal dominant FSP, including SPG4 (chromosome 2p), SPG3 (chromosome 14q), and SPG8 (chromosome 8q). We describe two early-onset autosomal dominant SPG families. In both families, clinical findings are similar with preponderant lower extremity spasticity and less important muscular weakness. However, subtle sensitive clinical differencies directed us to SPG3 in family 1 and SPG4 in family 2. Linkage analysis were performed in both families using microsatellite polymorphism on SPG3 and SPG4 loci. In family 1, linkage was established with SPG3 locus in accordance with clinical findings. However, in family 2, no linkage was found with either SPG3 or SPG4. Our results confirms genetic heterogeneity of autosomal dominant FSP, and classification difficulties according to chromosome location or age at onset symptoms.

P1269. Dipeptidyl carboxypeptidase 1, estrogen receptor alpha and interleukin 1 alpha polymorphisms in Finnish late onset Alzheimer«s disease

M. Pirskanen[1], *M. Hiltunen*[1], *S. Iivonen*[1], *A. Mannermaa*[2], *S. Helisalmi*[1], *M. Lehtovirta*[1], *A. M. Koivisto*[1], *M. Laakso*[3], *H. Soininen*[1]
[1]*Department of Neuroscience and Neurology, University and University Hospital of Kuopio; Kuopio, Finland;* [2]*Chromosome and DNA Laboratory, University Hospital of Kuopio; Kuopio, Finland;* [3]*Department of Medicine, University and University Hospital of Kuopio; Kuopio, Finland*
Mia.Pirskanen@uku.fi

Alzheimer«s disease (AD) is the most common form of dementia occurring in the later decades of life. The only risk gene that has been indisputably shown to associate with AD is the e4 allele of the apolipoprotein E (APOE) gene. In addition to APOE, other polymorphic genes are likely to operate as risk factors in late onset AD (LOAD). We have studied the role of the polymorphism in three different genes that have been implicated in LOAD. The studied polymorphism were the insertion/deletion polymorphism of dipeptidyl carboxypeptidase 1 (ACE) gene, the PvuII and XbaI polymorphisms of estrogen receptor alpha (ESR1) gene and the C-to-T transition polymorphism at -889 of the interleukin 1 alpha (IL1A) gene. ACE, ESR1 and IL1A polymorphisms were genotyped from 86 LOAD patients and 200 control subjects. 46 of the LOAD patients and all of the 200 control subjects were from a population-based study investigating cardiovascular risk factors and glucose metabolism in elderly people. The baseline study was conducted in Kuopio in 1986-1988 and it included 1300 subjects aged 65-74 years who were randomly selected from the inhabitants of the town of Kuopio. The additional 40 LOAD patients genotyped were from the same geographic area. No association was found between the AD and control ACE (p = 0.81), ESR1 PvuII (p = 0.85), ESR1 XbaI (p = 0.78) or IL1A (p = 0.80) genotype. Our data suggest that there is no association between the studied ACE, ESR1 and IL1A gene polymorphisms and LOAD.

P1270. Investigation of association of polymorphisms in TNFR1 and TNFR2 in Alzheimer s disease patients

R. T. Perry[1], *J. S. Collins*[2], *L. E. Harrell*[1], *D. Blacker*[3], *R. E. Tanzi*[3], *M. G. McInnis*[4], *S. S. Bassett*[4], *R. C. P. Go*[1]
[1]*University of Alabama at Birmingham; Birmingham, AL United States;* [2]*Greenwood Genetic Center; Greenwood, SC United States;* [3]*Massachusetts General Hospital and Harvard Medical School; Boston, MA United States;* [4]*Johns Hopkins University Medical Institutions; Baltimore, MD United States*
rperry@uab.edu

Using non-parametric analyses in 266 sib-pair families from the NIMH sibling dataset, we previously reported results of a collaborative genome wide scan where polymorphic markers located at 1p36 and 6p21 were associated with Alzheimer s disease (AD) (Collins et al, 2000; Go et al, 1998; Collins et al, 1998). Several inflammatory genes, such as interleukin 1 (IL1)-a and IL1-b (Grimaldi et al, 2000, Nicoll et al, 2000) located at 2q14-21, and tumor necrosis factor (TNF), located at 6p21, have been found to be associated with AD. We recently reported a haplotype containing alleles of the -238 and -308 promoter polymorphisms of TNF and the microsatellite TNFa, located ~16.5 kb upstream of TNF, was associated with AD in subset of the above families (Collins et al, 2000). From a subset of 320 families with an age of onset over seventy years (total NIMH sibling dataset = 481), we found suggestion of linkage at two regions on chromosome 1, including the same 1p terminal region. The GENEHUNTER program gave a peak NPL score of 1.66 (p-value=0.023) and SIBPAL analysis resulted in a peak IBD score at 0.52 (p=0.033) for the p terminal region. Because of the association of TNF to AD and the suggestion of the 1p36 region linked to AD, we looked at the tumor necrosis factor receptor 2 (TNFR2) gene, one of two main receptors for TNF, located at 1p36.2. We used family-based association testing to look at the T->G polymorphism at position 196 in exon 6 of TNFR2 that leads to a M->R substitution. However, we did not find any significant association between TNFR2 and AD in 150 families with at least two affected and one unaffected siblings (SIBAS-SOC, p=0.71, S-TDT, p=0.28, SDT, p=0.63) (Collins, 2000). The other TNF receptor, TNFR1, is located at 12 p13.2, a region where other genome wide scans have indicated as suggestively linked to AD (Kehoe et al, 1999; Rogaeva et al, 1998). We have genotyped an A->G silent change in codon 12 of TNFR1 in the same set of late-onset AD families and will present these results.

P1271. Benign familial temporal lobe epilepsy - suggestive linkage on chromosome 3.

S. Bourgoin[1], *M. Z. Labuda*[1], *B. Aboul-Khalil*[2], *E. Andermann*[3], *M. Pandolfo*[1]
[1]*CHUM Notre Dame Hospital; Montreal, PQ Canada;* [2]*Vanderbilt University Medical Center; Nashville, TN United States;* [3]*Montreal Neurological Institute; Montreal, PQ Canada*
sarahbourg@hotmail.com

We studied seven families of different ethnic origin with dominantly inherited temporal lobe epilepsy (TLE) or febrile convulsion. The TLE families were previously tested for known partial epilepsy loci on chromosomes 20q, 15q, 10q, 8q, 19q 22q. All families were subjected to the genome-wide scan using Weber set 9A, which contains about 160 markers spaced at an average genetic distance of 25 cM throughout the entire autosomal genome. We have identified a marker on chromosome 3 co-segregating with the TLE phenotype in an American family of European descent. There are seven affected individuals in this family, plus one of uncertain status, all presenting infrequent simple or complex partial seizures, some with vegetative aura or d j vu symptoms. Three had also generalized tonic clonic seizures. The maximum lod score of Z = 2.2 at theta = 0.0 was obtained for the marker D3S2500, assuming 80% penetrance and a 1% phenocopy rate. All affected family members segregate the same haplotype. The exons of two candidate genes in the region, KCNMB1 and KCNMB3, were sequenced but no mutation was found.

P1272. Towards the Localization of Genes involved in Developmental Dyslexia; The Dutch Study.

F. A. Hol[1], *A. J. G. Heister*[1], *C. Kuijpers*[2], *H. G. Brunner*[1], *G. W. Padberg*[3]
[1]*University Medical Center, Dept. Human Genetics; Nijmegen, The Netherlands;* [2]*Interfaculty Research Unit for Language and Speech; Nijmegen, The Netherlands;* [3]*University Medical Center Dept. Neurology; Nijmegen, The Netherlands*
f.hol@antrg.azn.nl

Recently, the Netherlands Organisation for Scientific Research (NWO) started a multidisciplinary research programme on the causes, effects and treatment of (specific) reading impairment. This programme basically consists of three parallel research projects. The first project is a prospective study of the early precursors of dyslexia. The second project concerns an intervention study, whereas the third component of the programme deals with the search for genetic factors that are involved in dyslexia. Several methods can be employed to localize genes influencing complex disorders like dyslexia. Major gene(s) with a strong effect on the phenotype will result in dominant inheritance of the trait in extended pedigrees and can be detected by classical parametric linkage analysis. Use of non-parametric affected-sibpair analysis allows detection of genes with smaller effect. The objective of the genetic study is to apply both strategies to identify predisposing genes for dyslexia in the Dutch population. Four extended pedigrees consistent with autosomal dominant transmission, with at least 9

dyslectics each, have been recruited. In addition, approximately 200 affected sibpairs from 165 nuclear families were sampled. The phenotype of each subject was assessed by use of a series of psychometric tests including single-word and non-word reading, non-word repetition, rapid naming and spelling. Genome-wide linkage analysis in the first extended pedigree with 14 dyslexic subjects, has been completed and results will be presented.

P1273. Natal Dispersal In Rhesus Macaques Is Related To Serotonin Transporter Gene Promoter Variation

A. Trefilov[1], *M. Krawczak*[2], *J. Schmidtke*[3]
[1]*Institute of Human Genetics, Hannover Medical School; Hannover, Germany;* [2]*Institute of Medical Genetics, University of Wales College of Medicine; Cardiff, United Kingdom;* [3]*Institute of Human Genetics, Hannover Medical School; Hannover, Germany*
Trefilov.Andrea@MH-Hannover.de

Natal dispersal in rhesus macaques is related to serotonin transporter gene promoter variation Andrea Trefilov, Michael Krawczak, J rg Schmidtke ABSTRACT To ascertain whether the serotonin system is genetically linked and/or associated to/with the age at male natal dispersal (an easily accesible measure of risk-taking/harm avoidance behaviour) in the Old World primate Macaca mulatta, 96 free-ranging rhesus macaques from Cayo Santiago, Puerto Rico were genotyped for five repetitive loci in three essential among mammalians highly conserved genes of the serotonin metabolism by the method of amplification fragment length polymorphism. The analysed repeat markers, that have already been associated with mood disorders in men, include the VNTRs in the promoter regions of the serotonin transporter (SLC6A4, 5-HTT) gene and monoamine oxidase A (MAOA) gene. Our results revealed that homozygotes for the short allelic variant of the 5-HTTLPR have left their natal groups significantly earlier (age 57.1–2.6 months) than carriers of the long allele (ll; age 71.5–2.1 months, ls; age 63.5–1.5 months). Since migration implies reproductive costs and benefits that change with age at first group transferal, migration at an intermediate age might have conferred a heterozygote advantage serving to maintain the VNTR polymorphism in the SLC6A4-promoter via overdominant selection.

P1274. Polymorphism of the serotonin transporter gene (SLC6A4) and the monoamine oxidase A gene (MAO A) in males with alcohol withdrawal delirium.

E. Gorbunova, *A. Galeyeva, E. Khusnutdinova*
Biochemistry and Genetics; Ufa, Russian Federation
ekkh@anrb.ru

A insertion-deletion polymorphism in promoter region of the SLC6A4 and EcoRV restriction polymorphism of the MAO A were studied for allelic association with alcohol withdrawal delirium in 96 Russian and 67 Tatar alcoholic males as well as in their ethnically matched nonalcoholic male controls (60 Russians and 52 Tatars) within aboriginal groups of the Volga-Ural region of Russia. The alcoholics were subtyped according to age of onset of alcoholic dependence. For the SLC6A4 no significant differences in allele and genotype frequencies between Russian and Tatar controls and between alcoholics in whole and controls of the same ethnic group were observed. However, a significantly higher frequency of the S/S genotype (0.43) and relatively lower frequency of the L/S genotype (0.29) were found in Russian early-onset alcoholics compared with Russian controls, Russian later-onset alcoholics and Tatar early-onset alcoholics. The S/S genotype frequency in Russian later-onset alcoholics (0.05) was significantly lower compared with healthy Russian control and Tatar later-onset alcoholic. For the EcoRV polymorphism of the MAO A there were no significant differences in allele frequencies between Russian and Tatar controls and between alcoholics and controls of the same ethnic group. The allele 456 frequency was significantly higher among Russian and Tatar early-onset alcoholics compared with nonalcoholic men. Thus, our results indicate that the S/S genotype is associated with susceptibility to early-onset alcoholism in Russian groups, and allele 456 MAO A is correlated with early-onset of the alcohol abuse among both investigated aboriginal groups of the Volga-Ural region of Russia.

P1275. Methylenetetrahydrofolate reductase (MTHFR) A1298C polymorphism as a NTD risk factor for the Italian population

V. Capra, *P. De Marco, A. Moroni, L. Arata, E. Merello, M. Calevo*
G. GASLINI; Genova, Italy
capraval@tin.it

Periconceptional folic acid intake prevents about 70% of all Neural Tube Defects (NTDs). It has been demonstrated that alterations in folate and homocysteine metabolism may play a role, since NTD patients as well as mothers have moderately increased levels of plasma homocysteine. Homozygosity for the C677T mutation of the methylene tetrahydrofolate reductase (MTHFR) gene, a key enzyme in the folates and homocysteine metabolism, is associated with an increased risk for NTD, even in Italian population, which has a relatively low prevalence of NTD. However, this genetic risk factor could not acount for all folic acid-preventable NTD. Another mutation in the same gene, the A1298C mutation, results in decreased MTHFR activity, which is more pronunced in the homozygous than heterozygous state. In this study, we studied the A1298C mutation in the Italian population to evaluate its role as risk factor. We used 200 children with sporadic myelomeningocoele, 99 mothers and 64 fathers and 190 unrelated normal volunteers as control group. The frequency of the mutated C allele was 0.29 in controls versus 0.40 in patients, 0.51 in the mothers and 0.41 in the fathers. Thus, we observed increased frequency of the mutated allele in the NTD patients and their parents when compared with controls. The determined odd ratios showed an increased risk of 2.25 (95% CI;1.14-4.47) for the prevalence of the C/C genotype in the affected children versus controls. These data indicate that homozygosity for the A1298C mutation in the MTHFR gene is an important risk factor for Italian NTD patients.

P1276. Possible Involvement of the Xp21.1-3 Region in the Etiology of 46,XX Sex Reversal in a Kindred with two XX Males

S. Kofman-Alfaro[1], *N. Monroy*[1], *M. Lopez*[2], *J. Berumen*[3], *J. Zenteno*[1]
[1]*Department of Genetics, Hospital General de Mexico-Facultad de Medicina, UNAM; Mexico City, Mexico;* [2]*Sistemas Biol gicos, UAM-X; Mexico City, Mexico;* [3]*Esc. Militar de Graduados en Sanidad; Mexico City, Mexico*
skofman@servidor.unam.mx

46,XX maleness is an uncommon form of human sex reversal characterized by testicular development in subjects lacking a Y chromosome. 46,XX males can be classified as Y positive or Y negative, depending on the presence or absence of Y-sequences. Approximately 90% of XX males carry the SRY gene, responsible for the testicular development. The remaining cases are SRY negative and testicular differentiation is probably due to mutations in autosomal or X-linked sex determining genes. Most 46,XX males occur sporadically but there are a few kindreds in which two or more affected individuals have been described, all being SRY negative. To determine whether XX sex reversal was X-linked, we performed linkage analysis using a total of 29 dinucleotide X-chromosome markers in a previously reported family including two SRY-negative XX male brothers. Our results show that the affected males and a 46,XX normal sister inherited the same maternal X chromosome, however at Xp21.1-3 the two brothers had in common 6 marker alleles in homozygous state, which differ from those observed in their sister. This data show that in this family XX sex reversal co-segregates with a 4.25 cM region at Xp, a chromosomal segment that includes the Dosage-Sensitive Sex reversal (DSS) locus. We propose that DSS may be involved in the etiology of XX sex reversal, either through molecular defects in DAX-1 or in a yet unidentified sex-determining gene located in this region.

P1277. Refinement of the locus for Oto-palato-digital syndrome type I (OPD-I)

T. Kosho[1], *K. Muroya*[2], *M. Tanimura*[3], *T. Uemura*[4], *M. Matsuo*[5], *H. Ohashi*[6], *N. Matsuo*[2], *T. Ogata*[2]
[1]*Department of Pediatrics, Tokyo Dental College Ichikawa General Hospital, Ichikawa; Japan;* [2]*Department of Pediatrics, Keio University School of Medicine, Keio; Japan;* [3]*Department of Child Ecology, National Children s Medical Research Center; Japan;* [4]*Department of Surgery, Division of Plastic Surgery, Saga medical school; Japan;* [5]*Division of Medical Genetics, Kanagawa Children s Medical Center; Japan;* [6]*Division of Medical Genetics, Saitama Children s Medical Center; Japan*
ktomoki@cocoa.ocn.ne.jp

Oto-palato-digital syndrome type I (OPD-I) (MIM31130) is a rare disorder characterized by broad distal digits with short nails, a peculiar face with a supraorbital ridge, and conductive deafness. X-linked transmission with intermediate expression in female and complete expression in males has been demonstrated. The previous study provided evidence for the linkage of OPD-I to the tightly linked markers DXS15, DXS52 and DXS305 at Xq28, and refined the critical gene for OPD-I to the approximately 10 Mb area distal to DXS539. Here, we report a linkage study with polymorphic microsatellite markers in a four-generation family consisting of two males with complete expression and three females with intermediate expression. Genotype analysis for 16 loci flanking at Xq26-28 revealed that the L1CAM locus was co-segregated with the OPD-I phenotype in all the male and female patients although the proximal BGN locus or the distal DXS1108 locus were not. Two-point linkage analysis revealed a maximum LOD score of 1.20 at theta=0 between OPD-I and L1CAM. The critical gene for

OPD-I was suggested to be refined to the approximately 3 Mb area between BGN and DXS1108.

P1278. Linkage Analysis In A Large Italian Family Affected By X-mental Retardation

A. Ciccodicola[1], *C. Lanzara*[1], *I. Conte*[1], *A. Zullo*[1], *I. Annunziata*[1], *A. Torino*[1], *S. Cocchia*[1], *D. Circolo*[1], *V. Ventruto*[1], *G. Casari*[2], *M. D Urso*[1], *M. G. Miano*[1]

[1]*International Institute of Genetics and Biophysics, CNR; Naples, Italy;* [2]*Stem Cells Research Institute; Milan, Italy*

ciccodic@iigb.na.cnr.it

Non-specific X-mental retardation (NXMR) is a very common and genetically heterogeneous disorder and affect approximately 1/600 males. Over the past two years, the repertoire of loci involved in NXMR has expanded rapidly. Linkage studies carried out on several X-linked mental retardation (XMR) families with informative meiosis and affected males distributed on two or three generations, mapped at least 60 XMR loci. Each of these accounts for only 0.5-1% of XMR cases. Through positional cloning effort six genes have been cloned. They are RSK2 (Xp22.2), IL1RAPL (Xp22), TM4SF2 (Xp11.4), OPHN1 (Xq12), PAK3 (Xq21.3) and GDI1 (Xq28). Here we report linkage analysis strategy applied to a large Italian pedigree affected by NXMR to identify the causative disease gene. Using 14 STRs covering the entire X chromosome, we have analysed on automatic sequencer the genotype of 23 individuals distributed on two generations of which 7 affected males have mild or severe mental retardation. Parametric linkage data carried out by MLINK program indicate a maximum lod score of 3.14 at theta = 0.00 for the polymorphic marker GATA72E05 located inside the pericentromeric region of 23 cM between Xp21.1 and q22.1. This linked portion of Xper has a similar position of other 23 XMR families already described in Xp11-q21. The XMR pericentromeric clustering suggests allelism and the presence at least of 2 XMR genes in this region. Multipoint linkage studies are in progress to restrict the XMR candidate region.

P1279. Association of CD14 polymorhism and monocyte receptor density.

K. Kalidas, *K. El-Sharnouby, S. A. Roberts, D. Irving, J. Sheldon, J. Poloniecki, S. Jeffery, J. C. Kaski*

St. George s Medical School; London, United Kingdom

kkalidas@sghms.ac.uk

Atherosclerosis is characterised by the formation of focal lesions which protrude into the arterial lumen, obstructing blood flow and producing stable and unstable angina, and acute MI. Monocyte CD14 receptor is activated by bacterial lipopolysaccharide to produce endothelial cell activation, cytokine secretion and pathophysiological effects associated with the initiation and development of atherosclerosis. It has been suggested that the T allele of a common polymorphism in the CD14 gene promoter is associated with an increased risk of MI, and that this effect may be due to an increased density of CD14 receptors on monocytes in TT homozygous individuals. We have examined the CD14 genotype and circulating monocyte receptor density in 60 individuals with differing states of cardiac disease, to determine whether such a correlation exists.

P1280. Analysis of associations of HindIII restriction fragment length polymorphism of lipoprotein lipase gene with myocardial infarction in Bashcortostan population

O. E. Mustafina, *E. Shagisultanova, T. Narsyb ullin, I. Tuktarova, A. Bikmeeva, E. Khusnutdinova*

Institute of Biochemistry and Genetics; Ufa, Russian Federation

ekkh@anrb.ru

Lipoprotein lipase (LPL, triacylglyceroprotein acylhydrolase, EC 3.1.1.34) plays a crucial role in plasma lipoprotein processing by catalyzing the hydrolysis of core triglycerides of chylomicrons and very low density lipoproteins. So LPL gene is a logical candidate gene of cardiovascular diseases (CVD). The aim of our study was to investigate whether common HindIII restriction fragment length polymorphism at intron 8 of LPL gene is associated with myocardial infarction (MI) in Russians and Tatars from Bashcortostan. Polymorphism of LPL gene was studied in unrelated males without symptoms of CVD (53 Russians and 80 Tatars) and coronary heart disease patients MI survives under 55 years (98 Russians and 68 Tatars) by the polymerase chain reaction. The frequencies of the genotypes HindIII(-/-), HindIII(+/-), HindIII(+/+) in Russians (3.77%, 49.06% and 47,17%) did not differ from that in Tatars (7.50%, 51.24%, and 41.25% accordingly); HindIII(-) allele frequency was 28.30% in Russians and 33.13% in Tatars. The HindIII(+/+) genotype was more common among MI survives then control subjects in Tatars (odds ratio; 1.56, $p<0.05$). It was also found that frequency of HindIII(+/+) genotype was higher ((odds ratio; 6.85, $p<0.05$) and frequency of HindIII(+/-) genotype was lower (odds ratio; 0.17, $p<0.05$) in russians patients with repeated MI then in no-CVD russians males.

P1281. Fine mapping of region 1p32 that contains the third major locus for autosomal dominant hypercholesterolemia

M. Abi-Fadel, *L. Villeger, S. Rochaud, A. Robin, J. Rabes, M. Devillers, C. Junien, C. Boileau, M. Varret*

INSERM UR-383, Hopital Necker-Enfants Malades; Paris, France

abifadel@necker.fr

Autosomal Dominant Hypercholesterolemia (ADH), one of the most frequent hereditary disorders, is characterized by an isolated elevation of LDL particles that leads to premature mortality from cardiovascular complications. It is generally assumed that mutations in LDLR and APOB genes account for ADH, however we have shown that ADH is genetically more heterogeneous. We identified 23 ADH families in which we excluded linkage to LDLR and APOB thus demonstrating the implication of a new locus we named FH3 . Genetic linkage was obtained in 6 pedigrees localizing FH3 in a 8 cM interval at 1p32-p34.1. This linkage result has been confirmed by S. Hunt et al. in a Utah pedigree. Taken together, the haplotype data define a 1 cM interval for FH3. By radiation hybrid mapping, 6 candidate genes (FABP3, SCP2, APOER2, PAFAH2, AMPK and EPS15) were located outside this interval demonstrating no identity with FH3. Through an e-mapping approach, we built a BAC contig that spans » 5 Mb and contains 1 non ovelapping area. The analysis of sequences draft reveals more than 30 genes (cloned and predicted). We are currently screening all these genes for liver expression and sequencing some of them in the FH3 families . Finally, heterogeneity tests estimated that 19% of 23 non-LDLR/non-APOB ADH families were linked to FH3, indicating the implication of a fourth locus called FH4 that we are currently mapping.

P1282. Linkage of Familial Combined Hyperlipidemia to chromosomes 1 and 13.

P. Hanifi Moghaddam[1], *J. de Graaf*[2], *L. A. Sandkuijl*[1], *M. J. Veerkamp*[2], *A. Tholens*[1], *A. F. H. Stalenhoef*[2], *R. R. Frants*[1]

[1]*Department of Human and Clinical Genetics, Leiden University Medical Center; Leiden, The Netherlands;* [2]*Department of Medicine, Division of General Internal Medicine, University Medical Center Nijmegen; Nijmegen, The Netherlands*

payman@lumc.nl

Familial combined hyperlipidemia (FCHL), the most common genetic form of dyslipidemia in man, affects 1-3% of the population and is found in up to 20% of premature myocardial infarction survivors. FCHL is defined by hypercholesterolemia and/or hypertriglyceridemia and the presence of coronary heart disease (CHD) in a first-degree relative < 60 years. Furthermore, FCHL is characterized by several traits that are associated with an increased risk of CHD including small dense LDL, insulin resistance and increased apolipoprotein B.

Segregation analysis suggests a complex inheritance pattern with major dominant genes influencing the major FCHL traits. Although several gene-mapping studies have surfaced candidate regions, no causative genes have been identified so far. Comparison of various linkage studies shows that several candidate loci are common in different populations, although often with mixed findings in replication studies, due to clinical, genetic and ethnic heterogeneity.

We have collected and characterized 40 multigenerational families with FCHL including 687 individuals with high clinical and diagnostic homogeneity. Recently, a genome wide linkage analysis with 405 microsatellite markers was performed with the support of the NIH in the 13 (n = 280) most informative families. Several candidate regions have been identified, two of which stand out as extremely promising with a joint lod score of 3.8 in a simultaneous search analysis. These two regions are located on chromosomes 1 and 13, respectively. Two other loci on chromosomes 4 and 20 showed promising lod scores between 1 and 2, individually. In order to enhance and pinpoint linkage evidence and eliminate false positive findings, fine mapping of candidate regions is being performed in the complete set of the 40 original families and three additional multigenerational FCHL families (n>850). As the results of the first stage genome screen already indicated locus heterogeneity for FCHL, it is extremely important to determine the exact contribution of each of the loci involved. For this purpose, quantitative trait analyses will be carried out for each of the traits that underlay FCHL. Noteworthy, we have recently completed a 5 years clinical and biochemical follow-up in the 40 FCHL families, giving important information on phenotype variation.

Insight in the genetic background of FCHL will form the basis for improved clinical care, including earlier and more effective preventive therapy.

P1283. Genotyping Of Factor V Leiden And Protrombin 20210 Mutations In Hemodialysis Patients With Multiple Arteriovenous Fistula Thrombosis

B. F. Ata *[1], N. zbek[2], N. zdemir[3], S. Sezer[3], K. B. Kazbey[1], D. Divanlioglu[1], A. Kara[1], M. Haberal[4]*
[1]Baskent University School of Medicine Department of Molecular Biology; Ankara, Turkey; [2]Baskent University School of Medicine Department of Pediatrics; Ankara, Turkey; [3]Baskent University School of Medicine Department of Nephrology; Ankara, Turkey; [4]Baskent University School of Medicine Department of General Surgery; Ankara, Turkey
batac@superonline.com

Vascular complications are one of the most comman problems which contribute to the morbidity of end-stage renal failure.After understanding the role of point mutations in the etiology of thrombosis, the high prevalance of Factor V Leiden (FVL) and Protrombin 20210 (Pt-20210) mutations began to gain more importance.Previously the incidence of Turkish heterozygous carrier status for FVL and Pt 20210 were described as7% and 2.3% respectively.This study was designed for the determination of FVL and Pt 20210 carrier status in hemodialysis patients with history of arteriovenous fistula (AVF) thrombotic complications. 46 adult hemodialysis patients (mean age 45.66+12.31years, mean HD duration 87.37+ 54.07months) who had AVF thrombosis at least three episodes were subjected .Single nucleotid polymorphism was searched in FVL (Arg506Gln) and Protrombin(G-A) genes by real time PCR and the fluorometric DNA melting point analysis. At the end of these molecular analysis mutation of FVL was found in 7 (15.2%)patients and Pt 20210 in 4 ((8.7%) patients. These results may indicate that the incidence of the both mutations were two fold increased in hemodialysis patients when compared with normal population. As a conclusion , in patients with frequent AVF thrombosis, in addition to medical and surgical reasons genetic factors should be investigated. Further thrombogenic complications can be prevented by early identification of this high risk group.

P1284. A recessive, most likely X-linked case of familial thrombocytosis in an Arab family

W. Kuehnau*[1], M. Stuhrmann[1], L. Bashawri[2], B. Al-Awamy[2], E. A. El-Harith[1]*
[1]Medizinische Hochschule Hannover; Hannover, Germany; [2]King Faisal University; Al-Khobar, Saudi Arabia
stuhrmann.manfred@mh-hannover.de

Familial thrombocytosis (FT) has previously been described as an autosomal -dominant disorder presenting with clinical symptoms similar to those of sporadic essential thrombocythaemia (ET). To date, three different mutations have been identified in the human thrombopoietin (THPO) gene of FT patients. We studied a Bedouin Arab family affected with an apparently recessive type of FT. In this family, all four brothers had markedly and/or moderately elevated platelet counts, while the two sisters and their parents had normal platelet counts. Plasma thrombopoietin levels were normal in all family members.The four brothers, aged 4-8 years, currently have no thrombotic or haemorrhagic complications. Mutation analysis at the THPO gene in the affected brothers failed to detect the major known mutation (intron 3 G->C) that had previously been described as causing FT. In addition, segregation analysis using a CA-repeat microsatellite marker tightly linked to THPO revealed completely discordant THPO alleles among the affected brothers, thereby excluding THPO as the disease locus in this family. We assume the existence of a new locus for FT, whereby the disorder is transmitted as a recessive, most likely X-linked trait.

P1285. Identification of polymorphisms in the 5 -UTR region of the TAFI gene; Relationship with plasma TAFI levels and risk of venous thrombosis

M. G. Fagundes*[1], M. A. Zago[2], J. Meijers[3], P. H. REitsma[4], D. Louren o[5], V. Morelli[5], F. H. Maffei[6], C. E. Piccinato[2], W. A. Silva-Jr[2], R. F. Franco[2]*
[1]Center for Research on Cell-based Therapy, School of Medicine of Ribeirao Preto, University of Sao; Ribeirao Preto-SP, Brazil; [2]Center for Research on Cell-based Therapy, School of Medicine of Ribeirao Preto, University of Sao Paulo; Ribeirao Preto-SP, Brazil; [3]Department of Hematology, Academic Medical Center, University of Amsterdam, the Netherlands.; Amsterdam, Netherlands Antilles; [4]Department for Experimental Internal Medicine, Academic Medical Center, University of Amsterdam, the Netherlands.; Amsterdam, Netherlands Antilles; [5]Federal University of Sao Paulo; Ribeirao Preto-SP, Brazil; [6]State University of Sao Paulo; Ribeirao Preto-SP, Brazil
gifag@ig.com.br

Thrombin-activatable fibrinolysis inhibitor (TAFI) is a potent fibrinolysis inhibitor. TAFI gene variations were not reported heretofore. We analyzed a 2083-bp region at 5 of the TAFI gene in 127 healthy subjects and identified 7 novel polymorphisms; -152 A/G, -438 A/G, -530 C/T, -1053 T/C, -1102 T/G,-1690 G/A, and -1925 T/C. The -152 A/G, -530 C/T and -1925 T/C transitions were found to be in strong linkage disequilibrium, as well as -438 A/G, -1102 T/G, -1053 T/C and -1690 G/A. Plasma TAFI levels (%) in subjects with the -438GG/-1102GG/-1053CC/-1690AA genotype (n=70) were 94–16.5; heterozygotes -438GA/-1053TC/-1102TG/-1690AG (n=51) had TAFI levels of 84.6–15.5, and in -438AA/-1053TT/-1102TT/-1690GG homozygous (n=6) levels were 72.2 – 8.8 (P=0.0003). TAFI concentrations in -152AA/-530CC/-1925TT homozygous (n=122) were 89.4–17.1, somewhat higher but not significantly different from levels observed -152AG/-530CT/-1925TC heterozygous (84–9.9; n=5). We also examined TAFI polymorphisms as risk factors for deep venous thrombosis (DVT) by determining their prevalence in 388 consecutive patients with DVT and in 388 matched controls. The -438GA/-1053TC/-1102TG/-1690AG and -438AA/-1053TT/-1102TT/-1690GG polymorphisms were detected in 166 patients and in 187 controls, yielding an odds ratio (OR) for DVT of 0.8 (95%CI; 0.6-1). Although the OR for DVT linked to -152AG/-530CT/-1925TC had been of 1 (95%CI; 0.5-2.2) in the whole group, in subjects aged <35ys the OR was 0.1 (95%CI; 0.01-0.9), indicating a significant protective effect for DVT. In conclusion, we identified variations in the TAFI gene and demonstrated that they influence plasma TAFI levels. The data also suggest that TAFI polymorphisms may influence the risk for DVT.

P1286. Exlusion of the ETM1 and ETM2 loci in a large Moroccan family with autosomal dominant essential tremor

L. Baala*[1], A. Sbiti[2], R. Nejjari[2], A. Munnich[1], S. Lyonnet[1], A. Sefiani[2]*
[1]Necker Hospital; Paris, France; [2]INH; Rabat, Morocco
baala@necker.fr

Essential tremor (ET) is a chronic neurological condition which may be regarded as the most common movement disorder in human. The main feature of ET is postural tremor of the arms, but the head, legs, trunk, voice, jaw, and facial muscles may be involved as well. ET can be either sporadic or familial (familial essential tremor, FET or ETM). In addition, the prevalence of Parkinson s disease and dystonia may be increased in families with ET. Autosomal dominant inheritance can be demonstrated in most families and two ET loci have been mapped on chromosomes 3q13 (ETM1, MIM 190300) and 2p25-p22 (ETM2, MIM 602134) respectively. Here we report on a large Moroccan family with isolated autosomal dominant ET. Interestingly, patients presented with a very early onset. They are mainly affected with fine rapid ET of both hands and arms. We tested microsatellites DNA markers at the ETM1 and ETM2 loci, and eventually excluded linkage to both regions suggesting further genetic heterogeneity of FET. Furthermore, the Parkinson s loci mapping to chromosomes 4 and 6 were also excluded. Ongoing gene mapping studies will hopefully shed ligh on the genetic variability of ET.

P1287. Exclusion of NPR1 as a candidate gene for autosomal dominant medullary cystic kidney disease type 1

M. Koptides*[1], R. Mean[1], C. Stavrou[2], A. Pierides[3], K. Demetriou[3], T. Nakayama[4], F. Hildebrandt[5], A. Fuchshuber[5], C. Deltas[6]*
[1]The Cyprus Institute of Neurology and Genetics; Nicosia, Cyprus; [2]Dept. of Nephrology, Paphos General Hospital; Paphos, Cyprus; [3]Dept. of Nephrology, Nicosia General Hospital; Nicosia, Cyprus; [4]2nd Dept. of Internal Medicine, Nihon Univ. School of Medicine; Tokyo, Japan; [5]University Children s Hospital; Freiburg, Germany; [6]The Cyprus inst. of Neurology and Genetics; Nicosia, Cyprus
Deltasco@mdrtc.cing.ac.cy

Autosomal Dominant Medullary Cystic Kidney Disease (ADMCKD) is an adult onset heterogeneous genetic nephropathy characterized by salt wasting and end stage renal failure. The gene responsible for ADMCKD1 was mapped on chromosome 1q21 and it is flanked proximally by marker D1S498 and distally by D1S2125, encompassing a region of ~8cM. Within this region there is a large number of transcribed genes including NPR1 that encodes the atrial natriuretic peptide receptor 1. This receptor plays a crucial role in regulation of blood pressure by facilitating salt excretion. Based on its presumed function we hypothesized this gene as a reasonable candidate for the MCKD1 locus. DNA mutation screening was performed on the entire NPR1 gene coding sequence, including the exon/intron splice junctions. The samples investigated belonged to patients of five large ADMCKD1 Cypriot families. The screening revealed two novel polymorphisms, one intragenic at aminoacid position 939, which was occupied by either arginine or glutamine, and a second one located in the 3 UTR, 29 nucleotides downstream the NPR1 stop codon. The latter was a single nucleotide C insertion/deletion in a stretch a three or four Cs. No relationship was present between any allele of the two polymorphisms and the disease, as both alleles were observed in both affected and healthy subjects. In addition, no association was observed between the

disease and another rare 8-bp deletion polymorphism at the 5 UTR of NPR1 and the disease. Based on these findings it is unlikely that NPR1 is the same as the MCKD1 gene although it is presently unknown whether it plays a disease modifying role.

P1288. Familial Ectrodactyly;An Autosomal Dominant Pedigree with Novel Skeletal Features and Exclusion of the p63 Locus

T. Roscioli[1], *P. J. Taylor*[2], *J. A. Donald*[3], *J. Masel*[4], *I. A. Glass*[5], *M. F. Buckley*[6]

[1]*Sydney Children s Hospital; Sydney, Australia;* [2]*Molecular and Cytogenetics Unit, South Eatern Area Laboratory Services; Sydney, Australia;* [3]*Department of Biological Sciences, Macquarie University; Sydney, Australia;* [4]*Royal Brisbane Hospital Radiology Department; Brisbane, Australia;* [5]*Division of Medical Genetics, University of Washington State; Seattle, WA United States;* [6]*Molecular and Cytogenetics Unit, South Eastern Area Laboratory Services; Sydney, Australia*

rosciolit@hotmail.com

We describe a family with AD variable ectrodactyly, encompassing the classical lobster claw deformity, through to partial absence of the thumb and radial ray, distal agenesis of the index finger and epiphysial coning. One individual has an unusual supernumerary ossicle with an articular surface opposed to the distal phalanx of the thumb. Two individuals had skeletal manifestations consistent with EEC; one with unilateral dysplasia of the nails of fingers 2-4 and another with cleft palate. Due to the presence of these features, a linkage study for the EEC Split Hand Split Foot (SHFM) 4 locus (p63)was undertaken. Two point Lod scores were calculated for three microsatellite markers that lie within 0.55cM of p63 and flank the gene. Linkage was excluded by Lod scores of <-2 within 8cM of D3S3549; 3cM of D3S3530; and 1cM of D3S1294 (Zmax is 4.9). Of the three other known SHFM loci, SHFM2 is X-linked; SHFM1 (7q21.3-q22.1) is usually sporadic comprising additional features of deafness, cleft palate and microcephaly, which were not present in this family. SHFM3 (10q24) contains a candidate F-box/WD40 gene Dactylin, which is currently being examined by other researchers. The HOXD cluster on the long arm of chromosome 2 has also been associated with ectrodactyly but our family does not have the imperforate anus or penoscrotal hypoplasia associated with deletion of the whole cluster. Currently, further linkage analyses are underway to determine whether linkage to 10q24 exists for our SHFM family or whether this represents a novel locus.

P1289. Polymorphisms in Positional Candidate Genes for Systemic Lupus Erythematosus, Mapped to 1q23-q42

A. S. A. Alansari[1], *A. G l*[2], *W. Ollier*[1], *J. Ordi*[3], *L. Teh*[4], *A. Hajeer*[1]

[1]*The University of Manchester; Manchester, United Kingdom;* [2]*University of Istanbul; Istanbul, Turkey;* [3]*Hospital General Vall d Hebron; Barcelona, Spain;* [4]*Blackburn Royal Infirmary; Blackburn, United Kingdom*

aliya@fs1.ser.man.ac.uk

Background; Family studies in different ethnic populations have shown that chromosome 1q23-q42 interval is linked with SLE. Several potential candidates are mapped to this region, including the gamma chain (FcRg), FcgRs, IL-10, CRP, SAP, PARP and TGFb2. Objectives; To investigate positional candidate genes polymorphisms and to study the possible association between identified polymorphisms and SLE in patients from three different populations. Methods; We have investigated the FcRg, FcgRIIb and TGFb2 genes for polymorphisms using PCR-SSCP and sequencing. Identified polymorphisms were typed using PCR-RFLP method. Cases and controls were available from three ethnic groups, Turkish (95), Spanish (120) and UK (100). All the patients fulfilled the 1982 ACR revised criteria for SLE. Results; Five SNPs have been identified in the FcRg gene, 2 SNPs in the promoter, one in intron four and 2 in the 3 UTR. Four out of the 5 SNPs were relatively common and therefore investigated in the 3 populations. Allele and genotype frequencies of all four SNPs investigated were not statistically different in cases and controls. In addition, polymorphisms has identified in the 5 UTR of TGFb 2 and in the 5 UTR and intron 3 of FcgRIIb. Discussion; The FcRg, FcgRIIb and TGFb2 genes are polymorphic. The identified FcRg polymorphisms do not contribute to SLE susceptibility. However, these polymorphisms may be useful in studying other diseases. The polymorphisms identified in the FcgRIIb and TGFb2 genes will be investigated in the three different ethnic case-control groups.

P1290. Linkage Study in families with febrile seizures

A. A. Pasqua[1], *A. Gambardella*[2], *P. Forabosco*[3], *C. Tomaino*[1], *F. Annesi*[1], *P. Spadafora*[1], *I. C. Cir Candiano*[1], *S. Carrideo*[1], *A. Labate*[2], *D. Pirritano*[2], *G. Annesi*[1]

[1]*CNR-Istituto Medicina Sperimentale e Biotecnologie; Mangone (CS), Italy;* [2]*Universit Magna Graecia; Catanzaro, Italy;* [3]*CNR-Istituto di Genetica; Alghero, Italy*

pasqua@imseb.cs.cnr.it

Febrile seizures (FS) are the most common form of childhood seizures. FS have both environmental and genetic factors involved in their pathogenesis. These convulsions probably have a variety of causes, but a genetic component has long been recognised. The genetics of familial FSs is somewhat ambiguous. Polygenic, autosomal dominant and autosomal recessive models have received support. Recently four putative FSs loci, FEB1 (chromosome 8q13-q21), FEB2 (chromosome 19p), FEB3 (chromosome 2q23-24) and FEB4 (chromosome 5q14-15), have been mapped. In this study we conducted a linkage analysis to the four known FSs loci in eight small families originating in Southern Italy that appeared to segregate FS as an autosomal dominant trait. Members of these families were genotyped using microsatellite markers linked to the previously identified febrile convulsion loci, FEB1, FEB2 , FEB3 and FEB4. The febrile seizures phenotype was analyzed as an autosomal dominant trait with an incomplete, but high, disease penetrance of 90% and with an estimated disease gene frequence of 0.001. Two-point linkage analysis was performed using the FASTLINK version MLINK of the LINKAGE 5.1 software package. Positive but not significant lod scores were obtained in seven families probably because of the small numbers of subjects examined. For one family two-point lod scores were negative and multipoint lod scores were below the threshold value of -2, excluding linkage with four known FC loci. These results indicate that there are at least five loci responsible for febrile convulsions

P1291. Genetic analysis of an Italian family with dominant focal distonia; evidence for further genetic heterogeneity

F. Brancati[1], *V. Caputo*[2], *G. De Fazio*[3], *A. Berardelli*[4], *L. Vacca*[4], *A. Pizzuti*[5], *G. Novelli*[6], *B. Dallapiccola*[5]

[1]*Universit di Roma La Sapienza , CSS Mendel Institute; Roma, Italy;* [2]*CSS, Mendel Institute; Roma, Italy;* [3]*Universit di Bari; Bari, Italy;* [4]*Universit di Roma La Sapienza ; Roma, Italy;* [5]*Universit di Roma La Sapienza , CSS Mendel Institute; Roma, Italy;* [6]*Universit di Roma Tor Vergata ; Roma, Italy*

frankbrank@libero.it

Idiopatic dystonias are neurological disorders characterized by involuntary twisting movements of affected parts of the body. Most dystonia cases are sporadic. However, familial dystonias, mostly with autosomal dominant transmission pattern, reduced penetrance and variable expression, not uncommonly come to the clinician attention. Seven genes for dystonias have been mapped so far and two of them have been identified (the TorsinA and the GTP-cyclohydrolase gene) carrying mutations in particular families. Two loci, DYT6 and DYT7 in chromosome 8p and 18p respectively, have been linked to single families with primary purely focal dystonias. The linkage has never been confirmed by other groups. Here we present a three generation family (15 members) from South of Italy with 8 patients affected by focal dystonia with age of onset between 20 and 50 years. We have analysed for linkage both the DYT6 and DYT7 regions by genotiping all the family members. Samples were loaded on a ABI PRISM 310 and analysed with the Genotyper 2.0 software. Furthermore, the DYT1 gene on 9q31 was screened for the GAGdel mutation. In this family, the study excluded linkage of the dystonia gene both for the DYT6 and DYT7 loci, and absence of the recurrent DYT1 mutation. The findings comfirm the high genetic heterogeneity of famlial idiopatic dystonias, in particular the focal and multifocal forms. (This work was supported by MURST)

P1292. Investigation of linkage at chromosome 2q in osteoarthritis of the hand

J. M. Stankovich[1,2], *H. M. Cooley*[1], *M. M. Sale*[1,2], *M. Bahlo*[3,2], *T. P. Speed*[3,2], *J. Dickinson*[1], *G. Jones*[1,2]

[1]*Menzies Centre for Population Health Research; Hobart, Australia;* [2]*Cooperative Research Centre for Discovery of Genes for Common Human Diseases; Melbourne, Australia;* [3]*The Walter and Eliza Hall Institute of Medical Research; Melbourne, Australia*

Jim.Stankovich@utas.edu.au

Twin studies have suggested a strong genetic component to osteoarthritis (OA), especially that of the hand. We have collected extensive phenotypic and genetic data on 470 subjects from 68 families containing at least two living affected individuals in Tasmania, Australia. Phenotypic assessment of hand OA utilised the Altman atlas for joint narrowing and osteophytes. This score was quantitative (range 0—58 out of a possible 60) and of high reproducibility (intra-class correlation 0.94—0.98). Subjects have been genotyped at 19 markers on chromosome 2q. We attempted to reproduce the findings of Lepp vuori et al. (Am. J. Hum. Genet. 65; 1060) by using an identical design and a non-parametric linkage (NPL) analysis on 2q of 69 individuals in 22 families with severe distal interphalangeal joint OA.

The maximum NPL score was 1.05 (at the same marker (IL1R1) identified by Lepp vuori et al.). These results do not provide confirmation of linkage at this site in our population. Subsequent fine mapping, QTL analysis or genome wide screens may be required for demonstration of linkage in this population.

P1293. CFTR gene polymorphisms in Latvia

L. Krumina[1], *V. Kroshkina*[1], *I. Tamane*[1], *M. Lazdins*[2], *V. Baumanis*[3], *A. Krumina*[1]
[1]*Medical Academy of Latvia; Riga, Latvia;* [2]*University of Latvia, faculty of Biology; Riga, Latvia;* [3]*University of Latvia, Biomedical Research Centre; Riga, Latvia*
liene777@hotmail.com

The aim of our study was to analyse CFTR gene polymorphisms useful in indirect DNA diagnosis of Cystic Fibrosis in Latvia. 31 patient with CF, 30 healthy family members and 146 healthy Latvians were subjected to DNA testing. DNA analysis was performed by PCR, restriction analysis and agarose or polyacrilamide gel electrophoresis. All DNA samples have been analysed for six CFTR gene polymorphisms. 3 extragenic diallelic polymorphic sites - KM-19/PstI, XV-2C/TaqI, J3.11/PstI and 3 intragenic polymorphic sites - [GATT]n in intron 6A, TUB18 in intron 18 and CA repeats in intron 1 were studied. Diallelic polymorphic sites are in Hardy - Weinberg equilibrium. The highest heterozygote frequency was detected for markers XV-2C/TaqI - 49,9% and KM-19/PstI - 42,8%. Absolute linkage disequilibrium was found between the mutation ˇF508 and polymorphic loci IVS 6a (GATT repeat) and XV-2C/TaqI; highly significant with loci TUB18 and KM-19/PstI. Result of haplotype analysis showed different distribution of haplotypes between normal, ˇF508 and non-ˇF508 CF chromosomes. The most common haplotypes associated with the ˇF508 mutation differ in the marker J3.11, located downstream (distance 1cM) from the CFTR gene. These findings are consistent with the data published for most European populations and will be useful in indirect DNA diagnosis for CF in Latvia and for tracing the origin of the ˇF508 mutation in the Baltic Sea region.

P1294. CF-like lung disease associated with the IVS8-5T allele in combination with the polymorphisms TG11 and M470V of the CFTR gene

B. Thamm-M cke[1], *D. Wand*[1], *T. Lietz*[2]
[1]*Gemeinschaftspraxis Dr. Reising-Ackermann & Koll.; Leipzig, Germany;* [2]*Universit tskinderklinik; Leipzig, Germany*
labrgenetik@aol.com

Cystic fibrosis (CF) is one of the most common autosomal recessive genetic disorders in the Caucasian population and is caused by mutations in the cystic fiobrosis transmembrane regulator (CFTR) gene. The 5T allele in intron 8 causes abnormal splicing of the CFTR gene by skipping of exon 9 leading to non-functional protein. In combination with an other CF mutation the 5T allele might be associated with congenital bilateral absence of the vas deferens (CBAVD) or chronic pancreatitis. However, the 5T variant has incomplete penetrance and variable expressivity, suggesting that some other regulatory factors may modulate the splicing of exon 9. Furthermore, the 5T allele alone has not been reported to cause lung disease. We describe a three years old male patient with CF-like lung disease. Sweat chloride conductance has been rising over the last three months (37,7 - 53,7 - 66,6 - 67,0 mmol/l) with increasing frequency of respiratory infections, but still no pneumonia. The digestive function appears normal. Complete sequencing of the CFTR gene revealed the TG11/ 5T allele in combination with the M470V polymorphism. No other mutation was present. These data suggest that the 5T polythymidine tract on specific haplotype backgrounds (number of TG repeats, presence of M470V) may cause mild CF-like lung disease with moderate values of sweat chloride conductance.

P1295. Genetic and Clinical Studies of Otosclerosis

X. C. Li, *D. Mills, P. Johnson, D. Brackmann, R. A. Friedman*
House Ear Institute; Los Angeles, CA United States
cli@hei.org

Clinical otosclerosis has a prevalence of 0.2 to 1% among white adults, making it the single most common cause of hearing impairment in this population. It is a disease of the otic capsule that is characterized by resorption and redeporsition of bony tissue. The majority of epidermiological studies indicate that the inheritance of otosclerosis is autosomal dominant with reduced penetrance. To date, only one otosclerosis locus, OTSC1, has been mapped on chromosome 15q. We studied a large multi-generational family in which otosclerosis has been inherited in an autosomal dominant pattern. Six of the affected persons have surgically confirmed otosclerosis. Exclusion study on this family by typing genetic markers that linked to OTSC1 indicates that a novel otosclerosis gene segregates in this family. We are now in the process of locating the disease-causing gene through whole genome scan and genetic linkage analysis. Once the gene is mapped, a correlation between the genotypes and phenotypes will be closely examined.

P1296. Rarity of molecular alterations in the promoter region of the androgen receptor gene

D. F. Cabral, *M. L. Ribeiro, A. dos Santos, C. Hackel*
Universidade Estadual de Campinas - UNICAMP; Campinas - SP, Brazil
dcabral@obelix.unicamp.br

The androgen receptor protein is essential for androgen action, by binding either testosterone (T) or its 5a-reduced metabolite, 5a-dihydrotestosterone. Thus, the androgen receptor gene (AR) plays an essential role in the development of male sexual characteristics. The AR gene has a relatively long 5 untranslated region (5 UTR). Since this region may play a role in translation control, protein expression could be modulated by polymorphisms in the normal male population. In addition, a previous report of two germline point mutations in the 5 UTR of the AR gene in men with prostate cancer1 suggest that these alterations may contribute to this disease. We therefore evaluate the variability of this region in healthy individuals and in prostate cancer patients from a Brazilian population. The screening for polymorphisms in the 5 UTR was performed by CSGE. Genomic DNAs from 100 male blood donors and from 100 patients with prostate cancer were amplified by PCR, with 4 pairs of primers designed to cover a 1,400 bp region. Since the AR gene is X-linked, each product was mixed with a previously sequenced normal fragment, in order to generate heteroduplexes in the presence of point mutations. Only one mutation was detected by this technique, in one individual from the blood donors sample, consisting of one base pair deletion (-T at position -1110) identified by sequencing. The deletion observed is situated far from the critical region, therefore its possible effects might be minimised. These results point out the highly conserved character of this region. 1Crocitto et al., J Urol 158;1599-1601,1997. supported by FAPESP.

P1297. Endothelial Nictric Oxide Synthase Minisatellite Polymorphism; analysis of associations with myocardial infarction and essential hypertension in Russians from Bashkortostan

E. I. Shagisultanova, *O. E. Mustafina, T. R. Narsyb?ullin, I. A. Tuktarova, A. M. Bikmeeva, E. K. Khusnutdinova*
Institution of Biochemistry and Genetics; Ufa, Russian Federation
ekkh@anrb.ru

Nitric oxide inhibits platelet aggregation, proliferation of vascular smooth muscle cells, and leukocyte adhesion to endothelial cells. An impaired synthesis of nitric oxide by the vascular endothelium has been implicated in the pathogenesis of coronary artery disease. Thus, genetic polymorphism of endothelial constitutive nitric oxide synthase (ecNOS) may be involved in the pathogenesis of coronary artery disease (CAD). The possible associations between variable number of tandem repeats polymorphism (VNTR, 27-bp repeat) at intron 4 of ecNOS gene, essential hypertension (EH), myocardial infarction (MI) were investigated in Russians from Bashkortostan. Polymorphism of ecNOS gene was studied in unrelated normotensive males without symptoms of CAD (102 subjects), CAD patients MI survives under 55 years (107 subjects), EH patients (107 subjects). The VNTR region of ecNOS gene was amplified by the polymerase chain reaction; two alleles, containing four (allele A) and five repeats (allele B), were identified. Distinctions between controls and hypertensive patients with left ventricular hypertrophy were found; in patients the genotypes eNOS4A/A, eNOS4A/B and eNOS4B/B were discovered in 4.17%, 52,08%, 43,75% of case, alleles A and B in 30.21% and 69.79% of case, respectively. In controls genotypes and alleles distributions were as follows; eNOS4A/A — 1.96%, eNOS4A/B — 29.41%, eNOS4B/B — 68.62% and A — 16.17%, B — 83.33%. But genotypes and alleles frequencies did not differ significantly between MI patients and controls. Thus, eNOS minisatellit polymorphism might be a genetic risk factor for the development of left ventricular hypertrophy among russian hypertensive patients.

P1298. Evaluation of markers on human chromosome 10, including the homologue of the rodent Rf-1 gene, for linkage to End-Stage Renal Disease (ESRD) in African-Americans.

B. I. Freedman[1], *H. Yu*[1], *M. Sale*[2], *B. H. Roh*[1], *D. W. Bowden*[1], *S. S. Rich*[1]
[1]*Wake Forest University School of Medicine; Winston-Salem, NC United States;* [2]*University of Tasmania; Hobart, Australia*
bfreedma@wfubmc.edu

African-Americans demonstrate familial clustering of hypertensive ESRD (H-ESRD). Animal models of H-ESRD also suggest that genetic factors make strong contributions to renal failure. In the Fawn-Hooded rat model, the majority of the risk for glomerulosclerosis appears to be on rat chro-

mosome 1 (the *Rf*1 and *Rf*2 genes). These renal failure genes appear to promote the development of nephrosclerosis. The corresponding syntenic regions of the human genome for *Rf*1 and *Rf*2 have been isolated to 10q24-26 and 9q21, respectively.
DNA was collected from 452 African-American sib pairs concordant for ESRD, 249 of these ESRD sib pairs are concordant for non-diabetic etiologies of ESRD (H-ESRD and chronic glomerulonephritis) and 203 ESRD sib pairs are concordant for diabetic ESRD. We evaluated the *Rf*1 region on chromosome 10 as a candidate gene for ESRD in black sibling pairs concordant for ESRD. We tested for genetic linkage between 21 polymorphic markers spanning chromosome 10 and ESRD using the GeneHunter (version 2) software.
When these 21 markers were genotyped in the 249 non-diabetic ESRD sib pairs, suggestive evidence for linkage was observed between adjacent markers D10S249 and D10S1435 on chromosome 10p (lod score ~ 1.7). Additionally, genes near the *Rf*1 region demonstrated weak evidence for linkage to all cause ESRD (lod score ~ 1.2). These results suggest that genes underlying human H-ESRD susceptibility may be found on chromosome 10p in man and also in the region homologous to the rodent *Rf*1 gene.

P1299. Comparative study of CYP2D6 polymorphism in healthy Bulgarian population and patients with Balkan endemic nephropathy (BEN)

B. Zaharieva[1], *S. Atanassova*[1], *A. Dimitrova*[1], *T. Dimitrov*[2], *D. Toncheva*[1]
[1]*Department of Medical Genetics, Medical University Sofia; Sofia, Bulgaria;* [2]*Department of Nephrology, Medical University - Sofia; Sofia, Bulgaria*
draga@sofiaonline.com

The cytochromes P450 play an important role in the metabolic activation of many precarcinogens. Different polymorphic variants are associated with cancer susceptibility. Development of urinary tract tumors (UTT) is a clinical problem for 38-40 % of the patients with BEN. The aim of the study is to compare CYP2D6 polymorphism in BEN patients and healthy persons. 125 samples are genotyped (55 from BEN patients and 70 samples from healthy persons) for three CYP2D6 mutant alleles, responsible for the PM phenotype. CYP2D6*A, CYP2D6*B and CYP2D6*D alleles were identified using allele-specific PCR (Heim and Meyer, 1991). The frequency of homozygotes BEN patients for mutant alleles is 5.45% versus 10% in healthy group. Significant differences between the frequencies of heterozygotes in BEN group (12.73%) and healthy persons (31.43%)($p<0.01$) were revealed. The frequency of homozygotes of wild type allele was significantly higher in BEN patients (81.82%) compared to the controls (58.57%)($p<0.01$). We suggest that wild type allele might be associated with genetic predisposition to development of urinary tract tumors in BEN patients.

P1300. Frequency of polymorphic (TAAA)n tandem repeat in ACPP locus in Bulgarian population

S. Atanassova[1], *E. Todorovska*[2], *T. Dimitrov*[3], *D. Toncheva*[4]
[1]*Medical University Sofia; Sofia, Bulgaria;* [2]*Institute of Genetic Engineering; Kostinbrod, Bulgaria;* [3]*Department of Nephrology, Medical University - Sofia; Sofia, Bulgaria;* [4]*Department of Medical Genetics, Medical University - Sofia; Sofia, Bulgaria*
draga@sofiaonline.com

A polymorphic (TAAA)n tandem repeat is located in an alu-repeat in the non-coding sequence of the ACPP (prostatic acid phospatase) gene in 3q21-3qter.
DNAs blood samples from 89 persons (178 alleles) were studied for polymorphism in ACPP locus - 34 from Bulgarian healthy persons (68 alleles) and 55 from patients (110 alleles) with Balkan endemic nephropathy (BEN).
Four different alleles C2, C3, C4 and C6 in the studied healthy Bulgarian individuals were identified. Their respective frequencies were as follows; C2 - 0.088, C3 - 0.441, C4 - 0.309 and C6 - 0.162. Our analysis showed that the frequency of the detected allelic variants in Bulgarian population is similar to those reported in the literature (Doak et al., 1991). The most frequent alleles in Bulgarian population were C3 and C4.
Most of the alleles observed in the controls were also found in BEN patients - C2 - 0.056, C3 - 0.427, C4 - 0.345, C6 - 0.136. Alleles C1 - 0.009, C5 - 0.018 and C7 - 0.009 (not reported so far in the literature) were detected only in BEN patients.
Three BEN patients were studied for LOH in ACPP locus by using normal and tumor DNAs. No LOH or any rearrangements were observed in the studied normal and tumor DNA samples.

Table 1. Results from the studies of polymorphism in ACPP locus in Bulgarian population

Allele (bp)	Frequency in BEN patients	Frequency in Controls	Frequency in the literature (Doak et al. 1991)
C6 (136)	0.136	0.162	0.153
C5 (140)	0.018	0	0.0267
C4 (144)	0.345	0.309	0.18
C3 (148)	0.427	0.441	0.52
C2 (152)	0.056	0.088	0.1067
C1 (156)	0.009	0	0.0133
New (112-116)	0.009	0	nonpublished

P1301. A new human mtDNA polymorphism ; ND6 14562 (C->T).

I. Manna[1], *V. Andreoli*[1], *A. La Russa*[1], *G. La Porta*[1], *A. Labate*[2], *R. L. Oliveri*[2], *D. Pirritano*[2], *R. Cittadella*[1]
[1]*Institute of Experimental Medicine and Biotechnology-National Research Council; Cosenza, Italy;* [2]*Institute of Neurology,University Magna Graecia; Catanzaro, Italy*
manna@imseb.cs.cnr.it

The observation of a multiple sclerosis (MS)-like illness in patients, who carry the most common Leber s hereditary optic neuropathy (LHON) mitochondrial DNA (mtDNA) mutation may indicate a contributory role for mitochondrial genes in genetic susceptibility to MS. During a screening study of Calabrian patients with MS-like illness for the pathogenic LHON mutations at positions 11778, 3460 and 14484 of human mithocondrial DNA, we observed a new genetic polymorphism caused by a variation of the base pair (bp) at position 14562, which is located in the gene for mitochondrial ND6. The variant is a C?T transition which creates one new restriction site ; Mbo I, leading to Valine substitution for Isoleucine.The restriction fragments were detected by electrophoresis on 3% agarose gel and visualised by ethidium bromide staining. This polymorphism was observed in a patient , 36-year-old male, clinically diagnosed MS. The molecular analysis of other asymptomatic members of proband s family confirmed the same nucleotide variation. The primary LHON mutations at nucleotides 11778, 3460 and 14484 were not present in this patient. We performed a mutational analysis in fifty normal subjects from the same geographic background , but this polymorphism was not present. Thus, this novel nucleotide transition is a neutral polymorphism.

P1302. A characteristic of polymorphism in a tetranucleotide short tandem repeat locus D9S768 in Siberian population

I. Goncharova, *T. Nikitina*
Institute of Medical Genetics RAMS; Tomsk, Russian Federation
masha@img.tsu.ru

Short tandem repeats (STRs) present polymorphic sequences which are widely distributed across human genome. They are highly informative genetic markers and are extensively used in such fields as population genetics, genetic councelling, forensic medicine, and oncology. However, allele frequencies may differ in the populations of different origin. So describing of allele frequencies distribution of such markers in different populations is of great interest. We have obtained population-genetic characteristics of tetra-nucleotide short tandem repeat D9S768 in the population of South-West Siberia (N=128, non-related, mainly of Slavic origin), The study was performed by analysis of separation of PCR products (amplimer UT5494 in GDB) in 7% acrylamide gel. 7 common alleles were identified in this locus. The distribution of their frequencies was consistent with Hardy-Weinberg equilibrium. The frequencies are; ?0 — 0.074; ? — 0.116; ? — 0.148; ? — 0.319; D — 0.130; ? — 0.148; F — 0.065. 25 different genotypes were observed, with the most frequent CC and CE (about 10% each). Allele sizes were determined (in base pairs); A0 — 308; A — 300; B — 288; C — 272; D — 268; ? — 252; F — 248. Observed heterozigosity was — 0.75, predicted value was 0.814. Probability of random match (pM) = 0.057. Power of discrimination (pD) = 0.943. Mean exclusion chance (W) = 0.813. Polymorphism information content (PIC) = 0.726. Our results suggest that this locus is highly informative and may be used in various fields of applied genetics.

P1303. Tsp509I-D1S80 subtypes frequency distribution in Romanian population

A. A. Ceacareanu
Cantacuzino Institute; Bucharest, Romania
alice_ceacareanu@yahoo.com

The D1S80 locus is one of the best known polymorphic loci, which shows a variable number of tandem repeats, exhibiting high heterozygosity in most populations and an associated high number of alleles. In the present work, we considered in parallel the frequencies of D1S80 alleles and the polymorphism of their subtypes generated by the presence of Tsp509 I restriction site in the 5« flank. A number of 74 genotypes were found among the 193 individuals. The most common genotypes in the total samples seems to be; 18-24 (17%), 24-24 (8.3%) and 18-18 (5.5%). The identified Tsp509I-D1S80 subtypes presented frequencies between 0.0024 and 0.2600, the most present were the subtypes 24+, 18-, 25+ and 31-. The observed heterozygosity of D1S80 alleles and of D1S80-Tsp509I subtypes was 0.84 and, respectively 0.87. Concordant to the constructed phylogenetic tree (DISPAN ? Genetic Distance and Phylogenetic Analysis program) on D1S80 allele frequencies, Romania is included into the cluster containing Slovenia, Russia and Belarus.

P1304. Transforming growth factor beta one (TGF b1) polymorphism and Dupuytren s disease

A. Bayat[1], *A. Alansari*[1], *M. Shah*[1], *J. S. Watson*[2], *J. K. Stanley*[1], *M. W. J. Ferguson*[1], *W. E. Ollier*[1]
[1]*Hand Surgery Units, Wrightington Hospital, Wigan & Withington Hospital; Department of ARC and Cells, Immunology and Development, University of Manchester; Manchester, United Kingdom;* [2]*Hand Surgery Units, Wrightington Hospital, Wigan & Withington Hospital; Department of ARC and Cells, Immunology and Development, University of Manchester; Macnhester, United Kingdom*
ardeshir.bayat@man.ac.uk

Dupuytren s disease (DD) is a benign fibroproliferative tumour of unknown cause. It is a familial condition and commonly affects Northern European Caucasian men. Genetic studies have yet to identify the genes involved in DD formation. Transforming growth factor beta one (TGF-b1) is a multifunctional cytokine, which plays a central role in wound healing and fibrosis. TGF-b1 has been shown to stimulate fibroblast proliferation and extracellular matrix deposition. Previous immunohistochemical studies have implicated TGF-b1 in DD. In the light of above evidence, it is feasible that TGF-b1 is a candidate gene in the pathogenesis of DD. The aim of this study was to investigate the association of four common single nucleotide polymorphisms in TGF-b1 with the risk of DD formation. A polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used for genotyping TGF-b1 polymorphisms. DNA samples from 135 DD patients and 199 controls were examined. There was no statistically significant difference in TGF-b1 genotype or allele frequency distributions between cases and controls for the codons 10, 25 and, -509, -800 polymorphisms. These results suggest that common TGF-b1 polymorphisms are not associated with a risk of DD formation. However, these data should be interpreted with caution as the lack of association was only shown in one cohort of cases (n=135) with only known common polymorphisms of TGF-b1. We are presently increasing our sample size and looking for novel polymorphisms in the TGF-b1 for further investigations.

P1305. Superoxide Dismutase (SOD) as a genetic marker for rheumatic diseases

P. Mokkapati[1], *P. Arumugam*[1], *R. Babu*[2], *P. Nallari*[1]
[1]*Osmania University, Department of Genetics; Secunderabad, India;* [2]*King Koti Hospital; Hyderabad, India*
pariplavi@eth.net

Rheumatic disorders,clinically and genetically heterogeneous in nature, affect all ages, sexes and ethnic groups. The commonest forms are Rheumatoid Arthiritis (age of onset-3rd-5th decade), Juvenile Chronic Arthiritis (age of onset < 16 years)and Osteoarthrosis (age of onset > 40 years).Our study aims at identifying polymorphic variations of superoxide dismutase (SOD) in the local population and with reference to arthiritis. Blood samples from 136 patients suffering from rheumatoid arthiritis, juvenile arthritis and osteoarthritis (54,16,and 66 respectively) referred from King-Koti Hospital Hyderabad were analysed alongwith 133 age and sex matched controls for phenotyping SOD following PAGE of red cell-membrane. The frequency of allelic distribution,Hardy-Weinberg equilibrium(HWE) and test for association were studied in the individual groups and as a whole and compared with the controls. The haplotype frequencies of alleles 2 and 1 of SOD were 0.656 and 0.344 (controls); 0.740 and 0.259 (rheumatoid arthritis); 0.787 and 0.212 (osteoarthrosis) and 0.781 and 0.219 (juvenile chronic arthiritis) respectively, with a significant deviation from the HWE in the arthritic group compared to the control group. Individuals of 2-2 phenotype were found to be at a higher risk for arthritis compared to the control group. They were also at a higher risk compared to the 1-1 and1-2 phenotypes within the arthritic group. Our findings strongly suggest that SOD phenotyping can be used as a genetic marker for risk prediction in rheumatoid arthritis and to help formulate counseling strategies.

P1306. Polymorphism of Alpha-1 Antitrypsin (AAT) and association of alleles with Duodenal Ulcer (DU)

P. Nallari[1], *S. Sulekha*[2], *P. Mokkapati*[2]
[1]*Osmania University; Secunderabad, India;* [2]*Osmania University; Hyderabad, India*
prathinallari@yahoo.com

Duodenal Ulcer (DU) a common structural disorder of the gastrointestinal mucosa was found to result due to an imbalance between the aggressive and defensive mechanism. Since studies so far have clearly pinpointed the role of genetic factors in the etiology of DU, the present study aims at identifying the polymorphic variation of AAT in the 173 healthy individuals and 210 endoscopically confirmed cases of DU. Sourcing samples from these individuals was phenotyped for AAT following PAGE and immunoblot techniques. Increased preponderence of Z & S alleles in the disease group compared to control group was observed. A significant deviation from the Hardy-Weinberg equilibrium was observed with respect to the gene and genotypic frequencies in comparison to controls. The relative risk estimates also revealed that homozygous ZZ and SS genotypes were at an increased risk for ulceration, suggesting that deficiency of serum AAT in DU cases as reported earlier could be accounted by the S & Z alleles in the disease group, because of point mutations

P1307. Nonsyndromic Xlmr With Inheritance Through A Normal Male

H. A. Lubs[1], *B. Hane*[2], *J. Collins*[2], *G. Arnold*[3], *J. F. Arena*[1], *D. A. Cabezas*[1], *R. E. Stevenson*[2], *C. E. Schwartz*[2]
[1]*University of Miami; Miami, FL United States;* [2]*J.C. Self Research Institute, Greenwood Genetic Center; Greenwood, SC United States;* [3]*University of Rochester; Rochester, NY United States*
hlubs@peds.med.miami.edu

A large five-generation family with nonsyndromic X-linked mental retardation (XLMR), has been mapped to the pericentric region at Xp11-q21. Twenty-five of sixty-five family members were studied, including the six living affected males. None of the obligate carriers was retarded. Clinical findings included a small head circumference in affected males and very large testes in two affected adults. Affected males had mild to moderate mental retardation and several had accomplished independent living. Linkage analysis detected linkage to Xq11-q21 with a maximum Z=1.69 at DXS1216 with a q=0.00. Multipoint linkage analysis by GENEHUNTER yielded a maximal Lod score of 3.84 in a 30 cM region between DXS1199 (Xp11) and DXS986 (Xq21).. Oligophrenin I is a known XLMR gene within this region and mutational analysis is in progress. Most interestingly, the haplotype analysis indicated that the mutation must have been transmitted through the founding male in generation I. This family, therefore, represents the first instance of nonsyndromic XLMR in which there was inheritance through a normal male. The Fragile X Syndrome is the only other known XLMR disorder with inheritance through a normal male.

P1308. A genome scan for loci influencing anti-atherogenic serum bilirubin levels

F. Kronenberg[1,2], *H. Coon*[1], *A. Gutin*[3], *M. E. Samuels*[3], *D. G. Ballinger*[3], *P. N. Hopkins*[1], *S. C. Hunt*[1]
[1]*Cardiovascular Genetics; Salt Lake City, UT United States;* [2]*Institute of Medical Biology and Human Genetics; Innsbruck, Austria;* [3]*Myriad Genetics, Inc.; Salt Lake City, UT United States*
Florian.Kronenberg@uibk.ac.at

Epidemiological studies have shown an association of decreased serum bilirubin levels with coronary heart disease. Two segregation analyses in large pedigrees have suggested a major gene responsible for high bilirubin levels occurring in about 12% of the population. Based on a recessive model from segregation analysis of 1967 individuals in 50 Utah pedigrees, we performed a genome scan using 589 markers to detect loci significantly linked to bilirubin levels. Two regions suggestive for linkage were found by multipoint linkage analysis. The first region is on chromosome 2q with a maximum multipoint LOD score of 3.2 for marker D2S1363. This region contains a previously described gene, uridine diphosphate glycosyltransferase 1, which has been associated with high bilirubin levels. A second region was found on chromosome 18 with marker D18S818 having a maximum multipoint LOD score of 2.7. These results provide evidence that loci

influencing bilirubin variation exist on chromosomes 2q and 18p.

P1309. Linkage and association of HLA class II genes with Vitiligo in Dutch population

M. Zamani[1], *M. Spaepen*[2], *S. S. Asghar*[3], *C. H. Huang*[2], *W. Westerhof*[4], *L. Nieuweboer-Krobotova*[5], *J. J. Cassiman*[2]
[1]*Center for Human Genetics, University of Leuven, Leuven, Belgium; Department of Human Genetics, School of Public Health, Tehran University of Medical Sciences; Tehran, Islamic Republic of Iran;* [2]*Center for Human Genetics, University of Leuven; Leuven, Belgium;* [3]*Department of Dermatology, University of Amsterdam, Academisch Centrum; Amsterdam, The Netherlands;* [4]*Department of Dermatology, University of Amsterdam, Academisch Centrum; Netherlands Institute for Pigmentary Disorders; Amsterdam, The Netherlands;* [5]*Netherlands Institute for Pigmentary Disorders; Amsterdam, The Netherlands*
Mzamani@excite.com

Family based and case-control association studies and linkage disequilibrium analysis were performed on 50 Dutch vitiligo families (150 individuals) and 204 healthy controls. They were genotyped for HLA- class II genes by PCR and sequence specific oligonucleotides (SSO s). Both linkage disequilibrium and case-control analysis showed the linkage and association of DRB4*0101 allele with vitiligo (pc = 0.0016, relative risk =2.21). The family-based association study also provided evidence for linkage and association of DQB1*0303 allele with vitiligo disorder (l2 = 7.36, p=0.006). We measured the clinical relevance of the test by calculating prevalence corrected positive predictive values (PcPPV) of test. The PcPPV of disease for DRB4*0101 allele was 0.017 and for DRB4*0101/0101 genotype was 0.0358. Which means that when a random person has DRB4*0101/0101 genotype who has a 3.58% risk to develop vitiligo. Taking all together, our results show that both DRB4*0101 and DQB1*0303 alleles are provide significant susceptibility for vitiligo disorder and able to explain findings of other studies on the association of the HLA class II with vitiligo.

P1310. Secondary Merosin Deficiency CMD Unlinked to Chromosomes 6,9 and 1 in Three Tunisian Families

F. H. Fakhfakh[1], *C. Triki*[2], *N. Louhichi*[1], *M. Meziou*[2], *H. Ayadi*[1]
[1]*laboratoire de genetique moleculaire humaine; Sfax, Tunisia;* [2]*service de neurologie chu H.Bourguiba; Sfax, Tunisia*
f.fakhfakh@gnet.tn

Congenital muscular dystrophies(CMD)are a heterogenous group of muscular disorder characterised by an autosomal recessive mode of inheritance.The CMD have been classified according to the involvment of the brain and the eyes.It is apparent that defects in different genes are responsable for each type.A deficiency of the alpha 2 chain of laminine 2(Merosin)occurs in about 40-50% of cases with the classical form of CMD.It is a primary phenomenon caused by defects in the gene for merosin(LAMA2)on chromosome 6q22.Recently a group of new separate entities of CMD have been reported.It encompasses unclassified CMD forms with merosin deficiency with or without structural brain abnormalities,mental retardation, absence of severe eye defects and unlinked to chromosomes 6,9 and 1.We have studied 3 cases affected with CMD and belonging to threeTunisian families. These patients had benefied from complete clinical investigation. Immunohistochemical and western blot analysis on muscles biopsies was performed using monoclonal antibodies against human merosin (80 and 300 fragments).Linkage analysis was undertaken using microsatellite markers spanning LAMA2locus on 6q22,FCMD locus on 9q31,MEB locus on 1p32 and CMD1B locus on 1q42.Clinical investigation of the three patients showed severe motor delay and moderate mental retardation in all cases and calf hypertrophy in one case and pontocerebellar hypoplasia associated to cerebellar cysts and white matter abnormalities in the other cases.Immunohistochemical and western blot analysis revealed a partial merosin deficiency in all cases.DNA analysis exclude any linkage to chromosome6,9 and1.In conlusion, these patients showed a CMD form with secondary merosin deficiency unlinked to chromosomes 6,9 and 1.

P1311. Microsphere-based readout technology for high throughput multiplexing SNP analysis

Y. He, *J. Chen, M. Weiner, P. Rivers, H. King, D. Taylor, Q. Nguyen, M. Wagner, M. Li, F. Ye, D. Briley*
GlaxoSmithkline; RTP, NC United States
yh42558@glaxowellcome.com

A rapid, multiplexing, high throughput read-out technology for single nucleotide polymorphism (SNP) analysis was developed employing both flow cytometric analysis of fluorescent microspheres and enzyme-based allele detection. An array of fluorescent microspheres (each population of microspheres is identified by its unique profile of red and orange fluorescence) was coupled to unique DNA oligonucleotide sequences termed complementary ZipCodes (cZipCodes). Allele detection was based on either single-base chain extension assay (SBCE) where a synthetic capture oligonucleotide probe was designed to contain both a ZipCode sequence at the 5 end and an SNP-specific sequence at the 3 end. A DNA polymerase adds a fluorescently labeled nucleotide to the capture oligonucleotide which was then hybridized to its cZipCode on the microsphere. Flow cytometric analysis of the microspheres simultaneously identified both the microsphere type and the fluorescent signal associated with the SNP genotype. This system is automated by the integration of many pieces of unique equipment for HTP SNP analysis.

P1312. 99 Genome-wide searches for genes predisposing to complex human diseases between 1993 and 2000; Comparison of study-design and number of probands

J. Altm ller, *G. Fischer, M. Wjst*
Institut f r Epidemiologie; Oberschlei§heim, Germany
altmueller@gsf.de

Extensive efforts have been made in recent years to search for predisposing genes of complex diseases using genome-wide scans, but only very few genes were identified yet. The purpose of this study was to analyse the data of 99 genome-wide scans done between 1993 and 2000 found by medline. In addition to statistical models and threshold definitions, trends in the study-design reflect the experiences of real studies and will be helpful for further research. All scans used microsatellite markers. The number of investigations increased rapidly in the observation time (from 6 in 1993/1994 to 45 in 1999/2000). With regard to year of publication, quantitative and qualitative traits (1;6) showed a proportionally equal increase as did the relation of isolated and non-isolated populations (1;5). The investigation of sibpairs increased steadily in the last years while the affected relatives and extended pedigrees design decreased. The most remarkable observation is a shift in the number of probands per study from average 323 in 1993-1996 (n=18) to 456 in 1999/2000 (n=38). No conclusions can be derived from these results, for example which study-design proves to be superior, because neither the investigation of isolated populations nor the calculation with quantitative traits has shown advantages in gene localisation. The experiences of the last 8 years, however, lead to the insight, that an investigation of far more families per study is necessary to identify predisposing genes.

P1313. New insights on the advantages of isolated versus outbred populations for the genetic dissection of complex traits

A. Darvasi[1], *S. Shifman*[2]
[1]*The Hebrew university of Jerusalem, idGENE Pharmaceuticals Ltd.; Jerusalem, Israel;* [2]*The Hebrew University of Jerusalem; Jerusalem, Israel*
arield@cc.huji.ac.il

Much attention has recently been focused on the relative values of isolated and outbred populations in identifying genes affecting complex traits. In particular, the extent of linkage disequilibrium (LD) in outbred and isolated populations was recently studied. In those studies it has been suggested that on average there is little difference between isolated (Finns and Sardinians) and outbred populations (UK, US and CEPH samples).
We have explored this question by looking at Ashkenazi Jews, a known isolated population and also by re-examining the results presented in Taillon-Miller et al. (2000). As anticipated and previously reported, for SNPs at distances up to 200kb there is no significant difference between the levels of LD for isolated and outbred populations. However, when the distance between SNPs was greater than 200kb, the level of LD (represented by r^2) was higher in the Finnish, Sardinian and Ashkenazi samples by 4.7, 6.1 and 7.0 fold, respectively. It should be noted that r^2 is proportional to sample size required to identify a specific gene. Thus in some instances a five to sevenfold increase in sample size might be required if outbred samples are used as compared to an isolated population.
Independent of the extent of LD, isolated populations have other important advantages, mainly in reducing genetic heterogeneity which can significantly increase genotypic relative risk and hence increase statistical power. Consequently, in some instances the use of isolated populations will allow the discovery of genes that could not be discovered otherwise.

P1314. Use Of Isolated Inbred Human Populations For The Study Of Complex Traits; The Carlantino Project

P. Gasparini[1], *P. Palancia*[2], *F. Lo Vecchio*[1], *M. Villella*[1], *A. Villella*[3], *M. Urbano*[2], *M. Di Giovine*[1], *A. D Adamo*[1], *P. Stanziale*[1], *F. Bertoldo*[4], *L. Zelante*[1]

[1]*IRCCS Hospital CSS; San Giovanni Rotondo, Italy;* [2]*ASL FG3; Carlantino, Italy;* [3]*Lucera Hospital; Lucera, Italy;* [4]*Verona University; Verona, Italy*

genetcss@fg.nettuno.it

The use of isolated inbred populations to reduce disease heterogeneity of complex disorders has already proved to be useful since it is expected that an association can be detected with a smaller sample of patients in an inbred population than in a panmictic one. We identified a small village, named Carlantino, characterized by 1417 inhabitants and located in Southern Italy. The village has been settled 5 centuries ago by few founders, and during last century the endogamy has been calculated to be 99.5%. Three different surnames accounts for the majority of the living people. In addition birth registers are available from 17th century, and in a more detailed way from 19th century. The project is characterized by a full clinical examination of all individuals including anamnesis, blood pressure measurements, electrocardiogram and bone mineral density evaluation, a clinical chemistry evaluation (blood count plus 20 different biochemical parameters), a development of DNAs and sera banks. The first part of the project has been completed since a) the whole population has been already enrolled in the project and sampled, b) the DNA and sera banks have been already established, c) the inclusion of all the data (historical, clinical, biochemical and instrumental) in the database has been finished. Preliminary epidemiological data suggest an increased frequency of osteporosis, hypercolesterolemia, hypertension, heart stroke pedigrees, cancer families, and miopia. Construction of DNA haplotypes of Y and X chromosomes, and of mithocondrial DNA indicates the presence of 6 couples as founders

P1315. The feasibility of conducting whole-genome scans to search for multiple low-penetrance cancer susceptibility genes

A. M. Dunning, *F. Durocher, M. D. Teare, C. S. Healey, F. Carlomagno, D. F. Easton, B. A. J. Ponder*

University of Cambridge; Cambridge, United Kingdom

alisond@srl.cam.ac.uk

Presently, association studies, used to find low-penetrance cancer susceptibility alleles, can only be performed in candidate genes. Ultimately the aim is to conduct genome-wide scans to find numerous loci in one experiment, using markers (SNPs) spaced throughout the genome. The success of such scans will be critically dependent on their design. We have examined the extent of linkage disequilibrium (LD) in relation to physical distance using 38 SNPs in 3 autosomal genomic regions and 4 different European populations. We find a similar inverse relationship between LD and distance in each region and in each population. Although statistically significantly LD exists between marker-pairs >200Kb apart, only 50% of marker-pairs at <5Kb display sufficient LD to be useful for association studies. These results indicate that a genome-wide scan would require SNPs spaced at least every 5Kb, or 600,000 markers per scan. Previous results, suggesting more extensive LD may be the result of biases due to small sample sizes. However, the estimated 40,000-100,000 genes in the human genome will be sequenced by 2003. It might be more feasible to conduct a scan solely using SNPs in genes, particularly since there is some evidence that LD may be more extensive within genes than between them. We are examining the extent of LD between SNPs within different genes and will compare this with the LD observed in random genomic regions.

P1316. Haplotype analysis in 5q31 indicates the discrete nature of linkage disequilibrium

M. J. Daly, *J. D. Rioux, E. S. Lander, S. Schaffner*

Whitehead Institute/MIT Center for Genome Research; Cambridge, MA United States

mjdaly@genome.wi.mit.edu

A major obstacle to the systematic analysis of SNP variation and its association to phenotype (even across relatively small genomic regions) is the lack of an analytic framework for interpretation of population haplotype patterns (linkage disequilibrium). Through the development of new mathematical approaches, we show here a comprehensive exploration of the patterns of variation across ~350 kb in the 5q31 region as reported in an accompanying abstract (Rioux, et. al.). We observe surprisingly limited haplotype diversity across significant distances discretely punctuated by sites of multiple historical recombinations. This leads us to propose a hypothesis for the nature of haplotype variation across short genomic regions (10s-100s of kb) and a model for interpreting and using such variation that may provide a powerful framework for future genetic studies.

P1317. A Genetic Hypothesis for Chiari Type 1 Malformation with or without Syringomyelia. Speer MC, George TM, Enterline DS, Franklin A, Wolpert CM, Milhorat TH

M. C. Speer

Duke University Medical Center; Durham, NC United States

marcy@chg.mc.duke.edu

The Chiari 1 malformation (CM1) is defined as herniation of the cerebellar tonsils through the foramen magnum, but recently we and others have proposed a volumetrically too small posterior fossa as causative. CM1 is the leading cause of syringomyelia (S). We propose a genetic hypothesis to at least a subset of CM1/S cases based on familial clustering in a rare disease, co-segregation with known genetic syndromes, and concordance in like-sex twins. We have identified 67 multiplex pedigrees. Some families demonstrate male-to-male transmission and transmission across more than two generations. Additional support for a genetic hypothesis for CM1/S comes from co-segregation of CM1 with at least 18 known genetic syndromes including, among others, achondroplasia, Klippel Feil sequence, Hadju-Cheney syndrome, Albright hereditary osteodystrophy (pseudohypoparathyroidism), hypophosphatemic rickets, Williams-Beuren syndrome, and renal-coloboma syndrome. Lastly, we identified 8 sets of like-sex twins in our family ascertainment efforts in CM1/S (7 female; 1 male); 7 of 8 are concordant for CM1 (one female twin pair is discordant for CM1). 3 are concordant for associated syringomyelia. One unlike-sex twin pair includes a female affected with CM1/S and a male unaffected. Provisional determinations of ls range from 4-21 across a broad range of prevalence estimates. We hypothesize that the underlying gene or genes for CM1/S will have pleiotropic effects influencing the extent of tonsillar herniation, posterior fossa volume, and/or other variables such as bony abnormalities in the base of the skull and/or syringomyelia. These data are consistent with a genetic hypothesis in at least a subset of CM1/S.

P1318. Familial Pulmonary Fibrosis in the USA.

M. M. Wahidi, *M.Speer, M. Steele, K. K. Brown, M. I. Schwarz, D. A. Schwartz*

Duke University Medical Center; Durham, NC and NJC Denver, CO, United States

marcy@chg.mc.duke.edu

Pulmonary fibrosis is rare form of lung disease that affects the interstitium of the lungs and often fatal within 3-5 of diagnosis. Families with at least two members affected by PF are recruited through a network of collaborators and advertisement on the Internet (www.fpf.duke.edu). Data on affected members includes a standardized clinical history and risk factor questionnaire, pedigree, DLCO, CXR, HRCT, and lung biopsy (when available). Asymptomatic relatives are screened with a questionnaire, DLCO, and CXR. Thirty families including 108 affected individuals have been identified. Males were more frequently affected than females (55%). The predominant race was Caucasian (94% Caucasian, 6% Hispanic). Smoking was identified in 42 % of cases with available data. Exposure to known fibrotic agents such as asbestos, silica, or wood dust was recorded in 19% of cases. Mean age at diagnosis was 59 (sd=15.8) and mean age of death was 61 (sd=15.5). The diagnosis of PF was confirmed by an open lung biopsy or autopsy in 24 patients (22%). A high resolution CT scan of the chest was obtained in 47 patients (43%). In the 24 families with at least 2 documented cases, PF occurred in multiple family members in 15 families. Ten families have at least one affected sibling pair, 4 have affected parent-child pairs including one pair of affected father and son, and 4 pedigrees include more distantly related affected relative pairs. The familial aggregation documented in these families is consistent with a genetic basis in at least a subset of PF cases.

P1319. European Twin and Sibling study; Genetic modulators of Cystic Fibrosis

M. Ritzka[1], *F. Mekus*[1], *U. Laabs*[1], *H. Veeze*[2], *B. T mmler*[1]

[1]*Medizinische Hochschule; Hannover, Germany;* [2]*Erasmus University; Rotterdam, The Netherlands*

ritzka.margit@mh-hannover.de

A major purpose of the European Twin and sibling study is the search for genetic modifiers for Cystic Fibrosis (CF) apart from the basic defect in the CFTR gene. Evaluation of the clinical status of 114 ˇF508 homozygous twins and sibpairs recruited from CF centres of 14 European countries revealed major variabilities in the clinical phenotype of this homogenous group (referred to their CF genotype), that cannot be due to environmental factors only. We searched for genetic modulators of disease by a pragmatic hypothesis driven genome wide association study in highly informative sib pairs who were ranked according to their clinical phenotype beforehand. In selecting candidate genes, we concentrated on three classes; First, there are molecules that could compensate for the basic defect. As

CFTR is an chloride channel and regulator of ion channels, alternative ion channels and regulators thereof are included in this group. Second, genes which mediate an increased susceptibility to airway infections are suitable candidates. We concentrate on genes mediating the innate and systemic immune response. The third group was chosen for practical clinical reasons; Genes regulating the metabolism of xenobiotics might have a large effect on the individual long range therapy success. Therefore, pharmacogenomics are a major subject of our study. In this poster, we provide information about the candidate regions and genes examined so far.

P1320. Cystic Fibrosis as a Genetically Complex Disease

F. Mekus[1], *M. Ballmann*[1], *I. Bronsveld*[2], *J. Bijman*[3], *H. Veeze*[3], *B. T mmler*[1]
[1]*Medizinische Hochschule Hannover; Hannover, Germany;* [2]*Sophia Children s Hospital; Rotterdam, The Netherlands;* [3]*Erasmus University; Rotterdam, The Netherlands*
Mekus.Frauke@MH-Hannover.de

The clinical phenotype of Cystic Fibrosis (CF) is characterised by a broad spectrum of disease severity and variation of the clinical course. The frequency of one disease-causing allele, i.e. deltaF508-CFTR, allows the recruitment of a sufficiently large number of patients with identical CFTR genotype in order to study the cause of CF disease variability that is unrelated to the major disease-causing lesion. The European CF Twin and Sibling Study collected data from 158 CF clinics from central European countries for a cohort of 277 sibling pairs, 12 pairs of dizygous twins and 29 pairs of monozygous twins. Monozygous twins were significantly more concordant than dizygous patient pairs. We could demonstrate that shared factors in twins and siblings outweigh the individual factors for anthropometry, but not for the pulmonary status (Twin Research 2000, in press). The search for inherited factors that modulate CF equals an assessment of CF disease severity as a quantitative trait. Under this condition, individuals with extreme phenotypes are most informative. To identify the extreme phenotypes among our cohort of 114 deltaF508 homozygous patient pairs, a computer-assisted method was executed to rank patient pairs within the categories concordant mild disease (CON+), concordant/severe disease (CON-) and discordant (DIS). We characterised these three patient pair cohorts as phenotypically distinct entities with respect to pulmonary function and nutritional state of the CF patients. An on-going association study in these informative deltaF508 homozygous sibpairs with extreme phenotypes has identified several chromosomal regions that encode modulators of basic defect and clinical phenotype.

P1321. Spontaneous attenuation of basic defect in inbred Cftr(tm1Hgu) CF mutant mice.

N. Charizopoulou[1], *S. Jansen*[2], *R. Farley*[3], *E. W. Alton*[3], *J. Dorin*[4], *H. J. Hedrich*[5], *B. T mmler*[2], *M. Dorsch*[5]
[1]*Institute for Laboratory Animal Science, Medical School, Klinische Forschergruppe, Medizinische Hochschule; Hannover, Germany;* [2]*Klinische Forschergruppe, Medizinische Hochschule; Hannover, Germany;* [3]*Ion Transport Laboratory, National Heart and Lung Institute; London, United Kingdom;* [4]*Medical Research Council Human Genetics Unit, Western General Hospital; Edinburgh, United Kingdom;* [5]*Institute for Laboratory Animal Science, Medical School; Hannover, Germany*
Charizopoulou.Nikoletta@MH-Hannover.DE

The basic defect in cystic fibrosis relates to abnormalities of ion transport in affected tissues, such as the respiratory and gastrointestinal tracts. A transgenic mouse model, *Cftr*^{tm1Hgu}/*Cftr*^{tm1Hgu} was generated following targeted insertional mutagenesis into exon 10 of the murine *Cftr* gene in embryonal stem cells. These mutant mice differ from other mutant mice that although they displayed the characteristic CF electrophysiological defects, they produced low levels of wt cftr mRNA. Using 4 of these animals as a starting population, we have generated four inbred (over more than 20 generations) *Cftr*^{tm1Hgu}/*Cftr*^{tm1Hgu} mutant strains (CF/1, CF/2, CF/3, CF/4). Surprisingly, all four strains did not display the expected electrophysiological defect of their progenitors, but NPD measurement revealed that these mice had shifted towards a normal range in respect to their Cl^- ion conductance across their airway epithelia. Furthermore, in respect to their wt cftr mRNA CF/1 had a residual activity of ~10%, in contrast with the remaining three strains which had a residual activity of less than 1%. Hence, these mice must have different pathway(s) for rescue which at the same time result in a uniform phenotype. A genome wide scan has been initiated in order to identify the modulators responsible for this compensation. This study is in synergy with the CF twin and sibling study which aims to identify the genetic factors, apart from the *CFTR* gene itself, which modulate the disease and are responsible for the variation of the clinical picture.

P1322. Mutations in the CFTR and SPINK1 genes in patients with idiopathic pancreatic disease

M. Stuhrmann, *J. Ockenga, M. P. Manns, T. D rk*
Medizinische Hochschule; Hannover, Germany
stuhrmann.manfred@mh-hannover.de

Up to 30% of the patients with idiopathic pancreatitis (IP) carry a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Recently, mutations of the serine protease inhibitor Kazal type 1 (SPINK1/PSTI) gene have been identified to be associated with IP. Mutations in both the CFTR and SPINK1 genes are very likely to act as predisposing or disease modifying genetic factors in IP. To elucidate whether double heterozygosity of CFTR and SPINK1 mutations may be responsible for IP, we investigated 24 patients (12 female, 12 male, mean age at disease onset 27 years) with adult onset of IP. We screened genomic DNA for the presence of 40 mutations and variants in the CFTR gene. In addition, the four exons of the SPINK1 gene were investigated by SSCP analysis and sequencing. Seven patients (28%) had at least one abnormal CFTR allele, as compared with an expected frequency of 4% in the general German population. Two patients were compound heterozygous. Mutations in the SPINK1 gene were found in two patients, one of which was homozygous for the known predisposing mutation N34S, while the second patient was heterozygous for the new SPINK1 mutation R65Q. The latter patient was also heterozygous for the CFTR mutation Y1092X. However, segregation analysis revealed that the patients healthy mother and sister as well carried both the CFTR and SPINK1 mutations. Hence, a pathogenic role of double heterozygosity for SPINK1 and CFTR gene variations could not be confirmed in this family, but deserves further investigation in larger cohorts

P1323. Multiplex Family Autism Research Resource

R. T. Johnson[1], *A. Mars*[2], *J. C. Beck*[1]
[1]*Coriell Institute for Medical Research; Camden, NJ United States;* [2]*Robert Wood Johnson Medical School; New Brunswick, NJ United States*
rjohnson@cimr.umdnj.edu

A genetic resource of DNA samples to support the study of autism in families where more than one child is affected or where one child is affected and one demonstrates another significant and related developmental disorder has been built at the Coriell Institute for Medical Research in collaboration with the clinical services at the University of Medicine and Dentistry Robert Wood Johnson Medical School. An open bank of anonymously collected materials documented by a detailed clinical diagnosis forms the basis of this growing database of information about the disease. Three criteria were used to assess the autistic phenotype. These include the ADI (Autism Diagnostic Interview), the ADOS (Autism Diagnostic Observational Schedule-Generic), and the DSM IV Diagnostic Criteria for 299.00 Autistic Disorder. All clinical interviews were conducted face-to-face. For each donor subject tested, a representative Autistic Diagnostic Criteria Score Sheet used to collect data is provided. Currently, the resource contains 20 families in which 41 individuals have been examined. Thirty-three of these individuals have a diagnosis of autistic disorder by two or more criteria; 28 of these satisfy all three criteria. There are 20 affected sib pairs with a diagnosis of autistic disorder by two or more criteria; 9 of these pairs meet the criteria for autistic disorder by all three criteria. Further information about this resource, including information on ordering, can be found at http;//locus.umdnj.edu/autism or by contact with the Coriell Cell Repositories.

P1324. Genome linkage analysis using affected sibling pairs for autism

G. H. Barnby[1], *I. M.G.S.A.C*[2]
[1]*The Wellcome Trust Centre for Human Genetics,University of Oxford; Oxford, United Kingdom;* [2]*http;//www.well.ox.ac.uk/~maestrin/iat.html; London, United Kingdom*
gaby@well.ox.ac.uk

Autism is a severe neurodevelopmental disorder characterised by deficiencies in three domains; reciprocal interaction, communication and stereotyped/repetitive behaviour. Evidence for a strong genetic basis for the disorder stems from twin studies and the increased rate of autism in siblings of autistic probands compared to the population prevalence. IMGSAC have previously published a whole genome screen for 36 sib-pairs, with a subset of 175 markers typed in a further 49 sib-pairs and 3 sibling trios. Five regions were identified with multipoint Maximum Lod Score (MLS) > 1 (chromosomes 4, 7, 10, 16 and 22). These regions have been characterised further and the results are presented here along with new findings. A whole genome screen has now been carried out for 86 sib-pairs using 394 microsatellites. A subset of 119 markers have been typed in 153 sib-pairs in 12 regions giving a multipoint MLS above 0.80. Three

chromosomes generated a multipoint MLS above 3 after 153 sib-pairs had been genotyped, 2q, 7q and 16p. Chromosome 2 gave the highest result with a multipoint MLS of 3.58 at D2S2188 followed by chromosome 7 at D7S477 with a multipoint MLS of 3.37 and then chromosome 16 with a multipoint MLS of 3.08 at D16S3102. Analysis of linkage data on the basis of sib-pair proband status has been carried out and the parental origin of alleles contributing to linkage was also investigated across the regions studied.

P1325. Multy - Disciplinary Evaluation Of Possible Aetiology Factors In Autism, Hyperactivity Disorder And Specific Language Impairment

S. Nikolic, *S. Brankovic-Niksic, G. Radivojevic-Pilic*
Institute of Mental Health; Belgrade, Yugoslavia
nbrankov@eunet.yu

Recent studies suggest that those complex diseases likely result from several different aetiologies or a combination of pathological mechanisms. The search we performed is based on the comprehensive multy - disciplinary evaluation which consisted of psychiatric examination of the child, speech and language evaluation, psychological testing and behaviorial assessment. Those patients who met the ICD - 10 criteria for autism, hyperactivity disorder and specific language impairment and had a full scale IQ under 70 performed a highly defined group (No 157) in which we undertook a cytogenetic analyses. A systematic obstetric and developmental complications data family history, and environmental factors were also obtained. Different medical conditions may be found in the developmental histories of patients with those complex diseases, and we proposed some important distinction versus control group (No 55; healthy infants followed up five years after amniocentesis). On the contrary, we found no scientific occurrence of chromosome abnormalities among probands in comparison with matched control group. The objective of this review is to summarize our findings of the specifically contributing factors and their implications on the aetiology of those complex diseases.

P1326. Molecular analysis of t(5;7) in a patient with autism

D. Tentler[1], *G. Anner n*[2], *C. Gillberg*[3], *C. Betancur*[4], *G. Brandberg*[5], *N. Dahl*[1]
[1]*Dep. of Genetics and Pathology, Uppsala University; Uppsala, Sweden;* [2]*Dep. of Genetics and Pathology, Uppsala University; Uppsala, Sweden;* [3]*Child Neuropsychiatry, Sahlgren University Hospital; G teborg, Sweden;* [4]*INSERM U. 513, Facult de M decine de Cr teil; Paris, France;* [5]*Falun County Hospital; Falun, Sweden*
dmitry.tentler@genpat.uu.se

The results of several linkage studies have provided support for an autism susceptibility locus on the long arm of chromosome 7. We report a girl with autistic features carrying a balanced translocation t(5;7)(q14;q34). Fluorescent in situ hybridisation (FISH) analysis with chromosome 7q specific YAC clones showed that the chromosome 7 breakpoint is located between markers D7S684 and D7S661. The region coincides with the candidate region for autism on chromosome 7 from previous linkage studies. A BAC clone of 82 kb spanning the translocation breakpoint was identified, and the breakpoint was mapped to 2 kb region within the BAC. Mutation screening of the 2 genes closest to the breakpoint was performed in a set of 30 autistic patients. We found no sequence variant which predicts aminoacid alteration associated with the phenotype. Two polymorphic nucleotides were identified in one of the genes, and the significance of the polymorphism in autism remains to be clarified. Study of the methylation pattern of the breakpoint region failed to find any difference between patient with the translocation and normal individuals. A positional effect of the translocation is not excluded, and further investigation of the 7q methylation pattern in the patient is in progress.

P1327. Infantile autism - association studies in candidate regions of interest with focus on 7q

S. M. Klauck[1], *K. S. Beyer*[1], *A. Benner*[2], *S. Epp*[1], *F. Poustka*[3], *A. Poustka*[1], *. Imgsac*[4]
[1]*Deutsches Krebsforschungszentrum, Molecular Genome Analysis; Heidelberg, Germany;* [2]*Deutsches Krebsforschungszentrum, Biostatistics; Heidelberg, Germany;* [3]*Department of Child and Adolescent Psychiatry, J.W. Goethe University; Frankfurt, Germany;* [4]*International Molecular Genetic Study of Autism Consortium; http;//www.well.ox.ac.uk/~maestrin/iat.html, United Kingdom*
s.klauck@dkfz.de

Autism is a severe developmental disorder with an onset in early childhood, characterized by marked social deficits, deviant language and a restricted range of stereotyped repetitive behaviors. A genetic etiology is strongly indicated by twin and family studies with a risk to siblings of idiopathic cases which is 75 times greater than the general population prevalence of 4/10,000 births. A two-stage genome search by IMGSAC (1998) identified regions on six chromosomes (4, 7, 10, 16, 19, 22) with the region on chromosome 7q31-q35 as the most significant result (maximum multipoint lod score (MLS) of 2.53). In a follow-up fine mapping of chromosome 7q was carried out by typing 104 additional markers in the interval D7S524 -D7S483. Association studies using linkage disequilibrium analysis in the consortium sib-pair and the German singleton sample provided further support for an autism susceptibility locus on chromosome 7q. In parallel, screening of several neurotransmitter system genes located in other genome regions were performed in the German singleton sample, but did not reveal a clear candidate.

P1328. De novo partial duplication of chromosome 7q in a male patient with infantile autism

K. S. Beyer, *S. M. Klauck, A. Poustka*
Deutsches Krebsforschungszentrum, Molecular Genome Analysis; Heidelberg, Germany
k.beyer@dkfz.de

Autism is a neurodevelopmental disorder with a strong genetic component as shown in family studies. The disease is characterized by impairments in reciprocal social interaction and communication together with restricted and stereotyped patterns of interests and activities. The genetics of the disorder is complex, probably involving the interaction of several genes. The neurobiological basis of the disorder is unknown and there are no strong candidate genes. Several genome screens were undertaken by different autism consortia identifying different regions of interest, defined by a multipoint maximum lod score (MLS) greater than 1. A region on chromosome 7q31-35 was the most significant region (MLS of 2.53) identified first by the International Molecular Genetic Study of Autism Consortium (IMGSAC), which we are part of. Meanwhile four other genome screens were published showing positive linkage on different chromosomal regions. The only common region of all analyses remained on chromosome 7q. One possibility to narrow down the candidate region and to identify new candidate genes are systematic karyotype analyses of autistic patients. One male patient showed a duplication on chromosome 7q in 11% of leukocytes in peripheral blood and in approximately 40% of the nuclei in the corresponding lymphoblastoid cell line. In order to determine the exact localization and orientation of the duplication FISH experiments using several YACs were performed.

P1329. Screening for MECP2 Mutations in Females with Autistic Disorder

R. J. Carney[1], *J. M. Vance*[1], *R. D. Dancel*[1], *C. M. Wolpert*[1], *G. R. DeLong*[1], *C. McClain*[2], *L. von Wendt*[3], *J. R. Gilbert*[1], *S. L. Donelly*[1], *S. A. Ravan*[4], *H. L. Abel*[1], *R. K. Abramson*[4], *H. H. Wright*[4], *H. Y. Zoghbi*[5], *M. L. Cuccaro*[4], *M. A. Pericak-Vance*[1]
[1]*Duke University Medical Center; Durham, NC United States;* [2]*University of New Mexico; United States;* [3]*University of Helsinki; Helsinki, Finland;* [4]*University of South Carolina; United States;* [5]*Baylor College of Medicine; United States*
mpv@chg.mc.duke.edu

Autistic Disorder (AD) is categorized as a Pervasive Developmental Disorder (PDD) in DSM-IV. Rett Disorder (RD) is also a PDD with unique clinical features including microcephaly and loss of purposeful hand movements followed by development of stereotyped, repetitive hand movements. Mutations in the MECP2 gene cause RD. Because of the phenotypic similarity between RD and AD, we screened 69 females in families ascertained for AD gene mapping studies. Patients had a clinical diagnosis of AD, confirmed using the ADI-R. Patients with RD features such as microcephaly were excluded. Patients were screened using DHPLC to detect variants followed by sequencing. Two were found to have mutations in the MECP2 gene. Patient one (age 10 years) developed normally until age 30 months when she regressed in language and motor skills. Head circumference is at the 25th percentile. She did not lose purposeful hand movements and never developed RD stereotyped hand movements. She has a 880C>T, relative to the start of the CDS, nonsense mutation resulting in an R294Xaa change and premature protein truncation. Patient two (age 16 years) developed normally until 18 months when problems in response to social and language stimuli were noted. Head circumference is at the 50th percentile. She never developed RD sterotyped hand movements. She is heterozygous for a 41 bp deletion at 1157-1197del, resulting in a frameshift and protein truncation. Mutations in both patients are de novo. These results indicate the need to screen the MECP2 in all female patients presenting with a diagnosis of AD.

P1330. Alzheimer s disease in a genetically isolated population; the GRIP study.

K. Sleegers[1], *G. Roks*[1], *P. Heutink*[2], *J. C. Van Swieten*[3], *L. A. Sandkuijl*[1], *P. J. L. Snijders*[1], *C. Van Broeckhoven*[4], *B. A. Oostra*[2], *C. M. Van Duijn*[1]
[1]*Epidemiology & Biostatistics; Rotterdam, The Netherlands;* [2]*Clinical Genetics; Rotterdam, The Netherlands;* [3]*Neurology; Rotterdam, The Netherlands;* [4]*Flanders Interuniversity Institute of Biotechnology (VIB); Antwerp, Belgium*
Sleegers@epib.fgg.eur.nl

Genetic factors play an important role in Alzheimer s disease (AD). Mutations in the amyloid precursor protein gene (APP) and the presenilin genes (PSEN1/PSEN2) cause autosomal dominant early-onset AD. The most important genetic determinant for AD in the general population is the apolipoproteinE gene (APOE) which explains about 17% of the occurrence of AD in the general population. Several genome wide studies identified regions of interest of which the chromosome 10 and 12 regions are the most promising. There is increasing interest in identifying genes in genetically isolated populations such as the Finnish and Icelandic populations. In this study we evaluated the feasibility of studying the genetics of AD in a Dutch recently isolated population of 20,000 inhabitants. This population was founded 300 years ago by 150 subjects and is characterized by minimal immigration. We ascertained 74 probable AD patients with a mean onset age of 73 years. The patient population comprised 65% females. Family history in first degree relatives was positive in 58%. We studied genealogy up to 15 generations, which revealed that at least 63 patients (79%) were related within 14 generations. We found no causal mutations in APP, PSEN1, and PSEN2. The APOE e4 allele frequency was 38% which is comparable with Caucasian AD patients (37%). Our study shows that AD in this isolated population is not fully explained by the known AD genes, and given the high percentage of patients with a positive family history other AD genes are to be expected. At present a genomic screen is performed on a cluster of closely related patients. Results on chromosome 10 and 12 will be presented.

P1331. Genomic Screen of 726 Sibpairs with Late-Onset Alzheimer Disease (AD)

M. A. Pericak-Vance[1], *L. R. Bailey*[2], *K. Nicodemus*[1], *D. Hedges*[1], *J. Grubber*[1], *P. T. Xu*[1], *S. Walters*[1], *K. De Sombre*[1], *M. B. Gardiner*[2], *A. Reed*[2], *J. L. Hall*[2], *A. M. Saunders*[1], *A. D. Roses*[1], *G. W. Small*[3], *W. K. Scott*[2], *P. M. Conneally*[4], *J. M. Vance*[1], *J. L. Haines*[2]
[1]*Duke University Medical Center; Durham, NC United States;* [2]*Vanderbilt University Medical Center; Nashville, TN United States;* [3]*UCLA Medical Center; Los Angeles, CA United States;* [4]*Indiana University Medical Center; Indianapolis, IN United States*
mpv@chg.mc.duke.edu

Two relatively small genomic screens (n=54, Pericak-Vance et al., JAMA 1997; 278;1237-1241; n=230 Kehoe et al., Hum Mol Gen 1999; 8;237-245) have been published for late-onset AD. These have identified several potential regions for novel AD genes, including the as yet unidentified chromosome 12 gene. However, the modest sample sizes provided minimal power to detect effects that might arise in only a subset of families. We undertook the largest AD genomic screen to date using 455 families (from DUMC, VUMC, UCLA, IU, and NIMH), a total of 726 affected sibpairs (ASPs). Over ? of these families have not been previously genotyped. We had sufficient sample size to examine the data independently in a subset of autopsy-confirmed (>1 autopsy-confirmed case per family; CONF) families. We used 2-point and multipoint parametric lod score and non-parametric analyses. We identified several potential regions (n=14; MLOD or MLS > 1.00) as locations for novel AD genes. The most interesting novel region is on 9p22 (MLOD = 3.45; MLS=3.30 in the overall and MLOD = 3.97; MLS = 4.42 in the CONF). We also found evidence supporting the recently reported linkage to chromosome 10q22 (MLOD=2.65; MLS=2.12 in the overall and MLOD=2.94; MLS=1.96 in the CONF. Linkage to 9p22 was independent of APOE genotype. These data suggest chromosomes 9p22 and 10q22 contain potential AD risk genes. Stratification by autopsy confirmation strengthened the results of the linkage studies. Haplotype and candidate gene analysis of the novel chromosome 9 region is ongoing and will be presented.

P1332. Genome-wide Linkage Disequilibrium Mapping Of Late Onset Alzheimer s Disease In Finland

M. Hiltunen[1], *A. Mannermaa*[2], *D. Thompson*[3], *D. Easton*[3], *M. Pirskanen*[1], *S. Helisalmi*[1], *A. M. Koivisto*[1], *M. Lehtovirta*[1], *M. Ryyn nen*[4], *H. Soininen*[1]
[1]*Department of Neurology, Kuopio University and Kuopio University Hospital; Kuopio, Finland;* [2]*Department of Clinical Genetics, Kuopio University Hospital; Kuopio, Finland;* [3]*CRC Genetic Epidemiology Group, University of Cambridge, Strangeways Research Laboratory; Cambridge, United Kingdom;* [4]*Department of Obstetrics and Gynaecology, University Hospital of Oulu; Oulu, Finland*
mikko.hiltunen@kuh.fi

Alzheimer s disease (AD) is a complex neurodegenerative disorder, for which several disease-associated loci have been located on various chromosomes. In order to find novel susceptibility genes for late onset AD, we have performed a population based genome-wide search using linkage disequilibrium (LD) mapping. To avoid population stratification, 47 late onset AD patients and 51 age-matched controls were carefully chosen from the same geographical area in Eastern Finland. Initial genome-wide screening with 366 polymorphic microsatellite markers revealed 22 chromosomal loci associated with AD with P-values in the range of 0.05 > P > 0.001. Subsequent comparison of single allele frequencies of the microsatellite markers in the AD and control groups indicated the presence of risk alleles displaying suggestive association with AD (odds ratios ranging from 2.0 to 6.3). In addition, certain markers revealed significantly lower frequencies of particular alleles in the AD group than in the control group suggesting a protective effect conferred by these alleles against the development of AD (odds ratios ranging from 0.0 to 0.4). Screening of the 22 LD regions with additional microsatellite markers revealed eight chromosomal loci in 1p36.12, 2p22.2, 3q28, 4p13, 10p13, 13q12, 18q12.1 and 19p13.3 to be associated with AD with in more than one microsatellite marker. Chromosome regions found to be associated with AD in the present study will provide the primary targets for future genetic and functional studies into AD.

P1333. The Cystatin C gene and late onset Alzheimer s disease.

F. C. Crawford[1], *J. Schinka*[1], *M. Freeman*[1], *L. Abdullah*[1], *M. Morris*[1], *R. Hartman*[2], *M. Gold*[3], *D. Richards*[1], *R. Duara*[4], *R. Anand*[2], *M. Mullan*[1]
[1]*Roskamp Institute; Tampa, FL United States;* [2]*Novartis Pharmaceuticals; East Hanover, NJ United States;* [3]*Bristol Myers Squibb; Princeton, NJ United States;* [4]*Wien Center; Miami, FL United States*
fcrawfor@com1.med.usf.edu

Cystatin C is a cysteine protease inhibitor which is found to colocalize with A§ in plaques and cerebrovascular deposits in Alzheimer s Disease (AD), cerebral amyloid angiopathy (CAA), and transgenic mouse models of AD. A mutation in the gene (CST3) enhances dimerization, reduces biological activity, and causes hereditary Icelandic CAA. A common Ala/Thr substitution in the signal peptide has also been reported, and we investigated this variant in a clinic and population based group of Caucasian AD cases (n=309, age range 60-90, mean 75.1) versus population based Caucasian controls (n=134, age range 60-90 years, mean 76.1). Our data showed no association between AD and CST3, but the expected association of AD with APOE-e4 genotype. However, logistic regression analyses revealed a significant interaction between CST3 G/G status and age/age of onset on AD diagnosis (p=.003) suggesting that the genetic risk changes differentially for CST3 as a function of age. We therefore stratified our sample based on age (of controls) or age of onset (of cases); 60-69, 70-79 and 80+ years. In the youngest age groups an APOE e4-carrying genotype conferred significant risk for AD (p<.001) while CST3 genotype was not a significant risk (p>.18). In the oldest age group (controls n=49; cases n=79) the effect of APOE e4-carrying genotype on AD was not significant (p=.28), while CST3 genotype, specifically the G/G homozygous genotype, becomes a significant risk factor (p=.04) with an odds ratio of 2.2 compared to A-carrying genotypes. We further investigated this association in Hispanic AD cases (n=146, age range 61-89, mean age of onset 72yrs) and controls (n=182, age range 60-90, mean age 70) and again found no association between CST3 and AD in the total Hispanic dataset, but a tendency towards an increase in the GG genotype in cases versus controls in the over 80 dataset. These data suggest that variation at the cystatin C locus is a novel risk factor for late onset Alzheimer s disease, particularly at an age when APOE genotype no longer confers increased risk. It is likely that variation at this locus, or one with which it is in linkage disequilibrium, confers risk for AD via effects on cystatin C trafficking and/or function, contributing to vascular AD pathology and possible exacerbation of §-amyloid pathogenic mechanisms.

P1334. Amyloid beta-secretase gene (BACE) is neither mutated nor associated with early-onset Alzheimer s disease.

B. Dermaut[1], *R. Rademakers*[1], *G. Roks*[2], *M. Van den Broeck*[1], *G. Munteanu*[1], *C. M. van Duijn*[2], *C. Van Broeckhoven*[1], *M. Cruts*[1]
[1]*Flanders Interuniversity Institute for Biotechnology (VIB); Antwerpen, Belgium;* [2]*Erasmus Medical Center; Rotterdam, The Netherlands*
bdermaut@uia.ua.ac.be

The major hallmark of Alzheimer s disease (AD) pathology is beta-amyloid (Abeta) deposition in the brain. Three protease activities named alpha-,

beta- and gamma-secretase are crucial in the proteolytic cleavage of the amyloid precursor protein (APP) to generate Abeta. Recently, the beta-secretase was identified and named beta-site APP-cleaving enzyme (BACE). Since all known familial early-onset AD (EOAD) related mutations in APP and presenilin (PSEN) increase Abeta production by affecting one of the secretase activities, we hypothesized that genetic variations within BACE might be similarly involved in the etiology of EOAD. We performed genetic linkage analyses in families of 9 autosomal dominant probands that had no mutation in APP or PSEN using markers D11S1327, D11S939, D11S1336 located near BACE. No family showed conclusive linkage and in 3 families linkage with BACE was excluded. cDNA and genomic mutation analysis revealed no mutations except for a frequent silent c.786C>G polymorphism (V262) and a c.840+5G>T variation at the fifth nucleotide of intron 5 that was observed in only 1 patient. Next, the V262 polymorphism was analyzed in a sample of 101 presenile AD patients and 185 control subjects. Allele and genotype distributions were not significantly different in cases and controls. Also, no differences were found when the sample was stratified for positive family history or the presence of an APOE4 allele. Together these results show that BACE is not genetically involved in the etiology of EOAD.

P1335. Screening For Presenilin-1 Mutations In Finnish Early Onset Alzheimer s Disease Patients

S. Iivonen[1], *M. Hiltunen*[1], *A. Mannermaa*[2], *S. Helisalmi*[1], *M. Pirskanen*[1], *I. Alafuzoff*[3], *M. Lehtovirta*[4], *A. M. Koivisto*[4], *S. Tuisku*[5], *H. Soininen*[4]
[1]*Department of Neurology and Department of Clinical Genetics, University Hospital and University of Kuopio; Kuopio, Finland;* [2]*Department of Clinical Genetics, University Hospital of Kuopio; Kuopio, Finland;* [3]*Department of Neurology and Department of Pathology, University Hospital and University of Kuopio; Kuopio, Finland;* [4]*Department of Neurology, University Hospital and University of Kuopio; Kuopio, Finland;* [5]*Kokkola Central Hospital; Kokkola, Finland*
susan.iivonen@kuh.fi

Mutations in the presenilin-1 (PSEN-1) gene on chromosome 14 are known to cause familial early onset Alzheimer s disease (AD). Over 70 different PSEN-1 mutations have been found so far and the highly penetrant PSEN-1 gene mutations are more frequent cause of AD than mutations in two other early onset AD genes, PSEN-2 and amyloid precursor protein. Here we present our PSEN-1 screening results among Finnish early onset AD patients. The major findings were as follows; We identified a novel 4.6-kb genomic deletion in PSEN-1 in an early onset AD family (mean onset age 43 years), which leads to an inframe exclusion of exon 9 from the mRNA transcript. The clinical and neuropathological features of patients in this family resembled those of the typical AD (Hiltunen et al., EJHG (2000) 8, 259-266). We also found a missense mutation P264L in exon 8 in an early onset AD family (mean onset age 54 years). In addition, E318G substitution was found in AD patients and controls indicating that this substitution is noncausative for AD. However, the allele frequency of the E318G variant was significantly increased in both familial (P = 0.005; OR 7.6, CI 2.2-25.7) and sporadic (P = 0.03; OR 3.1, CI 1.1-8.2) AD groups when compared to the control group, suggesting that the substitution may be a risk factor for AD. It is therefore possible that the E318G substitution is in linkage disequilibrium with yet another change located in the regulatory regions of the PSEN-1 gene.

P1336. Donauspital-study on the prediction of memory loss and dementia in Vienna; Is Alzheimer«s disease related to Mutations in the mitochondrial DNA?

H. Hombauer[1], *R. E. Bittner*[2], *P. Fischer*[3], *K. Huber*[1], *K. H. Tragl*[3]
[1]*Ludwig Boltzmann-Institut f r molekulargenetische Laboratoriumsdiagnostik; Vienna, Austria;* [2]*Institute of Anatomy, Neuromuscular Research Department, Univ. of Vienna; Vienna, Austria;* [3]*Ludwig Boltzmann-Institut f r Altersforschung, Donauspital; Vienna, Austria*
Klaus.Huber@SMZ.magwien.gv.at

Alzheimer«s disease (AD) is the most common neurodegenerative disorder of aging, accounting for an estimated two-thirds of all cases of senile dementia. Epidemiological studies suggest a complex etiology, with environmental and genetic factors influencing the pathogenesis. Considerable advances have been made in the last years in the understanding of the genetic factors causing the disease; Several mutations have been found in the amyloid precursor protein gene on chromosome 21. Two other dominantly operating genes on chromosome 1 and 14 were cloned recently (presenilin I and II) and a susceptibility gene for AD (apolipoprotein E gene) was found. Furthermore, several other hypotheses have been proposed to explain the age-relationship of this disease, including systemic metabolic changes, altered calcium homeostasis, neurotransmitter dependency, and oxidative stress. Pathological mutations of mitochondrial DNA (mtDNA) cause oxidative stress due to impaired respiratory capacity. This age-related decrease in the capacity to produce energy is proposed to be caused by the accumulation of somatic mtDNA mutations, which have been shown to cause a variety of other neurodegenerative diseases.
The Donauspital-study on AD focuses on a cohort (1,200 persons) of apparently healthy 75 year old volunteers, whose neurophysiologic status is examined and related to multiple clinical analyses. In the course of this task, we try to correlate the occurence of mtDNA mutations with the onset of AD. In the first phase, the mtDNA of 80 probands is sequenced and sequence changes are catalogued. In three years from now, an expected percentage (5%) of the probands will show obvious signs of AD. The mtDNA of these persons will then be re-sequenced, to obtain data on possible pathogenic mtDNA mutations causing or at least related to late-onset Alzheimer«s disease.

P1337. Prevalence of human leukocyte antigen (HLA) DRB1 alleles in Kuwaiti Arabs with schizophrenia

M. Z. Haider[1], *M. A. Zahid*[2], *M. A. Razik*[3]
[1]*Clinical Genetics Group, Kuwait University; Jabriya, Kuwait;* [2]*Department of Psychiatry Kuwait University; Jabriya, Kuwait;* [3]*Hospital for Psychological Medicine; Sulebikhat, Kuwait*
haider@hsc.kuniv.edu.kw

The frequency of human leukocyte antigen (HLA) DRB1 alleles has been determined in a cohort of 194 Kuwaiti Arabs consisting of 80 schizophrenia patients and 114 ethnically matched healthy controls, using a polymerase chain reaction-sequence specific primers (PCR-SSP) method. A total of twelve DRB1 alleles were identified in this Kuwaiti cohort. A statistically significant difference was detected in the frequency of alleles DRB1*04 and DRB1*13 between the schizophrenia patients and controls. Allele frequency of DRB1*04 in schizophrenia patients was 14 % compared to nearly 7 % in controls (P = 0.028). For DRB1*13, the allele frequency was found to be 18 % in schizophrenia patients compared to 9 % in the controls (P = 0.015). For alleles, DRB1*03, DRB1*07 and DRB1*16, the frequency was higher in controls compared to the schizophrenia patients. The frequency of DRB1*01, DRB1*08, DRB1*10, DRB1*11 and DRB1*15 alleles was almost identical in schizophrenia patients and controls. For the remaining alleles, the differences amongst the two groups were not statistically significant.

P1338. Association analysis of a 5-HT2A receptor polymorphism in schizophrenia and associated tardive dyskinesia

E. C. Tan[1], *S. A. Chong*[2], *R. Mahendran*[2], *C. H. Tan*[3]
[1]*Defence Medical Research Institute; Defence Science and Technology Agency, Singapore;* [2]*Institute of Mental Health; Woodbridge Hospital, Singapore;* [3]*National University of SIngapore; Kent Ridge, Singapore*
nmiv27@nus.edu.sg

Schizophrenia is a serious and debilitating psychiatric disorder with a lifetime prevalence of 1%. Neuroleptics are the mainstay of treatment, but long-term use may result in the development of tardive dyskinesia (TD). Besides age and cumulative neuroleptic dosage, the incidence of TD is higher among patients with a family history of mental illness, indicating the presence of genetic susceptibility factors.
We genotyped 97 healthy controls and 221 patients with schizophrenia (87 with TD, 134 without) for the 5-HTR2A T102C polymorphism by PCR-RFLP. Between controls and patients, there was an excess of the T-containing genotypes but the difference did not reach statistical significance. There was also little difference at the allelic level.
Among the patients, there was an excess of the T/T genotype for the TD group compared to those without TD (c^2=6.517, df=2, p=0.038). There was also significant difference in allele frequencies between the two groups (c^2=4.189, df=1, p=0.044) with the odds ratio of 1.54 (95% CI=1.02-2.33). For genotype distribution, the significance level is lower (p=0.055) after adjusting for age and daily neuroleptic dose in CPZ eq. Significant difference in allele frequency remains even after adjusting for the two significant variables (p=0.041) with the odds ratio at 1.64 (95% CI=1.02-2.62).
The C allele has been associated with susceptibility to schizophrenia and non-responsiveness to clozapine. Our results showed that it might also be associated with increased risk for neuroleptic induced-TD. This polymorphism might thus be a useful marker to screen for patients who might be at higher risk of developing TD.

P1339. On the role for glutamate in the pathogenesis of schizophrenia; association study with the N-methyl-D-aspartate receptor 2B

E. Di Maria[1], *R. Gulli*[1], *S. Begni*[2], *R. Pioli*[2], *E. Bellone*[1], *F. Ajmar*[1], *M. Gennarelli*[2], *P. Mandich*[1]
[1]*University of Genova; Genova, Italy;* [2]*I.R.C.C.S. Fatebenefratelli; Brescia, Italy*
edimaria@hgmp.mrc.ac.uk

Glutamate is an excitatory neurotransmitter of the central nervous system. Its action is mediated by different classes of ionotropic receptors. Among them, the N-methyl-D-aspartate receptors (NMDARs) are involved in neuronal plasticity and in glutamate excitotoxicity. The pathophysiological model of NMDAR-mediated glutamate hypofunction as a mechanism leading to the development of schizophrenia is supported by the evidences that administration of non-competitive NMDAR antagonists trigger psychotic symptoms in schizophrenic patients, and induce psychotic-like behaviours in normal subjects. Functional NMDARs are composed of the ubiquitous NR1 subunit and one of four NR2 subunits. The 2B subunit gene, named GRIN2B, is expressed in human brains in the hyppocampus, basal ganglia and cerebral cortex. We tested the hypothesis that a certain genetic background of the NMDAR2B is associated with susceptibility to develop schizophrenia. The genomic sequence of the GRIN2B gene was inferred from the known cDNA sequence through in silico analysis. Subsequently, three informative polymorphisms were identified; a silent G/C single nucleotide polymorphism (SNP) in exon 12 (SNP2664); a C/T SNP in the 3 UTR (SNP890); a multiallelic short tandem repeat (STR) in intron 2 (D12S364). Allele and genotype frequencies were estimated on a series of 105 unrelated patients with schizophrenia (DSM-IV) and on 150 normal controls. No significant difference between patients and controls was observed in allele and genotype frequencies of SNP2664 and D12S364. The T allele of SNP890 was found to be less frequent in patients than in controls ($p<0.05$), resulting an odds ratio associated with the G/G genotype equal to 3.39. These results did not allow to reject the hypothesis of association between the NMDAR2B genetic background and the susceptibility to schizophrenia. In order to confirm the preliminary analysis, the case control study on the extended haplotypes is in progress.

P1340. Association of polymorphisms of the human Ra

P1-targeting cAMP-GEFI gene with Japanese schizophrenia patients

H. Kawasaki[1], *H. Mitsuyasu*[1], *A. Tahara*[1], *T. Tahira*[2], *H. Ninomiya*[3], *Y. Fukumaki*[4], *D. E. Housman*[5], *A. M. Graybiel*[6], *N. Tashiro*[1], *K. Hayashi*[2]
[1]*Kyushu University, Graduate School of Medical Sciences, Dept. Neuropsychiatry; Fukuoka, Japan;* [2]*Kyushu University, Institute of Genetic Information, Div. Genome Analysis; Fukuoka, Japan;* [3]*Fukuoka Prefectural Dazaifu Hospital, Psychiatric Center; Fukuoka, Japan;* [4]*Kyushu University, Institute of Genetic Information, Div. Disease Genes; Fukuoka, Japan;* [5]*MIT, Center for Cancer Research; Cambridge, United States;* [6]*MIT, Dept. Brain and Cognitive Sciences; Cambridge, United States*
hkawasaki@aol.com

We previously reported the cloning of novel genes of a second-messenger regulated Rap1-GEF (guanine nucleotide exchange factor) gene family whose GEF activities are regulated by the binding of second-messenger molecules such as cAMP, calcium (Ca^{2+}) and diacylglycerol (DAG) (Kawasaki et al., 1998) indicating that three major second messengers transduce their signals to targets different from protein kinases. This gene family includes cAMP-GEFI (identical to Epac, de Rooji et al., 1998), cAMP-GEFII, CalDAG-GEFI and CalDAG-GEFII (identical to RasGRP, Ebinu et al., 1998). Both cAMP-GEFs have binding domains for cAMP molecules as well as for Rap1-targeting GEF domains. As antipsychotics have antagonistic actions at dopamine receptors regulating intracellular cAMP concentrations in the central nervous system, cAMP-GEFs could be good candidates for molecular studies of schizophrenia. We determined the exon-intron structure of human cAMP-GEFI gene based on sequence information from cosmids we isolated and draft sequence files in GenBank. The result indicate that there are 28 exons in the locus. All 28 exons were amplified by PCR to isolate single nucleotide polymorphisms (SNPs) using 12 Japanese individuals. Initial screenings were by dHPLC; subsequently, the SNPs were evaluated by PLACE-SSCP (Inazuka et al. 1997) and direct sequencing. By genotyping these SNPs in the schizophrenia patients and normal controls (Japanese population), the association between schizophrenia phenotype and these markers were evaluated. We also analyzed the linkage disequilibria between markers. PLACE-SSCP, single nucleotide extension, RFLPs and direct sequencing were employed as genotyping methods. Both individual and pooled DNAs were used for PLACE-SSCP analysis.

P1341. Association analysis of polymorphisms of the human dopamine D4 receptor gene (DRD4) with Japanese schizophrenia patients

H. Mitsuyasu[1], *H. Kawasaki*[1], *A. Tahara*[1], *T. Tahira*[2], *H. Ninomiya*[3], *Y. Fukumaki*[4], *K. Hayashi*[2], *N. Tashiro*[1]
[1]*Kyushu University, Graduate School of Medical Sciences, Dept. Neuropsychiatry; Fukuoka, Japan;* [2]*Kyushu University, Institute of Genetic Information, Div. Genome Analysis; Fukuoka, Japan;* [3]*Fukuoka Prefectural Dazaifu Hospital, Psychiatric Center; Fukuoka, Japan;* [4]*Kyushu University, Institute of Genetic Information, Div. Disease Genes; Fukuoka, Japan*
hrmitsu@npsych.med.kyushu-u.ac.jp

The human dopamine D4 receptor (DRD4) is of major interest in molecular studies of schizophrenia, because an atypical antipsychotic drug, clozapine has a relatively high affinity to DRD4 (Van Tol et al., 1991), and elevations in the density of DRD4 and DRD4 mRNA expression were found postmortem in the brains of schizophrenia patients (Seeman et al., 1993, Stefanis et al., 1998). Many polymorphisms have been reported in the DRD4 gene locus. Previously we have reported 9 novel polymorphisms (7 single nucleotide polymorphisms (SNPs) and 2 insertion/deletion polymorphisms) in the upstream region of the DRD4 gene in the Japanese population (Mitsuyasu et al., 1999). We genotyped 5 of the polymorphisms including one known RFLP in 208 Japanese schizophrenia patients and 210 normal controls (Mitsuyasu et al., 2001 in press). Including these, we collected 22 SNPs and 6 other polymorphisms (repeat polymorphisms; 4, deletion polymorphisms; 2) from a 4.9 kb region of entire DRD4 gene, based on our experiments, database, and literatures. Most of them were clustered within an approximately 1 kb area containing exon 1 and the promoter region. In this study, we report evaluation of these polymorphisms and genotyping of them using the Japanese schizophrenia patients and normal controls above mentioned. The methods adopted in the evaluation and genotyping were PLACE-SSCP (Inazuka et al., 1997), single nucleotide extension, RFLP, and direct sequencing of PCR products. We analyzed the linkage disequilibria between these polymorphisms and the association between these polymorphisms and schizophrenia patients in Japanese population.

P1342. Candidate genes for familial catatonic schizophrenia on chromosome 22q13.33

J. Meyer, *G. Ortega, I. Ulzheimer-Teuber, O. Grimm, S. Jatzke, A. Huberth, J. Gross, K. P. Lesch*
University of Wuerzburg; Wuerzburg, Germany
nerk108@mail.uni-wuerzburg.de

We have recently reported a 5 cM region on chromosome 22q13.33 with suggestive evidence for linkage with familial, catatonic schizophrenia (periodic catatonia according to Leonhard s classification; St ber et al., AJHG 67; 1201-1207, 2000). The region has been reduced to at least 4 cM by genotyping additional polymorphic markers. Gene annotation in this region shows a total of 30 genes and 47 small transcripts of viral origin or detected very rarely in cDNA libraries. One of the the strong candidate genes in this region is CELSR1, a gene encoding a neuronal seven-pass transmembrane receptor cadherin. Routing, branching, and final destination of developing neurons is mediated by cell-surface proteins and members of the cadherin cell adhesion protein family such as CELSRI. We have studied CELSR1 in detail and found a number of allelic variants within the coding region. Additionally, a polymorphic intronic repeat has been detected. These variants provide a powerful tool to investigate the role of CELSR1 in the etiopathogenesis of complex psychiatric disorders. Some of the other genes with unknown function in this region are expressed exclusively in the brain. Further studies will show if the variants of these genes detected so far may alter brain function and contribute to the pathogenesis of psychiatric disorders. Comparative sequencing of the coding regions of candidate genes in this chromosomal region may lead to the detection of a major gene involved in the etiopathogenesis of schizophrenia.

P1343. The Dopamine Transporter Gene (DAT1) And Schizophrenia; A Family Based Association Study

L. Georgieva[1], *I. Nikolov*[1], *A. Z. Dimitrova*[1], *S. Koleva*[1], *D. Toncheva*[1], *M. J. Owen*[2], *G. Kirov*[2]
[1]*Medical University Sofia; Sofia, Bulgaria;* [2]*Neuropsychiatric Genetics Unit at UWCM; Cardiff, United Kingdom*
bbcollaboration@hotmail.com

Schizophrenia is a severe psychiatric disorder which affects 1% of the general polulation. The aetiology is not known but neuroleptic drugs improve the symptoms in the majority of patients. These drugs block the dopamine receptors in the brain and therefore dopamine is supposed to play a role in the pathophysiology of the disease. The Dopamine transporter gene

(DAT1) encodes a protein which re-uptakes synaptic dopamine, making it a stong candidate gene to study in schizophrenia. A 40-bp VNTR in the 3 UTR of the gene has been exmined in previous case-control studies but no positive results were found. We used the more robust method of family controls from parent-offspring trios to test for association with disease.
We genotyped 178 trios with Schizophrenia (SZ) of Bulgarian origin. The results were analysed with the Extended Transmission Disequilibrium Test. We observed alleles of 5 6 8 9 10 and 11 repeats of the VNTR. The allele frequencies and transmissions from heterozygous parents are shown in the table.
We confirm previous results that the VNTR in DAT1 does not increase susceptibility for schizophrenia.
We examined the data sample for correlation between DAT1 genotypes and certain characteristics of auditory hallucinations, which are among the most important clinical features of Schizophrenia. We found a statistically significant positive correlation between DAT1 genotype and the severity of voices reported by patients; Spearman s rho=0.183, p=0.01, n=176, however this result is not corrected for multiple testing. By now, the importance of this relationship is unclear. We suggest that the occurrence of allele 10 could modify the severity of certain symptoms of Schizophrenia.
Table 1. Allele frequencies

	Allele number					
	5 repeat	6 repeat	8 repeat	9 repeat	10 repeat	11 repeat
	Allele frequencies					
Parents	0.1%	0.4%	0.3%	31.1%	67.1%	0.8%
Children	0.3%	0.8%	0.3%	30.1%	67.9%	0.6%
	Transmission from heterozygous parents					
Transmitted	1	3	1	77	85	2
Not transmitted	0	0	1	84	80	4
p-value				0.58	0.69	

P1344. Evidence For An Involvement Of The Notch4 Locus In The Development Of Schizophrenia

S. Cichon[1], *J. Schumacher*[1], *R. Ivo*[1], *F. Macciardi*[2], *T. G. Schulze*[3], *D. J. M llr*[3], *I. Kremer*[4], *M. Dobrusin*[5], *M. Mujaheed*[6], *I. Murad*[6], *M. Blanaru*[3], *I. Bannoura*[6], *A. Reshef*[4], *R. Bachner-Melman*[4], *R. H. Belmaker*[5], *R. P. Ebstein*[7], *W. Maier*[3], *M. Rietschel*[3], *P. Propping*[1], *M. M. N then*[1]
[1]*Institute of Human Genetics, University of Bonn; Bonn, Germany;* [2]*Center for Addiction and Mental Health (CAMH), Section of Neurogenetics, University of Toronto; Toronto, Canada;* [3]*Department of Psychiatry, University of Bonn; Bonn, Germany;* [4]*Emek Hospital; Afula, Israel;* [5]*Beersheva Mental Health Center; Beersheva, Israel;* [6]*Dr. Kemal Hospital; Bethlehem, Israel;* [7]*Herzog Hospital; Jerusalem, Israel*
cichon@mailer.meb.uni-bonn.de

Genetic epidemiology has provided consistent evidence that schizophrenia has a genetic component, and that this genetic component is complex. Molecular genetic studies, however, have so far failed to reproducibly identify any DNA variant conferring susceptibility to this common neuropsychiatric disorder. Recently, the NOTCH4 locus on chromosomal region 6p has been reported to be highly associated with schizophrenia in the British population [Wei & Hemmings, Nature Genet. 25; 376-377 (2000)]. This locus contains the gene encoding the NOTCH4 receptor which is involved in neurodevelopmental processes. Because credibility of association findings in genetically complex traits strongly depends on replication in independent samples, we examined four variants [(TAA)n repeat, SNP1, SNP2, (CTG)n repeat] in the 5 -flanking region and in the coding region of the NOTCH4 gene in parent-offspring trio samples from two different populations; 159 trios of German and 209 trios of Palestinian Arab descent. Linkage disequilibrium analysis was performed using the transmission disequilibrium test (TDT). In concordance with the British trio sample, we found a susceptibility locus for schizophrenia in significant linkage disequilibrium with the (TAA)n repeat in the Palestinian Arab sample (p=0.0082) and observed a borderline significant trend in the German sample (p=0.0547). Furthermore, the four-marker haplotype (TAA)n-SNP1-SNP2-(CTG)n was associated in both German (p=0.0038) and Palestinian Arab (p=0.0111) trio samples. Thus, our results provide further support for the hypothesis that the NOTCH4 gene or a nearby locus is involved in the etiology of schizophrenia.

P1345. molecular genetic analysis of bipolar disorder in South Africa

C. Cupido, *E. Pietersen, R. S. Ramesar*
Department of Human Genetics,University of Cape Town; Cape Town, South Africa
cc@anat.uct.ac.za

Bipolar disorder is a severe neuropsychiatric condition that affects approximately 1% of the U.S. population. The condition is complex with a strong genetic influence. The objective of this study was; (1) to assess the familiality of bipolar disorder in South Africa, (2) to assess the significance of association of other psychiatric disorders with bipolar disorder (in a familial setting), and (3) to identify the possible genetic loci which predispose to bipolar disorder in the families under investigation. A total of 130 families were recruited, who met the criteria for a diagnosis of either bipolar I disorder (BPI), bipolar II disorder (BPII) or major depressive episode recurrent (MDE-R), as confirmed by the Structured Clinical Interview for DSM-IV Axis I disorders (Version 2.0). In a review of the 130 families, BPI was seen to cluster with BPII and MDE. Compared to males a greater number of females with a mood disorder were observed. 51% of the cohort met the criteria for BPI and BPII, 44% for MDE (recurrent and single) and 5% for other mood disorders. 19% of the cohort failed to meet the criteria for any of the mood disorders. Current molecular genetic analysis involves screening a cohort of 12 South African families with fluorescent microsatellite markers, covering a comprehensive set of candidate loci/genes thought to play a role in the aetiology of bipolar disorder. It is likely that similar/common genetic factors underlie mood disorders and other psychiatric conditions.

P1346. Investigation of Notch 3 as a candidate gene for bipolar disorder suing brain hyperintensities as an endophenotype. Ahearn EP, Speer MC, Steffens DC, vanMeter S, Cassidy F, Chen YT, Provenazle J, Weisler R, Krishnan R.

E. P. Ahearn
Duke University Medical Center; Durham, NC United States
marcy@chg.mc.duke.edu

Genetic linkage studies in psychiatric disorders like bipolar disorder (BPD) have been complicated by extensive genetic and phenotypic heterogeneity. One way to maximize the power of linkage analysis is to identify endophenotypes that may predict the genetic basis of a phenotypic subset. Several studies suggest an increased prevalence of MRI hyperintense foci in BPD; these foci are located in both subcortical white matter and grey nuclei. Between 25-40% of young bipolars (< 45 years) demonstrate these MRI findings, which are similar to those in CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy), which has been associated with mutations in Notch3. We identified a large pedigree in which all 9 BPD patients were positive for MRI hyperintense foci; 6/10 relatives with no affective disorder were also positive for these MRI changes. Linkage analysis (parametric and non-parametric) using markers flanking Notch3 demonstrated negative lod scores across a variety of disease models, except for slightly positive lod scores for D19S923 when all MRI lesion-positive individuals, regardless of BPD status, are affected (z(q)] 0.86). Since Notch3 is a compelling functional candidate, we investigated exons 3 and 4, where mutations in CADASIL cluster, with SSCP. We found no evidence for gel shift in these two exons, providing further evidence that this gene is not involved in either the bipolar or MRI phenotype in this family. In addition to this large family, we have identified two other sets of sib pairs concordant for BPD and hyperintense foci and further investigations are on-going.

P1347. Linkage Disequilibrium Study Of The Serotonin Transporter Gene (SLC6A4) In Bipolar Disorder.

E. Mundo[1], *M. Walker*[1], *T. Cate*[1], *S. Parikh*[2], *F. Macciardi*[1], *J. L. Kennedy*[1]
[1]*Neurogenetics Section, CAMH, University of Toronto; Toronto, ON Canada;* [2]*Bipolar Disorders Clinic, CAMH, University of Toronto; Toronto, ON Canada*
Emanuela_Mundo@CAMH.net

Bipolar Disorder (BP) is a severe psychiatric condition with a strong genetic component (Gershon, 1990). The serotonin transporter gene (SCL6A4) is a good candidate for BP as the serotonin transporter protein is the site of action of selective serotonin re-uptake inhibitors (SSRIs) that successfully treat bipolar depression (Potter, 1998). The SLC6A4 is located on chromosome 17q11.1-q12 and it has two known polymorphisms; a 44bp deletion/insertion in the promoter region (5HTTLPR) (Lesch et al, 1996) and a variable number of tandem repeats (VNTR) in the second intron (Ogilvie et al, 1995). Results from previous association studies on SCL6A4 and BP are conflicting (Furlong et al, 1998; Mundo et al, 2000). To investigate for the presence of linkage disequilibrium (LD) between SCL6A4 and BP, we studied 280 subjects with BP I, BP II, or Schizoaffective Disorder, bipolar type, and their living parents. Diagnoses were assessed by a structured interview for DSM-IV (American Psychiatric Association, 1994) (SCID-I). Genotyping data were analyzed using the transmission disequilibrium test (TDT) (Spielman et al, 1993). With respect to the 5HTTLPR,

206 triads were informative for the analysis. The two alleles of the 5HTTLPR were transmitted equally to the affected (chi-square=0.262, df=1, p=0.608). For the VNTR polymorphisms 185 families were informative and no biases in the transmission of the alleles were found. The two polymorphisms were in LD (chi-square=43.115, df=2, p<.00001), but a Haplotype-TDT analysis (Chiano & Clayton, 1998) did not show any association with BP (overall chi-square=1.3514, df=4, p=0.8525). It appears unlikely that the SLC6A4 plays a fundamental role in the pathogenesis of BP. However, further studies focusing on the role of the gene in determining susceptibility to alternative phenotypes related to BP (e.g., the response to SSRIs) might be worthwhile.

P1348. Linkage and TDT analyses of markers on chromosome 22q in Bulgarian families with affective disorder

V. M. Chorbov[1], *R. P. Kaneva*[1], *V. K. Milanova*[2], *C. Kostov*[2], *V. Stoyanova*[2], *S. Krastev*[2], *I. Kremensky*[1], *A. Jablensky*[3]
[1]*Laboratory of Molecular Pathology, Sofia Medical University; Sofia, Bulgaria;* [2]*First Psychiatric Clinic, Department of Psychiatry; Sofia, Bulgaria;* [3]*Department of Psychiatry and Behavioural Science, University of Western Australia; Perth, Australia*
mitkov@excite.com

Several studies have provided evidence of a susceptibility locus for bipolar disorder on chromosome 22q. We analysed 18 Bulgarian pedigrees with affective disease for linkage with 4 polymorphic markers from 22q (D22S427, COMT, D22S264, D22S278), covering 17.4 cM. Two point linkage analysis under assumption of homogeneity and a dominant model with reduced penetrance produced small positive values only for marker D22S278 with maximum Lod=1.12 for marker D21S278 (q=0.05) and broad phenotype definition. Nonparametric linkage analysis under a phenotype model, including bipolar I, II and schizoaffective disorder, gave positive NPLall values (p<0.05) over the distal part of the studied region peak at D21S278 (Npl Zall=1.73, p=0.02). The multipoint Lod score analysis, performed by GENEHUNTER produced small positive score at the same locus (Lod=0.63). The transmission disequilibrium test was applied and preferential transmission could not be found for the analysed markers. The combined data does not support the hypothesis for the role of COMT and the adjacent region in the susceptibility to affective disorder in the analysed sample. The significant NPL scores and small positive Lod scores at locus D22S278 confirm previous findings and focus the attention on the distal part of chromosome 22q for further investigations.

P1349. Linkage disequilibrium analysis of affective disorder and loci on chromosome 21q

R. P. Kaneva[1], *V. Chorbov*[1], *V. Milanova*[2], *C. Kostov*[2], *V. Stoyanova*[2], *S. Krastev*[2], *I. Kremensky*[1], *A. Jablensky*[3]
[1]*Laboratory of Molecular Pathology; Sofia, Bulgaria;* [2]*First Psychiatric Clinic, Department of Psychiatry; Sofia, Bulgaria;* [3]*Department of Psychiatry and Behavioural Science, University of Western Australia; Perth, Australia*
radiva@techno-link.com

We analysed 18 Bulgarian pedigrees with affective disorder with 6 polymorphic markers on chromosome 21q (D21S65, D21S211, D21S1252, D21S1255, D21S1260, D21S416) implicated in previous studies. The region encompassed by the markers was 7.7 cM. We genotyped 42 patients (27 BPI; 3 BPII; 1 schizoaffective (manic); 7 recurrent major depression; 4 major depressive disorder, single episode) and 28 unaffected persons. Two affection models were tested; narrow including only BP I, BP II and schizoaffective manic and broad , including in addition to the narrow recurrent depression and major depressive episodes. The transmission disequilibrium test (TDT) and the ETDT tests were applied. Significant results were obtained for two of the studied markers and the narrow model of the disease; marker D21S1252 (allele-wise p=0.03, genotype-wise p=0.02) and D21S416 (allele-wise p=0.005, genotype-wise p=0.02). There was also evidence for LD between marker D21S416 and the broad affection status (allele-wise p=0.003, genotype-wise p=0.002). Genotypic disequilibrium between the used markers was tested in the group of affected and their healthy relatives using the program Genepop 3.1b. Almost all pairs of markers revealed significant disequilibrium in the affected, compared with lack of disequilibrium in the healthy group. The strongest level of genotypic disequilibrium was observed for marker pairs; D21S65-D21S1252, D21S1252-D21S416, D21S1252-D21S1260. The combined data from the current study provide additional support for the existence of a susceptibility gene for bipolar affective disorder on chromosome 21q22.3.

P1350. Association Between Violent Suicidal Behavior And The Low Activity Allele Of The Serotonin Transporter Gene

C. M. N. Buresi[1], *P. Courtet*[2], *D. Mouthon*[3], *P. Courtet*[2], *P. Baud*[3], *D. Mouthon*[3], *A. Malafosse*[3], *D. Mouthon*[3], *P. Baud*[3], *A. Malafosse*[3]
[1]*Geneva University Hospital; Chene-Bourg, Switzerland;* [2]*Lapeyronie Hospital, INSERM E99-30; Montpellier, France;* [3]*Geneva University Hospital, Neuropsychiatry; Geneva, Switzerland*
Catherine.Buresi@hcuge.ch

There is compelling evidence that serotonin system dysfunction is associated with certain behavioral disorders, such as suicidal behavior and impulsive aggression. A functional polymorphism in the promoter region of the serotonin transporter gene (5-HTTLPR) was recently identified and the presence of the short allele found to be associated with a lower level of expression of the gene, lower levels of 5-HT uptake, suicidal behavior and anxiety-related traits. We genotyped 51 West European Caucasians who had made violent suicide attempts and 139 controls of the same ethnic origin, with no history of suicidal behavior. The frequencies of the S allele and the SS genotype were significantly higher in the violent suicide attempters than in the controls. The odds ratio for the SS genotype vs. the LL genotype was 3.63 (95% CI [1.27-10.40]). The association was even stronger in those with a history of major mood disorder (odds ratio for SS genotype vs. LL genotype; 5.6, 95% (CI [1.36-23.06]), whereas no difference was observed between non suicide attempters with and without a history of major mood disorder. This suggests that a change in expression of the gene encoding the 5-HT transporter may be involved in a subgroup of suicide attempters, in whom violent suicidal behavior is combined with major mood disorder. Possible interaction between 5-HTTLPR and other genes of the serotonin pathway in these behavioral disorders will be presented.

P1351. Psychotic Disorders And The Serotonin Transporter Gene; A Family Based Association Study

A. Dimitrova[1], *G. Kirov*[2], *I. Nikolov*[1], *L. Georgieva*[1], *S. Koleva*[3], *N. Poriazova*[4], *M. J. Owen*[2], *D. Toncheva*[1]
[1]*Department of Medical Genetics, Medical University - Sofia; Sofia, Bulgaria;* [2]*Neuropsychiatric Genetics Unit at UWCM; Cardiff, United Kingdom;* [3]*Medical University Sofia; Sofia, Bulgaria;* [4]*Plovdiv Medical University; Plovdiv, Bulgaria*
draga@sofiaonline.com

Background. The human Serotonin transporter gene (5-HTT) is a strong candidate for involvement in the pathogenesis of mood disorders, as it is the target of antidepressants blocking the re-uptake of Serotonin. Previous studies have proposed that certain alleles of a 16/17bp VNTR in intron 2, might increase susceptibility to Bipolar affective disorder 1 (BP1). Association studies using case-control designs can, however produce spurious results. We used the more robust method of family controls from parent-offspring trios.

Methods. We genotyped 255 trios of Bulgarian origin. The distribution of DSM-IV diagnoses in the affected offspring is as follows; BP1- 72 families, Schizophrenia (SZ)- 157 families, Schizoaffective disorder (SA)- 26 families. The results were analysed with the Extended Transmission Disequilibrium Test.

Results. We observed alleles of 9, 10 and 12 repeats of the VNTR. The frequencies are shown in the table. For the whole sample, the 9 repeat was passed 9 times, not passed 7 times (c2=0.25,p=0.6); allele 10 was passed 110 times v. 129 times (c2=1.51, p=0.2); and allele 12 was passed 130 times v. 113 times (c2=1.19, p=0.3). None of the three diagnostic samples showed significant preferential transmission of any alleles. Some, but not all previous studies reported that allele 12 is more common in patients with BPI (Kirov et al, *Psychol Med*, 1999, **29**;1249-54). The only trend for the 12 repeat to be preferentially transmitted was in SA disorder (passed 17, not passed 10 times, c2=1.82, p=0.178). There was no significant correlation between the presence of allele 12 and any of the psychiatric symptoms recorded.

Conclusions. We could not confirm previous results that the 12 repeat in the VNTR in intron 2 in 5-HTT increases susceptibility to BP1. The weight of evidence now points against such an involvement. It appears that this polymorphism in the 5-HTT gene is not a risk factor in the pathogenesis of Schizophrenia either. Its role in behavioural or personality traits, such as anxiety, remains more plausible.

Table 1. Allele frequencies

		Allele frequencies		
Diagnosis	Member	9 repeat	10 repeat	12 repeat
SZ, n=157	Parents	1.4%	30.7%	67.8%
	Children	1.3%	29.6%	69.1%
SA, n=26	Parents	1.9%	37.5%	60.6%
	Children	3.8%	28.8%	67.3%
BP1, n=72	Parents	1.7%	34.8%	63.4%
	Children	2.1%	33.8%	64.1%

P1352. Characterization of a novel gene at 7q31 disrupted in a patient with Tourette s Syndrome

E. Petek[1], *J. B. Vincent*[2], *C. Windpassinger*[1], *H. M. D. Gurling*[3], *P. M. Kroisel*[1], *K. Wagner*[1], *S. W. Scherer*[2]
[1]*University of Graz; Graz, Austria;* [2]*The Hospital for Sick Children; Toronto, ON Canada;* [3]*Windeyer Institute of Medical Science; London, United Kingdom*
petek@kfunigraz.ac.at

Gilles de la Tourette syndrome (GTS) is a complex neuropsychiatric disorder characterized by multiple motor and phonic tics. We identified a male patient with GTS and other additional anomalies carrying a de novo duplication of the long arm of chromosome 7 [46,XY,dup(7)(q22.1-q31.1)]. The distal chromosomal breakpoint occurred between the two markers D7S515 and D7S522, a region previously shown to be disrupted in a familiar case of GTS. Sequence analysis of the distal breakpoint determined that the rearrangement occurred within a novel gene named mitochondrial inner peptidase subunit 2 (IMP2) due to its similarity to yeast IMP2. The human gene encodes 175 amino acids and spans about 900 kb of genomic DNA. An ubiquitous expression pattern could be demonstrated on several tissues by performing Northern blot and RT-PCR analysis. In yeast, IMP is required for proteolytic processing of the mitochondrially encoded protein Cox2 and the nucleus-encoded Cyt b2, Mcr1, and Cyt c1. Mitochondrial proteins have previously been associated with the appearance of neurodegenerative disorders such as Friedreich s ataxia, MERRF and MELAS, and have been hypothesized to be involved in neuropsychiatric disorders. To confirm of whether a defective IMP2 gene may predispose Tourette s syndrome or related disorders we are in the process of examining the IMP2 gene for mutations in unrelated Tourette s syndrome patients.

P1353. No evidence for variations of the cannabinoid receptor gene (CNR1) in German IV drug users

D. Heller*, U. Schneider, J. Seifert, M. Stuhrmann*
Medizinische Hochschule Hannover; Hannover, Germany
stuhrmann.manfred@mh-hannover.de

To date, no human disease has been definately proven to be caused or predisposed by mutations within the human central cannabinoid receptor (CNR1) gene. In 1997, Commings et al. reported on a significant association between homozygosity for a group of certain alleles of a polymorphic triplet repeat (AAT) marker for the CNR1 gene, prompting us to investigate this marker in 40 German unrelated opioid addicts (27 males and 13 females; mean age 37.9 Years) and 81 age and sex matched controls. In contrast to Comings et al., we found no association of AAT repeat alleles with IV drug use in our study group. In addition, no association was observed for alleles of an intragenic biallelic CNR1 polymorphism (1359G/A). Finally, by SSCP analysis and direct sequencing of the entire CNR1 gene of all probands, we did not detect any sequence variation which could confer susceptibility to IV drug abuse. Hence, in contrast to previous investigations, we found no evidence for an involvement of the CNR1 gene in a cohort of German IV drug users.

P1354. The mu-Opioid Receptor Gene and Risk for Use of Alcohol and Other Substances

M. J. Mullan[1], *T. Town*[1], *L. Abdullah*[1], *F. Crawford*[1], *P. Ordorica*[2], *E. Francis*[2], *P. Hughes*[2], *A. Graves*[3], *J. Mortimer*[3], *J. Schinka*[1,2]
[1]*Roskamp Institute; Tampa, FL United States;* [2]*Veterans Administration Medical Center; Tampa, FL United States;* [3]*Institute on Aging; Tampa, FL United States*
mmullan@com1.med.usf.edu

We have previously reported association of the +118A allele of the human mu-opioid receptor gene (OPRM1) with alcohol dependency in a population recruited through the Veterans Administration. However, other genetic association studies investigating the role of this functional polymorphism in risk for alcohol dependency have produced inconsistent findings, possibly because of the failure to recognize sampling methodology difficulties inherent in association studies of polygenic disorders. We therefore examined the frequency of the AA genotype and A allele in several groups of substance dependent cases, unscreened controls, and super controls screened for the use of alcohol and cigarettes. Our findings and analyses suggest that the OPRM1 +118 polymorphism is a general risk gene for substance dependence, but is not specific to a particular substance. The nature of the conferred risk is likely to be in use of multiple substances, but it is not yet determined if the risk could be expressed in severity of use of any particular substance. The contribution of the gene to risk for substance dependence is small, and is detected most easily in studies that use control samples that are screened for all forms of substance dependence.

P1355. Insertion-deletion ACE gene polymorphism is age dependent

O. Milosserdova*, P. Slominsky, S. Limborska*
Institute of Molecular Genetics; Moscow, Russian Federation
omil@img.ras.ru

The angiotensin I-converting enzyme (ACE) is key element of the renin-angiotensin system. However, data on relative contributions of alleles and genotypes of this gene into vascular disorders and survival are contradictory. We have investigated the frequency of ACE insertion/deletion (I/D) polymorphism in 4 group of subjects living in Moscow; a sample of children (mean age is 2.9+3.4 y.o), and 3 samples of adults aged 30-80 years (means age are 34.2+2.37, 56.4+4.5, 83.17+3.39 y.o). We observed the tendency of increased the frequency D-allele ($p<0.05$) and DD genotype ($p<0.01$) in centenarians. For example, a frequency of D allele was 0,48 for children group and 0,68 for centenarians group. Such results can have population-specific effect. Thus, genotype differences between age-group should be taken into account and the control group thoroughly selected when association are studied.

P1356. A candidate genetic marker of outstanding sport achievements

I. Nazarov[1], *V. Kazakov*[1], *V. Rogozkin*[2], *O. Shneider*[2], *N. Tomilin*[1]
[1]*Institute of Cytology of the Russian Academy of Sciences; Saint Petersburg, Russian Federation;* [2]*Institute of Physical Culture; Saint Petersburg, Russian Federation*
i_naz@mail.ru

The angiotensin-converting enzyme (ACE) gene insertion/deletion polymorphism may play a role of genetic marker of physical performance and athletic excellence (Montgomery et al., 1998, Gayagay et al., 1998). However, Rankinen et al. (2000), and Taylor et al. (1999) do not confirm this hypothesis. In this study was examined possible association of the ACE gene insertion (I) and deletion (D) allele frequencies and ACE genotypes (II,ID,DD) with sport specialization in Russian athletes on different sport levels. Athletes specialized in swimming, track and field, triathlon, and ski participated in the study. All athletes was divided into three groups. The group A consisted of sprinters who make their competition load into 1 minute. The group B consist of athletes who make competition load in time period from 1 to 20 minutes. And the group C included marathoners who perform their competition load during more then 20 minutes. Moreover, the athletes was classified as middle level athletes, standing (members and candidates of Russian national teams) and outstanding (champions of Russia, Europe and higher level) athletes. DNA was extracted from buccal epithelia. The ACE I/D polymorphism was genotyped by polymerase chain reaction as reported earlier (Evans et al. 1994). Significance was assessed by chi squared analysis. The results of this investigation are shown in the table 1. It is seen from the table 1 that at the middle and the standing athletes there are no significant differences of the ACE genotypes or I/D alleles frequencies in all groups compared to control group. However, significant increase of the D allele was detected among outstanding sprinters (group A). It accords to data that the response to strength training effect is increased by the presence of the D allele in ACE genotype (Folland et al., 2000). Significant increase of the I allele is detected among outstanding athletes of group B. This possibly can be explained by some preference owing to better oxygen delivery to skeletal muscles during exercises at submaximal intensity. This point is confirmed that among marathoners was not found significant association with frequencies of ACE gene I and D alleles, so as an intensity of physical load in this group lower and the oxygen delivery rate is not limiting factor for their exercising.

Table 1 Frequencies of ACE gene I and D alleles and ACE genotype distribution in athletes were gro

Group	n	ACE genotype				ACE allele		
		II	ID	DD	P value	I	D	
Middle level								
A	39	0,13	0,45	0,42	0,124	0,36	0,64	0,069
B	23	0,17	0,39	0,43	0,247	0,37	0,63	0,133
C	16	0,25	0,56	0,19	0,807	0,53	0,47	0,670
Standing (Intermediate)								
A	10	0,10	0,40	0,50	0,241	0,30	0,70	0,101
B	24	0,12	0,71	0,18	0,259	0,47	0,53	0,092
C	26	0,19	0,58	0,23	0,747	0,48	0,52	0,894
Outstanding								
A	18	0,06	0,44	0,50	0,061	0,28	0,72	0,017*
B	15	0,64	0,29	0,07	0,007*	0,79	0,21	0,002*
C	26	0,15	0,65	0,19	0,341	0,48	0,52	0,894
Controls								
-	111	0,24	0,50	0,26	-	0,49	0,51	-

P1357. ACE genotype - making of an elite athlete?

B. Schimmel[1], *R. Dahse*[1], *H. Wagner*[2], *S. Thaller*[3], *M. Sust*[3]
[1]*Institute of Human Genetics and Anthropology; Jena, Germany;* [2]*Institute of Sportsciences, Friedrich-Schiller-University; Jena, Germany;* [3]*Institute for Sport Sciences, Karl-Franzens University; Graz, Austria*
bschi@mti-n.uni-jena.de

Human physical performance and fitness phenotype is influenced by genetic factors. A variation in the structure of the human angiotensin - converting enzyme (ACE) gene has been detected in which the absence (deletion, D allele) rather than the presence (insertion, I allele) of a 287 bp fragment is associated with high ACE activity in tissues. Lower enzyme activity (associated with the longer I allele) has been reported to be linked to enhanced endurance performance possibly by improving the mechanical efficiency of trained muscle (Williams AG et al., Nature 403; 614, 2000). The I variant was associated with greater training response also in other studies and it was reported that the frequency of the DD genotype was significantly lower in elite athletes like Olympic rowers or mountaineers. In contrast, data from fitness phenotypes in a huge HERITAGE Family Study with 700 individuals did not support the hypothesis that the ACE polymorphism plays a major role in cardiorespiratory endurance (Rankinen T et al., J. Appl. Physiol. 88; 1029-1035, 2000). The aim of our study was to contribute to this controversial discussion by determining the influence of the ACE genotype on special muscle properties with the hypothesis, that individual muscle characteristics rather than parameters from the above named studies are associated with the ACE genotype. The ACE genotype was determined by PCR from 0.9% saline mouthwash samples. Muscle properties were measured in 72 subjects (II; n= 25; ID; n= 17; DD; n= 30) by determining the individual force-velocity relation described by an Hill-type muscle model. The subjects performed maximum voluntary single-joint leg-extension sitting on a leg-press. The dynamics and kinematics have been used to determine the individual muscle properties using a non-linear regression analysis. We found no significant correlation between the ACE genotype and the single parameters of Hill s equation. This result indicates that our hypothetical model of single Hill type parameters in correlation to the ACE genotype can not contribute to the discussion whether ACE genotype is making of an elite athlete.

P1358. Angiotensin-converting enzyme gene polymorphism in Saudis - association with hypertension.

M. A. F. El-Hazmi
College of Medicine, King Saud University; Riyadh, Saudi Arabia
mohsen@ksu.edu.sa

Angiotensin-converting enzyme (ACE; kininase II; EC 3.4.15.1) plays an essential role in the maintenance of vascular tone as it activates angiotensin I to the vasoconstrictory peptide- angiotensin II, and inactivates the vasodilatory peptide, bradykinin. Variations in the levels of ACE have been reported in different individuals and are related to occurance of different alleles of ACE gene.A well studied polymorphism in the intron 16 of the ACE gene due to insertion (I) or deletion (D) of a 287 bp sequence has been reported to occur producing three genotypes; DD, DI and II. The highest ACE levels occur in individuals with DD genotype and the lowest in the II homozygous. We investigated ACE polymorphism in 300 Saudi males and females. Primers spanning the polymorphic site in intron 16 of ACE gene were used to amplify the polymorphic site by PCR and the PCR product was subjected to agarose gell electrophoresis. Based on the fragments produced each individual was classified as DD, ID and II and the frequency of each genotype and the D and I alleles were determined in the males and females. The overall genotype frequency was 0.560, 0.026 and 0.414 for DD, II and ID genotypes, while the allele frequency were 0.767 and 0.233 for D and I alleles. The individuals with different genotypes were seperated and the prevalence of hypertension was calculated in each group. In addition prevalence of obesity and serum lipid abnormalities were investigated in each genotype. This paper will present our findings of ACE gene polymorphism in normotensive and hypertensive Saudi individuals and will discuss the role of ACE in the development of hypertension.

P1359. Non parametric linkage analysis of dopamine D2 receptor and essential hypertension in Singaporean Chinese affected sib -pairs

H. Wu[1], *E. P. H. Yap*[1], *E. Taylor*[2], *V. Oh*[2]
[1]*Defence Medical Research Institute; Singapore, Singapore;* [2]*Department of Medicine, National University of Singapore; Singapore, Singapore*
nmiv14@nus.edu.sg

The several genes that together predispose individuals to essential hypertension are unknown. Abnormalities in the dopaminergic system, which occur in rat models of hypertension, are implicated in human hypertension. Dopamine produces several effects, such as the control of renal vasomotor tone and natriuresis, and regulation of the secretion of renin, angiotensin, and aldosterone. The effects are mediated through two classes of receptors, D2/D3/D4 and D1/D5. We investigated the role of the dopamine D2 receptor (DRD2) in a genetic linkage study of hypertension. We enrolled Chinese hypertensive persons, whose mean pretreatment blood pressure was >140/90 mmHg on 24-hour ambulatory recording. We selected for disease onset <60 years, and excluded diabetes and a body mass index > 30. Forty-nine sib-pairs concordant for hypertension were enrolled. Two polymorphisms in the coding region (Ser311 and NcoI) and a 3 marker (TaqIA) were genotyped by polymerase chain reaction-restriction fragment length polymorphism. We carried out non-parametic linkage analysis of each marker. There was an increased sharing of alleles identical by state (hypertension) at the TaqIA locus (p=0.0025). The result suggests that DRD2 may be linked to essential hypertension in this Chinese population, and justify further studies of larger sample sizes and more polymorphic markers.

P1360. An angiotensin-converting enzyme and angiotensinogen gene polymorphisms, ischaemic stroke and carotid stenosis; association and sibs study

P. A. Slominsky[1], *T. Tupitsina*[1], *E. Koltsova*[2], *V. Skvortsova*[2], *S. Limborska*[1]
[1]*Institute of Molecular Genetics; Moscow, Russian Federation;* [2]*Russian State Medical Academy; Moscow, Russian Federation*
slomin@img.ras.ru

Family and twins study demonstrated that genetic factors may be involved in stroke. Previously, insertion/deletion (I/D) Alu-polymorphism in the angiotensin-converting enzyme (ACE) gene and M235T polymorphism in the angiotensinogen gene has been suggested as a risk factors for some cardiovascular diseases. There fore, cardiovascular factors are well-known risk factors for ischaemic cerebrovascular disease (ICVD). We have investigated the distribution of alleles of ACE and angiotensinogen genes in group of patients with ischaemic and their sibs. All patients and sibs with ischaemic stroke were investigated for the stenosis of the internal carotid artery (CS). There was a significant difference ($P < 0.05$) in the distribution of ACE alleles, homozygosity for the presumed susceptibility deletion allele being more common in patients with profound (>50%) CS than in healthy control subjects and healthy sibs. There was also a significant difference ($P < 0.05$) in patients with CS in comparison with matched ICVD patients without CS, both in allelic frequencies and in homozygosity for the deletion allele. However, I\D allele distibution demonstrated no evidence for statistically significant differences in frequencies of I/D alleles or II/DD/ID genotypes between ICVD patients and healthy control subjects. Our results indicate that the ACE gene polymorphism may be a risk factor for the development of CS - but not for ICVD. M235T angiotensinogen gene polymorphism is not risk factor for ICVD or CS in our patient s group - we don t find any significant differences in allele and genotype distribution between ICVD and CS patients and healthy control subjects.

P1361. Family based investigation of the role of the C677T polymorphism of methylenetetrahydrofolate reductase (MTHFR) gene in ischaemic heart disease (IHD)

M. S. Spence[1], *P. G. McGlinchey*[1], *C. Patterson*[2], *C. Belton*[3], *G. Murphy*[1], *D. McMaster*[3], *D. Fogarty*[3], *A. Evans*[2], *P. P. McKeown*[1,3]
[1]*Regional Medical Cardiology Centre, Royal Victoria Hospital; Belfast, United Kingdom;* [2]*Department of Epidemiology and Public Health, The Queen s University; Belfast, United Kingdom;* [3]*Department of Medicine, The Queen s University; Belfast, United Kingdom*
cardiac.research@royalhospitals.n-i.nhs.uk

Background; elevations in plasma homocysteine levels are associated with IHD. A C677T polymorphism in the MTHFR gene results in reduced folate-dependent enzyme activity and reduced methylation of homocysteine to methionine. The homozygous (TT) mutant genotype is associated with mild hyperhomocysteinemia, particularly in those populations with low plasma folate levels. There is controversy as to whether the TT genotype is associated with an increased risk of IHD and theoretically it may even protect against endothelial damage.
Aims; to investigate the presence of linkage disequilibrium between the C677T MTHFR polymorphism and IHD in a well defined population.
Methods; A total of 129 families were recruited (353 individuals). The presence of linkage disequilibrium between the C677T MTHFR polymorphism and IHD was tested for using the transmission disequilibrium test (TDT) / sib TDT and pedigree disequilibrium test (PDT). These tests are unaffected by population admixture. We tested for excess transmission of the C or T allele to affected individuals using two-tailed tests.
Results;TDT / sib-TDT; 47 of 112 discordant sibships and 12 of 20 trios were informative (one sibship or trio per family). There was a statistically

significant excess transmission of the C allele to affected individuals, (p=0.016).
PDT; 59 families were informative. There was a statistically significant excess transmission of the C allele to affected individuals, (p=0.021).
Conclusion; using newly developed family-based association methods we have demonstrated excess transmission of the C allele to individuals affected with IHD, suggesting that the T allele may be protective against IHD.

P1362. Association of common noncoding mtDNA polymorphisms with the status of the cardiovascular system

V. P. Puzyrev[1], *M. V. Golubenko*[1], *M. B. Freidin*[1], *V. B. Salukov*[1], *E. R. Eremina*[2]
[1]*Institute of Medical Genetics of Russian Academy of Medical Sciences; Tomsk, Russian Federation;* [2]*Buryat division of th Institute of pediatry and human reproduction of Russian Academy of Medical Sciences; Ulan-Ude, Russian Federation*
valery@img.tsu.ru

Mitochondrial DNA is a very variable locus of human genome. Traditionally, mtDNA polymorphisms are considered as neutral, especially those in noncoding regions. We have studied restriction polymorphism in D-loop of mtDNA and V region length polymorphism in several Siberian populations as well as some affected groups (hypertension, heart blocks, complicated pregnancy). Several traits concerning cardiovascular system function also have been investigated in the samples (blood pressure, lipid levels, ECG parameters) By comparing allele frequencies, we have found some significant (P<0.05) differences in the HaeIII 16517 frequency between case and ethnically matched controls; in Buryats with complicated pregnancy (42% vs 62% in controls) and in Russians with heart blocks (77% vs 58% in controls). Also, this polymorphism contributes in blood pressure variability in healthy Tuvinian population, with HaeIII+ individuals having lower blood pressure levels. V region length polymorphism contributes in ECG intervals and peaks variability in Tuvinians (PQ, QT, RR-ratio), where individuals harboring deletion or insertion in this region have lower values of these parameters. Some differences were revealed also for haplotype frequencies. These findings suggest possible involving of mtDNA in the function of cardiovascular system and allow it to be considered as susceptibility locus for common cardiovascular diseases. In particular, non-coding polymorphisms which were investigated may also have functional consequences. Major noncoding region of human mtDNA contains regulatory elements which are necessary for mtDNA expression. Hence, sequence polymorphisms may change the structure of these elements, that in turn contributes to mitochondrial function alterations.

P1363. Investigation of genetic variants of the tumor necrosis factor alpha (TNF-a) and their importance in the risk profile of CAD

U. Schagdarsurengin[1], *P. Greiser*[1], *S. Schulz*[1], *U. M ller-Werdan*[2], *A. Kabisch*[3], *D. Rehfeld*[1], *C. Schn rer*[2], *T. S §*[1], *L. Weidhase*[2], *A. Nordwig*[2], *K. Werdan*[2], *C. Gl ser*[1]
[1]*Inst. of Human Genetics & Med. Biology, University Halle; Halle, Germany;* [2]*Dep. of Internal Medicine, University Halle; Halle, Germany;* [3]*Inst. of Transf. Medicine/Bloodbank, University Halle; Halle, Germany*
christiane.gl ser@medizin.uni-halle.de

TNF-a, a potent cytokine, plays an important role in atherosclerotic development and could been identified in many tissues involved in the progression of atherosclerosis. Interested in the effect of genetic variants of TNF-a on CAD we investigated 5 polymorphic sites (C-863A, C-857T, G-308A, G-238A and A+1312G) in 234 patients (76,6% male, mean 50,3y) with angiographically proven severe CAD and 161 healthy controls (63% male, mean 42,3 y). The patient group was analyzed regarding; age at 1.MI (<=or>=45y), survival time after 1.MI (<=or>=1y), number of affected vessels (1or more) and therapy (PTCA or ACVB). C-863A; we found a significantly increased frequency of A-allele carriers (dominant-model for mutant A-allele) in the patient group with 1-vessel disease compared to patients with 2 or more affected vessels (0,4 vs. 0,2; p<0,02). G-308A; the frequency of A-allele carriers (dominant-model for mutant A-allele) was significantly increased in the patient group with MI (0,297 vs. 0,197; p<0,05) compared with healthy controls. G-238A; MI patients, who had the 1.MI <=45y, have significantly increased frequency of A-allele carriers (dominant-model for mutant A-allele) compared with MI patients, who had the 1.MI >45y (0,162 vs. 0,049; p<0,02). A+1312G; the patient group with 1-vessel disease had a significantly decreased frequency of mutant G-allele carriers (dominant-model for the mutant G-allele) compared to patients with 2 or more affected vessels (0,057 vs. 0,192; p<0,02). Our results suggest an assoziation of genetic variants of TNF-a to the progress and severity of CAD and should be considered in the genetic risk profile of CAD.

P1364. Variant alleles in methionine cycle; association study in patients with coronary artery disease (CAD)

B. Janosikova[1], *J. Sokolova*[1], *E. Richterova*[1], *M. Vackova*[1], *J. Krijt*[1], *L. Meixnerova*[1], *K. Vesela*[2], *A. Vitova*[2], *P. Kraml*[2], *M. Andel*[2], *V. Kozich*[1]
[1]*Institute of Inherited Metabolic Disorders, Charles University; Prague, Czech Republic;* [2]*2nd Clinic of Internal Medicine, Hospital Kralovske Vinohrady; Prague, Czech Republic*
bjano@lf1.cuni.cz

Numerous studies demonstrated that CAD is associated with mild hyperhomocysteinemia. Plasma homocysteine concentrations are modulated in part by variants in genes coding for enzymes of the methionine cycle. We performed a case-control study to analyze the role of 7 variants in selected genes of the methionine cycle; cystathionine beta-synthase (CBS), methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MS) and methionine synthase reductase (MSR). We genotyped 591 healthy controls (48% men, median age 50, range 18-65 years) and 296 patients with coronarographically verified CAD (82% men, median age 56, range 33-65 years) using ARMS-PCR. All subjects underwent standard methionine loading test, their fasting and post-load concentrations of aminothiols were determined by HPLC method. The calculated odds ratios (and 95% confidence interval) for the rare variants in each locus are as follows; MSR524, TT vs. CT+CC, 1.05 (0.7-1.57); MSR66, GG vs. AG+AA, 0.94 (0.68-1.28); MS2756, GG vs. AG+AA, 1.32 (0.64-2.68); MTHFR1298, CC vs. AC+AA, 0.78 (0.45-1.32); MTHFR677, TT vs. CT+CC, 1.37 (0.87-2.15); heterozygosity for CBS844ins68bp vs. CBS844del/del, 0.53 (0.33-0.85, p=0.005). The impact of the 844ins68 on aminothiol concentrations was assessed by non-parametric test; the heterozygotes for 844ins68 had insignificantly lower fasting and postload concentrations of plasma homocysteine and fasting blood glutathione, the median post-load decrease of plasma cysteine concentrations was 23 and 15 micromol/l in del/del and ins/del individuals, respectively (Man-Whitney, p= 0.0002). Our data show that the 844ins68 variant is associated with a significantly decreased risk of coronary artery disease, its mechanism remains obscure and may be mediated through modulation of cysteine metabolism.

P1365. The study of the 4G/5G genetic polymorphism in the promoter of the plasminogen activator inhibitor-1 (PAI-1) gene in different groups of myocardial infarction patients.

M. V. Volkova[1], *O. A. Berkovich*[2], *E. A. Bazhenova*[2], *V. I. Larionova*[3], *E. V. Fomicheva*[1], *E. V. Schlyakhto*[2], *E. I. Schwartz*[1]
[1]*Petersburg Nuclear Physics Institute; St.Petersburg, Russian Federation;* [2]*Pavlov s State Medical University; St.Petersburg, Russian Federation;* [3]*Petersburg Pediatric Medical Academy; St.Petersburg, Russian Federation*
wolf@sp.ru

We have investigated the PAI-1 4G/5G polymorphism in different group of myocardial infarction (MI) patients and controls. Group 1 - 289 male MI patients (mean age 60 years) were collected from emergency department during 1-2 days after acute MI. Group 2 - 100 male patients with MI before 45 years (mean age 41 years) were recruited from Medical University Clinic at least 6 months after their MI. As a control group 284 randomly sampled schoolchildren were used. A prevalence of the 5G allele in MI group 1 compared to controls was found (p<0.001). In MI group 2 higher frequency of the 4G/4G genotype compared to control subjects was determined (p<0.01). The comparison of 4G/5G alleles distribution between MI group 1 and 2 showed a strong prevalence of 5G allele in MI patients collected during 1-2 days after their MI. Recently Heymans S. et al. (Nat.Med.,1999) found out that exogenic expression of PAI-1 in mice could prevent cardiac rupture. Such differences in the PAI-1 alleles distribution among MI patients, who were taken into analysis during different time period after their MI, allow us to make a following conclusion; MI patients carrying the 4G allele of the PAI-1 gene and, accordingly, higher PAI-1 levels, could have more chances to get rid of such MI complication as a cardiac rupture. But this supposition requires more detailed prospective study concerning survival after MI.

P1366. The Asn291Ser, Asp9Asn and Ser447Ter mutations in the lipoprotein lipase gene in myocardial infarction survivors.

D. V. Demidova[1], *E. A. Bazhenova*[2], *O. A. Berkovich*[2], *V. I. Larionova*[1], *E. V. Schlyakhto*[2], *E. I. Schwartz*[3]
[1]*Petersburg Pediatric Medical Academy; St.Petersburg, Russian Federation;* [2]*Pavlov s State Medical University; St.Petersburg, Russian Federation;* [3]*Petersburg Nuclear Physics Institute; St.Petersburg, Russian Federation*
wolf@sp.ru

Prevalent mutations in the lipoprotein lipase gene (LPL) Asn291Ser and Asp9Asn had been shown to be associated with increased plasma triglyc-

erides and decreased HDL cholesterol, whereas Ser447Ter on the contrary with decreased triglycerides and increased HDL cholesterol levels. Hence these variations may affect cardiovascular risk. The aim of this study was to investigate the frequency of these substitutions in the LPL gene in myocardial infarction (MI) survivors (cases) and in the general population (controls) and to examine whether the carriers of Asn291Ser and Asp9Asn are at an increased risk of MI. We examined 158 cases (males survived after MI before 45 years) and 124 controls. The frequencies of mutations carriers in the cases were 3,8% for Asn291Ser, 0,6% for Asp9Asn and 16,4% for Ser447Ter and in the controls 0,8%, 1,6% and 19,4%, respectively. All differences between the groups were not significant. Thus our results did not confirm the role of investigated LPL gene mutations in modulation of MI risk.

P1367. The association of the Q191R polymorphism of the paraoxonase gene with myocardial infarction in young men (<45 years).

S. N. Pchelina[1], *N. Grudinina*[2], *E. Bazhenova*[3], *O. Berkovich*[3], *D. Cherkashin*[3], *S. Boitsov*[3], *E. Schlyakhto*[3], *V. Gorbunova*[1], *E. Schwartz*[1]
[1]*Petersburg Nuclear Physics Institute; St.Petersburg, Russian Federation;* [2]*Petersburg State Technical University; St.Petersburg, Russian Federation;* [3]*Pavlov s State Medical University; St.Petersburg, Russian Federation*
wolf@sp.ru

Myocardial infarction (MI) remains the main cause of mortality and morbidity in developed countries. The role of genetic factors is thought to be more likely in premature MI. We studied the Q191R and L54M polymorphisms of the paraoxonase (PON1) gene and the C677T mutation of the methylenetetrahydrofolate reductase (MTHFR) gene in 216 men survived after MI at the age of 45 years. The age-marched control group consists of 157 unrelated healthy men from the same community. PON1 is a high-density lipoprotein-associated enzyme capable of hydrolysing lipid peroxides. C677T MTHFR is responsible for reduced MTHFR activity and associated with increase in plasma homocysteine levels. The genotype distributions and allele frequencies of the L54M PON1polymorphism and C677T MTHFR did not differ in patients compared to the controls. The genotype distributions of the Q191R PON1 polymorphism were significantly different between the groups (?2=8.85, df=2, P<0.01). The R191 allele (B allele) was significantly increased in patients (?2=8.62, df=1, P<0.003). The odds ratio of MI for subjects homozygous for the R191 allele was 3.3 [95% CI; 1.46-7.34] compared to the Q allele carriers. The paraoxonase B isotype might not protect well against lipid oxidation, a major atherogenic pathway. Thus, the RR genotype of the PON1 gene may be considered to be an independent risk factor for MI in men at the age of 45 years.

P1368. Factor XIII V34L, factor V Leiden, and prothrombin G20210A; relationship of common gene variants of blood coagulation factors to risk of myocardial infarction.

H. Zheng[1], *C. Butt*[1], *E. Randell*[2], *M. Porter*[1], *P. Parfrey*[3], *Y. Xie*[2]
[1]*Discipline of Laboratory Medicine, Memorial University of Newfoundland; St. John s, NF Canada;* [2]*Disciplines of Laboratory Medicine, Genetics, Pediatrics, Memorial University of Newfoundland; St. John s, NF Canada;* [3]*Discipline of Medicine, Memorial University of Newfoundland; St. John s, NF Canada*
yxie@morgan.ucs.mun.ca

Factor V leiden (FVL) and prothrombin 20210A (FII20210A) were suggested as candidate risk factors for myocardial infarction (MI) based on an association with increased thrombotic tendency. A common polymorphism, FXIII V34L has recently been associated with a protect role against the pathogenesis of MI. However, some studies have reported conflicting results. These inconsistencies may be due to a number of factors including the effect of gene-gene interactions, and the heterogeneous genetic background of samples used in studies, and a relatively small number of samples used for study. We are carrying out an extensive study of genetic prediposition in MI using a relative isolated Newfoundland population, which has a relatively homogeneous genetic background. The goal of this study is to evaluate the possible roles of these three gene variants in the pathogenesis of MI and examine any potential interaction that may occur between them. As part of a pilot study, we concurrently analyzed the prevalence of FXIII V34L, FVL, FII 20210A, in approximately 221 patients with MI and 154 normal controls from the Newfoundland population. The details of the carrier frequency, allele frequency of these three gene variants in both MI patients and controls are summarized in Tab-1. The incidence of FXIII 34L homozygotes in the MI patient group (5.4%) is lower than in normal controls (6.9%), but the difference is not statistically significant. No homogygotes for FVL or FII 20210A were found. Conclusions; These results clearly show the prevalence of the FII 20210A allele to be lower in the Newfoundland population (0.6%) compared with that usually reported for the general Caucasian population (2%). Nevertheless, the FII 20210A mutation is associated with an increased risk for MI in the Newfoundland population. Based on the limited number of samples analyzed so far our results have failed to support the FXIII 34L allele as a strong protective factor, or FVL as a risk factor for MI.

	FXIII 34L		FVL		FII20210A	
	CF	AF	CF	AF	CF	AF
Patients (n = 221)	46.2%	25.8%	4.6%	2.3%	3.2%	1.6%
Controls (n = 159)	43.5%	25.0%	4.5%	2.3%	0.6%	0.3%
P value	N.S.	N.S.	N.S.	N.S.	<0.05	<0.05
OR (95% CI)	1.1(-2.1,4.4)	1.1(-1.9,4.1)	1.0(-17,19)	1.0 (-42,44)	5.1 (4.1,6.1)	5.1 (4.1,6.0)

Tab -1 (note; CF-carrier frequency; AF-allele frequency; N.S.-Not Significant)

P1369. Cholesterol-lowering gene affects Lp(a) plasma levels

B. Kunz[1], *A. Lingenhel*[1], *O. Eliav*[2], *E. Leitersdorf*[2], *G. Utermann*[1], *H. G. Kraft*[1]
[1]*Institute of Medical Biology and Human Genetics; Innsbruck, Austria;* [2]*Hadassah University Hospital; Jerusalem, Israel*
Birgit.Kunz@uibk.ac.at

A locus for a cholesterol lowering gene (CLG) which lowers the cholesterol, LDL, and HDL levels was recently identified on chromosome 13q in a family with familial hypercholesterolemia (FH).(Am.J.Hum.Genet. 66;157-166,2000) Since Lipoprotein(a) (Lp(a)) is assembled from LDL and apolipoprotein(a) (apo(a)) we here addressed the question whether the CLG affects also Lp(a) levels. Lp(a) concentration is extremely variable (>1000 fold differences among subject) and is controlled by a major gene, the structural gene for apo(a). We have previously shown that in addition FH causing mutations in the LDL-receptor gene have an influence on Lp(a) levels. We analysed Lp(a) levels and apo(a) phenotypes in the original family (65 individuals) in which the CLG had been detected. Lp(a) concentrations were measured by ELISA and apo(a) isoforms were specified by immunoblotting. In addition to the K-IV polymorphism in the apo(a) gene and the FH genotype also the CLG had a significant independent effect on Lp(a) levels. Employing ANOVA the K-IV polymorphism explained 70% of the variation of Lp(a) levels and the LDL-R and the CLG explained 17,3% and 6,6% of the residual variation, respectively. Therefore we conclude that the cholesterol-lowering gene affects also Lp(a) levels.

P1370. Family based association study (qTDT) on lipid abnormality-candidate genes on an isolated, admixtured population.

Z. Han[1], *W. Li*[1], *H. C. Simon*[2], *D. Shmulewitz1*[1], *S. B. Auerbach*[3], *T. Lehner*[1], *J. Ott*[1], *M. Stoffel*[1], *J. M. Friedman*[1], *J. L. Breslow*[1]
[1]*the Rockefeller University; New York, NY United States;* [2]*Mathematical Genetics, MSKCC; New York, NY United States;* [3]*HRSA,Department of Health & Human Services; New York, NY United States*
hanz@rockvax.rockefeller.edu

Traditional linkage analyses lack power and precision when applied to complex traits such as cholesterol (chol) level. Association may be more successful at identifying genes of small effect, but are often controversial due to problems such as population stratification (PS). We examined 8 polymorphic sites, previously shown to be associated with abnormal lipid and lipoprotein levels, on the Kosrae Island where the population is an admixture of native Kosraeans and Caucasian from late 19th century. The 1102 individuals collected in this study aggregate along a main branch of the Kosraean pedigree. The traits studied included triglyceride (Tg), chol, apolipoprotein-AI (apoAI), apolipoprotein-AII (apoAII), apolipoprotein-B (apoB), body mass index (BMI) and blood pressure (BP) levels. The 8 markers were common polymorphisms in following candidate genes; apo AI-CIII-AIV gene cluster (apo CIII/SstI), apo AII (MspI), apo E (HhaI), cholesteryl ester transfer protein (CETP/TaqIB), cholesterol 7?-hydroxylase (CYP7/BsaI), hepatic lipase (HL/DraI) and microsomal triglyceride transfer protein (MTP/HhpI). The method we used was QTDT program by Abecasis GR et al. When analyzed under a variance component model, the apoCIII/SstI was associated with Tg (p=0.0031), and apoE/HhaI was associated with apoB level (p=0.0004, 0.0070 and 0.0285 for allele E4, E3 and E2). There was suggestive evidence for linkage of apoAII/MspI to systolic BP (p=0.0497), and the linkage evidence was accounted for by adding association to the model. Our study have the advantages of reducing likelihood of false-positive result from PS and increasing the chance of discovering relevant genetic factors due to a more homogenous environmental effect.

P1371. Predisposition to thrombotic complications in patients with varicose veins.

A. M. Sheydina[1], *O. L. Kolobova*[2], *S. S. Baranovskaya*[1], *V. I. Larionova*[2], *V. A. Kobilianskaya*[3], *K. A. Papayan*[3], *N. A. Bubnova*[4], *L. P. Papayan*[3], *E. I. Schwartz*[1]

[1]Petersburg Nuclear Physics Institute; St.Petersburg, Russian Federation; [2]Petersburg Pediatric Medical Academy; St.Petersburg, Russian Federation; [3]Russian Research Institute of Haematology and Transfusioligy; St.Petersburg, Russian Federation; [4]Pavlov s State Medical University; St.Petersburg, Russian Federation

oskar@mail.wplus.net

Despite intensive studies of predisposition to thrombotic complications in patients with varicose veins is disputed.We studied 158 patients with varicose veins without history of venous thrombosis (VV), 111 patients with vein thrombosis (VT), 50 patients with varicose veins, having complications of vein thrombosis (VV/VT) and 229 healthy controls. The C677T MTHFR gene, the R506Q FV gene, the C1565T GPIIIa gene were determined. The frequencies of the MTHFR gene TT genotype among patients with VV, VT, VV and VT, and control group were 5.7%, 10%, 16 %, 8.4%, respectively. This mutation is not independent risk factor for VT development in Russians. However, this genotype is a significant risk factor in patients with both VV and VT {OR2.1, CI [0.19 24.01]}. The frequencies of the GPIIIa gene s CT+TT genotypes were 18.4%, 33.3%, 36%, 21.8% in different groups, respectively. In patients with both VV and VT complications was higher then in subjects with VT 2.1, (CI [1.34 4.99]) and 1.79 (CI [1.12 2.67]), accordingly. Although, the R506Q FV gene mutation is not a risk factor for thrombotic complications development in patients with VV (6% vs 3.5% in control group). While this mutation has a strong association with VT (OR 4.99, CI [2.63 9.46]). Our results allow us to make a conclusion that varicose veins may be an additive factor for thrombotic complications development in patients with other risk factors.

P1372. Genetic thrombophilic background in thalassemia major patients

A. Iolascon[1], *S. Storelli*[1], *B. Coppola*[1], *G. Forni*[2], *P. Cianciulli*[3], *A. Piga*[4], *A. Maggio*[5], *S. Perrotta*[6], *P. Giordano*[1], *C. Magnano*[7], *A. Mangiagli*[8], *G. Sechi*[9], *H. H. Li*[10], *M. Devoto*[10]

[1]University of Bari; Bari, Italy; [2]University of Genova; Genova, Italy; [3]University of Rome; Rome, Italy; [4]University of Torino; Torino, Italy; [5]Ospedale Cervello; Palermo, Italy; [6]University of Naples; Napoli, Italy; [7]University of Catania; Catania, Italy; [8]Ospedale di Siracusa; Siracusa, Italy; [9]Ospedale Maggiore Milan; Milan, Italy; [10]DuPont Child. Hospital; Wilmington, DE United States

a.iolascon@bioetaev.uniba.it

It is well known that the frequency of thrombosis is increased in the thalassemia syndromes. Moreover a higher thrombotic risk has been described in patients with thalassemia intermedia, expecially in untransfused cases. The underlying hypercoagulable state has been attributed to widely variable hemostatic alterations including platelet hiperaggregability, particularly post splenectomy, and reduced levels of physiologic anticoagulants such as antithrombin III (ATIII) , protein C (PC) and protein S (PS) The aim of this study was to investigate the genetic background of the thrombotic thalassemic patients. We recruited 33 thalassemia major affected with thrombosis (venous and arterial), and a group of age and sex matched non-thrombotic thalassemia major patients and healthy control subjects. After informed consent a sample of 5 ml of blood was obtained. Molecular analysis of the factor V G1691A mutation (fV Leiden), the C677T mutation in the MTHFR gene, the prothrombin G20210A mutation in 3 - untranslated region, the hypervariable region 4 of intron 7 of the factor VII gene and the glycoprotein IIIa gene polymorphism was performed by means of PCR, restriction digestion and electrophoresis. Statistical analysis was performed by contingency table analysis using Fisher s exact test or chi-square test as appropriate. A p-value of 0.0001 was obained by comparing allele frequencies of the fVII polymorphism in thrombotic and non thrombotic thalassemia patients, suggesting that the H7 allele is protective against thrombosis in Cooley patients. All other p-values were > 0.05. Our results demonstrate that no one of the investigated factors seems to play a statistical significant role in the trombophilia status of thalassemic patients, with the exception of factor VII, which appear involved in thrombophilia status. The possible pathogenesis of this finding could be represented by modifying factor VII blood levels associeted with different haplotypes.

P1373. Inherited risk factors for cerebral vascular disease.

O. V. Sirotkina[1], *S. Kapustin*[2], *S. Lomakina*[3], *A. Elchaninov*[3], *L. Papayan*[2], *V. Gorbunova*[1], *E. Schwartz*[1]

[1]Petersburg Nuclear Physics Institute; St.Petersburg, Russian Federation; [2]Russian Research Institute of Hematology and Transfusiology; St.Petersburg, Russian Federation; [3]Sokolov Central Hospital; St.Petersburg, Russian Federation

wolf@sp.ru

Stroke and ischemic heart disease are the major causes of death and disability in industrial countries. Ischemic stroke is uncommon at young age and large proportions of stroke are of undetermined etiology. We investigated 111 relatively young individuals (mean age 36.5, range 15 to 56 years) with cerebral-vascular disease (CVD) and 94 subjects of control group was recruited from blood donors (mean age 41, range 24 to 60 years). Factor V Leiden mutation, 20210GA polymorphism in 3 -untranslated region of the prothrombin gene, G-455A in the ?-fibrinogene gene, the 4G/5G PAI-1 promoter polymorphism, C677T transition in the MTHFR gene, PlA1A2 polymorphism in GP III gene were analyzed. 41 patients with CVD had antiphospholipid antibodies (APA) — lupus anticoagulant or anticardiolipin. A significant increase in the prevalence of the T677 allele of the MTHFR gene in patients with cerebral-vascular disease carried antiphospholipid antibodies compared with subjects without APA was found (allele frequency 36% and 22%,respectively, p=0.02, OR=1.9, CI 95% 1.2-3.3). A significant increase in the prevalence of the 5G allele of the PAI-1 gene in non-carried antiphospholipid antibodies CVD patients compared to controls was also found (allele frequency 46% and 39%,respectively, p=0.036, OR=1.8, CI 95% 1.3-2.6). Platelet activation was increased in carriers of APA compared with non-carriers (p=0.01, OR=4.1, CI 95% 1.6-10.6) No other differences between patients and controls were observed.

P1374. A Familial Typical Migraine Susceptibility Region Localises to Chromosome 1q31

R. A. Lea[1], *R. A. Curtain*[1], *S. Quinlan*[1], *P. J. Brimage*[2], *L. R. Griffiths*[1]

[1]Genomics Research Centre, School of Health Sciences, Griffith University; Gold Coast, Australia; [2]Institute of Neurological Sciences, Prince of Wales Hospital; Sydney, Australia

r.lea@mailbox.gu.edu.au

Migraine is a genetically heterogeneous disorder. Currently, the number of genes involved in this common disorder and the mode of inheritance is unclear. However, some insight has been gained from genetic studies into a very rare and severe subtype of migraine known as familial hemiplegic migraine (FHM). In this study, we took a family-based linkage and association approach to investigate the FHM susceptibility region for involvement in typical migraine. Initial multipoint ALLEGRO analysis provided good evidence for linkage of Chr1q31 markers to migraine in a large multigenerational pedigree. The 1-LOD* unit support interval for suggestive linkage spanned ~18cM with a maximum allele sharing LOD* score of 2.04 obtained for marker D1S249, P = 0.001. Subsequent analysis of an additional sample of 82 independent pedigrees added support to the initial findings with a maximum LOD* of 1.07 (P = 0.013). Combining LOD* scores across families resulted in a maximum overall LOD* score of 3.11 at marker D1S249 (P = 0.00007). Utilising the independent sample of 82 pedigrees we also performed a Transmission Disequilibrium Test (TDT) using the TRANSMIT program. Results of this analysis indicated significant distortion of allele transmission at marker D1S205 (global X2(11) = 22.47, P = 0.021), located 8.2cM telomeric to D1S249. These positive linkage and association results will need further confirmation by independent researchers, but overall they provide good evidence for the existence of a typical migraine locus near these markers on Chr1q31 and support the idea of a common defective migraine gene within this genomic region.

P1375. Mapping of Migraine Genes to Chromosomes 1q, 19p and Xq.

L. R. Griffiths[1], *R. A. Lea*[1], *R. P. Curtain*[1], *P. J. Brimage*[2]

[1]Genomics Research Centre, School of Health Science, Griffith University; Gold Coast, Australia; [2]Institute of Neurological Science, Prince of Wales Hospital; Sydney, Australia

l.griffiths@mailbox.gu.edu.au

Migraine is a painful and debilitating neurovascular disorder affecting a large proportion (~12%) of Caucasian adults. The disease is genetically heterogeneous and the number of involved genes is currently not known. Studies in our laboratory have utilised the complementary strategies of family-based linkage analysis and allelic association testing to localise genes involved in migraine. We have incorporated techniques that employ both parametric and non-parametric statistical measures. To date we have localised three migraine susceptibility loci. One large family displayed significant excess allele sharing and cosegregation of chromosome 19p13 markers, thus localising a migraine susceptibility near the CACNA1A gene implicated in familial hemiplegic migraine, a rare and severe subtype of migraine. Further linkage analyses using families showing linkage to chromosome 19, have localised a second major migraine gene locus to chro-

mosome Xq. Here, we report results of a recent fine mapping project that has refined this localisation to Xq24-28. As well, we provide new evidence implicating a third migraine susceptibility region on chromosome 1q31 that may be partially interacting with the Xq region. Finally, we will also be presenting new genome scan data using a large independent sample of 92 migraine affected families and some positive case-control results from candidate gene studies. Overall, our findings show that migraine is a polygenic disorder and suggest that defective genes on different chromosomes may be interacting epistatically to influence this complex disorder.

P1376. Identification of epilepsy susceptibility mutations in a regulatory subunit of a calcium-activated potassium channel gene (KCNMB3) on 3q26.

M. Z. Labuda[1], *A. Siren*[2], *S. Bourgoin*[3], *S. Hu*[4], *H. McDermid*[4], *D. D Agostino*[5], *E. Andermann*[5], *A. Gambardella*[6], *M. St-Hilaire*[3], *H. Kwiecinski*[7], *M. Pandolfo*[3]

[1]*CHUM, Notre Dame Hospital; Montreal, PQ Canada;* [2]*Tampere University Hospital; Tampere, Finland;* [3]*CHUM; Montreal, Canada;* [4]*University of Alberta; Edmonton, AB Canada;* [5]*Montreal Neurological Institute; Montreal, PQ Canada;* [6]*School of Medicine; Catanzaro, Italy;* [7]*Medical University of Warsaw; Warsaw, Poland*

mlabuda@total.net

A recent study on 130 multiplex families with idiopathic generalized epilepsy provided evidence for susceptibility genes on chromosomes 2q36, 3q26 and 14q23. Prompted by this finding, we sequenced a gene on chromosome 3q26 that encodes a regulatory beta subunit (KCNMB3) of a calcium-activated potassium channel. We identified three variants in exon 3 of that gene; N161S, M226T, and a single base deletion introducing a stop codon which truncates the last 23 aminoacids, delA807. We compared the distribution of these polymorphisms between a group of 115 individuals with idiopathic epilepsy and a group of 165 controls. The N161S polymorphism was found on 8.3% of patients chromosomes (19/230) and on 4.9% of control chromosomes 16/330). delA807 was found on 10.9% (25/230) of patients and 8.5% (28/330) on control chromosomes. This difference is non-significant. However, the distribution of genotypes in the patients group was in departure from Hardy-Winberg equilibrium (p< 0.005) and differred significantly from the control group; 10/115 epileptic patients were homozygotes or compound heterozygotes for the polymorphisms (three N161S/N161S, one delA807/delA807, five N161S /delA807, one M226T/delA807), compared to only 2/165 controls (one N161S/N161S and one N161S/delA807). These patients had generalized as well as partial epilepsy. We analyzed genetic association and linkage of these variants in epileptic families by the transmission disequilibrium test (Tdt). N161S and delA807 were trasmitted significantly more often to affected offspring andthe wild type allele was trasmitted significantly more often to unaffected offspring (P=0.004). This analysis could not be carried out for M226T because this variant was found in only one family. We genotyped 33 individuals in five families segregating both N161S and delA807. We detected 14 homozygotes or compound heterozygotes, 11 of which have epilepsy. Among the 19 remaining subjects, 15 were heterozygotes (two affected) and four wild type homozygotes (none affected). KCNMB3, though expressed at a low level in the brain, may play a role in the regulation of neuronal excitability. It accelerates the opening of a calcium-activated potassium channel in response to an increase of intracellular calcium, facilitating repolarization after sustained firing. If the variants we identified affect its function, KCNMB3 may be a suceptibility gene that contributes to the multifactorial etiology underlying epilepsy.

P1377. An Association Study Of 3 Novel Single Nucleotide Polymorphisms In The Calcium Channel Gene CACNA1A And Idiopathic Generalised Epilepsy

B. A. Chioza[1], *H. J. Wilkie*[1], *B. Suarez-Merino*[2], *D. McCormick*[3], *J. Blower*[3], *L. Nashef*[3], *P. Asherson*[1], *A. J. Makoff*[1]

[1]*Institute of Psychiatry; London, United Kingdom;* [2]*University College London; London, United Kingdom;* [3]*Kent & Canterbury Hospital; Canterbury, United Kingdom*

spbcaja@iop.kcl.ac.uk

The CACNA1A gene encodes the main pore-forming ?1A subunit of P/Q-type voltage-gated calcium channels. These channels are expressed in a large variety of neurones, where they have important roles in the control of membrane excitability, neurotransmitter release and gene expression. Mutations in the mouse homologue of this gene are responsible for phenotypes mainly comprising absence type epileptic seizures and ataxia in the models tottering and leaner. In humans, mutations are involved in the pathogenesis of episodic ataxia 2 (EA-2), spinocerebellar ataxia type 6 (SPA6), familial hemiplegic migraine (FHM). In a previous association study for idiopathic generalized epilepsy (IGE) we conducted with CACNA1A, a single nucleotide polymorphism (SNP) present in exon 8 gave a significant result in a case-control study. We confirmed this result by HHRR and TDT, which excluded possible population stratification artefacts. We have investigated this association further using 3 novel SNPs identified by denaturing high performance liquid chromatography (dHPLC). These polymorphisms are in the introns adjacent to exon 8. Genotyping was carried out in 230 IGE patients unselected for subtype and 238 controls. All three SNPs gave significant p values by genotype and by allele (p=0.0003, p=0.0015 and p=0.0104 respectively). These results provide confirmation of the initial result. To date none of the SNPs appear to be functional and probably exert their effect by being in linkage disequilibrium with a putative functional mutation. Future work will involve screening CACNA1A for mutations at greater distances from exon 8 to define the region of interest more precisely and to identify functionally significant SNPs, which might identify the putative IGE susceptibility locus itself.

Positions and Genotype Frequencies of CACNA1A Polymorphisms in IGE Patients and Control Subjects

P1378. Significant association of the tumor necrosis factor-alpha and its receptor 2 genes with human narcolepsy

H. Hohjoh[1], *N. Terada*[1], *M. Kawashima*[1], *Y. Honda*[2], *K. Tokunaga*[1]

[1]*University of Tokyo; Tokyo, Japan;* [2]*Seiwa Hospital; Tokyo, Japan*

hohjohh@m.u-tokyo.ac.jp

Narcolepsy is a sleep disorder characterized by recurrent daytime sleep episodes and cataplexy, and also a multifactorial disorder, involving genetic and environmental factors. A genetic factor strongly associated with narcolepsy has been found in the human leukocyte antigen (HLA) class II region; the HLA-DRB1*1501-DQB1*0602 haplotype predisposes to the disorder. We have been searching for other genetic factors associated with the disorder, and our previous study, where the tumor necrosis factor-alpha (TNF-a) gene were investigated in narcoleptic patients and healthy controls, suggested that TNF-a could be a new susceptibility gene for human narcolepsy. In the present study, we focused on the tumor necrosis factor receptor 2 gene (TNFR2) and examined if there were any associations of the receptor with human narcolepsy. The TNFR2 gene is mapped on chromosome 1p36 and possesses the single nucleotide polymorphism (SNP) that participates in an amino acid substitution at position 196, i.e., generates two alleles having methionine (196M) and arginine (196R). Using the SNP (196M/R) as a marker, we investigated TNFR2 in narcoleptic patients and healthy controls. Results indicate that the frequency of the TNFR2-196R allele in the patients was higher than that in the healthy controls. We further examined the relationship of the TNFR2 and TNF-a genes with the susceptibility to human narcolepsy. Results suggested that the combination of TNFR2-196R and TNF-a(-857T) could increase predisposition to the disorder. Accordingly, these observations suggest the possibility that TNF-a-signal transduction system could participate in the pathogenesis and pathophysiology of human narcolepsy. Finally, we add that DRB1*1502 appears to have a negative association with narcolepsy, leading to the possibility that the haplotype carrying DRB1*1502 confers protection against human narcolepsy.

P1379. Further Studies on Association Studies Between OPRM1 and IGE

H. J. Wilkie[1], *B. Chioza*[1], *D. McCormick*[2], *L. Nashef*[2], *P. Asherson*[1], *A. J. Makoff*[1]

[1]*Institute of Psychiatry; London, United Kingdom;* [2]*Kent and Canterbury Hospital; Canterbury, United Kingdom*

h.goodwin@iop.kcl.ac.uk

Animal studies have provided compelling evidence for a role for opioid receptors in idiopathic generalised epilepsy (IGE), and in particular absence epilepsy. The WAG/Rij strain of rats exhibits both non-convulsive epileptic seizures similar to absences and a high density of mu-opioid receptors in the thalamic nuclei, striatum and cortex. The mu-opioid receptor can decrease neuronal excitability by activating G-protein coupled inwardly rectifying potassium channels. A number of polymorphisms in OPRM1 have been previously detected through mutation screening. An A to G substitution at position 118 in exon 1 results in the replacement of asparagine by aspartic acid (position 40). A recent study reported association between the Asp40 allele and an idiopathic absence epilepsy sample comprised of childhood and juvenile absence epilepsies (p=0.019). We undertook this study in order to replicate these findings. Genotyping was performed on 218 probands and 200 controls for A118G and a less frequent polymorphism, C17T. Only A118G was found to be significant (p=0.00028). Within family studies were performed for 104 probands and both parents. Both HHRR and ETDT analysis gave a significant difference between transmitted and untransmitted alleles (p=0.006 and p=0.0087 respectively). Mutation screening by denaturing high performance liquid

chromatography (dHPLC) has so far detected a number of mutations. These results confirm and improve upon the earlier findings. To date, it is unclear how the Asp40 allele is involved in the generation of absence seizures. We will be genotyping polymorphisms detected by dHPLC in exonic and intronic regions of OPRM1 soon.

P1380. Early Onset Familial Parkinson s Disease and Parkin Gene Mutations

A. Parsian[1], *B. Racette*[2], *R. Sinha*[1], *J. S. Perlmutter*[2]
[1]*University of Louisville; Louisville, KY United States;* [2]*Washington University; St. Louis, MO United States*
parsian@louisville.edu

Idiopathic Parkinson s disease (PD) is an age dependent, neurodegenerative condition. Although it is predominantly a sporadic disease, 20-25% of cases are familial suggesting a complex mode of inheritance. a-Synuclein and ubiquitin C-terminal hydrolase genes have been implicated in a few families with autosomal dominant form of PD. In addition, mutations in parkin gene have been identified as cause of autosomal recessive juvenile parkinsonism. Recently, Lucking et al., (2000) reported that 49% of their autosomal recessive PD families with early onset (7 to 58 years) had parkin mutations. In the isolated PD group, 77% of those with age of onset less than 20 and 3% with age of onset less than 30 years had mutations in the parkin gene. In order to determine the role of mutations in parkin gene in our familial PD sample, we screened a sample of 161 patients with a positive family history for PD and 108 matched normal controls with mutations in exons 4, 6, 7, and 8. The PD sample was divided into two groups based on the age of onset of 40 years. None of the reported mutations in parkin gene was detected in our PD subgroups or normal controls. We concluded that parkin gene dose not play a role in the development of early onset familial PD in our sample.

P1381. Detection of heterozygous exon deletions in a large family with parkinsonism using the LightCycler

K. Hedrich[1], *P. P. Pramstaller*[2], *C. Eskelson*[1], *B. Kis*[1], *M. Kann*[1], *J. Garrels*[1], *P. Vieregge*[1], *L. J. Ozelius*[3], *P. L. Kramer*[4], *E. Schwinger*[1], *C. Klein*[1]
[1]*Medizinische Universitaet zu Luebeck; Luebeck, Germany;* [2]*Regionalkrankenhaus Bozen; Bolzano, Italy;* [3]*Albert Einstein College of Medicine; Bronx, NY United States;* [4]*Oregon Health Sciences University; Portland, OR United States*
hedrich_k@neuro.mu-luebeck.de

Autosomal recessive parkinsonism is a progressive neurodegenerative disorder which is clinically similar to idiopathic Parkinson«s disease but associated with additional neurological signs. We studied a large family (194 members) from South Tyrol (Northern Italy) with parkinsonism in three successive generations (definitely affected; n=13, possibly affected; n=12) and other movement disorders, such as isolated postural and kinetic upper limb tremor (n=10) and restless legs syndrome (RLS; n=14). In the paternal branch, parkinsonism in four affected siblings was shown to be due to a compound heterozygous mutation previously reported as DC1 (del Ex1-7) and DC2 (del1072), respectively, while the origin of parkinsonism in the maternal branch was unknown. By haplotype analysis, linkage to loci for parkinsonism on 4p, 4q and 2p, as well as to the GCH1 gene could be excluded for both parkinsonism and RLS. However, incompatibilities at the markers D6S411 and D6S305 (located in intron 7 of parkin) suggested hemizygosity and thus a partial deletion of one allele of parkin. By quantitative PCR using the LightCycler (Roche), we identified a heterozygous deletion of exon 7 and in additon, redefined the DC1 mutation to be a deletion of exon 7 only. For this analysis we developed a new method of quantitative duplex PCR, involving PCR of all 12 exons of parkin and coamplification of beta globin as internal standard. Taken together, we present a new and accurate method to detect heterozygous exon deletions in parkin. Patients with parkinsonism should be tested for the presence of this kind of mutations.

P1382. Associtions Between Tnf Beta, Colorectal Cancer And Inflammatory Bowel Disease

M. R. Eichenberger, *R. Lewis, S. Plymale, S. Galandiuk*
University of Louisville; Louisville, KY United States
mreich01@athena.louisville.edu

Background; Ulcerative colitis and Crohn s disease are disorders that affect 1 million Americans and are collectively referred to as inflammatory bowel disease (IBD). In many patients, it is difficult to distinguish which disease is present and both are associated with increased risk of cancer. Tumor necrosis factor beta (TNF-beta)is involved in rapid host response and has been linked to IBD. Therefore, polymorphisms in this gene may be associated with IBD or cancer. A Nco1 RFLP in intron 1 of the TNF-beta gene, is such a site. The goal of this study was to determine whether genotype or allele frequencies reveal an association with colorectal cancer or IBD. Methods; DNA from 124 IBD patients (68 Crohn s disease, 56 ulcerative colitis), 33 colon cancer patients and 77 controls were PCR amplified using primers specific for the RFLP site in intron 1 of TNF-beta. This generated a 782bp product, which was digested with Nco1 and gel electrophoresed. All patients were Caucasians from a predominantly rural region. Results. The digest produced two alleles and three genotypes. Statistical analysis of the allele and genotype frequencies revealed a difference in the TNFB1/B1 genotype frequency between cancer and control patient populations (p=0.034). There were no significant differences between IBD patient groups and controls. Conclusions; The TNF-beta intron 1 polymorphism TNFB1/B1 genotype was more common in cancer patients than in controls, confirming results reported by Park et al (1998). It does not appear to be related to ulcerative colitis or Crohn s disease, supporting Hampe et al (1999).

P1383. Genome wide scan in celiac disease - confirmation of previous results, strengthens the hope for positional cloning in complex diseases.

. Torinsson Naluai[1], *S. Nilsson*[2], *L. Samuelsson*[1], *A. H. Gudj nsd ttir*[3], *H. Ascher*[3], *J. Ek*[4], *B. Hallberg*[1], *B. Kristiansson*[3], *T. Martinsson*[1], *O. Nerman*[2], *L. M. Sollid*[5], *J. Wahlstr m*[1]
[1]*Dept. of Clinical Genetics; G teborg, Sweden;* [2]*Chalmers University of Technology; G teborg, Sweden;* [3]*Dept. of Pediatrics, Queen Silvia Children s Hospital; G teborg, Sweden;* [4]*Dept.; Drammen, Norway;* [5]*Institute of Immunology, Rikshospitalet; Oslo, Norway*
asa.torinsson@sahlgrenska.se

Celiac disease(CD) is a common chronic inflammatory disorder of the small intestine. In fact, in Sweden and probably in the entire western world, it is the third most common chronic disorder among children. CD is associated to HLA but is strongly influenced by genetic factors other than the HLA-complex. We have performed a genome-wide scan in CD. Based on the relatively high risk of siblings to develop CD, also in comparison with other autoimmune and chronic inflammatory disorders, we believe that genome-wide screening is a very promising way to find important genes that predispose individuals to CD. We investigated familiar segregation at 400 microsatellite markers in 108 well defined Swedish and Norwegian families. We found several interesting regions. Strikingly, our best results coincide with earlier findings in independent families! This gives strong evidence that these regions are indeed true susceptibility regions in CD.

P1384. Localization of a susceptibility gene for atopic dermatitis to chromosome 3q21

Y. A. Lee[1,2], *U. Wahn*[2], *R. Kehrt*[2], *L. Tarani*[3], *L. Businco*[3], *D. Gustafsson*[4], *F. Andersson*[4], *A. P. Oranje*[5], *A. Wolkertstorfer*[5], *A. von Berg*[6], *U. Hoffmann*[7], *W. K ster*[8], *T. Wienker*[9], *F. R schendorf*[1,9], *A. Reis*[1]
[1]*Gene Mapping Center, Max-Delbr ck-Center; Berlin, Germany;* [2]*Dept. of Pediatric Pneumology and Immunology, Humboldt-University; Berlin, Germany;* [3]*Dept. of Pediatrics, University La Sapienza ; Rome, Italy;* [4]*Dept. of Pediatrics, rebro Medical Center Hospital; rebro, Sweden;* [5]*Dept. of Dermatology and Venereology, University Hospital; Rotterdam, The Netherlands;* [6]*Dept. of Pediatrics, Marienhospital; Wesel, Germany;* [7]*Dept. of Pediatrics, Technical University; Munich, Germany;* [8]*TOMESA-Fachklinik; Bad Salzschlirf, Germany;* [9]*Institute of Medical Biometry, Informatics and Epidemiology; Bonn, Germany*
yolee@mdc-berlin.de

Atopic dermatitis (eczema) is a chronic inflammatory skin disease with onset mainly in early childhood. It is commonly the initial clinical manifestation of allergic disease, often preceding the onset of respiratory allergies. Along with asthma and allergic rhinitis, atopic dermatitis is an important manifestation of atopy that is characterized by the formation of allergy antibodies (IgE) to environmental allergens. In the developed countries, the prevalence of atopic dermatitis is approximately 15% with a steady increase over the past decades. Genetic and environmental factors interact to determine disease susceptibility and expression. Family and twin studies indicate that the genetic contribution is substantial and previous studies reported associations of atopic dermatitis with several candidate genes. To identify susceptibility loci for atopic dermatitis, we ascertained 199 families of European origin with at least two affected siblings based on established diagnostic criteria. A genome-wide linkage study revealed highly significant evidence for linkage on chromosome 3q21 (Zall=4.31, P=8,42x10-6). Moreover, this locus provided significant evidence for linkage of allergic sensitisation under the assumption of paternal imprinting (hlod=3.71, a=44%) further supporting the presence of an atopy gene in this region. This locus has not previously been noted in genome wide studies investigating asthma or other atopic phenotypes. Our findings suggest that distinct genetic factors contribute to susceptibility to atopic dermatitis

and that the study of this disease opens new avenues to dissect the genetics of atopy.

P1385. Studies on linkage and association of asthma with the Interleukin 4-Receptor alpha chain gene (IL-4Ra) in Sardinian population.

G. Sole[1], *G. Pilia*[1], *L. Balaci*[2], *D. Altea*[2], *C. Maganucco*[2], *N. Olla*[1], *C. Spada*[2], *M. Fattori*[2], *S. Rassu*[2], *V. Brancolini*[3], *M. Devoto*[3], *A. Cao*[1]
[1]*Istituto di Ricerca sulle Talassemie ed Anemie Mediterranee, CNR; Cagliari, Italy;* [2]*Dipartimento di Scienze Biomediche e Biotecnologie, Universit di Cagliari; Cagliari, Italy;* [3]*Dipartimento di Oncologia, Biologia e Genetica, Universit di Genova; Genova, Italy*
G.Sole@irtam.ca.cnr.it

Asthma is a chronic inflammatory disease of bronchial epithelium and submucosa and it is one of the most common chronic diseases, affecting 2-4% of the population. It is a complex disease with multiple genetic and environmental determinants. Several studies have shown linkage or association of atopic asthma and IgE levels to the IL-4Ra gene in ethnically diverse populations, but not in all populations. The aim of this study was to determine the involvement of the IL-4Ra gene in the genetic determination of asthma and/or the production of IgE in Sardinian population, that is a isolated founder population. We studied the ILE50VAL variant and in addition we looked for and genotyped 3 highly polymorphic microsatellite markers of the IL-4Ra gene, in a sample of 121 affected sib-pairs belonging to 110 families of Sardinian ancestry. All the subjects were phenotyped for asthma, atopy and total serum IgE. We performed a linkage analysis by affected sib-pair method and an association study by the TDT (Transmission disequilibrium test), using Genehunter 2.0 program. Linkage analysis indicated no significant increase in allele sharing for asthma, atopy or IgE levels. No allele transmission disequilibrium was observed and neither particular 3 point haplotype was associated with any phenotype. We conclude from our data that IL-4Ra are unlikely to exert a major effect on the induction of the asthmatic phenotype in Sardinian population.

P1386. Searching for Candidate Genes for Asthma using Combined Linkage and Segregation Analysis

N. MANIATIS[1], *C. Lonjou*[2], *A. Collins*[2]
[1]*University of Southampton; Southampton S016 6YD, United Kingdom;* [2]*University of Southampton; Southampton, United Kingdom*
nikmaniatis@hotmail.com

Segregation analysis involves fitting a general model to the inheritance pattern of a trait. This method is used for parametric linkage analysis where the mode of inheritance must be specified exactly. Several non-parametric methods, however, have been proposed for linkage analysis of complex traits with unknown mode of inheritance and therefore comparison of parametric and nonparametric methods to search for candidate genes affecting asthma form the core of this study. Data on chromosome 5 from the Consortium on Asthma Genetics (COAG) were studied. Various analyses of individuals affected with asthma were compared. This study is ideal for comparison in that it has been previously analysed by COAG using nonparametric alternatives. Three phenotypes were used; a) affection dichotomy, where both normal and affected sibs were included in the analysis; b) analysis of affection status, which was based on affected sibs only and c) analysis with severity and diathesis, which were defined as a polychotomy or a quantitative trait within affected or normal individuals, respectively. Single-point analyses were performed using the COMDS program (combined segregation and linkage analysis with diathesis and severity). A number of models were employed throughout the study but emphasis was given to the additive single and two locus models. There is evidence to suggest that certain classes of parametric models are useful for linkage analysis of complex traits.

P1387. Linkage disequilibrium mapping of the novel psoriasis susceptibility region on chromosome 19

A. Reis[1], *P. Hensen*[2], *C. Windemuth*[3], *F. R schendorf*[3], *G. N rnberg*[4], *A. Stadelmann*[2], *V. Hoppe*[2], *D. Fennecker*[2], *M. St nder*[5], *M. Schmitt-Egenolf*[6], *T. Wienker*[3], *H. Traupe*[2]
[1]*Inst. of Human Genetics; Erlangen, Germany;* [2]*Dept. of Dermatology; M nster, Germany;* [3]*Inst. of Med. Biometry; Bonn, Germany;* [4]*Max-Delbr ck-Center; Berlin, Germany;* [5]*Fachklinik; Bad Bentheim, Germany;* [6]*Dept. of Dermatology; Trondheim, Norway*
reis@humgenet.uni-erlangen.de

Recently we identified a novel psoriasis susceptibility region on chromosome 19 by genome-wide linkage analysis. To further evaluate the role of the susceptibility gene(s) in this region, we performed linkage disequilibrium (LD) studies using densely spaced DNA markers. We recruited a completely new sample of 199 nuclear psoriasis families (trio-design). 49 polymorphic microsatellite markers covering the region from marker D19S216 to D19S412 were genotyped. To test for allelic association, we performed the family based association test (FBAT) and the classical transmission/disequilibrium test (TDT). Two regions of interest were identified. The first region comprises about 1.5 cM and is defined by the three closely linked markers D19S928 (p=0.0269); D19S414 (p=0.0027); D19S871 (p=0.0114), and by the adjacent D19S431 (p=0.0046). The second region is defined by D19S922 (p=0.0260), by D19S391 (p=0.0027), and by D19S916 (p=0.0094) and extends about 4.8 cM. P-values were corrected for multiple testing. We conclude that this LD profile confirms the existence of at least one psoriasis susceptibility locus in the newly defined region on chromosome 19. This locus maps between 51.7 cM and 53.2 cM from pTEL (nucleotide coordinates 36.9 Mb to 40.2 Mb). A second region of interest spanning from 26 cM to 30.8 cM also displays significant LD. Identification of this locus will require genotyping of more densely spaced markers iin order to further narrow this candidate gene region with positional cloning methods.

P1388. Refinement of the PSORS4 psoriasis susceptibility locus on chromosome 1q21

G. Novelli[1], *F. Capon*[1], *S. Semprini*[1], *S. Chimenti*[1], *G. Fabrizi*[2], *G. Zambruno*[3], *C. Carcassi*[4], *R. Mingarelli*[5], *B. Dallapiccola*[5]
[1]*Tor Vergata University of Rome; Rome, Italy;* [2]*Catholic University of Rome; Rome, Italy;* [3]*IDI IRCCS; Rome, Italy;* [4]*University of Cagliari; Cagliari, Italy;* [5]*CSS Mendel Institute; Rome, Italy*
novelli@med.uniroma2.it

Psoriasis is a chronic skin disorder affecting approximately 2% of the Caucasian population. Family clustering of the disease is well established and non-parametric linkage analyses have mapped disease susceptibility loci on chromosomes 6p (PSORS1) and 17q (PSORS2). Non confirmed evidence for linkage is also available for chromosomes 2q 3q, 4q (PSORS3), 8q, 16q and 20p. We mapped an additional susceptibility locus on chromosome 1q21 (PSORS4). In this study, we have carried out a linkage disequilibrium analysis, in order to achieve a finer localization. We recruited 79 triads from continental Italy and typed them at five loci spanning the 1.6 Mb region generating the highest multipoint LOD scores in our previous linkage study. We observed significant evidence for association with D1S2346 marker (p = 0.004). Results consistent with this data were obtained by typing an independent sample that included 28 patients and 56 controls, originating from Sardinia. In fact, p values of 0.02 were observed with both D1S2346 and D1S2715 markers. We sought further confirmation of our results by typing both samples with two novel markers (140J1C and 140J1D) flanking D1S2346. Marker 140J1D generated a p value of 0.003 in the continental Italy sample where a D1S2346/140J1D haplotype was found with a higher frequency among patients chromosomes. Altogether our data indicate that the 1q21 susceptibility gene may be localized in the genomic interval spanned by D1S2346 and 140J1D. This is the first evidence supporting the refinement of a non-HLA psoriasis susceptibility locus.

P1389. A haplotype-based case-control approach to mapping multiple sclerosis susceptibility genes in the Tasmanian population

J. P. Rubio[1], *M. Bahlo*[1], *H. Butzkueven*[1], *J. Dickinson*[2], *T. Dwyer*[2], *P. Groom*[2], *T. J. Kilpatrick*[1], *M. Ring*[2], *M. Sale*[2], *R. Simmons*[3], *T. P. Speed*[1], *I. van der Mei*[2], *R. Williamson*[4], *S. J. Foote*[1]
[1]*The Walter and Eliza Hall Institute of Medical Research; Melbourne, Australia;* [2]*The Menzies Centre for Population Health Research; Hobart, Australia;* [3]*The Australian National Register of Multiple Sclerosis Families; Canberra, Australia;* [4]*The Murdoch Children s Research Institute; Melbourne, Australia*
rubio@wehi.edu.au

Multiple sclerosis (MS) is the most common autoimmune disorder of the nervous system. Its onset is usually in young adulthood, and it affects mainly Caucasians of North European origin. Previous studies have identified a number of suggestive linkages with association between MS and human leukocyte antigen (HLA) polymorphisms on chromosome 6p21, thus far, the only consistently replicated finding. Genetically isolated populations such as Finland, Iceland and Sardinia offer attractive environments for the study of complex diseases. There is good evidence to suggest that the Tasmanian population is also a genetic isolate, which is more homogeneous than mainland Australia. A haplotype-based case-control strategy has been developed to search genome-wide for shared MS susceptibility genes in people of Tasmanian ancestry. Our recruitment strategy has focussed on identifying individuals (cases and controls) who are descendants of the initial settlers, from the early to mid 1800 s. To date, we have collected samples for 180 MS patients, 100 controls and a close constel-

lation of relatives for both. Theoretically, the expected size of a shared segment between two people in such a young population is 25 centimorgans (cM), with a standard deviation of 18 cM. Therefore, we are performing a genome-wide search at a resolution of 5 cM to detect shared chromosomal regions inherited identical-by-descent in distantly related MS patients using 811 genetic markers from the ABI PRISM“ HD-5 set. In addition, we are attempting to replicate findings for some regions of suggestive linkage identified previously using more densely spaced markers.

P1390. Analysis of the NRAMP1 gene implicated in iron transport; association with multiple sclerosis and age effects

N. de Villiers, *L. du Plessis, R. Rooney, C. Bouwens, J. Carr, M. Kotze*
University of Stellenbosch; Tygerberg, South Africa
jnpdv@gerga.sun.ac.za

Multiple sclerosis (MS) is believed to be an autoimmune process occurring in genetically susceptible individuals after an appropriate environmental exposure. We have exploited the homogeneous Afrikaner population of European ancestry to investigate the likelihood that iron dysregulation, in association with infectious and/or autoimmune disease susceptibility, may underlie the MS phenotype in a subgroup of patients. The functional Z-DNA forming repeat polymorphism of the natural resistance-associated macrophage protein-1 (NRAMP1) gene was analyzed in 104 patients diagnosed with MS and 522 Caucasian controls. A family-based control group consisting of 32 parental alleles not transmitted to MS offspring was additionally studied to exclude the likelihood of population substructures. Statistically significant differences in allelic distribution were observed between the patient and control samples drawn from the same population (P<0.01). Evidence is furthermore provided that alleles considered to be detrimental in relation to autoimmune disease susceptibility may be maintained in the population as a consequence of improved survival to reproductive age following infectious disease challenge. Although it remains to be determined whether the disease phenotype in MS patients with allele 5 of the NRAMP1 promoter polymorphism is directly related to dysregulation of iron or modified susceptibility to viral infection and/or autoimmunity, a combination of these processes most likely underlie the disease phenotype in these patients. In view of the emerging role of polymorphic variants in complex diseases and minimizing of possible confounding factors in this association study, we conclude that allelic variation in the NRAMP1 promoter may contribute significantly to MS susceptibility in the South African Caucasian population.

P1391. Mapping the murine hindshaker (hsh) gene locus; the story so far.

D. A. Vouyiouklis, *H. King, D. Kirkham, J. T. Anderson, S. A. Karim, K. J. Johnson, I. R. Griffiths*
University of Glasgow; Glasgow, United Kingdom
D.Vouyioukus@vet.gla.ac.uk

Spontaneous myelination-defective mutants may help identify individual genes involved in myelination or disorders affecting myelin. The autosomal recessive hsh mouse displays hindquarter and tail tremors during the period of maximum CNS myelination (P5-P20), that largely disappear in the adult. Ultrastructural examination showed hypo-myelination that was most pronounced in the spinal cord and brain stem . This has implications for the regional control of myelination by local oligodendrocytes, by comparison with their brain hemisphere counterparts. Initial experiments identified the chromosomes which harbour the hsh gene and a major modifying locus. Fine mapping originally placed the hsh mutation at an interval of 2.8cM. Recent experiments have reduced this interval to 1.2cm, with the mutation positioned up-stream of a calcium-binding protein gene cluster. At least two genes present within this family are up-regulated at the mRNA transcription level when the hsh phenotype is most strongly expressed (P20), which may suggest that the hsh gene is involved in their regulation. Current experiments are aimed at generating novel polymorphic markers within the area of interest, in order to identify putative candidate genes.

P1392. Genetics of Non-Insulin-Dependent-Diabetes mellitus

M. L. H pler[1], *T. Egger*[1], *K. Huber*[2], *K. Bauer*[2], *R. Schr der*[3], *K. H. Tragl*[3]
[1]*Ludwig Boltzmann-Institut f r Altersforschung; Vienna, Austria;* [2]*Ludwig Boltzmann-Institut f r Molekulargenetische Laboratoriumsdiagnostik, Donauspital, SMZ-Ost; Vienna, Austria;* [3]*Intitut f r Mikrobiologie und Genetik, Universit t Wien; Vienna, Austria*
Klaus.Huber@SMZ.magwien.gv.at

Non Insulin Dependent Diabetes Mellitus (NIDDM) is one of the most frequent diseases and patient numbers are rising worldwide. While the molecular basis of the common form of NIDDM has not been elucidated, it is thought to result from genetic defects that cause both insulin resistance and insulin deficiency. Insulin resistance can be demonstrated early in the course of the disease, at a later stage plasma levels of insulin fall and can no longer compensate for the insulin resistance.
Specific mutations have been identified that cause diabetes, e.g., mutations in the insulin receptor, mutations that decrease the number of receptors, mutations in the insulin gene and many others. The aim of this project is to find genes involved in the pathogenesis of NIDDM by gene-profiling of tissue samples from patients and controls with microarrays. In a second step the contribution of allegedly critical genes to the disease will be validated on all patients. In a first phase of this project, methods for the representative amplification of mRNA isolated from various tissues were compared. To this end, a one-step RT PCR protocol was found fit to amplify mRNA from sparse tissue samples. Subsequently, minute samples of adipose tissue will be collected during surgery for RNA extraction and gene-expression profiles be investigated with micro-arrays.

P1393. An association between NIDDM and GAA trinucleotide repeat polymorphism in the X25/frataxin (Fridreich s ataxia) gene in Russia

M. Shadrina[1], *P. Slominsky*[1], *O. V. Milosserdova*[1], *M. Balabolkin*[2]
[1]*Institute of Molecular Genetics; Moscow, Russian Federation;* [2]*Institute of Endocrinology; Moscow, Russian Federation*
shadrina@img.ras.ru

Multiple and different genetic defects may be associated with the development of non-insulin dependent diabetes mellitus (NIDDM). Friedreich s ataxia (FA) is an autosomal recessively inherited neurologic disease associated with a high prevalence of diabetes. This disease is caused by the decreased expression of a mitochondrial protein, frataxin, encoded by the X25 gene. A variable expansion of a GAA repeats is present in the fist intron of the X25 gene. Long repeats (> 66 repeats) are present in patients with Friedreich s ataxia, while a shot repeats may be associated with NIDDM. Using a polymerase chain reaction-based assay, We evaluated expansions of the triplet repeat in the X25 gene in two populations; control random group and NIDDM patients group from Russia. We found 11 allelic variants with a variable number GAA repeats (from 1 to 15 repeats). Sufficient differences in distribution of alleles between control population and patients were observed (X2=37,14, p<0,0001). A frequency allele contained 4 GAA repeats, especially, was distinguished between control subjects (69%) and patients (44%). We conclude that the X25/frataxine GAA polymorphism, probably, is associated with the pathogenesis of NIDDM in Russia.

P1394. Two-locus linkage-allelic association model using grade-of-membership analysis; IDDM recurrence risk in multiplex families.

E. H. Corder[1], *D. K. Madsen*[1], *L. L. Field*[2]
[1]*Duke University; Durham, NC United States;* [2]*University of British Colombia; Vancouver, BC Canada*
beth@cds.duke.edu

Recurrence risk was investigated for 578 sibling pairs derived from multiplex IDDM families (Field et al., 1996) using a pattern recognition approach called grade of membership analysis. The genetic information used to construct model-based groups included 1) the number of alleles shared IBD within sib pairs for each of 8 markers spanning the IDDM11 locus on chromosome 14, 2) IBD sharing for the HLA-DQ locus, and 3) the specific shared HLA-DQ alleles. Sex and disease status for the pair (AA; both affected; AU; discordant) were also input into the model. Six latent groups best represented the data. Each variable was highly informative. The groups had distinct profiles of model-based frequencies for the input variables. Group 1; male AA pairs, no IDDM11 region sharing, both HLA-DQ alleles shared IBD (usually 4-4, 3-3, or 1-3), and 18% prevalence. Group 2; no recurrence risk or sharing for either loci. Groups 3 and 4; exactly one IDDM11 region allele shared from the mother or father, respectively, recurrence risks of 34% and 50%, and similar prevalences of 20%. Group 5; female AA pairs most of whom shared the high-risk HLA-DQ 3-4 haplotype (6% prevalence). Group 6; complete IDDM11 region IBD sharing and 80% recurrence (26% prevalence). Seventy-six pairs (13%) belonged entirely to a single group. The remainder divided membership. We conclude that the distinct genetic patterns for the IDDM11 and HLA-DQ loci predict recurrence risk for IDDM in multiplex families.

P1395. Leptin - is it a marker for obesity in Saudis?

A. S. Warsy[1], *M. A. F. El-Hazmi*[2]
[1]*College of Science, King Saud University; Riyadh, Saudi Arabia;* [2]*College of Medicine, King Saud University; Riyadh, Saudi Arabia*
aswarsy@ksu.edu.sa

Leptin, the protein product of the obesity(OB) gene, is produced by the adipose tissues and plays an important role in regulation of body weight and

energy expenditure through CNS feedback mechanisms. Levels of leptin are elevated in patients with obesity and this is believed to result from leptin resistance. We conducted a Nation wide study to determine the prevalence of obesity in Saudi Arabia and screened 14805 adult males and female Saudis. Using Body Mass Index (BMI) values the individuals were classified as overweight(BMI; 25-29.9), obese (BMI >30) and normal (BMI <25). The overall prevalence of overweight and obesity in the Saudi males was 27.23% and 13.0% and in Saudi females was 25.20% and 20.3%, respectively. We then conducted a study of serum leptin level, plasma lipids (cholesterol and triglycerides) and glucose on randomly selected 173 individuals. 75 of these were obese, 49 were overweight and the rest were normal weight individuals. Serum leptin was estimated by RIA and the other parameters on autoanalyser. The serum leptin was significantly higher in the obese group compared to the non obese both in the males and females(p<.001). A significant correlation was obtained with BMI. However no correlation could be established with age, plasma cholesterol, triglycerides and blood glucose. This paper will present a comprehensive coverage of leptin functions, its genetics and its role in obesity in Saudis.

P1396. A balanced translocation t(4;15) associated with severe obesity

J. Klar[1], *C. Rehnman*[2], *G. Ann ren*[1], *N. Dahl*[1]
[1]*Department of Genetics and Pathology; Uppsala, Sweden;* [2] *rebro Regional Hospital; rebro, Sweden*
joakim.klar@genpat.uu.se

Obesity is a highly prevalent, multigenic trait that predicts increased morbidity and mortality and contributes to many health problems in the Western society.

We have identified a family in which a balanced reciprocal translocation between chromosome 4 and 15 is associated with severe obesity. Chromosome analysis revealed the translocation in the mother and her two children. Clinical evaluation and investigation of family members revealed a body mass index (BMI) of >= 45 kg/m^2, but no other pathological features in individuals carrying the translocation. Metabolic and dietary factors behind the increased BMI were excluded.

We suggest that the phenotype in this family is caused by a disruption of a functional gene or a positional effect at one of the two breakpoints. There are no identified genes corresponding to obesity close to the translocation breakpoints at chromosome 4 or 15 in our family. The Prader-Willi region is located on chromosome 15, but not close to the breakpoint.

To further map the translocation breakpoints, we are in using fluorescence *in situ* hybridisation (FISH) to metaphase chromosomes from affected family members.

P1397. Analysis of candidate gene region for polycystic ovary syndrome (PCOS) on Chr19p13

M. Urbanek[1], *A. Woodroffe*[1], *J. McAllister*[2], *R. S. Legro*[2], *D. A. Driscoll*[1], *J. F. Strauss*[1], *A. Dunaif*[3], *R. S. Spielman*[1]
[1]*University of Pennsylvania; Philadelphia, PA United States;* [2]*Pennsylvania State University; Hershey, PA United States;* [3]*Brigham and Women s Hospital; Boston, PA United States*
murbanek@mail.med.upenn.edu

PCOS is a common cause of infertility in reproductive age women and is characterized by hyperandrogenism and chronic anovulation. One promising candidate region for PCOS identified by a candidate gene screen is the insulin receptor gene (INSR) region on Chr19p13. This region has evidence for both linkage and association with PCOS. The strongest evidence for linkage with PCOS in 95 affected sister pairs was with short tandem repeat polymorphism D19S884 (IBD = 0.62; Chi Square = 7.04). The strongest evidence for association with PCOS as measured by the transmission disequilibrium test (TDT) in a sample of 347 parent-affected offspring trios was observed with allele 8 of D19S884 (Chi Square = 14.58) and allele 12 of D19S922 (Chi Square = 10.38). D19S884 and D19S922 are separated by 13 kb and both markers are located approximately 1 Mb centromeric to INSR. Association in the presence of linkage as determined by the TDT is dependent on the presence of disequilibrium between the phenotype and marker allele tested, and disequilibrium generally is not maintained over such extensive regions in outbred populations. It is therefore unlikely that the observed association is due to a variant at the INSR gene itself. More likely the association is due to a variant in an unidentified gene or a very distal regulatory element of INSR. Six known genes and 12 unnamed mRNAs or ESTs have been mapped within 250 kb of D19S884 and D19S922. We are currently evaluating these genes and ESTs as candidate genes for PCOS.

P1398. Association with polycystic ovary syndrome and functional analysis of the follicle stimulating hormone receptor (FSHR) gene G2039A polymorphic site

S. K. Sahota, *C. Bell, I. Huhtaniemi, N. E. Haites*
University of Aberdeen; Aberdeen, United Kingdom
s.sahota@abdn.ac.uk

Polycystic ovary syndrome (PCOS) is a common endocrine disorder affecting up to 10% of women of a reproductive age characterised by polycystic ovaries, hyperandrogenism and chronic anovulation. Consequently, it is a major cause of infertility. The heterogeneity of symptoms presented has contributed to an elusive genetic aetiology. We performed a case-control study showing that the AA genotype of the G2039A FSHR gene polymorphism confers a reduced risk of PCOS (OR = 0.04;P < 0.05) in the control population (n=75) in comparison to PCOS women (n=29). This concurs with a recent study suggesting that women with the 2039G FSHR allele require higher ampoules of FSH during ovarian stimulation therapy with comparison to those with the 2039A FSHR allele. We are currently performing expression studies of each of the FSHR alleles (2039A & 2039G) in COS-7 cells in order to identify functional effects of the polymorphic locus on ligand-binding receptor-mediated second messenger signalling. The identification of genes involved in the pathogenesis of PCOS will assist in the accurate diagnosis of this disorder and improve clinical management of these patients, with particular significance in fertility treatment.

P1399. A Full Genome Screening in a large Tunisian family affected with Thyroid Autoimmune Disorders

H. Ayadi[1], *A. Maalej*[1], *H. Makni*[1], *F. Ayadi*[1], *M. Bellassoued*[1], *J. Jouida*[2], *N. Bougacha*[1], *M. Abid*[1]
[1]*Facult de M decine; Sfax, Tunisia;* [2]*Dispensaire Bir Elhfa ; SidiBouzid, Tunisia*
hammadi.ayadi@fmsf.rnu.tn

The autoimmune thyroid diseases (AITDs) including Graves disease (GD) and Hashimoto s thyroiditis (HT) are inherited as complex traits. We initiated a whole genome linkage study of patients with AITDs, in order to identify the susceptibility genes involved in their pathogenesis. We have studied 39 patients affected with GD or HT and 68 related controls, who belonged to a large consanguinous family composed of more than 200 members. Linkage analysis was performed using the lod score method under two arbitrary models, dominant and recessive ones. A positive lod score was found for D2S171, assuming a recessive mode of inheritance and 50% penetrance, which suggests the presence of a major AITDs susceptibility gene on chromosome 2p21. The examination of genes mapped to this region showed that the hFKBP-12 gene which modulates the immune system was mapped near D2S171 and could be therefore a candidate gene in AITDs pathogenesis. Genetic and functional studies of the hFKBP-12 gene in AITDs patients are being accomplished.

P1400. CTLA4 polymorphisms in a large Tunisian family affected with thyroid autoimmune disorders

A. Maalej
Faculty of Medecine; Sfax, Tunisia
abdellatifmaalej@eudoramail.com

We investigated the role of CTLA-4 gene in the autoimmune thyroid diseases (AITDs) development. We used three polymorphic microsatellites D2S311, D2S143 and the intronic CTLA-4 (AT)n marker to look for linkage and two CTLA-4 dimorphisms located in the promotor region at position -318 (C/T) and in exon 1 (49 A/G) to test an association. Forty-two patients and fifty-one related controls who belong to a large Tunisian family were investigated. We obtained no significant lod scores for each microsatellite assuming either dominant or recessive mode of inheritance. Looking for a genetic association, the allelic, genotypic and phenotypic frequencies corresponding to the CTLA-4 49 (A/G) position were compared. No significant differences between patients and controls were found. In addition, the CTLA-4 alleles transmission showed no statistical deviation from the expected 50% distribution. Therefore, CTLA-4 was neither a major nor a minor contributing gene to AITDs susceptibility.

P1401. CTLA-4 gene polymorphisms in Tunisian patients with Graves Disease

H. Hadj Kacem[1], *M. Bellassoued*[2], *N. Bouguacha*[1], *M. Abid*[2], *H. Ayadi*[1]
[1]*Facult de M decine; Sfax, Tunisia;* [2]*Service d Endocrinologie, CHU H di Chaker; Sfax, Tunisia*
hammadi.ayadi@fmsf.rnu.tn

Graves disease (GD) is organ-specific autoimmune disorder of multifactorial aetiology with a polygenic mode of inheritance. A recent report has demonstrated a linkage and an association between a genetic markers of CTLA-4 gene on chromosome 2q33 and GD. To confirm this association in

Tunisian population, three polymorphisms of CTLA-4 gene were analysed; one at -318 position from the ATG start codon consisting of a C/T change, a second in position 49 of the exon 1, which lies in a A/G transition and third in the 3 untranslated region with variant lenghts of dinucleotide (AT)n repeat. The genomic DNA from 87 patients with GD and 205 healthy individuals were genotyped after specific polymerase chain reaction (PCR) amplification. Comparative analysis using chi(2) test showed significant differences in allele and genotype frequencies of A/G dimorphic marker between patients and controls. A significant increase of A/A homozygous individuals among patients (25.3% vs 12.7%, p=0.007, OR=2.33) was found. Analyses of CTLA-4 A/G polymorphism with respect to sex showed a significant difference in AA genotypes between female patients and controls (OR=2.52; 95%, 1.22<OR< the of that The and was with in Population. Tunisian GD to susceptibility confers it, associated closely one or gene, CTLA-4 confirm results these conclusion, In 2.23<OR

P1402. Linkage And Association Analysis Of 70 Candidate Genes For Diabetic Nephropathy

K. G. Ewens, *K. E. O Brien, M. M. Kelly, F. N. Ziyadeh, R. S. Spielman*
University of Pennsylvania; Philadelphia, PA United States
ewens@mail.med.upenn.edu

Familial clustering of diabetic nephropathy (DN) suggests the existence of susceptibility genes that contribute to the development of kidney disease in diabetic patients. In order to identify these genes, we have analyzed 70 candidate genes for linkage and association with DN. Families were ascertained through a proband with diabetes and ESRD. DN in diabetic sibs of the proband was defined by elevated albumin (ACR >300 ug/mg) in two of three urine samples. Diabetic sibs were considered unaffected for DN if they had normoalbuminuria (ACR<30 ug/mg), duration of diabetes >15 years, and were not taking ACE inhibitor medications. Microsatellites <1cM from each candidate gene were genotyped in 32 parent-child trios and 47 multiplex families. Nominally significant TDT results were obtained at several genes (e.g., lamiminC1 and integrinA2). We focus here on the findings with two microsatellites in the region of the angiotensin receptor 1 gene (AGTR1; chromosome 3q) where Moczulski et al (Diabetes, 47;1164, 1998) found evidence for linkage with DN in discordant sibs (DSPs) but not from TDT. Although we found no evidence for linkage in DSPs, there was an elevated TDT at D3S1308 (allele 7; 64% of 44 transmissions; chi-square=3.3) and ATCA (allele 6; 72% of 18 transmissions; chi-square=3.6). When these results are combined with transmissions to unaffected offspring (diabetic but non-DN), the results for D3S1308 become more striking; allele 7 was transmitted to only 7 of 23 non-DN offspring, resulting in a contingency chi-squared of 6.7. In view of the small sample size these results must be considered preliminary; results from additional families will be presented.

P1403. Evidence for an association of the endothelial nitric oxide synthase gene polymorphism in intron 4 and progression to end-stage renal failure in a Cypriot population

K. Lamnisou[1], *M. Koptides*[2], *K. Demetriou*[3], *A. Pierides*[4], *C. Deltas*[2]
[1]*The Cyprus Institute of Neurology and Genetics; Nicosia, Cyprus;* [2]*The Cyprus Inst. of Neurology and Genetics; Nicosia, Cyprus;* [3]*Dept of Nephrology, Nicosia General Hospital; Nicosia, Cyprus;* [4]*Dept. of Nephrology, Nicosia General Hospital; Nicosia, Cyprus*
Deltasco@mdrtc.cing.ac.cy

A variable number tandem 27-bp repeat in intron 4 of the endothelial nitric oxide synthase (ecNOS) gene has been found to affect the plasma levels of NO metabolites. Two alleles are of varied frequencies in different populations (a and b). The shorter allele, a, has been associated in Japanese populations with the pathogenesis of coronary artery disease and progression of renal failure. The progression of renal failue was tested in patients with ESRD of various etiologies or in patients with various nephropathies, excluding diabetic nephropathy. Here we tested the putative association of this polymorhism in a Cypriot population of patients with ESRD, by studying the genotypes in 70 ESRD patients and 92 non renal patients or healthy subjects. The two alleles were of similar frequencies in the two populations, 0.15 and 0.85 for alleles a and b respectively. The frequencies of aa, ab, and bb genotypes were 0% (0/92), 33.7% (31/92), 66.3% (61/92) in the control group, and 8.6% (6/70), 15.7% (11/70), 75.7% (53/70) in the patient group. The data between patients and controls were analysed by chi square test, and showed that the frequency of ecNOS4 aa genotype in the patient population was significantly higher than that in the control group (P<0.005). Our work perhaps represents the first study supporting the association of ecNOS gene polymorphism with ESRD in a Caucasian population. Not surprisingly, the frequency of allele a is somewhat increased (15%) compared to Japanese populations reported (~10%). Also of interest is the finding that in our study population the aa genotype is a predisposing factor to ESRD progression regardless of etiology, not excluding diabetics.

P1404. Variance component analysis of a candidate region for bone mineral density in 1p36

C. Specchia[1], *H. Li*[2], *A. Tenenhouse*[3], *H. Rodriguez*[4], *L. D. Spotila*[4], *M. Devoto*[2,1]
[1]*Universita degli Studi di Genova; Genova, Italy;* [2]*AI duPont Hospital for Children; Wilmington, DE United States;* [3]*McGill Bone Centre; Montreal, PQ Canada;* [4]*Drexel University; Philadelphia, PA United States*
claudia@eco.unipv.it

Osteoporosis (OP) is a common condition characterized by reduced skeletal strength and increased susceptibility to fracture. It is estimated that 10 million people are affected with OP in the USA alone, and each year more than 1.5 million people worldwide suffer from fractures of the hip, the most debilitating consequence of OP. Low bone mineral density (BMD) is the single most important risk factor for OP. The risk of fracture approximately doubles with each 1 SD below the normal (young adult) BMD. The importance of genetic factors in variability in BMD is suggested by several epidemiological studies showing increased concordance in monozygotic vs. dizygotic twins, increased risk of low BMD with parental history of low BMD, and the results of segregation analysis consistent with major gene influences on BMD and other related traits. Our initial genome screen in seven large Canadian pedigrees suggested a candidate region for BMD in 1p36, with a peak multipoint lod-score for hip BMD of 2.29 near marker D1S450 obtained with the Haseman-Elston test. In order to verify this finding, we typed the same families and an additional sample of 42 Canadian nuclear families for 9 markers spanning ~30 cM around D1S450. Variance component analysis using the Genehunter program gave a maximum multipoint lod-score of 1.2 for the old families, of 1.1 for the new families, and of 2.1 for all families combined for hip BMD. These results seem to confirm that a locus for BMD may be located in this region of chromosome 1.

P1405. Two new SNPs in the COL1A1 promoter are involved in specific binding to osteoblast nuclear factors.

N. Garcia-Giralt[1], *X. Nogu s*[2], *A. Enjuanes*[2], *S. Quintana*[2], *A. Bay Jensen*[1], *L. Mellibovsky*[2], *A. D ez-P rez*[2], *D. Grinberg*[1], *S. Balcells*[1]
[1]*Department of Genetics, Universitat de Barcelona; Barcelona, Spain;* [2]*URFOA, Hospital del Mar, UAB; Barcelona, Spain*
ngarcia@porthos.bio.ub.es

In an effort to identify genes involved in bone mineral density determination, the 800 bp region of the human COL1A1 promoter containing cis-elements important for in vivo osteoblast expression was screened searching for new allelic variants. Two new SNPs (PCOL1 and PCOL2) were found. PCOL1was in strong linkage disequilibrium with the previously described Sp1 polimorphysm of intron 1, whereas PCOL2 showed association to BMD (P< 0.05) in a cohort of 256 postmenopausal women. Electrophoretic mobility shift assays (EMSA) were performed using single or double-stranded oligonucleotides containing the PCOL1 or the PCOL2 sites and showed, in both cases, a specific binding to osteoblast nuclear proteins. PCOL1 is a deletion of a T in a tract of eight T residues. In the EMSA results, the binding seems to specifically involve the T-bearing strand. This result suggests that poly-pyrimidine single-strand DNA-binding proteins are involved. Several studies show the capacity of these sequences to adopt alternative DNA conformations. EMSAs perfomed using several competitions of double and single—stranded oligonucleotides seem to agree with this possibility. PCOL2 is a G to T transversion that lies within putative and overlapped GKLF and Sp1 binding sites. When comparing the two alleles, the affinity of the binding is stronger for the G allele. This might result in a different rate of gene transcription. Interestingly, the oligonucleotide bearing the consensus Sp1 binding site is able to compete in the EMSA analyses. This result suggests that either Sp1 or a related factor is involved in the binding to PCOL2.

P1406. Analyses of the developmental genes TFAP2a, MSX2 and SLUG potentially involved in human neural tube defects (NTDs).

M. C. Koch, *B. Richter, K. Stegmann*
Center of Human Genetics; Marburg, Germany
koch2@mailer.uni-marburg.de

Little is known about the identity of genetic factors involved in the complex aetiology of non-syndromic neural tube defects (NTDs). Reasonable human NTD candidate genes are the human homologs of mice exencephaly genes and other vertebrate genes, for which a functional role in neural tube closure is well established. TFAP2a, MSX2 and SLUG are expressed in the dorsal part of the developing neural tube and were therefore tested as candidates contributing to the aetiology of human NTD. The

coding sequence of the three genes was screened for mutations in 200 NTD patients using single strand conformational analysis (SSCA). We identified sequence variants in all three genes; Two TFAP2a point mutations in individual patients were silent on amino acid level (C308C, T396T). On nucleic acid level, these mutations change evolutionary conserved codons and thus may influence mRNA processing and translation efficiency. One NTD patient displayed an exonic 9 bp deletion in MSX2 leading to a shortened and possibly less functional protein. In yet another patient a missense mutation (D119E) was found in the Slug subfamily-defining region preceding the first zinc finger. Seven polymorphisms detected in TFAP2a and MSX2 were equally distributed in patients and controls. Patients with combined heterozygosity of a MSX2 and a TFAP2a polymorphism were at a slightly increased risk for NTD (OR 1.71; 95% CI 0.57-5.39). The present study defines new genetic variants, which may act in concert with other yet unidentified factors to increase susceptibility to human NTD. Further association and functional studies are necessary to support these observations.

P1407. Chromosome 17; Gene Mapping Studies of Cleft lip With or Without Cleft Palate in Chinese Families

S. Peanchitlertkajorn[1], *M. E. Cooper*[1], *Y. Lui*[2], *L. L. Field*[3], *M. L. Marazita*[1]
[1]*University of Pittsburg; Pittsburgh, PA United States;* [2]*Zhabei Eye Hospital; Shanghai, China;* [3]*University of British Columbia; Vancouver, BC Canada*
supst5+@pitt.edu

Loci on chromosome 17 (including RARA) have shown allelic association with non-syndromic oral clefts in Caucasian populations, although never investigated in Asian populations. Furthermore, there is a major oral cleft gene on murine chromosome 11, homologous to human chromosome 17. 17 markers spanning chromosome 17 (10cM apart, including RARA), were assessed in 36 multiplex families from Shanghai, China. LOD scores (single point and multipoint), model free linkage analyses (SimIBD), and allelic association tests (TDT, Fisher s exact test and Chi square tests) were performed on the total sample, plus families whose probands had either cleft lip and cleft palate (CLP subgroup, n=23), or cleft lip only (CL subgroup, n=13). LOD scores from single point analyses were inconclusive, multipoint LOD scores rejected linkage except for a few regions in the CL subgroup. However, positive results were found for the RARA region and nearby loci using the model-free methods, with variable results for the CL and CLP subgroups. Therefore, genetic variation within or near RARA appears to be involved in cleft formation in this population. Furthermore, based on the differing pattern of results in the CL versus CLP subgroups, it appears that the formation of CL and CLP is either due to differing alleles at the same genetic locus, or to different but related (and/or linked) genes, that modify the severity and expression of oral clefting.

P1408. G protein beta3 subunit gene C825T polymorphism in patients with vesico-ureteric reflux

B. Zagradisnik, *K. Bracic, A. Gregoric, N. Kokalj-Vokac*
Maribor Teaching Hospital; Maribor, Slovenia
boris.zagradisnik@sb-mb.si

A C825T polymorphism in the GNB3 gene encoding a subunit in heterotrimeric G proteins was found to correlate strongly with the activity of the G proteins. This difference in cell signaling could participate in the development of a variety of clinical entities. The aim of this study was to investigate the association of the polymorphism C825T with primary congenital vesico-uretric reflux (VUR). Genotyping was done in a sample of 65 children diagnosed with third- or fourth-degree reflux and in 50 healthy controls. Following genotype frequencies were detected; 23 (35.4%) patients with VUR were CC homozygotes, 35 (53.8%) were CT heterozygotes and 7 (10.8%) were TT homozygotes; 26 (52%) healthy controls were CC homozygotes, 18 (36%) were CT heterozygotes and 6 (12%) were TT homozygotes. A significant overrepresentation of CT and TT genotypes in patients with VUR in comparison with healthy controls was found (chi square 8.4, p= 0.015). Therefore, C825T polymorphism in the GNB3 gene might have a role in the development of primary congenital vesico-ureteric reflux.

P1409. Exclusion of a major role of the IGF signal transduction axis members IRS1 and GRB2 in the aetiology of growth retardation and Silver-Russell syndrome

K. Eggermann[1], *P. Kloos*[1], *S. Mergenthaler*[1], *M. B. Ranke*[2], *H. A. Wollmann*[2], *T. Eggermann*[1]
[1]*Institute of Human Genetics; Aachen, Germany;* [2]*University Children«s Hospital; T bingen, Germany*
teggermann@post.klinikum.rwth-aachen.de

Insulin receptor substrate-1 (IRS1) and the growth factor bound protein 2 (GRB2) play an important role in the IGF and insulin signal tranduction pathway by being the major substrates of IGF1 receptor tyrosine kinases. Several members of the IGF pathway have been proposed as candidates for growth retardation, particularly in Silver-Russell syndrome (SRS). Due to their role in intracellular IGF signalling and their genomic localisation (2q36 and 17q24-25), we searched for genetic variations in IRS1 and GRB2. Loss of function of these proteins might contribute to the clinical feature of growth retardation. Aberrations in the regions on chromosomes 2 and 17 have been described to be involved in growth restriction. Our screening approach included 17 patients with idiopathic pre- and postnatal growth retardation and 10 SRS patients. Applying single strand conformation polymorphism analysis, restriction assays and sequencing, we found two amino acid substitutions (D90E and G971R) and two silent variants (c1722G>A and c3432G>A) in IRS1. The variants D90E and c3432G>A have not been reported before. All of these variants occurred in similar frequencies in both patient groups and in controls. In GRB2, we did not find any mutation or polymorphism. Thus, it is strongly conserved underlining its important in role in the IGF signal transduction. In conclusion, mutations in the coding regions of IRS1 and GRB2 do not play a major role neither in the aetiology of growth retardation. However, the new polymorphisms in IRS1 are powerful tools for association studies in other diseases related to the IGF and insulin signal transduction axis.

P1410. The study of beta myosin heavy chain gene in patients with hyperthrophic cardiomyopathy from Russian population

M. V. Goloubenko[1], *V. P. Puzyrev*[1], *K. V. Puzyrev*[2], *V. B. Salukov*[1]
[1]*Institute of Medical Genetics, Tomsk Scientific Center of Siberian Branch of Russian Academy of Medical Sciences; Tomsk, Russian Federation;* [2]*Institute of Cardiology, Tomsk Scientific Center of Siberian Branch of Russian Academy of Medical Sciences; Tomsk, Russian Federation*
masha@img.tsu.ru

Hyperthrophic cardiomyopathy (HCM) is in most cases genetically determined disease with high level of allele and locus heterogeneity. There are several candidate genes for HCM. The most often mutated one is the beta myosin heavy chain gene (MYH7), which is responsible for about half of genetic cases. To study causes of HCM in Russian population, we have collected the sample of patients with idiopathic HCM. Searching for functional mutations and SNPs in this gene was performed by means of automated sequencing with dye-terminator chemistry. Exons 3-24 of the gene were screened, which frequently mutate in HCM. Some presumable modifying loci also were investigated (i.e., NOS3, ACE, mtDNA). We have found several MYH7 sequence alterations (SNPs) in the affected sample. Although representing common polymorphisms, some of them may be relevant to the disease. SNPs may be in linkage disequilibrium with unidentified mutations that contribute to the HCM phenotype. We have found that novel silent substitution in exon 24 (T15753C, with frequency of rare allele C 27% in affected sample) is associated with the extent of cardiac hyperthrophy. Left ventricular mass index mean value was 161.75 in the group of carriers of rare allele, whereas it was 229.44 in the group of non-carriers (F=6.73, P=0.0212). Our findings confirm high genetic heterogeneity of HCM. Association of silent SNP with extent of hyperthrophy suggests presence of other mutations which are in linkage disequilibrium with this SNP and which may be either causal for HCM or having modifying effect on the phenotype.

P1411. Vitamin D and Breast Cancer; Interaction between dietary vitamin D and genetic variation in the Vitamin D receptor may be implicated the aetiology of breast cancer

Z. H. Miedzybrodzka[1], *L. Sharp*[1], *J. Richardson*[1], *A. Schofield*[2], *F. McGuigan*[1], *S. D. Heys*[1], *J. Little*[1], *N. E. Haites*[1]
[1]*University of Aberdeen; Aberdeen, United Kingdom;* [2]*Univeristy of Aberdeen; Aberdeen, United Kingdom*
zosia@abdn.ac.uk

The active form of vitamin D has been shown to induce apoptosis and inhibit breast cancer cell growth, mediated by the vitamin D receptor (VDR). Although at least three groups have studied VDR polymorphisms in breast cancer, we are not aware of any published study of interactions. We describe a pilot case-control study. Sixty-five breast cancer cases were recruited through the breast service at Aberdeen Royal Infirmary, Scotland. 58 female controls were selected at random from local general practitioner registers. Subjects completed a food-frequency questionnaire and provided a DNA sample (Fok1 and Bsm1 VDR polymorphisms). Women in the highest tertile of vitamin D intake were at two-fold raised risk of breast cancer, compared to the lowest tertile (OR=2.18; 95% CI 0.88-5.39), but this did not reach statistical significance. No association was found between breast cancer risk and the BsmI polymorphism. There was a trend of

reducing risk with increasing number of FokI variant alleles; compared to homozygous wild-type, heterozygous women had an almost 30% reduced risk (OR=0.72; 95% CI 0.30-1.70) and homozygous mutants a 60% lower risk (OR=0.40; 95% CI 0.08-1.95). To investigate interactions, subjects were stratified by presence or absence of the FokI variant allele and odds ratios for vitamin D intake computed within each strata. Compared with lowest vitamin D intake levels, higher intake was associated with increased risk in both strata; among women FokI homozygous wild-type OR high vs low=1.90 (0.32-11.31); among Fok1 heterozygotes, OR high vs low=1.50 (0.32-11.31). This finding requires confirmation in other studies.

P1412. Vitamin-D Receptor Gene Polymorphism Influences Mortality Risk in Hemodialysis Patients

J. Fibla[1], *M. Marco*[2], *L. Craver*[2], *E. Fernandez*[2]
[1]*Dep. of Basic Medical Sciences University of Lleida; Lleida, Catalonia, Spain;* [2]*Service of Nephrology. Hospital Universitary Arnau de Vilanova; Lleida, Catalonia, Spain*
joan.fibla@cmb.udl.es

Vitamin-D receptor (VDR) gene polymorphisms had been proven to have an influence on mineral metabolism and on the course of cancers and infections. This study is a four-year-survival analysis of 143 hemodialysis (HD) patients who were genotyped for polymorphisms located at the 5 and 3 regions of the VDR gene. The Chi-square test was applied to compare genotype distribution among survivors and non-survivors. Survival was analysed with Kaplan-Meier according to Bsm-I genotypes. Cox regression analysis was performed, including as variables the 5 polymorphic locus Fok-I and the two 3 polymorphic loci Bsm-I and PolyA, adjusting for; age, time on HD, hours of HD per week, serum calcium, phosphorus, albumin and iPTH, presence of; diabetes mellitus, ischemic heart disease and calcitriol treatment. Bsm-I and PolyA polymorphisms were in strong linkage disequilibrium. The bb genotype was over-represented among survivors (bb 38/83; 45.7%), compared to non-survivors (bb 13/60; 21.6%). Cox analysis showed a significant influence of Bsm I polymorphism (but nor Fok I nor Poly-A), age, calcitriol treatment, and diabetes mellitus on mortality. The BB and Bb genotypes were independent predictor of mortality (Hazard Ratios [HR] 3.7; 95% Confidence Interval [CI]; 1.7-8 and 2.75; 95%CI; 1.4-5.4, respectively). Survival means by Kaplan-Meier analysis were; 983 days (95%CI; 785-1181) for BB, 1152 days (95%CI; 1030-1274) for Bb and 1290 days (95%CI; 1183-1396) for bb; log-rank; p=0.01. Similar results were obtained when the analysis was performed to the patients who had been on hemodialysis for less than 5 years (n=94), indicating that the genotype influence survival rate irrespective of the duration of hemodialysis previously to enrolment. In conclusion, this study shows that Bsm-I polymorphism of the VDR gene is an independent predictor of mortality in hemodialysis patients.

P1413. Analysis of environmental genes polymorphisms in patients with recurrent early pregnancy miscarriage

O. N. Bespalova*, T. E. Ivaschenko, O. N. Arjanova, V. S. Baranov*
Ott s Institute of Obstetrics&Gynecology; St.-Petersburg, Russian Federation
TIV@ti2629.spb.edu

The genetic polymorphisms of glutathione-S-transferase M1 (GSTM1) and glutathione-S-transferase T1 (GSTT1) genes responsible for xenobiotic conjugating enzymes of Phase II detoxification system were studied by PCR-RFLP in 40 matrimonial couples with histories of pregnants with REPM in anamnesis. The control group consisted of 66 individuals of Russian origin, living in St. Petersburg. The analysis of the genes, encoding Phase II detoxification system enzymes, have shown that the frequency of GSTM1 gene deletion was significantly higher for group with REPM (58% versus 41,0% in control; p=0.05). Proportion of glutathione-S-transferase theta null genotypes (GSTT10/0) increased in the investigated group (35%) compared to controls (23,5%), but these differenses were not significant. Concordance of both GSTM1 0/0 and GSTT10/0 homozygotes was recorded in 26 % of genotypes in the group with REPM and only in 9,1% of the control group. The comparable OR in the presence of both null genotypes was 3.56 (95%CI=1,39-9,08).

P1414. PCR-detection of the Glutathione S-transferase M1 0/0 genotype in patients with Iron Anemia in Bashkortostan.

Z. Soultanaeva[1], *A. Morozova*[2], *T. Victorova*[1], *E. Khusnutdinova*[1]
[1]*Institute of Biochemistry and Genetics; Ufa, Russian Federation;* [2]*Bashkir medical university; Ufa, Russian Federation*
ekkh@anrb.ru

Polymorphisms at the glutathione S-transferase M1 (GSTM1) gene locus have attracted much interest because the homozygous GSTM1 deletion (GSTM1 0/0) seems to modify the risk for different types of cancer, allergy and other diseases. The iron deficiency anemia is one of the widespread diseases; on a Globe 700 - 800 million persons suffer iron anemia or latent deficit Ferri lactas. This disease most frequently meets for children, adolescents and women. Iron anemia is complex disease caused by a combination of both genetic and environmental influences. Polymorphism at the GSTM1 gene locus in 93 patients with iron anemia from Bashkortostan and in 90 healthy individuals from control group was studied by PCR method. The frequencies of the GSTM0/0 genotypes were 0,40 in patients with iron anemia and 0,54 in control group. The data of this study demonstrate that the differences in distribution of GSTM1 allele frequencies between patients with iron anemia and control individuals were not significantly (p=0,05). Our findings indicate that the GSTM10/0 genotype was not linked with iron anemia in Bashkortostan.

P1415. IL-4 promoter polymorphism -590C/T does not associate with severe malaria in Thailand

J. Ohashi[1], *I. Naka*[1], *J. Patarapotikul*[2], *H. Hananantachai*[2], *S. Looareesuwan*[2], *K. Tokunaga*[1]
[1]*The University of Tokyo; Tokyo, Japan;* [2]*Mahidol University; Bangkok, Thailand*
juno@m.u-tokyo.ac.jp

IL-4 is one of important anti-inflammatory cytokines. The -590C/T polymorphism in the promoter of the IL-4 has been reported to be associated with asthma and atopy. Although an exact role of the -590C/T polymorphism in the pathogenesis of these diseases is still unclear, this variation may affect the expression of the IL-4. Recently, the serum level of IL-4 was found to be inversely correlated with development of severe malarial anaemia in Zambia. Thus, we examined a possible association of the -590C/T polymorphism with malaria severity in 219 mild malaria, 164 non-cerebral severe malaria, and 110 cerebral malaria patients living in the northwest of Thailand. The genotyping was performed by PCR-SSCP method. Our result showed no significant difference in the frequency of the -590C/T polymorphism among three groups. Interestingly, in this population, the frequency of -590T allele was higher than that of -590C allele. Further variation analysis of other cytokine genes, IL-6, IL-10, and IL-13, is currently underway for our patient samples.

P1416. Human Immunodeficiency Virus Type 1 Disease Progression among Seropositive Intravenous Drug Users in relation to Chemokine Receptor (CCR5) and Vitamin-D Receptor (VDR) gene Polymorphisms

A. Velasco[1], *Y. Barber*[1], *C. Rubio*[2], *E. Fernandez*[3], *M. Rubio*[2], *J. Fibla*[1]
[1]*Dep. of Basic Medical Sciences. University of Lleida; Lleida, Catalonia, Spain;* [2]*Service of Internal Medicine. Hospital Universitary Arnau de Vilanova; Lleida, Catalonia, Spain;* [3]*Service of Nephrology. Hospital Universitary Arnau de Vilanova; Lleida, Catalonia, Spain*
joan.fibla@cmb.udl.es

The D32 insertion-deletion polymorphism of the CCR5 chemokine receptor gene and the Bsm-I RFLP of the Vitamin-D Receptor (VDR) gene has been studied in relation to human immunodeficiency virus type 1 (HIV-1) infection and disease progression in a cohort of seropositive intravenous drug users (HIV+/IVDU). No HIV+/IVDU patients homozygous for D32 allele were detected, indicating that those individuals are strongly protected against HIV-1 infection. CCR5-wtD32 heterozygotes were not associated with slow progression to AIDS or CD4 cell count <200 cell/ L. In contrast, a bad progression of the disease was associated with VDR-BB genotype. A higher proportion of this genotype was found in patients with CD4 cell count <200 cells/ L (p=0.007). In addition, VDR-BB patients show a faster progression to both, AIDS CDC 1993 (hazard ratio [HR]; 1.7; 95% confidence interval [CI]; 1.02-2.8) and CD4 cell count <200 cell/ L (HR; 2.1; 95%CI; 1.2-3.5). When the analysis was restricted to those patients with a VDR-bb genetic background, CCR5-wtD32 heterozygosity confers protection to disease progression. CCR5-wtD32 heterozygotes were over-represented in both, CDC 1993 non-progressors (odds ratio [OR], 0.28; 95%CI; 0.08-0.96) and those with CD4 cell count >200 cells/ L (OR, 0.26; 95%CI; 0.06-1.15). In addition, CCR5-wtD32 heterozygotes show a slow progression to AIDS CDC-1993 (HR; 0.29; 95%CI; 0.08-0.97). Further studies will be required to clarify the role of VDR polymorphisms in HIV-1 pathogenesis and their co-operative interaction with chemokine receptor polymorphisms so far involved in HIV-1 disease progression. The confirmation that certain VDR genotypes confers a bad progression of the disease and that other genotypes allow CCR5-wtD32 heterozygotes to manifest a delayed progression of the disease will offer new opportunities for the rational design of therapeutic interventions.

P1417. Increased skewing of X inactivation in elderly females may be determined by genes linked to the G6PD gene

K. Orstavik[1], *M. Kristiansen*[2], *H. Hagen-Larsen*[3], *G. Knudsen*[1], *J. W. Vaupel*[4], *L. Bathum*[4], *A. K. Naumova*[5], *K. Christensen*[4]
[1]*Department of Medical Genetics, National Hospital; Oslo, Norway;* [2]*Institute of Medical Genetics, University of Oslo; Oslo, Norway;* [3]*Department of Medical Genetics, University of Oslo; Oslo, Norway;* [4]*The Danish Twin Registry, Section for Epidemiology and the Danish Center for Demographic Research, Odense University; Odense, Denmark;* [5]*Departments of Obstetrics and Gynecology and Human Genetics, McGill University Health Center; Montreal, PQ Canada*
k.h.orstavik@ioks.uio

Elderly females have a higher frequency of skewed X inactivation in peripheral blood cells. G6PD-linked genes have been implicated in this age-related skewing of X inactivation in Safari cats. In a study of X inactivation in 71 elderly monozygotic twin pairs, we found a strong tendency for the same cell line of blood cells to be the predominant cell line in twin pairs. In the present work we tested the possibility that age-related skewing of X inactivation is related to a locus linked to the G6PD gene in humans. We analyzed X inactivation in 101 dizygotic twin pairs aged 73-93 years. The X inactivation pattern was classified as skewed when 80% or more of the cells had the same X-chromosome active.The frequency of skewed X inactivation in the twins (37%) was increased compared to blood donors aged 19-65 years (7%). The correlation coefficient for the X inactivation pattern was 0.23 (P=0.02) for the dizygotic twin pairs and 0.52 (P<0.01) for the monozygotic twin pairs, confirming a genetic influence on X inactivation phenotype in elderly females. Analysis of markers in the G6PD region (F8C and DXS15) showed a significant tendency for twins who had inherited different maternal alleles in these markers to be discordant for X inactivation phenotype (one twin having a skewed and the other twin having a non-skewed pattern). We conclude that skewing of X inactivation in elderly females may be influenced by a locus linked to the G6PD region, most probably through a selection mechanism.

P1418. The Use of Pharmacogenetics to Optimize Drug Development and Therapy

D. M. Grant
Orchid BioSciences Inc; Princeton, NJ United States
dgrant@orchid.com

The completed sequence of the human genome provides the starting material for genome-wide discovery of DNA sequence variants among individuals in human populations and their correlation with drug response, toxicity and disease predisposition. Pharmacogenetic correlation studies are expected to be valuable in target discovery and validation, in preclinical and clinical phases of drug development, and for designing the post-marketing genetic diagnostic tests for individualizing drug dosing in the future. Such studies require a range of technical capabilities both in sample genotyping and data handling, along with access to phenotypically well-defined clinical populations exhibiting variable drug response. Many methods are becoming available for the scoring of single nucleotide polymorphisms (SNPs), the most common form of genetic variation, in pharmacogenetic correlation studies. However, most of these methods are not practical for large-scale studies, and thus have limited ability to meet industry demands for i) accuracy; ii) reliability; iii) operational simplicity; iv) reproducibility; v) automatability; vi) low cost; and vii) flexible formatting. Orchid BioSciences has developed a proprietary SNP genotyping technology, based on the principle of single-base primer extension, which is amenable to a variety of manual and automated platforms so as to enable a wide spectrum of pharmacogenetic study designs ranging from large-scale gene mapping and new drug target discovery programs through to highly focused, hypothesis-based clinical pharmacogenetic correlation studies. Orchid is currently using its technology to conduct pharmacogenetic association studies in collaboration with academic thought leaders across a broad range of therapeutic areas, through our rapidly expanding Clinical Genetics Network (CGN) program. Progress in selected trials underway to investigate disease predisposition and drug efficacy variability in more than 2200 patients in therapeutic areas that include cardiovascular, inflammatory and CNS disorders will be presented.

P1419. Tree-based linkage and association analyses of complex diseases

H. Zhang
Yale University; New Haven, CT United States
heping.zhang@yale.edu

Tree-based linkage and association analysis methods will be described and applied for complex diseases and traits such as asthma, cancer, and Obsessive Compulsive Disorders. The statistical methods are developed from the recursive partitioning technique as described by Breiman, Friedman, Olshen, and Stone (CART, 1984) and Zhang and Singer (Recursive Paritioning in the Health Sciences, 1999). These methods can simultaneously accommodate a genome wide scan of polymorphic markers and are useful for identifying multiple candidate genes, gene-gene, and gene-environment interactions for diseases and traits of complex inheritance.

P1420. Psychological well-being in women with Turner syndrome; Is there a correlation to karyotype?

U. Wide Boman[1], *I. Bryman*[2], *A. M ller*[2], *C. Hanson*[2]
[1]*Department of Psychology, G teborg University; G teborg, Sweden;* [2]*Department of Obstetrics and Gynecology, G teborg University; G teborg, Sweden*
ulla.wide-boman@psy.gu.se

Turner syndrome (TS) occurs in approx. 1 of 2500 female births, and is caused by a total or partial deletion of one of the sex chromosomes in all or in some cells. Main clinical features are short stature and accelerated rate of atresia of ovarian follicles causing gonadal insufficiency, incomplete puberty and impaired fertility. Intelligence is usually in the normal range, although impairments in spatial processing are common. An increased risk for emotional and social difficulties has been reported, but also a considerable variability. The aim of the present study was to investigate psychological well-being in women with TS compared with reference data, and to investigate whether there was a relation between karyotype and well-being. Method; 63 women with TS (mean age 31.5 years) completed the Psychological General Well-being Index (PGWB), covering the states of anxiety, depressed mood, positive well-being, self-control, vitality and general health. The scores were compared with a female reference group. To examine the possible relation to karyotype, the participants with one copy of the p-arm of the X chromosome according to karyotype (45,X and strucutural abnormalities) (n=52) were compared to those with two copies of the p-arm (45,X/46,XX) (n=11). Results; The TS group rated their psychological well-being at a similar level as the reference group. There was no significant difference in psychological well-being according to karyotype group, however, there was a tendency for the group with two copies of the p-arm to report higher levels of anxiety. Conclusion; This study did not find evidence for impaired psychological well-being in women with TS. The psychological well-being did not differ according to karyotype group, however, this questions should be investigated in studies with larger sample size.

P1421. Familial occurrence of ring chromosome 15

O. Bushueva[1], *N. Nikitina*[1], *G. Pavlov*[2], *E. Nikolaeva*[1], *M. Devaikina*[1]
[1]*Medico-Genetic Centre; Ekaterinburg, Russian Federation;* [2]*Ural Medical Academy; Ekaterinburg, Russian Federation*
gvp@online.ural.ru

Ring chromosome 15 was detected in a 31-year-old woman and her son. The mother had short stature (150 cm), slight mental retardation, triangular face and dislocation of both hip joints. She married at the age of 27 and became pregnant 3 years later without examination and treatment. Her son was born at 40 weeks of gestation by c-section. His weight was 2400 g, his length was 47 cm. Developmental abnormality was first suspected at 12 months. He had short stature; his weight was 6100 g, length - 65 cm, occipitofrontal circumference - 43 cm. Muscular hypotonia was obvious and early milestones of development were retarded. The boy sat at 8 months, crawled by 12 months, walked unaided by 18 months, and spoke his first words at the age of 18 months. At the age of 5 his height was 88 cm, weight 9380 g, OFC - 47,6 cm. Dysmorphic features included a triangular face,a big mouth hypotelorism, thin hair, white coffee spots on the body, hypogenitalism and a hypoplastic penis. Speech and mental development was retarded. Chromosome analysis of the mother and her child revealed ring chromosome 15, the break points in the ring chromosome being located at the same sites. The inheritance of the ring chromosome has been reported rather infrequently. This occurrence is the third report (both the first and the second were from Japan), and the present case is the first to be published in Russia.

P1422. Methylation imprints on human chromosome 15 are established around or after fertilization

K. Buiting[1], *O. El-Maarri*[2], *E. G. Peery*[3], *P. M. Kroisel*[4], *B. Balaban*[5], *K. Wagner*[4], *B. Urman*[5], *J. Heyd*[2], *C. Lich*[1], *C. I. Brannan*[3], *J. Walter*[2], *B. Horsthemke*[1]

[1]*Institut f r Humangenetik, Universit tsklinikum Essen; Essen, Germany;* [2]*Max-Planck-Institut f r Molekulare Genetik; Berlin, Germany;* [3]*Department of Molecular Genetics and Microbiology, University of Florida College of Medicine; Gainesville, FL United States;* [4]*Institut f r Medizinische Biologie und Humangenetik, Universit t Graz; Graz, Austria;* [5]*Assisted Reproductive Technology Unit, Amerikan Hastanasi; Istanbul, Turkey*

karin.buiting@uni-essen.de

Prader-Willi syndrome (PWS) is a neurogenetic disorder, which results from the lack of transcripts expressed from the paternal copy of the imprinted chromosomal region 15q11-q13. In some patients, this lack is associated with a deletion of the SNURF-SNRPN exon 1 region inherited from the paternal grandmother and the presence of a maternal imprint on the paternal chromosome. Assuming that imprints are reset in the germline, we and others have suggested that this region constitutes part of the 15q imprinting centre (IC) and is important for the maternal to paternal imprint switch in the male germ line. To investigate the effect of an IC deletion on the imprinting process in the male germline, we analyzed sperm samples from two males carrying such a deletion on their maternal chromosome. On examination of six differentially methylated sites at the MKRN3, NDN, u1D, u1B, PW71/u1A, and YL48E loci, which are located upstream and downstream of the deletion, we found that spermatozoa DNA from these two males had a normal paternal methylation pattern. Similar findings were made in a mouse model harboring a microdeletion of the Snurf-Snrpn exon 1 region. These findings indicate that the incorrect maternal methylation imprint in IC deletion patients is established de novo after fertilisation. Furthermore, we found that CpG-rich regions in the SNURF-SNRPN and NDN genes, which in somatic tissues are methylated on the maternal allele, are hypomethylated in unfertilized human oocytes. These results suggest that the normal maternal methylation imprints in 15q11-q13 also are established around or after fertilisation.

P1423. A mtDNA mutation, 14453A->G, in the NADH dehydrogenase subunit 6 associated with a severe congenital case of MELAS syndrome

K. Ravn[1], *F. Wibrand*[2], *F. Juul Hansen*[1], *N. Horn*[2], *M. Schwartz*[1]

[1]*Rigshospitalet; Copenhagen, Denmark;* [2]*John F. Kennedy institute; Glostrup, Denmark*

K_Ravn@yahoo.com

We report a novel point mutation in the mitochondrial encoded ND6 subunit of the NADH ubiquinone oxidoreductase (complex I) in a patient with MELAS syndrome. The mutation is a missense mutation, changing the amino acid alanine to valine in a highly conserved region of the ND6 subunit. The mutation was heteroplasmic and found in both muscle and blood, but was not detected in the patient«s mother. A marked reduction of complex I activity was found in the patients muscle. This is first case of a mutation in the ND6 subunit causing MELAS. Our data confirm the genetic heterogeneity in mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes syndrome, and confirms that MELAS can be caused by mutations in polypeptide-coding mtDNA genes.

P1424. Silver-Russell syndrome; quantitative PCR and FISH approaches to detect rearrangements in 7p11.2-p13

S. Mergenthaler[1], *A. Sharp*[2], *C. Joyce*[2], *M. Ranke*[3], *K. Temple*[4], *V. Kalscheuer*[5], *H. Wollmann*[3], *T. Eggermann*[1]

[1]*Institute of Human Genetics; Aachen, Germany;* [2]*Wessex Regional Genetics Laboratory; Salisbury, United Kingdom;* [3]*University Children s Hospital; T bingen, Germany;* [4]*Southampton University Hospitals; Southampton, United Kingdom;* [5]*Max Planck- Institute of Molecular Genetics; Berlin, Germany*

smergenthaler@post.klinikum.rwth-aachen.de

Silver-Russell syndrome is characterised by intrauterine and postnatal growth retardation (IUGR/PNGR), typical craniofacial abnormalities combined with asymmetry of head and limbs, and other less frequent abnormalities. The majority of the 400 cases occurred sporadically, but some familial cases indicate a genetic origin of the disease. Few cytogenetic aberrations have been published; five cases involved chromosome 7, which is also subject to maternal uniparental disomy (matUPD) in 10% of all SRS cases. The finding of matUPD7 indicates the involvement of at least one imprinted gene on this chromosome. Three recently published SRS patients with cytogenetic duplications focussed the interest on 7p11.2-p13; this chromosomal segment harbours the putative candidate genes EGFR, GRB10, IGFBP1 and IGFBP3, which, by mutation and imprinting analysis, have already been excluded from playing a major role in the etiology of SRS. To estimate the frequency of duplications in 7p11.1-p13 we analysed 32 SRS patients by quantitative PCR. A multiplex PCR with the CFTR gene as control and an internal standard as reference for the amount of DNA was performed. We tested the genes EGFR, GRB10, IGFBP1 and IGFBP3 and one microsatellite within the possibly duplicated region, but we did not detect any rearrangement. Additionally we have started to investigate SRS patients and patients with idiopathic IUGR/PNGR by FISH. We are using 3 YACS hybridising to the regions 7p11.2, 7p13 and 7p13-14. These probes were shown to be duplicated in previous studies. So far, no triple-signals were observed, thus confirming the results obtained by the quantitative approach.

P1425. A molecular, cytogenetic, pathology, endocrine and genetic view of hermaphroditism, the SRY gene and female gender; A study of five atypical patients

M. J. Sutcliffe[1], *A. Root*[1], *R. Haire*[2], *F. Faccio*[2], *D. P. Dumont*[3], *A. Lacson*[1], *K. Washington*[3], *W. Chamizo*[3], *P. Ferre*[3], *M. M. Haag*[4], *O. T. Mueller*[1], *B. B. Bercu*[1], *F. Diamond*[1], *D. Shulman*[1], *R. Sutphen*[1], *J. D. Ranells*[2], *B. G. Kousseff*[2]

[1]*All Children s Hospital/Univ. of South Florida; St. Petersburg, FL United States;* [2]*Univ. of South Florida; Tampa, FL United States;* [3]*All Children s Hospital; St. Petersburg, FL United States;* [4]*Shodair Children s Hosspital; Helena, MT United States*

sutcliff@allkids.org

True hermaphroditism is defined as the presence of both ovarian and testicular tissue in one individual. Gonadal histology usually demonstrates a range between undifferentiated gonads with ovarian and testicular features to ovarian stroma with oocytes and seminiferous tubules with spermatogonia. The SRY male determining gene may be present or absent, the karyotype may be XX, XY or mosaic and genitalia may be female, male or ambiguous. We present a series of five atypical patients.

Patient	Karyotype	SRY Gene	Genitalia	Ductal Organization	Gonadal Tissue
103	45,X/ 46,idic(Y)(q11.21)	copy x2	female	bilat. fallopian tubes	ovarian
101	46,XX/ 46,XY/ 47,XYY	copy x2	female	bilat fallopian tubes/uterus	ovarian
105	46,XY	copy x1	ambig	lt epididymal tube rt fallopian tube	lt testicular/ gc* rt ovarian
102	46,XX	none	ambig	lt fallopian tube rt fallopian/epididymal tubes	lt ovarian/ gc rt ovotestis/gc
104	46,XX	none	ambig	bilat fallopian/epididymal tubes	bilat ovotestes/gc

*gc = germ cell Sequencing of the SRY gene, where present, failed to demonstrate any mutation. The karyotype and internal ductal/gonadal and external genitalia differentiation of our patients will be reviewed in the context of current knowledge of the genes upstream and downstream of the SRY male determining switch.

P1426. Russell-Silver Syndrome; Establishment of a possible critical region on 7p14 and characterization of putative candidate genes.

K. Nakabayashi[1], *B. A. Fernandez*[1], *R. Edge*[1], *I. Teshima*[1], *C. Shuman*[1], *K. Mitsuya*[2], *M. Meguro*[2], *M. Oshimura*[2], *R. Weksberg*[1], *S. W. Scherer*[1]

[1]*Department of Genetics, The Hospital for Sick Children; Toronto, ON Canada;* [2]*Department of Molecular and Cell Genetics, Faculity of Medicine, Tottori University; Yonago, Japan*

kazuhiko@genet.sickkids.on.ca

Russell-Silver syndrome (RSS) is a form of congenital dwarfism characterized by severe growth retardation and variable dysmorphic features. RSS is considered to be an imprinting disease, because 10% of sporadic patients demonstrate maternal uniparental disomy (mUPD) of chromosome 7. Although 3 genes on chromosome 7 (MEST, gamma2COP and GRB10) are shown to be imprinted in human or mouse, several lines of evidence exclude their involvement in RSS, suggesting the existence of additional imprinted gene(s) on chromosome 7 which contribute to the disease. Two RSS patients were identified with de novo cytogenetic abnormalities involving the short arm of chromosome 7. One had a partial duplication [46, XX, dup(7)(p12p14)] and the second contained a paracentric inversion [46, XY, inv(7)(p14p21)]. Fluorescence in situ hybridization (FISH) mapping revealed that the rearrangement breakpoints on 7p14 in the two patients were located within an interval of less than 500kb. Seven candidate genes identified in the vicinity of the breakpoints were subjected

to allelic expression analysis using mouse A9 somatic cell hybrids containing paternal or maternal human chromosome 7, in which the imprinting of MEST was maintained. All seven genes showed biallelic expression in A9 hybrids, although the results don t exclude the possibility of tissue-specific imprinting of the genes. The exon-intron structure of the positional candidate genes (CDC2L and others) were determined and being subjected to mutation screening in RSS patients.

P1427. Family-Based Tests of Association in the Presence of Linkage

S. Lake[1], *D. Blacker*[2,1], *N. M. Laird*[1]
[1]*Harvard School of Public Health; Boston, MA United States;* [2]*Massachusetts General Hospital; Boston, MA United States*
slake@hsph.harvard.edu

Linkage analysis may not provide the necessary resolution for identification of the genes underlying phenotypic variation. This is espicially true for studies that focus on complex diseases. One positional genomic strategy involves application of association methodology to areas of identified linkage. Detection of association in the presence of linkage localizes the gene(s) of interest to more-refined regions in the genome than is possible through linkage analysis alone. This strategy introduces a statistical complexity when family-based association tests are used; the marker genotypes among siblings are correlated in linked regions. Ignoring this correlation will compromise the size of the statistical hypothesis test, thus clouding the interpretation of test results. We present a method for computing the expectation of a wide range of association test statistics under the null hypothesis that there is linkage but no association. To standardize the test statistic an empirical variance-covariance estimator that is robust to the sibling marker-genotype correlation is used. For example, we analyze a deletion in the A2M gene at the 5 splice site of 'exon ii of the bait region in Alzheimer disease (AD) discordant sibships. Since the A2M gene lies in a chromosomal region (chromosome 12p) that consistently has been linked to AD, association test should be conducted under the null hypothesis that there is linkage but no association.

P1428. Molecular diagnostics of hereditary angioneurotic edema in Hungary

L. Kalmar[1], *A. Bors*[1], *H. Farkas*[2], *B. Fandl*[1], *L. Varga*[1], *H. Andrikovics*[1], *G. Fust*[2], *A. Tordai*[1]
[1]*Institute of Haematology and Immunology; Budapest, Hungary;* [2]*Semmelweis University Kutvolgyi Clinical Center; Budapest, Hungary*
lkalmar@biomembrane.hu

Hereditary angioneurotic edema (HANE) is an autosomal dominant disorder characterised by episodic local subcutaneous and submucosal edema affecting the upper respiratory and gastrointestinal tracts. It is caused by the deficiency of activated C1 esterase inhibitor protein (C1-INH, type I deficiency; reduced serum level, type II deficiency; reduced activity) whose function is preventing unnecessary activation of the complement system. The aim of the present study is to determine the disease-causing mutations among Hungarian HANE patients. The estimated number of affected HANE-families in Hungary is approximately 40-50, out of which 30 families (type I;25, type II;5) are managed in a single center in Budapest. To detect large deletions and insertions, which supposedly occur exclusively in type I HANE patients with an approximate frequency of 20%, we use Southern-blotting analysis with *Bcl*I and *Bgl*II digestions. In the absence of large structural changes, we employ single strand conformational polymorphism (SSCP) analysis covering the whole coding region and splicing sites of the C1-INH gene by 15 primer-pairs (overlapping PCR-products shorter than 200 bp), with silver staining detection. The determinations are currently in progress, so far large deletions were detected by Southern-blotting in 3/19 HANE type I families. The SSCP analyses completed so far showed altered patterns in exon 8 in eight HANE patients, in seven type II patients we identified the disease causing mutation (Arg444Cys) by sequencing. In the remaining type I patient a Val458Met amino acid change was found. Our program provides definite molecular diagnosis and opens the possibility for prenatal diagnostics.

P1429. Mutation analysis of the CX26 gene in sporadic cases with moderate to profound deafness

S. Kupka[1], *T. T th*[2], *S. Aberle*[3], *N. Blin*[3], *I. Sziklai*[2], *H. Zenner*[1], *M. Pfister*[1]
[1]*HNO, Univ.-Klinikum; T bingen, Germany;* [2]*DEOEC F l-Orr-G geklinika; Debrecen, Hungary;* [3]*Molekulare Genetik, Univ. T bingen; T bingen, Germany*
susankupka@yahoo.de

Non-syndromic neurosensory recessive deafness (NSRD) is one of the most common human sensory disorders. Mutations in the connexin 26 gene have been established as a major cause of inherited and sporadic non-syndromic deafness in different populations. The CX26 gene encodes the gap junction protein connexin 26 (beta-2, GJB2) whose expression was shown in several tissues and in the cochlea. The 30delG mutation is the most frequent mutation in the CX26 gene. It represents a deletion of a guanosine (G) in a sequence of six G extending from position 30 to 35 of the CX26 cDNA. The deletion creates a frameshift resulting in a premature stop codon and a non-functional intracellular domain in the protein. The 30delG mutation can be detected at the molecular level using PCR followed by BsiYI-digestion. We now screened 100 control individuals and 250 patients with non-syndromic sporadic deafness for this mutation to determine their distribution in the German and Hungarian populations. The frequency of the 30delG mutation in the German pool of sporadic cases was 0.11 wheras in controls it was 0.04. While studying 24 small-sized Hungarian families, this frequency was 0.38.
DNA from individuals showing a heterozygous status for 30delG was sequenced. This study revealed several new patient-related mutations and new gene variants resulting in e.g. amino acid substitutions (A->G; basic to acidic; G->A; nonpolar to polar; A->C; acidic to nonpolar). Moreover, one deletion and one insertion was noted. In summary, more than 20 new allelic changes were detected and for most of them, patterns of inheritance were documented.

P1430. Establishing Denaturing High Performance Liquid Chromatography (DHPLC) for Mutation Detection in the Elastin Gene

C. Kraus[1], *A. Rauch*[1], *M. Hofbeck*[2], *H. Singer*[3], *A. Reis*[1]
[1]*Institute of Human Genetics, Friedrich-Alexander University of Erlangen-Nuremberg; Erlangen, Germany;* [2]*University Department of Pediatrics; T bingen, Germany;* [3]*Department of Pediatrics; Erlangen, Germany*
cokraus@humgenet.uni-erlangen.de

Loss of function mutations in the Elastin gene (ELN) have been demonstrated to be responsible for nonsyndromic supravalvular aortic stenosis (SVAS). SVAS is an obstructive vascular disorder that causes hemodynamically significant narrowing of large arteries. Although the aorta is the most frequently diseased, any artery can be affected, including the pulmonary, carotid and coronary arteries. The onset and severity of the vascular disease in SVAS is variable. If untreated, this disorder can lead to heart failure, myocardial infarction and death. SVAS occurs with an incidence of 1 in 20 000 life births. It can be inherited as an isolated autosomal dominant trait or as one symptom of Williams-Beuren syndrome, a complex developmental disorder characterized by cardiovascular, neurobehavioral, facial, connective tissue, and metabolic abnormalities. In nonsyndromic SVAS patients up to now 14 different mutations have been reported in the Elastin gene. As mutations are distributed all over the gene, mutation analysis is time consuming and cost intensive. Therefore we established the DHPLC method for mutational screening of all 34 coding exons and flanking introns. In order to reduce false positive heteroduplex formations PCR conditions had to be optimized for DHPLC. Temperature and buffer gradient conditions were elaborated for each exon to optimize elution profiles. DHPLC analysis were performed in five unrelated SVAS patients and available family members. Fragments with normal (wild type) sequence were used as controls. We identified the same disease causing mutation (exon 10; K176X) in two non consanguineous patients by DHPLC and sequencing. In addition elution profiles with heteroduplex formation were observed in 8 out of 34 exons analyzed. Whether these heteroduplex pattern are due to a mutation or a polymorphism have to be proved by sequencing.

P1431. The Distribution of Huntington Disease Gene (IT-15) Polymorphic Features on Normal and Mutant Alleles

H. G. Karabulut[1], *T. Tuncali*[2], *A. Tukun*[1], *H. Ilgin Ruhi*[1], *I. Bokesoy*[1]
[1]*Ankara University School of Medicine, Department of Medical Genetics; Ankara, Turkey;* [2]*Ankara University School of Medicine, Department of Medical Biology; Ankara, Turkey*
karabul@dialup.ankara.edu.tr

The underlying mutation causing Huntington Disease is the expansion of a CAG trinucleotide repeat in the first exon of IT-15 gene beyond a certain repeat number. IT-15 has also some other polymorphic features that may be associated with CAG repeat number. In this study, the distribution of polymorphic features identified in the first exon and 5 promoter region of the gene was investigated on normal and mutant alleles seperately. Polymorphic features included in the study are 6 and 20 base-pairs (bp) repeats (GGGGGC and GGCCCCGCCTCCGCCGGCGC namely) and G?A and C?T point mutations located on 5 promoter region and, CAG and CCG repeats in the first exon of the gene. PCR and DNA sequencing was performed following genomic DNA isolation from peripheral blood samples of patients after obtaining their informed consent. The method of choice enabled a clear investigation of these polymorphic features both in wild

type and mutant alleles seperately. CAG repeat number was found to be in the range of 17-25 and 41-51 on normal and mutant alleles respectively. Only two alleles of CCG repeats, 7 and 10 repeats, were observed and 10 repeats of CCG was only seen in mutant alleles. 6 and 20 bp repeat numbers were 1 and 2 respectively for all normal and mutant alleles except for two normal alleles one with 2 repeats of 6 bp and one with 3 repeats of 20 bp. The nucleotides seen in G->A and C->T positions were G and C respectively in all normal and mutant alleles. To our knowledge, this is the first report on HD alleles investigating 5 promoter region polymorphic features relative to CAG repeat number on normal and mutant alleles seperately.

P1432. A novel test for the detection of truncating mutations

M. M. Matin[1], *A. Dalton*[2], *D. P. Hornby*[1]
[1]*The Transgenomic Research Laboratory, Krebs Institute for Biomolecular Sciences; Sheffield, United Kingdom;* [2]*North Trent Molecular Genetics Laboratory, Children s Hospital; Sheffield, United Kingdom*
MBP95MM@sheffield.ac.uk

Screening for mutations in the genome of individuals with a family history of diseases such as breast or ovarian cancer is a contentious issue since there is little prospect of fixing such genetic defects in the near future. In the vast majority of cases, those mutations, which significantly increase the risk of cancer susceptibility are truncating mutations, i.e. the protein encoded by the mutant gene is smaller in size than normal. We have established that engineered forms of DNA methyltransferase genes can be used in conjunction with certain strains of Escherichia coli for the direct selection of recombinant molecules. We have tested a number of DNA methyltransferase genes for their ability to accommodate DNA fragments of up to 1000 base pairs without loss of phenotype and yet to be susceptible to inactivation when a truncating mutation arises within the DNA fragment. This property was employed to construct a plasmid vector, pSPRX, for the detection of truncating mutations using M.SPRX a modified version of the gene encoding the cytosine-C5-specific DNA methyltransferase M.SPRI. M.SPRX, which has non-methyltransferase polypeptide sequence in place of TRD M, can accommodate an in frame insertion of up to 300 amino acids without any loss of activity, and yet insertion of fragments containing truncating mutations, reduce its activity.

P1433. Four novel mutations in ryanodine receptor gene (RYR1) found in patients with human stress syndrome and malignant hyperthermia

T. Krieger[1], *M. Steinfath*[2], *W. Wappler*[2], *S. Singh*[1]
[1]*Institute of Human Genetics, University of Hamburg; Hamburg, Germany;* [2]*Clinic for Anaesthesia, University of Hamburg; Hamburg, Germany*
singh@uke.uni-hamburg.de

Malignant hyperthermia(MH) is a rare autosomal dominant pharmacogenetic trait which can manifest itself in persons with genetic predisposition in an acute life threatening form during general anaesthesia. Porcine stress syndrome has similar symptoms like the MH and has been traced back to a point mutation in ryanodine receptor(RYR1)gene. Mutation analysis in human RYR1 gene has shown uptil now about 25 mutations which could be associated with MH predisposition. Human stress syndrome (HSS) is a very rare condition which is triggered by a very warm environment or excessive bodily stress leading to well known crisis similar to MH. In a large collective of MH and some HSS patients, we performed mutation analysis of 14 selected exons of RYR1 gene in DNA from blood by employing the techniques of SSCP and fluorescence based direct sequencing. Apart from already known mutations, we found four novel mutations A1435G, G6385A, A6566G, C7258T, two of them in patients expressing HSS. These results further confirm our previous suggestion that MH and HSS seem to be part of the same large syndrome. In order to further confirm these observations a screen in cDNA from muscle from a bigger collective of patients, especially HSS patients, is being attempted in order to examine all the exons of RYR1 gene.

P1434. SpliceXpress; expression and analysis of splice site mutations in the fibrinogen alpha and gamma genes accounting for congenital afibrinogenemia.

C. Attanasio[1], *P. de Moerloose*[2], *S. Antonarakis*[1], *M. Morris*[1], *M. Neerman-Arbez*[1]
[1]*Division of Medical Genetics, University Medical School and University Hospital; Geneva, Switzerland;* [2]*Division of Angiology and Hemostasis, University Hospital; Geneva, Switzerland*
Attanas5@etu.unige.ch

Congenital afibrinogenemia (MIM # 202400) is a rare, autosomal recessive disorder characterized by the complete absence of circulating fibrinogen. Our recent studies on the molecular basis of the disease showed that the most common genetic defect is a donor splice mutation in FGA intron 4, IVS4+1G>T. Because it was impossible to directly study the effect of this mutation on mRNA splicing in patient hepatocytes, and illegitimate RT-PCR from leukocyte RNA was unsuccessful, we used a transfected cell approach. COS-7 cells expressing the control FGA genomic construct produced only correctly-spliced mRNA molecules, whereas cells expressing the IVS4 mutant construct produced only abnormal transcripts which were individually cloned for identification. Multiple cryptic donor splice sites situated in exon 4 and intron 4 were found to be utilized. One of these, situated 4 bp downstream of the normal site was used in 85% of transcripts resulting in a 4 bp insertion-frameshift leading to premature truncation of FGA. Our results confirm the validity of this approach to study mRNA splice-site mutations and demonstrate that the common FGA IVS4+1G>T variant is a null mutation leading to afibrinogenemia. Cloning the RT-PCR products allows the identification of rare aberrant transcripts that would have been missed by simply sequencing the total product. Two other mutations affecting donor sites in FGA; IVS1+3G>A and IVS3+1_+4delGTAA, and one acceptor site mutation in FGG; IVS2-3C>G have been identified in patients with congenital afibrinogenemia. The respective genomic constructs are currently being expressed in COS-7 cells to assess their effect on mRNA splicing.

P1435. Mucolipidosis type 4; Mutations in a novel gene.

G. Bach[1], *R. Bargal*[1], *N. Avidan*[2], *E. Ben Asher*[3], *D. Lancet*[2], *A. Raas-Rothschild*[1], *M. Zeigler*[1]
[1]*Hadassah University Hospital; Jerusalem, Israel;* [2]*Weizman institute; Rehovot, Israel;* [3]*Weizman Insitute; Rehovot, Israel*
Bach@hadassah.org.il

Mucolipidosis type IV (MLIV) is a recessive, neurodegenerative, lysosomal storage disorder manifested with psychomotor retardation and ophthalmological abnormalities, including cornea opacities, retina degeneration and strabismus. The disease is found in relatively high frequency among Ashkenazi Jews. MLIV is one of the few lysosomal storage disorders in which neither the basic metabolic defect nor the relevant gene had been identified hitherto. We undertook positional cloning which led to the identification of a novel gene on human chromosome 19p13.2-13.3. The gene - MCOLN1 is expressed in all the human tissues examined and encodes a putative protein - Mucolipin1 that was found to be a member of a new family - the mucolipins. The putative protein of 580 amino acids is a calcium channel containing 6 transmembrane domains and a serine lipase and nuclear localization signal motives. MLIV-causing mutations were identified in MCOLN1, two of which were found in 95% of the MLIV alleles in the Jewish Askenazi families (72% for the first mutation and 23% for the second). The 2 mutations correlated with specific haplotypes in this region. Homozygotes for each of these mutations as well as compound heterozygotes showed all similar clinical phenotype. Six other MLIV-causing mutations in the MCOLN1 gene were also identified mostly among non Jewish MLIV patients. The identification of the gene involved with MLIV finally permits accurate patients diagnosis, including prenatal diagnosis and heterozygotes identification. Heterozygote frequency was found to be 1/100 in the Ashkenazi population. Since 2 mutations accounts for 95% of the MLIV alleles in the Ashkenazi population it will enable population screening in this ethnic group for the ascertainment of high risk couples before the birth of the first affected child. The lysosomal storage in MLIV stems from abnormal endocytosis process from late endosomes to lysosomes. The characterization of the protein involved with this process will permit a deeper understanding for this process and the defect in this

P1436. Mutations in the AAAS gene encoding a novel protein with a peroxisome targeting signal 1 (PTS1) cause triple A syndrome

K. Handschug[1], *S. Sperling*[2], *H. Lee*[3], *S. Hennig*[2], *F. Oberender*[1], *C. Hilscher*[1], *H. Petzold*[1], *S. K. Yoon*[3], *A. J. L. Clark*[4], *A. Huebner*[1]
[1]*Children s Hospital, Technical University; Dresden, Germany;* [2]*Max-Planck-Institute of Molecular Genetics; Berlin, Germany;* [3]*Research Institute of Molecular Genetics, Catholic Research Institutes of Medical Sciences; Seoul, Republic of Korea;* [4]*Departments of Endocrinology, St Bartholomew s and the Royal London School of Medicine and Dentistry; London, United Kingdom*
Katrin.Handschug@mailbox.tu-dresden.de

The triple A syndrome (MIM*231550) is a rare autosomal recessive disorder characterized by adrenocorticotropic hormone (ACTH) resistant adrenal failure, achalasia and alacrima as well as a variety of neurological features. Haplotype analysis of 47 triple A families allowed us to refine the critical region from 6 cM to a genetic segment of 0 cM between KRT8 and D12S1651. After construction of a high resolution BAC/PAC contig of this region we identified a novel gene, designated AAAS (Achalasia-Addisonianism-Alacrima-Syndrome gene) encoding a protein of 546 amino acids

which is mutated in all affected individuals. We found 19 different mutations scattered over the coding sequence including 3 splice mutations. Most mutations lead to a truncated protein suggesting loss of function. The protein shows high similarities (over 90 %) to putative proteins from Sus scrofa, Bos taurus, Rattus norvegicus and Mus musculus suggesting a conserved structure and function in mammals. RNA blotting experiments revealed ubiquitous expression with a higher level in neuroendocrine and gastrointestinal structures which are predominantly affected in triple A syndrome. The predicted protein belongs to the WD-repeat family of regulatory proteins. At the C-terminal end it contains the tripeptide Ser-His-Leu, a specific peroxisome targeting signal 1 (PTS1) raising the possibility that the AAAS gene product may function as a peroxisomal protein.

P1437. Demethylation, reactivation, and destabilization of human fragile X full mutation alleles in mouse embryocarcinoma (EC) cells

D. W hrle, *U. Salat, H. Hameister, W. Vogel, P. Steinbach*
Department of Human Genetics, University Hospital of Ulm; Ulm, Germany
peter.steinbach@medizin.uni-ulm.de

The major cause of fragile X syndrome is mutational expansion of the CGG repeat in gene FMR1, hypermethylation, and transcriptional silencing. Most fragile X embryos develop somatic mosaicism of disease causing full expansions of different lengths. Homogeneity of the mosaic patterns among multiple tissues indicates that these unstable expansions aquire mitotic stability in early fetal life. As mitotic stability is found strictly associated with hypermethylation in adult tissues, current theory fixes the time of instability to developmental stages when fully expanded CGG repeats exist in an unmethylated state. Murine EC cells (PC13) were used as a model system of pluripotent embryonic cells. Hypermethylated and unmethylated full expansions on human chromosomes were transferred from murine A9 hybrids into EC cells by microcell fusion. As demonstrated for the first time, even full expansion alleles that were fully methylated and stable in the donors fibroblasts and in A9 as well, became demethylated, reactivated, and destabilized in undifferentiated EC hybrids. When destabilized expansions were reintroduced from EC cells into A9, instability was reversed to stability. Our results strongly support that fully expanded alleles are initially unstable and unmethylated in the human embryo, and gain stability upon genetic or epigenetic change of the embryonic cells.

P1438. Mutations in the transcription factor FOXC2 in families with lymphoedema distichiasis.

R. Bell[1], *G. Brice*[1], *A. H. Child*[1], *S. Mansour*[1], *V. A. Murday*[1], *J. R. O. Collin*[2], *P. S. Mortimer*[1], *K. Burnand*[3], *S. Jeffery*[1]
[1]*St George s Medical School; London, United Kingdom;* [2]*Moorfields Eye Hospital; London, United Kingdom;* [3]*St Thomas Hospital; London, United Kingdom*
rbell@sghms.ac.uk

Primary lymphoedema is chronic tissue oedema, most frequently of one or both lower limbs, resulting from an intrinsic fault in lymph conducting pathways. There is a strong genetic component, with 35% of patients showing a positive family history, and there are 3 three main autosomal dominant forms of the disorder; Milroy s disease (congenital), and Lymphoedema-distichiasis (LD) and Meige s disease, where the onset is at or after puberty. In LD, features associated with the tissue swelling include eyelashes arising inappropriately from the meibomian glands (distichiasis), heart anomalies and cleft palate. We established linkage of LD to 16q24.3 (Mangion et al, Am. J. Hum. Genet. 1999;65;427-432), and the gene mutated in LD has very recently been established as FOXC2, a member of the forkhead/winged helix family of transcription factors. (Fang et al, Am. J. Hum. Genet. 2000;67;1382-1388, R. Bell, unpublished data). Fang et al reported prematurely terminating mutations in FOXC2 in two families with LD, and we have analysed the 10 LD families on our database for mutations in this gene. We have found 4 deletions and 5 insertions, scattered throughout the gene, all of which produce frameshifts that would lead to premature termination of the protein. These data support the proposition that FOXC2 mutations in LD act in a haploinsufficient manner.

P1439. Mutation in the FOXC2 gene causing lymphedema distichiasis in a German family

N. Tilgen, *B. Halliger-Keller, W. Kress*
Institute of Human Genetics; W rzburg, Germany
nikola.tilgen@mail.uni-wuerzburg.de

Lymphedema distichiasis (LD; MIM #153400) is a rare autosomal dominant disorder with variable penetrance, presenting mainly with lymphedema of the limbs and a double row of eyelashes. Recently Fang et al. (Am. J .Hum. Gen, 67; 1382-1388, 2000) described two unrelated families suffering from LD and carrying inactivating mutations in one allele of the FOXC2-gene. FOXC2 is a member of the forkhead/winged-helix family of transcription factors, whose members are involved in diverse development pathways. The gene produces a 2.2-kb transcript encoded by a 1.5-kb single exon that is highly GC rich. To test the hypothesis whether FOXC2-mutations cause lymphedema distichiasis in general, we studied a family with LD in two generations presenting a variable phenotype. By SSCP-analysis and subsequent sequencing, we identified an insertion of guanin within a stretch of five guanins beginning at position 867 of the FOXC2-gene in the two clinical affected children. This frame-shift mutation results in a stop codon, leading to a truncated form of the FOXC2-protein. The mutations described by Fang et al. also resulted in stop-codons and were localized at positions 297 and 1093. Our results confirm the hypothesis that an inactivating mutation of one allel of the FOXC2-gene is causing LD. Also further studies have to confirm that there is no evidence for heterogeneity in LD. Because of the variable clinical features, a mutational analysis of FOXC2-gene may give better insights in the pathogenesis of the disease, e.g. the identification of a phenotype-genotype correlation.

P1440. FOXC2; Confirming its role in Lymphedema-Distachiasis Syndrome.

M. A. Kimak[1], *E. C. Lawrence*[1], *K. L. Levinson*[1], *D. N. Finegold*[2], *R. E. Ferrell*[1]
[1]*Department of Human Genetics, University of Pittsburgh; Pittsburgh, PA United States;* [2]*Department of Pediatrics, University of Pittsburgh; Pittsburgh, PA United States*
height@pop.pitt.edu

Lymphedema-Distachiasis syndrome (LD {MIM153400}) is a diverse, heritable condition whose primary components, edematous limbs and an extra row of eyelashes, are often seen amid a spectrum of other features including craniofacial, cardiovascular, and vertebral abnormalities. FOXC2, a member of the winged-helix forkhead transcription factor family, contains a highly conserved 110 bp DNA binding domain which has been implicated in such developmental processes as determining embryonic cell fate, initiating and maintaining tissue differentiation, and tumorigenesis later in life. Recent identification of FOXC2 mutations in 2 LD families (AJHG 2000 Dec; 67(6)1382-88) has placed this gene in a favorable position to explain the phenotypic complexity found in LD. Here we report identification of five additional FOXC2 mutations in six lymphedema families, four of which exhibit distachiasis. Of the two not reporting distachiasis, one family demonstrates an additional feature consistent with the LD syndrome, namely cleft palate. Onset of lymphedema was pubertal in nearly all cases. Direct sequencing of FOXC2 revealed both single nucleotide and insertion/deletion mutations occurring in or near the forkhead domain. All mutations observed are predicted to cause early termination of the FOXC2 protein. We speculate that the clinical heterogeneity seen in Lymphedema-Distachiasis syndrome is the result of haploinsufficiency of this developmental gene and further investigation into the FOXC2 expression pattern may elucidate its role in lymphatic development and its interaction with other developmental genes.

P1441. Phenotypic heterogeneity in lymphedema families with FOXC2 mutations.

K. L. Levinson[1], *R. E. Ferrell*[1], *M. A. Kimak*[1], *E. C. Lawrence*[1], *J. W. Dunlap*[1], *D. N. Finegold*[2]
[1]*Department of Human Genetics, University of Pittsburgh; Pittsburgh, PA United States;* [2]*Department of Pediatrics, University of Pittsburgh; Pittsburgh, PA United States*
klevinso@helix.hgen.pitt.edu

Lymphedema-Distichiasis is a syndrome which includes ocular abnormalities, pubertal onset lymphedema, congenital heart defects, and cleft palate (OMIM 153400). Other lymphedema syndromes include Yellow Nail Syndrome (OMIM 153300) and Lymphedema-Ptosis (OMIM 153000). We report on the phenotype of six families in our study as well as two published families, all with mutations in the FOXC2 gene on 16q24. The six caucasian families from our study were ascertained through our Lymphedema Family Study website (www.pitt.edu/~genetics/lymph) or GeneTests, Inc. (www.genetests.org). Included in these eight families were 42 affected individuals and/or mutation carriers. Two were aborted fetuses with hydrops, and two mutation carriers were completely normal. Lymphedema, with an average onset age of 15, was present in 31 individuals. Among the 9 individuals without lymphedema, 7 were between the ages of 6 and 14, and presumably presymptomatic. Distichiasis was present in 21 individuals from six families. Tetralogy of Fallot, cleft palate, ptosis, cystic hygroma, and fetal hydrops were observed in at least one member of these families. Yellow nails was present in one family with lymphedema, distichiasis, and ptosis. Phenotypic and allelic heterogeneity and variable expression characterize families with mutations in FOXC2. The occurrence of

phenotypic features associated with three syndromes, previously classified as distinct genetic entities, suggests the need for reclassification of the lymphedema syndromes. Five families with lymphedema and distichiasis, but no detectable mutations in the FOXC2 coding sequence, indicate a high likelihood of further locus heterogeneity.

P1442. A missense mutation that causes Primary Congenital Lymphoedema by interfering with VEGFR-3 signalling

A. L. Evans[1], *M. J. Karkkainen*[2], *G. Brice*[1], *A. Crosby*[1], *P. Comeglio*[1], *P. Mortimer*[1], *K. Burnand*[3], *M. Sarfarazi*[4], *A. Child*[1]
[1]*St George s Hospital Medical School; London, United Kingdom;* [2]*University of Helsinki; Helsinki, Finland;* [3]*St. Thomas Hospital; London, United Kingdom;* [4]*University of Connecticut Health Center; Farmington, CT United States*
aevans@sghms.ac.uk

Primary lymphoedema is a chronic tissue swelling, most frequently of the lower limbs, resulting from deficient lymphatic drainage. We have previously reported a locus for primary congenital lymphoedema, (PCL), where onset is at birth, to the most telomeric portion of chromosome 5q35.3. Mutations have been reported in the vascular endothelial growth factor-3 receptor (VEGFR-3) gene, which lies within this region. We have analysed an additional 5 families with PCL and shown them to be consistent with linkage to 5q35.3. Furthermore, we describe a G to A transition in VEGFR-3, which results in Q for R substitution at amino acid 1041. This substitution affects a residue in a highly conserved region of the catalytic loop in the kinase domain of VEGFR-3 and by in vitro studies, has been shown to interfere with the receptor tyrosyl phosphorylation. Thus defective VEGFR-3 signalling seems to be one cause of PCL in families linked to 5q35.3.

P1443. ARSACS; Mutation detection and studies towards understanding the function of Sacsin.

A. M. Richter[1], *J. Mercier*[1], *J. C. Engert*[2], *C. LeBlanc*[1], *T. J. Hudson*[2]
[1]*Centre de Recherche, H pital Sainte-Justine, Universit de Montr al; Montr al, PQ Canada;* [2]*Montreal Genome Centre, McGill University Health Centre; Montr al, PQ Canada*
andrea.richter@umontreal.ca

Our overall aim is to understand the molecular pathogenesis of autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS/SACS MIM# 270550) a unique, early-onset disease. Although rare elsewhere, a founder effect increased disease frequency in the population of northeastern Quebec in Canada. We mapped the gene to chromosome 13q11 (Richter et al, AJHG 64;768, 1999) . We identified the 11.7 kb single exon sacsin gene and detected two mutations g.6594delT (DT) and the rare g.5254C>T nonsense mutation (Engert et al, Nature Genetics 24; 120, 2000). Analyses of the 437 kD predicted sacsin protein with sequence comparison tools did not reveal extensive similarity to known proteins, although the presence of heat-shock domains suggest a role in chaperone-mediated protein folding. To facilitate molecular diagnosis and gene carrier detection, we developed an ASO based mutation detection method. The analysis of 164 independent ARSACS alleles revealed that 152 (92.7%) carried the DT and 6 (3.7 %) the C>T mutation. For 6 ARSACS alleles (3.7%), we have yet to identify the mutation. This unknown mutation(s) is/are always carried in the heterozygous form with the frequent, DT mutation. The identification of these rare alleles is now underway. Sacsin was identified entirely using a sequence-based approach. We cloned sacsin fragments in expression vectors and produced anti-sacsin antibodies to specific fragments. The cloning of full length sacsin to obtain Abs to the complete protein is underway. The use of these different Ab species to detect the presence and cellular distribution of sacsin protein in control and ARSACS patient cells will be described. Understanding the function of sacsin is the first step in the development of possible treatment options and may have relevance to the understanding of other progressive neurodegenerative diseases.

P1444. A new gene is disrupted by the t(2;14) translocation in a patient with mirror-image polydactyly

K. Yoshiura, *S. Kondo, K. Komatsu, N. Matsumoto, N. Niikawa*
Department of Human Genetics, Nagasaki University School of Medicine; Nagasaki, Japan
kyoshi@net.nagasaki-u.ac.jp

We previously reported that the 14q13 translocation breakpoint in a patient with mirror-image polydactyly lies between D14S75 and D14S306 within a complete BAC/PAC contig (Genomics, 1997). A novel gene, named mirror-image polydactyly gene 1 (MIPD1) was found to be disrupted by the breakpoint. The MIPD1 gene consists of 17 exons and spans a 450-kb genomic region. All exon-intron boundaries were determined using genomic sequences in this contig map. The expression is detectable in the adult heart, liver, skeletal muscle, kidney, and pancreas but not in the adult brain lung or placenta by Northern blot analysis. MIPD1 has a coiled-coil domain in the C-terminus but no homology to any known genes. Mutation analysis for two patients with mirror-image polydactyly and a whole mount in situ hybridization in the developing limb bud in mouse embryos are now in progress.

P1445. Genotype/phenotype correlation of NPHS1 and NPHS2 mutations advocate a functional interrelationship in renal glomerular filtration.

A. B. Koziell[1], *S. Hussain*[1], *U. Lenkkeri*[2], *K. Tryggvason*[3], *P. Scambler*[1]
[1]*Institute of Child Health; London, United Kingdom;* [2]*Biocenter; Oulu, Finland;* [3]*Karolinska Institute; Stockholm, Sweden*
A.Koziell@ich.ucl.ac.uk

Mutations of novel podocyte genes NPHS1 and NPHS2 are typically associated with Finnish type congenital nephrotic syndrome (CNF) and autosomal recessive focal segmental glomerulosclerosis (FSGS) respectively. We examined genotype/phenotype correlation of NPHS1/NPHS2 mutations to a) characterise accurately the mutation phenotype and b) further identify their role in the pathogenesis of glomerular protein leak. Methods. 47 patients and 50 normal controls were analysed. NPHS1 and NPHS2 exons were PCR amplified and products sequenced on an ABI 377. Phenotypic diagnosis was based on clinical and family history and/or renal biopsy. 38/47 patients had CNF, 4/47 congenital FSGS and 5/47 early onset (1 year) FSGS. All presented with severe nephrotic syndrome, but disease progression was uncharacteristically mild in 9 CNF patients. Results. 30/38 CNF patients had NPHS1 mutations, and 2/30 carried a specific additional NPHS2 mutation, R229Q, a putative hypomorphic allele. 2 of 8 CNF patients lacking NPHS1 mutations had NPHS2 mutations. One NPHS1 mutation, R1160X, previously connected with severe disease, was consistently associated with the milder CNF phenotype. All patients with congenital FSGS had concomitant mutations in NPHS1 and NPHS2. Mutations were absent in early onset FSGS. Conclusions. Genotype/phenotype correlation detected overlap between NPHS1 and NPHS2 mutations, with co-existence of mutations in both genes in congenital FSGS and rarely, CNF. In addition, the association of R1160X with mild and severe CNF indicates genetic/metabolic interference, unconnected with NPHS2 mutations. Our findings advocate a functional interrelationship between NPHS1 and NPHS2 within the podocyte, further supporting their integral role in the pathogenesis of proteinuria.

P1446. Fine mapping of deletion breakpoints in Williams-Beuren syndrome patients

M. Bay s, *O. de Luis, L. F. Magano, L. A. P rez Jurado*
Genetics Unit, Dept. of Experimental Sciences, Universitat Pompeu Fabra; Barcelona, Spain
monica.bayes@cexs.upf.es

Williams-Beuren syndrome (WBS) is a genetic disorder resulting from a heterozygous deletion of contiguous genes at 7q11.23. Large blocks of region-specific low-copy repeat elements (LCRs A,B and C) predispose to a common ~1,6 Mb deletion through unequal crossing-over, mostly between the centromeric and medial LCR blocks B. These blocks share >99.5% sequence identity along ~120 Kb and contain three genes at the medial location (GTF2I, NCF1 and GTF2IL), with the corresponding pseudogenes at the centromeric side, as well as a high density of SINE (44%). A third block B with pseudogenes lies more telomeric. In this study, we have defined the deletion breakpoints in 35 WBS patients using copy-specific nucleotide differences. Most patients (33/35) exhibited the typical 1,6 Mb deletion caused by recombination between the centromeric and medial blocks B. Junction fragments were mapped within GTF2I/P1 or GTF2I-NCF1 intergenic in 19 cases (58%), NCF1/P1 in 9 (27%), NCF1-GTF2IL intergenic in 2 (6%), and GTF2IL/P1 in 3 (9%). Sequencing across the junction fragment in two cases revealed the presence of Alu repeats. Only 2 patients had a larger deletion likely due to recombination between blocks A. No correlation was found between the different deletions and the clinical phenotype. Our data indicate that; 1- hemizygousity at NCF1 and/or GTF2IL does not affect the WBS phenotype; 2- unequal homologous recombination causing WBS is a precise mechanism that preferentially occurs in LCR regions of extremely high sequence identity and rich in repeat elements, but without specific hot spots within them.

P1447. Mutation analysis of the MECP2 gene in Rett syndrome females from UK and Italy.

M. D Esposito
International Institute of Genetics and Biophysics, CNR; Naples, Italy
desposit@iigbna.iigb.na.cnr.it

Rett syndrome is an X linked dominant neurological disorder, which appears to be the commonest genetic cause of profound combined intel-

lectual and physical disability in Caucasian females. Recently, this syndrome has been associated with mutations of the MECP2 gene, a transcriptional repressor of still unknown target genes. We present the results of a MECP2 mutation analysis of Italian and British RTT females, the majority of which show the classical phenotype. An overall mutation frequency of roughly 70 % was detected, and several new mutations identified. We were able to trace a hot spot map for MECP2 mutations, analyzing our data and the published results for different populations. The populations analyzed show six most common mutations which account for the 62% of the cases. Using this strategy, we established the high occurrence of —COOH terminal deletions, in the patients analyzed. In this region of the MeCP2 protein, database comparisons identified a potential new structural domain, common to other regulatory genes, all significantly expressed in brain. The combined approach of mutational and bioinformatic analysis let us to add a number of observations. Such analysis could become the basis for a genotype-phenotype correlation. However, this was not feasible here, as the majority of cases in our study population have classical RTT.

P1448. Genotype-phenotype correlation of MECP2 mutations in Rett syndrome

I. Kondo[1], *T. Fukuda*[1], *R. Morishita*[1], *T. Matsuishi*[2], *Y. Yamashita*[3], *K. Kuwajima*[4], *H. Nitta*[5], *I. Ohmori*[6], *I. Horiuchi*[6]
[1]*Ehime University School of Medicine; Ehime, Japan;* [2]*Kurume University School of Medicine; Hukuoka, Japan;* [3]*Kurume University School of Medicine; Fukuoka, Japan;* [4]*Ibaraki Pref. Handicapped Children s Center; Ibaraki, Japan;* [5]*Niigata Pref. Hamagumi Rehabilit. Center for Handicapped Children; Niigata, Japan;* [6]*Asahigawa Jidoin; Okayama, Japan*
ikondo@m.ehime-u.ac.jp

Rett syndrome (RTT) is a neurodevelopmental disorder affecting exclusively females with regression loss of speech and purposeful hand use after 6 months of almost normal development. Since DNA mutations in the methyl-CpG-binding protein 2 gene (MECP2) were detected in patients with RTT in 1999, many different mutations in MECP2 have been reported not only in patients with classic RTT, but also in patients with non-specific mental retardation.However, genotype-phenotype correlation of MECP2 mutations has not been established. In this study, we have screened DNA mutations MECP2 in 100 Japanese sporadic patients with clinical diagnosis of RTT by child neurologists. We identified 35 different causative mutations in 85 patients (85% of patients examined) ; 13 missense mutations in 39 patients, 6 nonsense mutations in 30 patients and 15 frameshift mutations in 15 patisnts and one splicing anomaly in one patient with RTT. The common mutations of MECP2 were T158M and R168X in our patients, detected in 12 and 9 classic RTT patients, respectively. On the other hand, R133C and R294X were predominantly detected in patients with preserved speech vatiant form of RTT, detected in 4 in 6 patients with R133C and 3 in 6 patients with R294X. Skewed X- inactivation was not obserbed in these patients. Although effect of X-inactivation on phenotypes have been represented, the types of MECP2 mutations may also effect on phenotypes of RTT.

P1449. Diagnostic MECP2 mutational analysis in Korean patients with Rett syndrome

C. Kim[1], *I. Kim*[1], *K. Kwak*[1], *S. Kim*[1], *B. Son*[2], *T. Hwang*[3], *S. Park*[3], *C. Kang*[1], *S. Kim*[1], *B. Chung*[1]
[1]*Department of Biochemistry, College of Medicine, Pusan National University; Pusan, Republic of Korea;* [2]*Department of Pediatrics, St. Benedict Hospital; Pusan, Republic of Korea;* [3]*Department of Pediatrics, College of Medicine, Inje University; Pusan, Republic of Korea*
kimcm@hyowon.cc.pusan.ac.kr

Rett syndrome (RTT) is a neurodevelopmental disorder affecting 1 in 10,000 to 15,000 female births worldwide. Mutations in the MECP2 (methyl-CpG-binding protein 2) gene, mapped on chromosome Xq28, cause RTT. MeCP2 contains both a methyl-CpG binding domain (MBD) and a transcriptional repression domain (TRD), and participates in transcriptional silencing via DNA methylation. We analyzed three exons of the MECP2 gene from the peripheral blood of 30 Korean patients with RTT by PCR and direct sequencing. We detected mutations of MECP2 in 20 cases (66.7%) of the 30 RTT patients. The mutations consisted of 12 types including 9 missense and 3 nonsense mutations. Of these, three (L100V, G161E and T311M) mutations were newly identified, and five (T158M, R168X, R255X, R270X, R306C) mutations were frequently generated. Mutations involved MBD and TRD were showed 8 cases (40%) and 7 cases (35%) of 20 current mutations, respectively. Therefore these mutations found in the functional domains may critically affect the function of MECP2. We found that most MECP2 mutations were confined in the exon 3 (19 of 20 mutations). So it is suggested that the sequence analysis of exon 3 should be done at first to screen mutation in MECP2. The analysis of MECP2 gene should be expected an essential and useful tool to confirm the clinical diagnosis for RTT patients.

P1450. Analysis of MECP2 gene mutations in Turkish Rett Syndrome Patients

C. Akyerli[1], *A. Sayi*[1], *R. S. Kocoglu*[1], *M. Cimbis*[2], *M. Topcu*[2], *T. Ozcelik*[1,3]
[1]*Department of Molecular Biology and Genetics, Bilkent University; Ankara, Turkey;* [2]*Department of Pediatric Neurology, Hacettepe University Medical School; Ankara, Turkey;* [3]*Bilgen, Bilkent University; Ankara, Turkey*
akyerli@bilkent.edu.tr

Rett Syndrome (RTT) is a progressive X-linked dominant neurodevelopmental disorder, affecting 1/10,000-15,000 females. The disease-causing gene was identified as *MECP2* on chromosome Xq28, and mutations have been found in ~80% of patients diagnosed with RTT. We started mutational screening on *MECP2*, exon 2 and 3, in 52 RTT patients. As a first step, we screened for six known mutations; R106W, P152R, T158M, R306C, R168X, F155S, and one polymorphism; E397K. We found R106W in three patients, P152R in two patients, T158M in four patients, and R306C in four patients. Mutations R168X and F155S were not detected in our patients. Only one patient had E397K polymorphism, who also bears R306C mutation. In exon 3 of *MECP2*, there are several known deletion type of mutations. By PCR analysis, two patients were found to have ~ 26 bp deletion in exon 3. In order to confirm this mutation, we are performing sequencing analysis at the moment. As a result of this initial screening, *MECP2* mutations were identified in ~ 30 % of the Turkish patients diagnosed with RTT. We will further analyze *MECP2* by direct DNA sequencing in patients not found to have a mutation by restriction endonuclease analysis.

P1451. Mutations in the a3 subunit of the vacuolar H+-ATPase cause infantile malignant osteopetrosis

C. Kubisch[1], *U. Kornak*[2], *A. Schulz*[3], *W. Friedrich*[3], *S. Uhlhaas*[1], *B. Kremens*[4], *T. Voit*[4], *C. Hasan*[5], *U. Bode*[5], *T. J. Jentsch*[2]
[1]*Institut f r Humangenetik, Universit t Bonn; Bonn, Germany;* [2]*Zentrum f r Molekulare Neurobiologie Hamburg; Hamburg, Germany;* [3]*Universit t-skinderklinik, Universit t Ulm; Ulm, Germany;* [4]*Klinik und Poliklinik f r Kinder- und Jugendmedizin, Universit t-Gesamthochschule Essen; Essen, Germany;* [5]*Zentrum f r Kinderheilkunde, Universit t Bonn; Bonn, Germany*
kornaj@zmnh.uni-hamburg.de

Infantile malignant osteopetrosis is a rare and fatal disease that usually begins during the first postnatal weeks. Leading signs are a massive increase in bone density, absence of marrow cavities in the long bones and anemia due to insufficient extramedullary blood production, that further causes hepatosplenomegaly. The only currently available cure is bone marrow transplantation. As the osteoclast is thought to be the only cell capable of degrading bone, it was likely that the cause of the disease was a defect in osteoclast function. These cells degrade bone material in a tightly sealed extracellular compartment that is acidified by a V-type H+-ATPase. Recently, a new isoform of the membrane-spanning a-subunit of this H+-pump was cloned, shown to be expressed in osteoclasts and its gene was mapped close to a locus for infantile osteopetrosis. Further, mutations in the corresponding mouse gene were found to cause an osteopetrotic phenotype. In 9 out of 12 patients with infantile malignant osteopetrosis, we now demonstrate mutations in OC116/TCIRG1, the gene encoding the a3 subunit of the V-ATPase from osteoclasts. On the other hand, a patient from a consanguineous family was heterozygous at the OC116/TCIRG1-locus, suggesting that a mutation in a different gene underlies osteopetrosis in that pedigree. Analysis of further samples is currently being carried out. Our work shows that mutations in the gene encoding the a3 subunit of the proton pump are a rather common cause of infantile malignant osteopetrosis and suggests that this disease is genetically heterogeneous.

P1452. Molecular genetics of TRAPS (TNF receptor-associated periodic syndrome), a new hereditary periodic fever syndrome

I. Aksentijevich, *D. L. Kastner*
NIH; Bethesda, MD United States
aksentii@exchange.nih.gov

Mutations in the extracellular domains of the 55 kDa TNF receptor (TNFRSF1A) define a dominantly inherited periodic fever syndrome TRAPS that is characterized by prolonged attacks of recurrent fevers, sterile peritonitis, arthralgia, myalgia, rash, and/or conjunctivitis (MIM142680). TRAPS can be distinguished from familial Mediterranean fever (FMF) based on ethnic background, duration of attacks, and poor response to colchicine. We have previously described 6 disease-associated

TNFRSF1A mutations (C30R, C33Y,T50M, C52F,C88R, and C88Y), 5 of which disrupt conserved extracellular cysteines. In a survey of additional patients with unexplained periodic fevers (150), we have identified 6 new TNFRSF1A mutations in 28 patients ; H22Y, C33G, P46L, S86P, R92Q, c.193-14 G> A, and one mutation (C30S) that has been recently reported. This brings the total number of TRAPS mutations to date to 14, all of them missense substitutions with 7 involving cysteine residues. We also report reduced penetrance for several of the mutations; C33Y, T50M, P46L, R92Q, and c.193-14 G> A. The c.193-14 G> A, R92Q, and T50M mutations occurred at CpG hotspots and were observed in patients of diverse ethnic descent. Haplotype analysis showed evidence for a common intragenic (TNFRSF1A) founder for R92Q even among patients of very distinct background (9/9 shared a microsatellite/ SNP haplotype that is very uncommon in the general population). However, there were independent founders for the other two mutations. Our analysis thus far does not explain the skewed ethnic distribution of TRAPS patients (Irish / Scottish) on the basis of a common mutation or founder chromosome.

P1453. Analysis of mutations in a large Bardet-Biedl cohort reveals minor involvement of BBS6 and evidence for possible digenic inheritance.

P. L. Beales[1], *N. Katsanis*[2], *R. A. Lewis*[2], *S. J. Ansley*[2], *N. Elcioglu*[3], *J. Raza*[4], *M. O. Woods*[5], *J. S. Green*[5], *P. S. Parfrey*[5], *W. S. Davidson*[6], *P. J. Scambler*[1], *J. R. Lupski*[2]
[1]*Molecular Medicine Unit, Institute of Child Health, University College London; London, United Kingdom;* [2]*Baylor College of Medicine; Houston, TX United States;* [3]*Department of Pediatric Genetics, University Hospital; Istanbul, Turkey;* [4]*National Institute of Child Health; Karachi, Pakistan;* [5]*Faculty of Medicine, Memorial University of Newfoundland; St. John s, NF Canada;* [6]*Department of Molecular Biology and Biochemistry, Simon Fraser University; Burnaby, BC Canada*
pbeales@hgmp.mrc.ac.uk

Bardet-Biedl syndrome (BBS) is an autosomal recessive disorder characterised by obesity, post-axial polydactyly, retinal dystrophy, hypogonadism, cognitive deficit and renal disease. There exists substantial genetic and phenotypic heterogeneity, which until now has hindered efforts to positionally clone the six known BBS loci. Our collection of 163 pedigrees of diverse ethnic origin has been evaluated for mutations in the recently discovered BBS6 gene (MKKS) on 20p12 and for potential assignment to any of the other known BBS loci. Through haplotype analysis of all pedigrees, we report substantially reduced critical intervals for BBS2, 3 and 5. Furthermore, we identified nine missense alterations, each potentially pathogenic in MKKS from eight pedigrees, each of which segregate with the disease. In only one of these pedigrees were we able to identify both mutations. However, three of the remaining pedigrees in which alterations were found, were also compatible with linkage to a separate BBS locus. Moreover, through autozygosity mapping of a further cohort of consanguineous kindreds from Turkey, Iraq and Pakistan, we noted that a single Pakistani pedigree mapped to two separate BBS loci. These data suggest that although BBS6 is a minor contributor to the syndrome, some BBS6 alleles may act in conjunction with mutations at other BBS loci to cause or modify the BBS phenotype.

P1454. Mutations in MKKS cause obesity, retinal dystrophy and renal malformations associated with Bardet-Biedl syndrome.

N. Katsanis[1], *P. L. Beales*[2], *M. O. Woods*[3], *R. A. Lewis*[1], *J. S. Green*[3], *P. S. Parfrey*[3], *S. J. Ansley*[1], *W. S. Davidson*[4,3], *J. R. Lupski*[1]
[1]*Baylor College of Medicine; Houston, TX United States;* [2]*Institute of Child Health; London, United Kingdom;* [3]*Memorial University of Newfoundland; St. John s, Canada;* [4]*Simon Frasier University; Vancouver, BC Canada*
katsanis@bcm.tmc.edu

Bardet-Biedl syndrome (BBS) is an autosomal recessive disorder predominantly characterized by obesity, retinal dystrophy, polydactyly, learning difficulties, hypogenitalism and renal malformations, with secondary features that include diabetes mellitus, endocrinological dysfunction and behavioral abnormalities. Despite an initial expectation of genetic homogeneity due to relative clinical uniformity, five BBS loci have been reported, with evidence for additional loci in the human genome. We performed a genome screen with BBS families from Newfoundland that were excluded from BBS1-5 and identified linkage with D20S189. Fine-mapping reduced the critical interval to 1.9 cM between D20S851 and D20S189, encompassing a chaperonin-like gene, in which mutations were reported recently to be associated with McKusick-Kaufman syndrome (MKKS). Given both the mapping position and clinical similarities of these two syndromes, we screened MKKS and identified mutations in five Newfoundland and two European-American BBS pedigrees. The majority are frameshift alleles which are likely to result in a non-functional protein. Our data thus suggest that a complete loss of function of the MKKS product, and thus an inability to fold a range of target proteins, is responsible for the clinical manifestations of BBS. Furthermore in contrast to adult tissues, where MKKS expression was widespread at stable levels, we have found the abundance of MKKS mRNA to vary significantly during early development, with strong expression correlating to sites of disease such as the heart, branchial arches and the developing limb bud, suggesting that the onset Bardet-Biedl syndrome may be as early as the fifth week of human development.

P1455. The common breakpoint of NF1 microdeletions interrupts a new gene

E. Legius, *T. Deraedt, H. Brems, P. Marynen*
Centre for Human Genetics; Leuven, Belgium
Eric.Legius@med.kuleuven.ac.be

We performed molecular studies in 38 NF1 microdeletion patients. Twenty-two out of 27 microdeletions where the parental origin could be studied were of maternal origin (81%) and 5 were of paternal origin. Thirty-seven of the 38 microdeletions have a 1.5 Mb size with breakpoints in flanking paralogous sequences (NF1-REPs).These paralogous sequences are >100 kb long and represent duplicated sequences in a direct orientation with 98% homology. At least 5 expressed sequences are present in the flanking paralogous sequences. Additional paralogous sequences are present on chromosome 17 and on other chromosomes. The same paralogous sequences are also present in Gorilla and Bonobo, but not in mice. Further molecular analysis of the 37 cases with breakpoints in the flanking paralogous sequences showed that 19 microdeletion breakpoints clustered in a 2 kb region. There was no specific structure present at the breakpoints responsible for the meiotic recombination process. Conversion events in the breakpoint region were documented in at least two cases. These data show that about 50% of NF1 microdeletions originate from an unequal recombination between paralogous sequences on the homologous chromosomes 17 during the first meiotic division. This recombinational process interrupts a gene in the flanking NF1-REPs and deletes 4 expressed sequences located in the telomeric NF1-REP. Aside from the NF1 gene with its three embedded genes and the expressed sequences in the paralogous regions at least 13 other expressed sequences are localized in the microdeletion region. We are currently investigating the role of these deleted genes for the specific phenotype in these microdeletion patients.

P1456. Evidence for a tumor-modifying gene in neurofibromatosis type 1.

K. G. Stephens[1], *M. O. Dorschner*[1], *K. A. Leppig*[2], *V. P. Sybert*[1]
[1]*University of Washington; Seattle, WA United States;* [2]*Group Health Cooperative; Seattle, WA United States*
millie@u.washington.edu

We present clinical, molecular, and mapping evidence for a novel gene that potentiates the formation of dermal neurofibromas in patients with neurofibromatosis type 1 (NF1). Patients carrying a l.5 Mb contiguous gene deletion that includes NF1 are remarkable for an early age at onset and a heavy burden of dermal neurofibromas relative to age. Using molecular mapping techniques and sequencing, we found that the NF1 gene is flanked by 80kb DNA elements (NF1REPs), which share >97% nucleotide identity and facilitate the homologous recombination event that generates the deletion. Unexpectedly, the deletion breakpoints of the majority of patients were clustered in a 6 kb recombination hotspot within the NF1REPs. Therefore, NF1 microdeletions occur predominantly at the same site and delete the same set of genes. We hypothesize that the severe tumor phenotype is due to the deletion of both NF1 and a novel neurofibroma-potentiating locus (NPL). The location of this putative tumor modifier has been narrowed to <700 kb by the identification of patients with novel shorter deletions. Our hypothesis is strengthened by a patient with somatic mosaicism for an NF1 microdeletion, who had rapid onset of dermal neurofibromas in her early 20s. Therefore, the clinical manifestations of the mosaic individual were ameliorated compared to those of the germline NF1 microdeletion patients; a result predicted by our hypothesis. Currently, we are investigating 5 functional genes within the 700 kb critical region as candidates for the NPL locus. Our data implicate for the first time a locus that functions to modify neurofibromagenesis in humans.

P1457. Establishment of an in vivo fusion protein assay in yeast for the detection of mutations in the NF1 gene

C. Gottesmann[1], *P. Zwirn*[1], *M. Krainer*[2], *M. Edelh user*[1], *C. Fonatsch*[1], *R. Wieser*[1], *K. Wimmer*[1]
[1]*Institut f r Medizinische Biologie, Universit t Wien; Wien, Austria;* [2]*Klinik f r Innere Medizin I, Universit tsklinik; Wien, Austria*

a9003816@unet.univie.ac.at
Neurofibromatosis 1 (NF 1) is one of the most common inherited disorders. The NF1 gene, mapped to chromosome 17q11.2, spans approximately 350 kb of genomic DNA and contains 60 exons. The condition appears to be fully penetrant but has highly variable expression. For the patients, only identification of the pathogenic germline mutation allows for presymptomatic and/or prenatal testing in offspring. However, mutation detection in the NF1 gene is laborious and complex due to the size of the gene, the existence of pseudogenes and the lack of mutational hotspots. Since the vast majority of pathogenic lesions in NF1 patients lead to truncated protein the protein truncation test (PTT) is one of the most widely used mutation detection technique. Recently, an alternative strategy has been developed for the detection of truncating mutations in large genes. This method exploits the high efficiency of homologous recombination in yeast to fuse the test sequence in-frame to a reporter gene. The method permits the analysis of a large number of samples in a minimum of steps and simplifies sequencing by the separation of wild type and mutant alleles. We have adapted this method for the analysis of the NF1 gene in 10 fragments. By analysing 13 different mutations we have shown that the sensitivity of the test is comparable to that of the PTT. Further, we have demonstrated that the use of the translation inhibitor puromycin increases the sensitivity of the test due to reduction of nonsense-mediated mRNA decay. Thus, this assay offers a rapid and reliable alternative method for genetic diagnosis in NF1 patients.

P1458. Molecular study of new candidate genes in non-syndromic X-linked mental retardation patients with disease-associated balanced chromosome rearrangements

V. Kalscheuer[1,2], *A. Schr er*[1], *H. G. Nothwang*[1], *S. K barth*[1], *J. Wirth*[1], *M. Platzer*[3], *C. Menzel*[1], *O. Hagens*[1], *M. Ross*[4], *J. Chelly*[2], *J. P. Fryns*[2], *C. Moraine*[2], *B. Hamel*[2], *U. Trautmann*[5], *M. Zenker*[5], *G. Barbi*[6], *N. Tommerup*[7], *H. H. Ropers*[2]

[1]*Max-Planck-Institute for Molecular Genetics; Berlin, Germany;* [2]*European MRX Consortium; ., France;* [3]*Institute for Molecular Biotechnology; Jena, Germany;* [4]*The Sanger Centre, Wellcome Trust Genome Campus; Hinxton, United Kingdom;* [5]*Institute for Human Genetics, University of Erlangen-Nuernberg; Erlangen, Germany;* [6]*Department of Medical Genetics, University of Ulm; Ulm, Germany;* [7]*Department of Medical Genetics, IMBG, University of Copenhagen; Copenhagen, Denmark*

kalscheuer@molgen.mpg.de

Severe mental retardation is found in 0.5% of the newborn population and roughly 2/3 of these cases are due to monogenic or chromosomal disorders. In 1/3 of the affected males, X-chromosomal gene defects are involved, and cryptic subtelomeric or interstitial deletions may be an equally important cause of mental retardation. In an attempt to study X-linked mental retardation in a systematic manner, we have set out to collect and characterize 24 mentally retarded patients with balanced X;autosome translocations. By employing FISH probes from a near-complete X-chromosomal YAC contig (manuscript in prep.), all but two of the X-chromosomal breakpoints could be mapped to an interval of 500 kb or less. For many of these, gene identification is in progress. Here we report on two patients, a familial inv(Xp22.2;q28) in a boy and a de novo t(X;8)(p11.2;p22.3) in a girl. Each of the three X-chromosomal breakpoints was found to disrupt an interesting candidate gene. Mutation analysis in the >200 unrelated patients of the European MRX consortium is in progress.

P1459. Evidence That A Missense Mutation (P312L) in FGD1 Results in Nonsyndromic XLMR.

C. E. Schwartz[1], *R. R. Lebel*[2], *M. M. May*[1], *H. A. Lubs*[3], *R. E. Stevenson*[1]

[1]*Greenwood Genetic Center; Greenwood, SC United States;* [2]*Genetic Services; Glen Ellyn, IL United States;* [3]*University of Miami School of Medicine; Miami, FL United States*

schwartz@ggc.org

The Aarskog syndrome (AS, MIM #305400) is an X-linked condition characterized by facial, genital and skeletal anomalies. It is caused by mutations in the FGD1 gene located in Xp11.21. Since many X-linked mental retardation (XLMR) conditions localize to a broad region encompassing Xp11.2, we began a mutational analysis of XLMR families linked to Xp11.2 as well as males from small, unstudied XLMR families. In one affected male in the families, a shift in exon 4 of FGD1 was detected by SSCP. Sequence analysis revealed a C1665T change, which gave rise to an amino acid substitution, P312L. The base change was confirmed by StyI digestion of the altered allele. Analysis of the family indicated the C1665T alteration segregated with XLMR in the family. The C1665T alteration was not detected in 250 normal X chromosomes. The phenotype of the affected males is non-syndromic. Except for a tendency towards short stature (at or below the 10 %tile), the affected males did not exhibit any of the hallmark features of AS; widow s peak, prominent forehead, hypertelorism,downslanting palpebral fissures, joint hyperextensibility, shawl scrotum or foot deformations . Furthermore, all three males had severe MR that is not a feature of AS. Utilization of several programs for secondary sequence analysis revealed that the substitution of a leucine for proline at position 312 appears to lengthen a coil region two-fold by removing a beta turn. FGD1 is now like RSK2 and MECP2 in that mutations in it give rise to both syndromic and nonsyndromic XLMR conditions.

P1460. Mutation Spectrum in Papillon-Lef vre syndrome.

C. A. Hewitt[1], *C. Toomes*[1], *C. L. Wu*[1], *J. Wood*[1], *D. McCormick*[2], *J. James*[1], *P. Sloan*[1], *M. Dixon*[1], *A. P. Read*[1], *N. S. Thakker*[1]

[1]*Manchester University; Manchester, United Kingdom;* [2]*Queens University Belfast; Belfast, United Kingdom*

Chelsee.Hewitt@man.ac.uk

Papillon-Lef vre syndrome (PLS) is a rare autosomal recessive condition characterised by palmoplantar hyperkeratosis and severe periodontitis that results in premature tooth loss. We recently identified the gene that is mutated in this condition (Toomes et al., Nat. Genet. 23, 421-424, 1999). This gene, CTSC, encodes the oligomeric lysosomal protease cathepsin C (also known as dipeptidyl aminopeptidase I) which plays an essential role in the activation of granule serine proteases expressed in bone marrow-derived effector cells of both myeloid and lymphoid series. These serine proteases are implicated in a wide variety of immune and inflammatory processes and a lack of functional CTSC in PLS may be associated with a reduced host response against bacteria in dental plaque. We have performed mutation analyses on 17 PLS families and identified 20 mutations. Affected individuals in two of the families were compound heterozygotes and one of these two families had three different mutations. Of the genetic changes, 13 were misense mutations, 3 were nonsense mutations, 2 splice site mutations, 1 frameshift insertion and 1 frameshift deletion. There was no obvious correlation with the phenotype. In 5 families that were available for further analysis, a functional assay showed almost total loss of CTSC activity in PLS patients, and reduced activity in obligate carriers.

P1461. Cathepsin B as a positional candidate on 8p22-23 for keratolytic winter erythema

S. Arndt[1], *S. Appel*[2], *A. Bergheim*[1], *D. McCormick*[3], *N. S. Thakker*[4], *W. Hide*[5], *A. Reis*[2], *H. C. Hennies*[2], *M. Ramsay*[1]

[1]*SAIMR and University of the Witwatersrand; Johannesburg, South Africa;* [2]*Max-Delbr ck Centre; Berlin, Germany;* [3]*Queens University of Belfast; Belfast, United Kingdom;* [4]*University of Manchester; Manchester, United Kingdom;* [5]*SANBI; Cape Town, South Africa*

silkea@mail.saimr.wits.ac.za

Keratolytic winter erythema (KWE) is an autosomal dominant disorder of unknown etiology, characterized by cyclical erythema, hyperkeratosis, as well as recurrent and intermittent peeling of the palms and soles, especially during winter. KWE has been localized to chromosome 8p22-23 and haplotype analysis in South African families placed the candidate gene in a 6cM interval between D8S550 and D8S552. Positional candidates in the 6cM region were identified through cDNA direct selection, exon trapping and database searches. This region contains several known genes, including cathepsin B (CTSB). This gene is a compelling candidate since an autosomal recessive disorder characterized by palmoplantar hyperkeratosis and periodontitis is caused by mutations in the cathepsin C gene (CTSC) on 11q14. CTSB mutation analysis was done on genomic DNA and by RT-PCR on patient lymphocytes. Several polymorphisms were identified in the coding region, including two amino acid substitution mutations, V26L and S53G, and one synonymous mutation, T140T (nt420 C?A). Several additional polymorphisms were observed in the introns. Each of the variant alleles in the coding region was found in both affected and unaffected individuals and therefore CTSB is unlikely to be the KWE gene. The CTSB promoter region is presently being examined for functionally significant mutations.

P1462. Transgressive palmoplantar keratoderma; evidence for genetic and clinical heterogeneity

H. C. Hennies[1], *G. G. Lestringant*[2], *P. M. Frossard*[2], *K. M. Eckl*[1], *A. Reis*[3]

[1]*Max-Delbr ck-Centrum; Berlin, Germany;* [2]*Tawam Hospital and Faculty of Medicine and Health Sciences; Al Ain, United Arab Emirates;* [3]*Friedrich-Alexander-Universit t; Erlangen, Germany*

hennies@mdc-berlin.de

Transgressive hyperkeratosis is a characteristic that may occur in various forms of palmoplantar keratoderma (PPK). It consists of hyperkeratosis spreading from the palms and soles onto the dorsa of the fingers, the toes,

the hands and the feet, the flexor aspects of the wrists and the heels. In particular, transgressive PPK is a feature of autosomal recessively inherited disorders such as Naxos disease (PPK with cardiomyopathy) and Papillon-Lef vre syndrome (PPK with periodontopathia). In contrast, Mal de Meleda (MDM; OMIM #248300), or keratosis palmoplantaris transgrediens of Siemens, is a transgressive and progressive PPK without associated involvement of other organs than skin. A locus for MDM has been described recently on chromosome 8q24-qter between the markers at D8S1751 and D8S2334. Here we present five patients from three consanguineous Arab families with autosomal recessive transgressive PPK and a prominent erythroderma. By homozygosity mapping and linkage analysis with microsatellites, we have excluded the MDM gene region from linkage in these families. Furthermore, several further candidate genes for hyperkeratosis, including type-I and II keratin genes, the plakoglobin gene, and the genes of the epidermal differentiation complex, were ruled out. Our findings reveal that non-syndromic transgressive PPK is genetically heterogeneous, and a genome-wide linkage scan is necessary to identify the new locus for the disorder in these families. Moreover, we have also seen the erythroderma characteristic of the patients described here in an MDM patient mapping to chromosome 8q24-qter. Hence there are currently no conclusive diagnostic criteria clearly correlating with the genetic etiology of MDM.

P1463. Genetic Heterogeneity in Erythrokeratodermia Variabilis; Pathogenic Mutations in the connexin gene GJB4 (Cx30.3)

G. Richard[1], *K. Butt*[1], *N. Brown*[1], *J. J. DiGiovanna*[2,3], *V. Sybert*[4], *J. Van der Schroeff*[5], *E. Bijlsma*[6], *L. F. Eichenfield*[7], *J. D. Hendrix*[8], *E. Siegfried*[9], *S. J. Bale*[10,11], *J. Uitto*[1]

[1]*Thomas Jefferson University; Philadelphia, PA United States;* [2]*NCI, NIH; Bethesda, MD United States;* [3]*Brown University; Providence, RI United States;* [4]*University of Washington; Seattle, WA United States;* [5]*Bronovo Hospital; Den Haag, The Netherlands;* [6]*University of Amsterdam; Amsterdam, The Netherlands;* [7]*Children s Hospital and Health Care Center; San Diego, CA United States;* [8]*University School of Medicine; Charlottesville, VA United States;* [9]*St. Louis University School of Medicine; St. Louis, MO United States;* [10]*GeneDx; Rockville, MD United States;* [11]*NIAMS, NIH; Bethesda, MD United States*

gabriele.richard@mail.tju.edu

Erythrokeratodermia variabilis (EKV) is an autosomal dominant genodermatosis characterized by persistent plaque-like or generalized hyperkeratosis and transient red patches of variable size, shape and location. Recently, we demonstrated that EKV is caused by mutations in GJB3 encoding the gap junction protein connexin-31 (Cx31) on 1p35.1. However, two-thirds of all families had no detectable mutations in GJB3 or the co-localized connexin genes GJB5 and GJA4 despite convincing evidence for locus homogeneity. We report here the identification of 4 distinct mutations in another connexin gene GJB4 (Cx30.3) at this cluster in 4 extended EKV families and a sporadic case. All heterozygous missense mutations completely co-segregated with the disease and altered highly conserved residues. One of them, G12D, lies in the N-terminus and may interfere with the gating polarity of Cx channels. Other mutations (T85P, F137L, F189Y) affected the transmembrane domains of Cx30.3, thus potentially hindering regulation of voltage gating and kinetics of channel closure. All GJB4 mutations resulted in localized hyperkeratosis, while identical mutations in GJB3 (G12D and F137L) were associated with severe, generalized skin involvement. Nevertheless, we observed considerable intrafamilial variability in 2 multiplex families, suggesting the influence of other genetic and environmental factors. Sequence analysis further revealed 3 missense substitutions and a common 4-bp frameshift deletion within the GJB4 coding region, which might represent either non-consequential polymorphisms or recessive mutations, and as such, could contribute to the phenotype. Our results demonstrate genetic heterogeneity in EKV, and emphasize that intercellular communication mediated by Cx31 and Cx30.3 is crucial for epidermal differentiation.

P1464. A novel mutation in the human connexin 50 gene (GJA8) underlies autosomal dominant zonular pulverulent cataract.

A. Polyakov[1], *I. Shagina*[1], *O. Khlebnikova*[2], *O. Evgrafov*[1]

[1]*Research Center for Medical Genetics; Moscow, Russian Federation;* [2]*Institute of eye deseases; Moscow, Russian Federation*

dnalab@online.ru

Inherited cataract is a genetically heterogeneous lens disease that most often presents as a congenital, autosomal dominant Mendelian trait showing considerable inter- and intrafamilial clinical variation and high penetrance. CZP1, autosomal dominant zonular pulverulent cataract, was mapped to chromosome 1. GJA8, the gene for gap- junction protein alpha-8, which encodes a connexin protein (Cx 50), has been recognized as the disease — causing gene in CZP1. We have investigated CZP1 family with three affected members in three generations. We used microsatellite markers D1S2696 and D1S252 to confirm linkage of the disease with CZP1 locus in this family. Maximum lod score was 0.90 at a recombination fraction of 0.0 The coding region of the GJA8 gene was screened for mutation by SSCP analysis. The both affected patients showed the same altered SSCP paterns. Sequencing analysis revealed a T to G transition resulting in Ile to Met substitution at the conserved amino acid position 247 and disappearance of HinfI site. Restriction analysis confirmed presence of this mutation in both affected individuals of the family.

P1465. A Unique Spectrum of Alterations in the Cx-26 Gene in Deaf Probands from Mongolia

A. Pandya[1], *M. Tekin*[1], *R. Erdenetungalag*[2], *X. Xia*[1], *B. Dangaasuren*[2], *J. Radnaabazar*[2], *S. H. Blanton*[3], *W. E. Nance*[1]

[1]*Virginia Commonwealth University; Richmond, VA United States;* [2]*Maternal and Child Health Research Center; Ulaanbaatar, Mongolia;* [3]*University of Virginia; Charlottsville, VA United States*

apandya@hsc.vcu.edu

Mutations in the connexin-26 (Cx 26) gene account for 20-30% of all prelingual deafness, in many populations. Certain mutations such as 35delG, 167delT and 235delC are rather frequent in probands of Western European, Ashkenazi Jewish and Oriental population respectively. Screening for Cx 26 mutations in a sample of 136 deaf Mongolian probands yielded only three 35delG heterozygotes. Two probands were homozygous for the 235delC mutation and three additional heterozygotes were detected, 2 for the 235delC mutation and one for a novel 2 bp deletion at nt 100. Thus the overall frequency of Cx-26 deafness is quite low ranging from 1.5% to 5.8% if all of the apparent heterozygotes are cryptic homozygotes. A large number of deaf probands carried the V27I substitution, 22% being heterozygous and 4.4% being homozygous for this change. Interestingly, when present the E114G substitution was always noted in cis with the V27I background in 11.6% of the deaf probands. Although this sequence change was observed in the normal population, its high frequency in the deaf probands as well as homozygosity for both polymorphisms in a deaf individual has prompted us to perform in vitro functional analysis to address its clinical relevance. The low frequency of Cx 26 mutations in a population where assortative mating amongst the deaf is rare supports the hypothesis that marriages amongst the deaf may affect the frequency of Cx 26 deafness in certain populations.

P1466. High Frequency of GJB2 Gene Mutations in Polish Patients with Prelingual Non-syndromic Deafness

W. Wiszniewski, *E. Szyrwinska-Nowakowska, T. Mazurczak, J. Bal*

Institute of Mother and Child; Warsaw, Poland

wojtekw@klif.amwaw.edu.pl

Partial or complete hearing loss is often of congenital origin. Among isolated forms of deafness, the autosomal recessive forms (DFNB) are markedly prevalent (80%). It is estimated that about one hundred different genes are involved in the non-syndromic form of deafness. Among those genes, one particular — GJB2 is responsible for a high proportion of cases. Mutations in GJB2 gene were observed in 50% of families affected with profound prelingual autosomal recessive deafness in many European countries. Herein we report analysis of 104 unrelated Polish patients with profound prelingual deafness for mutation in GJB2 gene. Mutations were found in 43/104 (41%) of subjects. Among mutated alleles, 35delG was prevalent and present in 90% ones. In 9 alleles mutations different than 35delG were found; M34T, Q47X, R184P and 313del14 (found in 6 patients). The subsequent results proved mutations in GJB2 gene are responsible to high degree for hereditary non-syndromic deafness in Poland with strong prevalence of 35delG. We have also found high carrier frequency (1/50) for 35delG mutation in Polish population.

P1467. Pre- and postlingual sensorineural deafness in sibs homozygous for the 35delG mutation in the connexin 26 gene (GJB2)

A. T. Maciel-Guerra, *C. A. Oliveira, E. L. Sartorato*

State University of Campinas; Campinas - SP, Brazil

gilguer@bestway.com.br

Mutations in the connexin 26 gene (GJB2) are responsible for the majority of nonsyndromic recessive deafness in many world populations, and are also a major cause of apparently sporadic congenital deafness. Three mutations are particularly common in specific populations; 35delG in Caucasoids, 167delT in Ashkenazi Jews, and 235delC in Orientals. GJB2 mutations are usually associated with prelingual hearing loss, though

severity may range from mild to profound even within the same family. We had the opportunity to examine a sibship composed of four sisters, two of them (20 and 17 years old) with prelingual sensorineural deafness and the other two (18 and 16 years old)with normal speech development and moderate sensorineural hearing loss which was detected only a few years ago. Mutation analysis of the GJB2 gene revealed that all four girls were homozygous for the 35delG mutation, while both parents were heterozygotes. Though this may be a rare finding, screening programs for genetic deafness in newborns should consider the possibility that the deaf phenotype associated with mutations in the connexin 26 gene may not develop until later in life.

P1468. Molecular basis of Greek DFNB1 deafness; Novel mutations and polymorphisms in the GJB2 gene

T. Antoniadi[1], *I. Konstantopoulou*[2], *A. Pampanos*[3], *K. Gronskov*[4], *D. Yannoukakos*[2], *K. Brondum-Nielsen*[4], *J. Economides*[5], *P. Leotsakos*[5], *N. Voyiatzis*[6], *T. Iliadis*[7], *P. Neou*[8], *M. Tsakanikos*[9], *M. Grigoriadou*[3], *A. Skevas*[10], *M. B. Petersen*[3]

[1]*Department of Genetic and Molecular Biology, MITERA Maternity & Surgical Center; Athens, Greece;* [2]*Molecular Diagnostics Laboratory, I/R-RP, National Center for Scientific Research Demokritos; Athens, Greece;* [3]*Department of Genetics, Institute of Child Health; Athens, Greece;* [4]*John F. Kennedy Institute; Glostrup, Denmark;* [5]*ENT Department, Aghia Sophia Children s Hospital; Athens, Greece;* [6]*Department of Pediatrics, Aristotle University of Thessaloniki; Thessaloniki, Greece;* [7]*ENT Department, Aristotle University of Thessaloniki; Thessaloniki, Greece;* [8]*Department of Pediatrics, P&A Kyriakou Children s Hospital; Athens, Greece;* [9]*ENT Department, P&A Kyriakou Children s Hospital; Athens, Greece;* [10]*ENT Department, University of Ioannina; Ioannina, Greece*

thantoniadi@yahoo.com

Hearing impairment affects 1/1000 children and at least 50% of these cases are inherited. The inherited forms of deafness are classified in syndromic (30%) and non-syndromic deafness (70%). Among the non-syndromic cases the major proportion is due to autosomal recessive inheritance (70%); 20% of the cases are due to autosomal dominant inheritance, 5% are X-linked and mitochondrial inheritance is responsible for about 1%. To date, 30 loci (DFNB1 to DFNB30) have been described for autosomal recessive inheritance. The DFNB1 locus accounts for approximately 80% of autosomal recessive deafness in Mediterranean families, due to mutations in the GJB2 gene. The GJB2 gene encodes the gap-junction protein connexin-26 and except the major 35delG mutation, more than 60 sequence alterations have been reported so far. This study presents the analysis of 18 families for the presence of GJB2 mutations other than the 35delG mutation. All probands were either heterozygotes or negative for the 35delG mutation. Sequencing of these patients revealed 2 novel mutations; 509del14 and 486insT (both in heterozygosity with the 35delG). A common novel polymorphism, 681+84C>T, was also detected in 11 patients. The previously reported K224Q mutation was identified as the sole mutation in one patient. The L90P mutation was identified in 2 patients and the W24X mutation in another 2 patients (all in heterozygosity with the 35delG). The characterization of the spectrum of the GJB2 mutations in Greek patients with non-syndromic sensorineural recessive deafness will reveal the molecular basis of the disease and provide the necessary information for genetic counseling.

P1469. A new connexin 32 missense mutation (Tyr65His) in a Czech Charcot-Marie-Tooth type X1 disease family.

P. Seeman[1], *R. Mazanec*[2], *M. Ctvrteckova*[2], *D. Smilkova*[3]

[1]*Charles University Prague, 2nd School of Medicine; Praha 5, Czech Republic;* [2]*Charles University Prague, 2nd School of Medicine, Dept of Neurology; Prague, Czech Republic;* [3]*Dept of Rehabilitation; Kladno, Czech Republic*

pavel.seeman@lfmotol.cuni.cz

Charcot-Marie-Tooth (CMT) disorders is group of clinicaly and geneticaly heterogeneus inherited peripheral neuropathies. The most common type is by far the autosomal dominant CMT1A caused by a 1,5 Mb duplication in 17p11.2-12 comprising the PMP22 gene. CMT type X is the second most common type of CMT. This type is inherited as a gonosomal dominant trait. Hemizygous males are earlier and more severly afected then the heterozygous females. No male to male transmision can be found in the CMTX pedigrees. Connexin 32 mutations are the most common cause of CMTX. We report a Czech family with a severe type of CMT 1 disease. A disorder with gait disturbancies, moderate footdrop and distal muscle weakness and foot deformities is present in at least 3 generations in this family. EMG showed signs of demyelination confirming type 1 of CMT disease. After exclusion of the CMT1A duplication at 17p, we directly sequenced the whole coding region of connexin 32 gene (Cx32). A T to C transition at nucleotide 193 causing a tyrosine to histidine aminoacid change in the first extracelular domain of the Cx32 protein was found in the mother and son of this family. This mutation was not yet reported. Detailed clinical and electrophysiological data from this two mutation carrier will be presented. Supported by IGA MH CR and IP Nr. 111300003

P1470. Analysis Of Mutations In The Gjb2 Gene In Spanish And Cuban Families

A. Alvarez[1], *A. Uriarte*[1], *M. Villamar*[1], *I. Del Castillo*[1], *I. Menendez*[2], *L. Romero*[1], *F. Moreno*[1]

[1]*Hospital Ramon y Cajal; Madrid, Spain;* [2]*Willam Soler; La Habana, Cuba*

mvillamar@hrc.insalud.es

The GJB2 gene codes for the gap junction protein connexin 26 (Cx 26). Mutations in this gene may cause autosomal recessive or dominant non-syndromic hearing loss. A group of 123 families with autosomal recesive deafness and 91 patients without familial history of deafness from Spain and Cuba, were analyzed for mutation in GJB2. In 39 familial cases and 28 sporadic cases we found two GJB2 mutant alleles which indicates that 31.3% of deafness cases are due to mutations in GJB2. In 12 familial and 8 sporadic cases only one mutation was found. The mutation 35delG accounts for 74% of the mutated alleles. In 37 cases, the patient was homozygous for the 35delG mutation and, in 22 cases was heterozygous for the mutation. Nineteen other different connexin 26 mutations were identified. Five of these appear in two or more patients, E47X (5), W77R (5), W24X (3), R143W (3) and R184P (2).

P1471. GJB2 Gene Mutations And Genetic Counseling In Neurosensory Non Syndromic Deafness

A. Caleffi[1], *R. Bertorelli*[1], *E. Mora*[2], *S. Santucci*[3], *R. Balli*[4], *A. Forabosco*[1], *D. A. Coviello*[1,5]

[1]*Servizio di Genetica Medica, Policlinico di Modena; Modena, Italy;* [2]*Clinica ORL, DISCAT, Universit di Genova; Genoa, Italy;* [3]*Genetica Medica, Sezione Salute Donna, AUSL Modena; Modena, Italy;* [4]*Divisione ORL, Universit e Policlinico di Modena; Modena, Italy;* [5]*Molecular Genetic Laboratory, Istituti Clinici di Perfezionamento; Milan, Italy*

coviello@unige.it

Non Syndromic Recessive Deafness (NSRD) is present in 1/500 of general population. The mode of transmission is autosomal recessive and the gene more often involved is GJB2. This gene encodes the connexin 26 (Cx26) protein, a component of cell junctions, the links that allow small molecules to pass from one cell to the next. More then 30 different mutations of this gene are described but one is particularly common, the 30delG (also known as 35delG), a deletion of one base in a sequence of six guanine residues that starts at position 30. Previous studies identified the 30delG mutation in 63% of NSRD patients from south Italy and in 69% of patients from mixed population. In the present study we perform a screening for mutations in the GJB2 gene in 114 NSRD patients and in 111 control subjects from the general population to assess the mutation s frequency in a sample of patients from north Italy. The analysis has been performed by direct sequencing of PCR products, using the automatic sequencer ABI377. We identified mutations of GJB2 gene in 38/228 chromosomes analysed; 47.4% (18/38) showed 30delG mutation, while the remaining showed 14 different mutations in10 subjects 52.6% (20/38 chromosomes). We identified one subject heterozygous for the 30delG mutation in the normal control population representing 1% (2/192 chromosomes). In conclusion our findings confirm that the 30delG mutation of the GJB2 gene is the most common cause of NSRD, but many other mutations are also present indicating that the complete sequence is needed for an appropriate molecular diagnosis. Genetic counselling for NSRD has been so far considerably impaired by the difficulty in distinguishing genetic and non genetic deafness. Molecular analysis extended on a larger patient population shall clarify the role of specific gene mutations in the pathogenesis of the disease and will be of considerable help in genetic counselling, prognosis and treatment of deafness.

P1472. Assess the prevalence /spectrum of connexin 26 mutations and their phenotype in children with congenital sensorineural hearing loss (SNHL)

B. Wu[1], *V. Lip*[2], *M. Kenna*[1], *G. Cox*[2], *B. Korf*[3]

[1]*Children s Hospital and Harvard Medical School; Boston, MA United States;* [2]*Children s Hospital; Boston, MA United States;* [3]*Partner s Center for Human Genetics and Harvard Medical School; Boston, MA United States*

wu_b@tch.harvard.edu

Congenital SNHL has an incidence in children of 1-2/1000 for bilateral severe-to-profound losses (>50 dB) and up to 4/1000 if mild/moderate and unilateral loss are included. Up to 60% of congenital SNHL can be attrib-

uted to genetic causes. Of those, autosomal recessive non-syndromic hearing loss accounts for approximately 80% and mutations in the connexin 26 (GJB2) gene are the most common known cause. We have analyzed a total of 152 unrelated families /patients with congenital SNHL, either recessive or sporadic, from a large pediatrics medical center. Most probands were age 1 week to 18 years, had no obvious etiology for the congenital SNHL, and were tested using a highly sensitive, unambiguous three-step PCR-based sequencing assay to detect all mutations in the entire coding region of Cx26 gene. Of the 304 unrelated Cx26 alleles analyzed from 152 probands, 39% (60/152) had Cx26 mutations; 25 patients with biallelic mutations (9 homozygotes and 16 compound heterozygotes), and 35 with a single mutation. Four different homozygous mutations were found; 35delG/35delG (3.4%), M34T/M34T (1.4%), 167delT/167delT (0.7%) and V37I/V37I (0.7%). Six different compound heterozygous mutations were detected; 35delG/167delT (3.4%), 35delG/M34T (2.7%), 35delG/G12V (1.4%), 35delG/E47X (1.4%), 35delG/N206S (1.4%), T8M/V153I (0.7%). Overall, mutations detected in this study are the 11 previously reported in the literature; 35delG, 167delT, E47X, M34T, G12V, V37I, L90P, V84L, R127H, R143W, and 310del14, and the 3 novel mutations newly reported here; N206S, T8M, and E129K. None of these new mutations were found in 100 normal controls. Hearing loss in these patients with biallelic Cx26 mutations ranged from unilateral high frequency to bilateral profound, except those with homozygous or compound heterozygous for M34T. These patients had mild hearing loss.

P1473. Contribution of GJB2 Mutations for Nonsyndromic Deafness in Portuguese Families

C. S. R. Rodrigues[1], *H. Vieira*[1], *M. Vitorino*[1], *A. Mena*[1], *H. Caria*[1], *I. Galhardo*[2], *A. O Neill*[3], *O. Dias*[2], *M. Andrea*[2], *C. Correia*[1], *G. Fialho*[1]
[1]*Centre of Genetics and Molecular Biology; Lisbon, Portugal;* [2]*Centre of ORL Research, University of Lisbon; Lisbon, Portugal;* [3]*Hospital Egas Moniz; Lisbon, Portugal*
catrodrigues@hotmail.com

The GJB2 gene encodes the connexin 26 (Cx26) component of gap junctions. Mutations in this gene have been found to be responsible for half of the severe-to-profound autosomal recessive nonsyndromic deafness (NSRD). One single mutation, 35delG, has been shown to be the most prevalent mutation in this gene and one of the most frequent mutations identified so far. The present study is based in a preliminary analysis of the portuguese population, previously reported, where 10 NSRD families and a sample of normal hearing individuals were screened for the 35delG mutation. Analysis of the entire coding region was carried out in both the heterozygous and 35delG negative families, revealing the presence, in one of the families, of a novel splicing mutation, IVS1-23G-T, in compound heterozygosity with M34T mutation. In the negative cases or those presenting a single coding sequence mutation we have searched for mutations in the Cx26 non-coding exon (exon 1) and flanking donor splicing site. A similar study has now been carried out in a total of 52 NSRD families. The results obtained will be presented and discussed in comparison with other european populations.

P1474. Screening for mutations in TMPRSS3 in non-syndromic recessive childhood deafness

M. Wattenhofer[1], *H. S. Scott*[1], *S. Alwan*[1], *C. Rossier*[1], *S. E. Antonarakis*[1], *R. Rabionet*[2], *B. Montserrat*[2], *M. Arbones*[2], *X. Estivill*[2], *A. Pampanos*[3], *M. B. Petersen*[3], *M. V. Di Iorio*[4], *A. Pasquadibisceglie*[4], *M. D Amelio*[4], *P. Gasparini*[4]
[1]*Medical Genetics, Geneva University, Medical School; Geneva, Switzerland;* [2]*Medical and Molecular Genetics Center, Institut de Recerca Oncologica; Barcelona, Spain;* [3]*Institute of Child Health; Athens, Greece;* [4]*Medical Genetics Service, IRCCS-CSS; Rotondo, Italy*
marie.wattenhofer@medecine.unige.ch

50% of early-onset deafness cases are genetic and 50% of the recessive case are due to mutations in the GJB2 gene. The TMPRSS3 gene encodes a novel transmembrane serine protease. It is mutated in the families used to describe both the DFNB10 and DFNB8 loci on chromosome 21 (Nat.Genet. Jan01). To determine if TMPRSS3 mutations are an important contributor to the etiology of childhood deafness, we performed SSCP screening of all 13 exons and intron-exon junctions and sequencing of DNAs of non-syndromic recessive deaf patients negative for GJB2 gene mutations. A total of 391 such unrelated patients (100 from Spain, 198 from Italy, and 93 from Greece) were enrolled in the study. The analysis has so far been completed in DNA samples from 293 patients. Several common exonic polymorphisms were found (V53I, G111S, V151V, I253V) as well as a series of intronic changes. In only 2 patients there were changes that are likely to be associated with the phenotype. A deletion of nucleotide 207C in exon 4 has been found in homozygosity in one Spanish patient resulting in a truncation of the TMPRSS3 protein after the Transmembrane Domain. In another Spanish patient, an amino acid substitution (A426T) has been identified in heterozygosity in an exon coding for the protease domain. A426 is well conserved in serine proteases. These two changes have not been found in any other patient of our sample. These results indicate that mutations in TMPRSS3 do not substantially contribute to the non-syndromic deafness in the Caucasian population.

P1475. Novel Mutations of TMPRSS3 in two Unrelated Tunisian Families with Non-Syndromic Autosomal Recessive Deafness, DFNB8/10.

S. Masmoudi[1], *S. Antonarakis*[2], *M. Grati*[1], *M. Pappasavvas*[2], *A. Ghorbel*[3], *A. Elgaied-Boulila*[1], *C. Rossier*[2], *M. Drira*[3], *H. Ayadi*[1], *M. Guipponi*[2]
[1]*Laboratoire de G n tique Mol culaire Humaine, Facult de M decine, Sfax; Sfax, Tunisia;* [2]*Division of Medical Genetics, University of Geneva Medical School; Geneva, Switzerland;* [3]*Service d O.R.L., C.H.U. H. Bourguiba, Sfax; Sfax, Tunisia*
Saber.Masmoudi@fss.rnu.tn

Tow loci for non-syndromic recessive deafness located on chromosome 21q22.3 have previously been reported, DFNB8 and DFNB10. Recently, The TMPRSS3 gene, which encodes a novel transmembrane serine protease, was found to be mutated in the families used to describe both the DFNB10 and DFNB8 loci(Nat. Genet. Jan. 2001. Our study included 2 unrelated consanguineous Tunisian families with congenital autosomal recessive form of sensorineural deafness; The audiometric tests showed a loss of hearing greater than 70 dB, in all affected individuals. Using DNA polymorphic markers we found linkage to the DFNB8/10 locus in these two families. Mutation screening of all 12 exons and the intron-exon junctions of the TMPRSS3 gene revealed two novel TMPRSS3 mutations. These missense mutations were found in exon 8 and 12, which encode part of the serine protease domain and affected highly conserved amino acid (W251C and P404L). Cosegregation of both mutations with the disease phenotype was demonstrated by PCR-restriction analysis. Both mutations were not found in 200 control Tunisian chromosomes. The detection of naturally occurring missense mutations in families with DFNB8/10 identifies important amino acids for the function of TMPR

P1476. ABCD syndrome is caused by a homozygous mutation in the EDNRB gene.

J. B. G. M. Verheij[1], *J. Kunze*[2], *J. Osinga*[1], *A. J. van Essen*[1], *R. M. W. Hofstra*[1]
[1]*Department of Clinical Genetics, University Hospital; Groningen, The Netherlands;* [2]*Klinikum Rudolf Virchow, Free University of Berlin; Berlin, Germany*
J.Verheij@medgen.azg.nl

In 1995 Gross and colleagues described a new autosomal recessive syndrome, named ABCD syndrome, characterised by Albinism, Black lock, Cell migration disorder of the neurocytes of the gut (Hirschsprung disease-HSCR) and Deafness. This phenotype clearly overlaps with the features of the Shah-Waardenburg syndrome, comprising sensorineural deafness, hypopigmentation of skin, hair and irides and HSCR. We therefore screened DNA of the index patient of the ABCD syndrome family for mutations in the endotheline B receptor (EDNRB) gene, a gene known to be involved in Shah-Waardenburg syndrome. A homozygous nonsense mutation in exon 3 (Arg201stop) of the EDNRB gene was found. This means that ABCD syndrome is not a separate entity, but a severe expression of Shah-Waardenburg syndrome.

P1477. Mutation of CDH23, encoding a new member of the cadherin gene family, causes Usher syndrome type 1D.

H. J. Bolz[1], *B. von Brederlow*[1], *A. Ram rez*[2], *E. C. Bryda*[3], *K. Kutsche*[1], *H. Nothwang*[4], *M. Seeliger*[5], *M. del C.-Salced Cabrera*[6], *M. Caballer Vila*[6], *O. Pelaez Molina*[6], *A. Gal*[1], *C. Kubisch*[2]
[1]*Institut f r Humangenetik, Universit tsklinikum Hamburg-Eppendorf; Hamburg, Germany;* [2]*Institut f r Humangenetik, Universt t Bonn; Bonn, Germany;* [3]*Department of Microbiology, Immunology and Molecular Genetics, Marshall University; Huntington, WV United States;* [4]*MPI f r Molekulare Genetik; Berlin, Germany;* [5]*Abteilung II, Augenklinik der Universit t T bingen; T bingen, Germany;* [6]*Centro Internacional de Retinosis Pigmentaria, Camilo Cienfuegos; La Habana, Cuba*
bolz@uke.uni-hamburg.de

Usher syndrome type I (USH1) is an autosomal recessive disorder characterized by congenital sensorineural hearing loss, vestibular dysfunction, and visual impairment due to early onset retinitis pigmentosa (RP). So far, only two USH1 genes have been identified. We identified a Cuban pedigree linked to the locus for Usher syndrome type 1D within the q2 region of chromosome 10. Affected individuals present with congenital deafness

and a highly variable degree of retinal degeneration. Using a positional candidate approach, we identified a new member of the cadherin gene superfamily, CDH23. It encodes a large protein with a single transmembrane domain and 27 cadherin repeats. In the Cuban family, we detected two different mutations; a severe course of the retinal disease was observed in individuals homozygous for what is probably a truncating splice-site mutation (c.4488G>C), whereas mild RP is present in individuals carrying a homozygous missense mutation R1746Q. A variable expression of the retinal phenotype was seen in patients with a combination of both mutations. In addition, we identified two mutations in a German USH1 patient, DM1281 and IVS51+5G>A (the latter resulting in skipping of exon 51). Our data show that different mutations in CDH23 result in USH1D with a variable retinal phenotype. The identification of this putative cell adhesion molecule, the first cadherin mutated in a monogenic sensory disorder, opens an exciting new field for genetic and physiological studies of the inner ear and retina.

P1478. A large deletion of the USH2A Gene in a Spanish family with Usher Syndrome type II

M. Baiget[1], *S. Bernal*[1], *M. Calaf*[1], *E. Del Rio*[1], *D. Valverde*[2]
[1]*Hospital Sant Pau; Barcelona, Spain;* [2]*Universidad de Vigo; Vigo, Spain*
mbaiget@hsp.santpau.es

Usher syndrome type II is an autosomal recessive disorder characterized by stable hearing impairment from childhood and progressive retinitis pigmentosa. Mutations in the USH2A gene, located on 1q41, were shown to be responsible for Usher syndrome type II. All reported mutations are scattered throughout the gene and are small point mutations causing nonsense (11) or missense (10) changes or splice site changes (1) or small insertions (3) or deletions (6) causing frameshift mutations. One of these mutations, 2299delG occurs at high frequency among Usher 2A patients. Here we report a Spanish family with Usher 2A syndrome and a large deletion of the USH2A gene. It is the first gross deletion of the USH2A gene encompassing exons 9 to 14. Intragenic deletion was initially suspected as a cause of the disease in the two affected siblings because a non-successful PCR amplification of the USH2A exon 13 containing the frequent 2299delG mutation. This simple PCR-based approach was further used to localize the approximate breakpoints of the deleted region. Our two patients are under 20 years old and have a clinical picture typical of Usher type II. Although it is difficult to predict the phenotypic consequences of the severely truncated USH2A gene, the deletion might bring about other phenotypic changes with older age.

P1479. The complete form of X-linked congenital stationary night blindness is caused by mutations in a gene encoding a leucine-rich repeat protein

A. Meindl[1], *C. M. Pusch*[2], *C. M. Zeitz*[3], *O. Brandau*[4], *K. Pesch*[2], *S. Feil*[3], *J. Ramser*[1], *J. Maurer*[5], *H. Hellebrand*[1], *A. Hardcastle*[6], *B. Wissinger*[2], *W. Berger*[3]
[1]*Ludwig-Maximilians University; Muenchen, Germany;* [2]*Universit ts-Augenklinik; Tuebingen, Germany;* [3]*Max Planck Institute for Molecular Genetics; Berlin, Germany;* [4]*University; Lund, Sweden;* [5]*Ressource Center; Berlin, Germany;* [6]*Institute of Ophthalmology; London, United Kingdom*
alfons@pedgen.med-uni-muenchen.de

X-linked congenital stationary night blindness (XLCSNB) is characterized by impaired scotopic vision with associated ocular symptoms like myopia, hyperopia, nystagmus, and reduced visual acuity. Genetic mapping in families with XLCSNB revealed two different loci on the proximal short arm of the X chromosome. These two genetic subtypes can also be distinguished on the basis of ERG responses and psychophysical testing in a complete (*CSNB1*) and an incomplete form (*CSNB2*). Recently we have been shown that CSNB2 is caused by mutations in the CACNA1F gene (Strom et al., Nature Genetics 1998). The *CSNB1* locus has been mapped to a 5 cM linkage interval in Xp11.4. Construction and analysis of a contig between the markers *DXS993* and *DXS228* led to the identification of a novel gene that is mutated in *CSNB1* patients. It is partially deleted in three families and mutation analysis in further 22 families detected another 14 different mutations. The gene, designated *NYX,* codes for a 481 amino acid protein (nyctalopin) and is expressed at low levels in various tissues including retina, brain, testis and muscle. The predicted polypeptide is a GPI-anchored extracellular protein with 11 typical and two cysteine-rich leucine-rich repeats (LRRs). This motif is important for protein-protein interactions and members of the LRR superfamily are involved in cell adhesion and axon guidance. Currently, antibodies against NYX are raised to determine the precise localization within the retinal tissues and to identify interacting proteins.

P1480. ORF15, coding for a glutamic acid rich domain, contains the majority of RPGR mutations

I. Bader[1], *O. Brandau*[2], *E. Apfelstedt-Sylla*[3], *H. Achatz*[4], *B. Lorenz*[5], *M. Hergersberg*[6], *B. Wissinger*[3], *B. Wittwer*[7], *T. Meitinger*[1], *A. Meindl*[4]
[1]*GSF research centre, Ingolst dter Landstr.1; D-85764 Neuherberg, Fax.; ++49-89-3187-3297, Germany;* [2]*Department of Experimental Pathology, Lund University; Lund, Sweden;* [3]*Department of Ophthalmogenetics, University of T bingen; T bingen, Germany;* [4]*Department of Medical Genetics at the University of Munich, Goethestr. 29; 80336 Munich, Germany;* [5]*Department of Ophthalmogenetics, University of Regensburg; Regensburg, Germany;* [6]*Institute of Human Genetics; Zurich, Switzerland;* [7]*Institute of Human Genetics; M nster, Germany*
ingrid@pedgen.med.uni-muenchen.de

Retinitis pigmentosa (RP) describes a group of diseases characterized by progressive retinal degeneration. X-linked forms (xlRP) account for 15 % of all hereditary RP cases and result in particulary severe phenotypes. Linkage data predict 60-80 % of all xlRP cases to be associated with the RP3 and 10-20 % with the RP2 locus. The recent identification of a novel 3'terminal RPGR exon (ORF15) which harbours a mutational hotspot raised the question of the total proportion of RPGR mutations in patients with xlRP. Mutation analysis of the novel RPGR exon, which extends exon 15 for 1554 nucleotides, and codes for a glutamic-acid-rich domain, turned out to be technically challenging because of an extremely purine-rich and highly repetitive stretch of 800 bp. Several strategies were persued to obtain reliable sequences including subcloning of PCR products, solid phase or direct sequencing of PCR products. Good results were obtained with sequencing a 1,6 kb fragment containing the purine-rich region. Several additives had to be used for PCR amplification and sequencing. We screened 64 unrelated xlRP patients for mutations in the complete coding regions of RPGR and RP2. Only three patients (4 %) had mutations in the RP2 gene, RPGR mutations were detected in 32 patients (52 %) with 22 mutations (36 %) clustering in the novel exon ORF15. Among a subgroup of 30 index-patients with pedigrees demonstrating clear X-linkage (several affected males in at least two generations connected by carrier females) 50 % had mutations in the novel exon ORF15.

P1481. Molecular analysis of the EYA1 gene and exploring genetic heterogeneity in families with Branchio-Oto-Renal Syndrome

S. Kumar[1], *H. A. Marres*[2], *C. W. R. Cremers*[2], *W. J. Kimberling*[1]
[1]*Boys Town National Research Hospital; Omaha, NE United States;* [2]*University Hospital Nijmegen; Nijmegen, The Netherlands*
kumar@boystown.org

Branchio-oto renal syndrome (BOR) is a human developmental disorder characterized by hearing loss, preauricular pits, structural defects of the external, middle and/or inner ear and renal anomalies. It is inherited as an autosomal dominant pattern and affect at least 2% of profoundly deaf children and have estimated prevalence of 1 in 40,000. The phenotypic expression of the branchial arch as well as audiologic and renal development can be quite variable, even within the same family. The first BOR gene has been localized to chromosome 8q13 and has been shown to be caused by mutations in the EYA1 gene. Recently, a second genetic locus (BGS2) associated with BOR syndrome has been localized to chromosome 1q. We have performed mutation analysis on more than sixty BOR families by heteroduplex followed by sequence analysis of sixteen EYA1 exons. To date, using this approach, we have identified fourteen novel mutations. About 65% of our large data set of BOR families do not show mutations in the EYA1 gene. This further complicates the issue and suggests that either most of the mutations lie in the untranslated region or several genes are involved in the branchiogenic disorder. Genetic linkage analysis was performed on several multigenerational BOR type families with 8q and 1q markers to explore the possibility of further genetic heterogeneity. Our current results indicate that two large BOR type families did not show linkage with 8q and 1q markers suggesting the involvement of third gene. A genome-wide search is being performed for the localization of third locus. Also, a candidate gene analysis is underway to identify genes in the critical region of BGS2 locus on chromosome 1q. The present results, together with mutation screening and genetic linkage study, demonstrate further genetic heterogeneity. These results provide the basis for a molecular-genetic testing that will help the clinical evaluation and genetic counseling of members of BOR families. Further characterization of EYA1 mutation and identification of other BOR genes will significantly help in defining the spectrum of defects associated with branchial and hearing anomalies.

P1482. A Novel Nonsense Mutation in the RP1 Gene Causing Autosomal Dominant Retinitis Pigmentosa in a Lithuanian Family

D. Kasperaviciute[1], *D. Ambrasiene*[1], *J. V. Arciuliene*[2], *V. Kucinskas*[1]
[1]*Human Genetics Center, Vilnius University; Vilnius, Lithuania;* [2]*Laboratory of Visual Rehabilitation, Kaunas Medical University; Kaunas, Lithuania*
dalia.kasperaviciute@gf.vu.lt

Retinitis pigmentosa represents a genetically heterogeneous group of disorders characterised by night blindness, visual field loss and retinal pigmentary changes. Mutations in the RP1 gene have recently been shown to account for 4 — 7 % autosomal dominant retinitis pigmentosa (adRP) cases. Ten different mutations have been reported so far, they seem to cluster in the 5 end of exon 4. The purpose of this study was to determine mutations in RP1 responsible for adRP in Lithuanian families. 22 patients from unrelated adRP families were screened for mutations in a segment of the RP1 gene from nucleotide 1038 to 1720 using heteroduplex analysis and single strand conformation polymorphism analysis. Direct sequencing revealed a nonsense mutation K792X (c.2374A>T) in one patient. This novel mutant allele, if expressed, would encode a severely truncated RP1 protein lacking approximately 63 % of its original length. All affected members of the family available for investigation had this mutation. The condition has a late onset at 40-45 years of age and slow progression. We also found this mutation in 3 asymptomatic subjects of the same family under the age of 18. Based on this study, mutations in the RP1 gene appear to cause 4 % of adRP cases in Lithuania. That frequency does not significantly differ from frequency in other populations, however, mutations frequent in other populations (R677X and 2280-2284del) were not found in Lithuania.

P1483. A missense mutation in the gene responsible for Usher syndrome type II is associated with nonsyndromic recessive Retinitis Pigmentosa in Spanish patients

S. Bernal[1], *C. Ayuso*[2], *G. Anti olo*[3], *S. Borrego*[3], *I. Marcos*[3], *M. Calaf*[1], *E. Del Rio*[1], *M. Baiget*[1]
[1]*Hospital Sant Pau; Barcelona, Spain;* [2]*Fundaci n Jimenez Diaz; Madrid, Spain;* [3]*Hospital Virgen del Rocio; Sevilla, Spain*
mbaiget@hsp.santpau.es

Several candidate genes involved in the visual transduction cascade have been studied in autosomal recessive Retinitis Pigmentosa (ARRP)Spanish families. Disease-causing mutations habe been only identified in the beta subunit of the rod cGMP-phosphodiesterase gene and in the ATP-binding cassette receptor gene. They account respectively for 8% and 6% of Spanish ARRP families. These data indicates that other than these genes may be involved in the remaining families, emphasising the genetic heterogeneity of the disease and reinforcing the hypothesis that in ARRP there is no a major gene but several genes may account individually for a small number of cases. A recent report(1) indicates that a missense mutation (Cys 759Phe) in the gene responsible of Usher syndrome type II (USH2A gene) is present in 4,5% of patients from North America with non-syndromic ARRP with a frequency of this allele of 0.027. We have analized the presence of this mutation in a set of ARRP Spanish families from all over the country that were collected by members of the Spanish Multicentric Group fro the Study of RP. The Cys759Phe mutation was found in heterozygous form in 4 out of 130 unrelated patients (allelic frequency; 0.015). This finding demonstrates the involvement of the USH2A locus in recessive non-syndromic RP in Spanish poulation. As previously described (1) this mutation was found to be always associated with an isocoding change at codon His772 (CAT-CAC).

P1484. TGFBI Gene Mutations in Japanese Corneal Dystrophies

K. Fujiki[1], *K. Nakayasu*[1], *T. Yamaguchi*[2], *N. T. Ha*[1], *A. Murakami*[1], *S. Endo*[3], *N. Ishida*[3], *T. Yajima*[4], *A. Kanai*[1]
[1]*Juntendo University School of Medicine; Tokyo, Japan;* [2]*St. Luke s International Hospital; Tokyo, Japan;* [3]*Ishida Eye Hospital; Jyouetsu, Japan;* [4]*Japanese Red Cross Medical Center; Tokyo, Japan*
fujiki@med.juntendo.ac.jp

Purpose. To investigate mutations of TGFBI (§IGH3, keratoepithelin) gene in Japanese patients with autosomal dominant corneal dystrophies (CD). Methods. After an appropriate informed consent was obtained from each subject, genomic DNA was extracted from the peripheral blood of 159 patients and 22 unaffected relatives from 120 families with granular CD (GCD), lattice CD (LCD), or Reis-B cklers CD (RBCD). Fifty normal volunteers served as controls. Each exon was amplified by polymerase chain reaction and directly sequenced. Results. Six different heterozygous missense mutations were detected in codons R124, L518, L527 and R555 of the TGFBI gene in 147 patients from 116 families. Among 89 families with GCD, Avellino CD (ACD) with R124H mutation was detected in 94% and Groenouw type I with R555W mutation in 6%, therefore, in Japan, ACD has been referred as GCD. Among 27 families with LCD, LCD type I (LCDI) with R124C mutation was detected in 48%, atypical LCDI with L518P mutation was in one family (3.7%), and LCD with deep stromal opacities (type IV) with L527R mutation was in 33%. No mutation was found in 4 families with LCD. Among 4 families with RBCD, a R555Q mutation was detected in 6 patients. Conclusions. We conclude that codons R124 and R555 of the TGFBI gene are also hot spots in Japanese patients with GCD, LCD and RBCD, where 94.4% of GCD was ACD with R124H mutation, and the remaining few percents (3.7%) were Groenouw I with R555W mutation. The L527R is the second common mutation in Japanese LCD.

P1485. Analysis of I-GlcNAc6ST gene (CHST5) in macular corneal dystrophy

N. Hasegawa[1], *S. Mita*[2], *K. Fujiki*[3], *A. Murakami*[3], *K. Nakayasu*[3], *O. Habuchi*[2], *A. Kanai*[3]
[1]*Japan Red Cross Medical Center; Tokyo, Japan;* [2]*Aichi University of Education; Kariya, Japan;* [3]*Juntendo University School of Medicine; Tokyo, Japan*
hasenobu@tkg.att.ne.jp

Macular corneal dystrophy type I (MCD) is an autosomal recessive inheritance disorder, which is caused by deficient enzyme activity of GlcNAc-6-sulfotransferase (GlcNAc6ST), a sulfotransferase needed in synthesizing corneal keratan sulfate. This disease manifests as corneal opacity, leading severe visual impairment. Previous linkage study revealed that MCD is mapped to chromosome on 16q21. The carbohydrate sulfotransferase gene (CHST5), encoding intestine GlcNAc6ST has cloned on 16q21 as same chromosomal location as MCD. We screened CHST5 in nine patients with MCD and ninety-five unrelated healthy controls. Genomic DNA was isolated from peripheral leukocytes, and 1658-bp genomic fragment of CHST5 amplified by PCR was directly sequenced. No responsible mutations which cosegregate with disease were found in CHST5 gene. Two polymorphisms of R163Q and T297M were identified. The R163Q was observed significantly higher frequency ($p<<0.001$) in the patients than that in the controls. The significant linkage disequilibrium of R163Q polymorphism indicates that the most critical gene should be located very near to CHST5. Recently Akama et al. reported a couple mutations in cornea specific GlcNAc6ST (CHST6) in MCD patients. CHST6 is located approximately 30kb downstream of CHST5. Our results are consistent with their findings and we postulates that the polymorphism of CHST5 may have relevant effects on the sulfotransferase activitiy together with a variation of responsible gene.

P1486. Cloning of a retinal gene encoding RFamide-related peptides; a candidate for cystoid macular dystrophy on chromosome 7p

H. L. Schulz[1], *M. van Driel*[2], *F. P. M. Cremers*[2], *H. St hr*[1], *B. H. F. Weber*[1]
[1]*Human Genetics; Wuerzburg, Germany;* [2]*Department of Human Genetics; Nijmegen, The Netherlands*
dhgpg@hotmail.com

To date, the cause of more than 60 Mendelian syndromic and non-syndromic retinal dystrophies is still unknown. Towards the goal to identifiy novel candidates for retinal disease genes, we are performing systematic expression profiling of established UniGene EST clusters showing a high level of representation in retinal cDNA libraries. Cluster Hs.60473 is an assembly of two retina 3 read ESTs and was confirmed to be exclusively expressed in retinal tissue by RT-PCR and Northern blot analysis. The screening of retinal cDNA libraries and 5 RACE experiments yielded more than 15 overlapping cDNA fragments that were assembled into a 1190 bp full length cDNA containing an ORF of 196 amino acids, termed C7orf9. Analysis of the deduced protein sequence revealed the presence of two conserved RFamide-related peptides. RFamides are a family of small bioreactive peptides known to be expressed in vertebrate and invertebrate nervous systems including the sensory retina. The C7orf9 gene contains three exons localized to the D7S2493-D7S529 interval on chromosome 7p15-p21, a region harbouring the gene for dominant cystoid macular dystrophy (CYMD). Due to its chromosomal location and retina-specific expression, C7orf9 represents an excellent candidate for the CYMD gene. A possible involvement of C7orf9 in disease is currently being assessed by mutation analysis in affected patients of a large Dutch CYMD family previously linked to chromosome 7p.

P1487. Molecular study of RS gene in three Spanish families affected with juvenile retinoschisis

C. Ayuso[1], *A. Gimenez*[1], *B. Garcia-Sandoval*[1], *J. Bueno*[1], *M. Trujillo*[1], *D. Cantalapiedra*[1], *J. Fernandez-Toral*[2], *M. Baiget*[3], *C. Ramos*[1]

[1]*Fundacion Jimenez Diaz; Madrid, Spain;* [2]*HOSPITAL GENERAL ASTURIAS; OVIEDO, Spain;* [3]*HOSPITAL SAN PABLO; BARCELONA, Spain*
cayuso@fjd.es
X-linked juvenile retinoschisis (XLRS, OMIM *312700) is a progressive hereditary retinopathy consisting of intraretinal splitting due to degeneration. The RS gene resposible for the disease is located on Xp22.2 —22.1 and has 6 exons. The encoded 224 amino acid protein is exclusively expressed in retina and contains a highly conserved motif implicated in cell-cell interaction and possibly active in cell adhesion processes. We analysed the entire coding region of RS gene, by PCR amplification followed by direct sequencing (ABIprism 310 Perkin Elmer) in 3 unrelated Spanish families affected with juvenile retinoschisis. Complete ophthalmological studies and Electroretinograms were recorded according to the standard testing protocols. We detected three missense changes; Gln154Arg Arg 213Gln, and Glu215Gln.There was co-segregation with the disease within each family. The mutations were located in exons 5 and 6 emphasizing the critical functional significance of the discoidin domain of the XLRS1 protein. The identification of the responsible mutations for the disease coupled with complete familiar analysis allowed us to detect carrier women and therefore provide better genetic counselling in these families.

P1488. Generation of a mouse model for X-linked juvenile retinoschisis

A. Gehrig[1], *N. Hemmrich*[1], *R. S. Molday*[2], *H. Schrewe*[3], *B. H. F. Weber*[1]
[1]*Human Genetics; Wuerzburg, Germany;* [2]*Department of Biochemistry; Vancouver, BC Canada;* [3]*School of Biosciences; Birmingham, United Kingdom*
gehrig@biozentrum.uni-wuerzburg.de
X-linked juvenile retinoschisis (RS) is an early-onset vitreoretinal disorder characterized by schisis, or splitting, of the nerve fiber and ganglion cell layers of the retina. RS is clinically variable with symptoms ranging from slightly reduced visual acuity to more significant complications such as retinal detachment or vitreous hemorrhage. The gene underlying RS was identified by positional cloning and is composed of 6 exons encoding a 224 amino acid precursor protein, termed retinoschisin, with a 23 amino acid hydrophobic leader sequence. Most of the protein consists of a discoidin-like domain which is common to a family of proteins implicated in cell adhesion. By immunohistochemistry, we show that retinoschisin is exclusively present in the membrane fraction of retinal tissue homogenates. Moreover, retinoschisin antibodies specifically label rod and cone photoreceptors and most bipolar cells. The protein appears to be active as a disulfide-linked oligomeric complex. To further analyze the role of retinoschisin in retinal cell biology, we have generated a knock-out mouse by targeting exon 3 of the murine Rs1h gene resulting in a true null allele. In addition, a lacZ reporter was introduced in-frame and has allowed us to establish the developmental pattern of gene expression, thus linking absent Rsh1 transcription to early retinal pathology. Our current model assumes that the retinoschisin complex binds to the surface of photoreceptors and bipolar cells where it may function as a cellular adhesion protein stabilizing the cellular organization of the retina.

P1489. Mutation screen in patients with Autosomal Dominant Optic Atrophy

K. Hoertnagel[1], *G. Rudolph*[2], *T. M. Strom*[1], *T. Meitinger*[1]
[1]*GSF Forschungszentrum f r Umwelt und Gesundheit; M nchen / Neuherberg, Germany;* [2]*Augenklinik der Ludwig-Maximilians-Universit t; M nchen, Germany*
konstanze@pedgen.med.uni-muenchen.de
Optic atrophy type1 (OPA1) is a dominantly inherited optic neuropathy with an onset in early childhood and is characterized by progressive loss of visual acuity. The predominant locus for the disease has been localized to 3q28-q29, a second locus has been mapped to chromosome 18q12.2-q12.3. Symptoms similar to those found in Leber hereditary optic neuropathy LHON are suggestive of a mitochondrial disease. Recently, the OPA1 gene on 3q28 has been identified as a dynamin related GTPase homologous to the yeast Mgm1 gene, which encodes a mitochondrial protein that plays a role for the distribution and morphology of mitochondria. We established the gene structure of the human Mgm1 homologue using the working draft sequence available from the public sequencing centres. Eight patients from non-related families were screened for mutations by amplifying and sequencing all 28 coding exons. Only in a single patient we detected a missense mutation 377A>C which changes an asparagine to an alanine. This mutation could not be found in 100 control chromosomes. A dosis Southern blot analysis revealed deletions in at least two more patients suggesting haploinsufficiency as the causative disease mechanism. The large number of exons, the existence of exon deletions which escape nonquantitative PCR detection and non-allelic heterogeneity pose difficulties to the molecular diagnosis of patients with this disorder.

P1490. Investigation of an extended Tasmanian pedigree with a novel PAX6 mutation

M. M. Sale[1], *J. E. Craig*[2], *J. C. Charlesworth*[1], *L. M. FitzGerald*[1], *I. M. Hanson*[3], *J. L. Dickinson*[1], *S. J. Matthews*[3], *V. van Heyningen*[3], *D. A. Mackey*[2]
[1]*Menzies Centre for Population Health Research, University of Tasmania; Hobart, Tasmania, Australia;* [2]*Centre for Eye Research Australia, University of Melbourne; Melbourne, Victoria, Australia;* [3]*MRC Human Genetics Unit, Western General Hospital; Edinburgh, United Kingdom*
Michele.Sale@utas.edu.au
Two distinct phenotypes - aniridia and primary open-angle glaucoma (POAG) - segregate within a large Tasmanian pedigree. We have identified a novel PAX6 mutation associated with highly variable phenotype within one branch of this pedigree, and wished to investigate its role (if any) in relatives with POAG. Eleven individuals in the aniridia branch of the pedigree were subjected to detailed phenotypic analysis, including anterior segment and fundus photography, automated perimetry and IOP measurement. Mutation analysis revealed a novel PAX6 1410delC mutation in 9 affected individuals, not present in 9 unaffected relatives or spouses. One family member believed to be mildly affected by the family, with visual acuity 6/9 and 6/7.5, was found not to carry the mutation. Clinical features in mutation carriers ranged from total aniridia to minimal anterior segment findings. Other findings included keratitis, cataract, glaucoma, optic disc anomalies and foveal hypoplasia. POAG individuals in two additional branches of the extended pedigree had IOP measured, Humphrey visual fields and stereo disc photographs taken, and clinical history documented. The PAX6 mutation was not detected in 7 individuals with POAG. Other genetic or environmental factors involved in anterior segment development may interact with the PAX6 gene to determine the severity of the phenotype in the aniridia branch, and may also account for the slightly reduced vision in a non-mutation carrier. Optic nerve anomalies were particularly common, and the same genes may modify the aniridia phenotype as well as contribute to POAG.

P1491. Autosomal recessive colobomatous micro/anophthalmia in a consanguineous Irish traveller family - further genetic heterogeneity

A. J. Green[1,2], *M. Ni Chroinin*[1], *P. Tormey*[3], *S. Ennis*[1,4]
[1]*National centre for Medical Genetics; Dublin 12, Ireland;* [2]*Dept. of Medical Genetics, University College Dublin; Dublin, Ireland;* [3]*Dept. Ophthalmology, Waterford Regional Hospital; Waterford, Ireland;* [4]*Dept. of Medical Genetics, University College Dublin; Dublin, Ireland*
andrew.green@ucd.ie
We describe 9 members of a consanguineous Irish traveller family, with severe visual impairment due to anophthalmia, microphthalmia or colobomatous microphthalmia. Two members have complete bilateral anophthalmia, five had unilateral anophthalmia and contralateral colobomatous microphthalmia, and two had microphthalmia. There was no history of chemical exposure, no other associated malformations, and chromosomal analysis in two affected individuals was normal. There is pseudodominant inheritance in one family, with an affected son born to an affected father in a consanguineous marriage. Only a small number of families with autosomal recessive colobomatous micro/anophthalmia have been described before. Several reports have been of families with a consistent expression of exclusively bilateral anophthalmia in homozygotes, in contrast to the family we describe with a more varied expression in homozygotes. This is the first description of this condition in the Irish traveller population, and represents a further disease allele for this population. A previously known 14q32 locus for autosomal recessive anophthalmia, and the recently described CHX10 gene on chromosome 14, known to cause autosomal recessive anophthalmia, have been excluded in this family suggesting at least a third locus for this condition. Homozygosity mapping for autosomal recessive anophthalmia is currently under way.

P1492. Two Thai families with Norrie Disease (ND); Association of two novel missense mutations with severe ND phenotype, epileptic seizures, and a manifesting female carrier

K. Yamada[1], *N. Kurotaki*[2], *J. Takano*[2], *T. Kayashima*[2], *N. Niikawa*[2], *T. Kishino*[2]
[1]*Departments of Human Genetics and Ophthalmology, Nagasaki University School of Medicine; Nagasaki, Japan;* [2]*Department of Human Genetics, Nagasaki University School of Medicine; Nagasaki, Japan*
f1198@cc.nagasaki-u.ac.jp
Two Thai families with Norrie disease (ND), including 10 affected males

and one manifesting female in three generations, are described. All affected males in each family had severely defective eye development with complete loss of vision. In addition, three male patients (one from a family 1 and two from the other family) suffered from epilepsy, and one female carrier from one family manifested blindness with phthisis bulbi in her right eye. Mutation analysis of the ND gene (NDP) revealed two different novel missense mutations (L16P and S75P) that co-segregated with ND in each family, suggesting that the newly appearing proline at codon 16 or codon 75 alters the conformation of the ND protein and contributes to the severe phenotype of ND in each family. It has been suggested that epileptic seizures or growth retardation that is associated with ND is the consequence of loss of contiguous genes, because most such patients had deletions extending beyond the Norrie locus. Our finding that the three affected males in the two families with the missense mutations have epilepsy does not support a contiguous gene effect, but favor the pleiotropism of NDP, as far as at least the epileptic manifestation is concerned. The female carrier with unilateral blindness may be due to non-random X-inactivation.

P1493. Analysis of the phosphatidylinositol transfer protein gene (PITPN) on chromosome 17p, in a South African family with retinal degeneration.

R. G. Goliath, *L. J. Greenberg, R. Ramesar*
Department of Human Genetics, University of Cape Town; Cape Town, South Africa
RG@ANAT.UCT.AC.ZA

Phosphatidylinositol transfer protein (PITPN) is involved with the regulation of membrane transport within photoreceptor cells and is essential for cell survival and recovery from light stimulation. A mutation identified in the Drosophila rdgB gene causes photoreceptor degeneration. It is, therefore, conceivable that defects in the PITPN gene (PITPN) could be involved in human retinal disease. Autosomal dominant retinitis pigmentosa (ADRP), a form of retinal degeneration, was localised to the same chromosomal region as PITPN (chromosome 17p), making this gene a good candidate for the disease phenotype. To date, no genomic DNA sequence of human PITPN has been published. This study involved investigation of PITPN through defining exon-intron boundaries for SSCP analysis of the coding the splice consensus regions. Performing electronic database comparisons elucidated the genomic structure of PITPN. The cDNA sequence was found to be homologous to a large genomic sequence file (AC006405). AC006405 contained all except the first 236bp at the 5 end and 68bp at the 3 end of the cDNA sequence. Eight exons were distinguishable for SSCP analysis using oligonucleotide primers derived from the intronic sequences flanking the exonic fragments for PCR amplification. To date, no disease-causing mutations have been identified in this analysis. The inability to detect a pathogenic alteration could be due to the method employed in the mutation screen. PITPN, however, remains a candidate for the other forms of retinal disease localised to chromosome 17p. Hense, these findings will assist in future investigations into disorders involving retinal dysfunction linked to chromosome17p.

P1494. Glaucoma phenotype in Australian pedigrees with Myocilin Thr377Met mutation

D. A. Mackey[1], *J. E. Craig*[1], *D. H. Healey*[1], *J. H. Fingert*[2], *E. M. Stone*[2]
[1]*Centre for Eye Research Australia; EAST MELBOURNE, Australia;* [2]*University of Iowa; IOWA, United States*
D.Mackey@utas.edu.au

INTRODUCTION; Mutations in the Myocilin gene account for 3% of primary open angle glaucoma with the Gln368STOP mutation being the most frequently observed. The next most frequently identified mutation to date in Australia is the Thr377Met mutation. We present detailed phenotype analysis in 4 glaucoma pedigrees with this mutation. METHODS; Four families were identified with the Thr377Met mutation from over 2,000 glaucoma samples from South Eastern Australia tested from the GIST project. Clinical notes, visual fields and stereo disc photographs were obtained on all subjects. RESULTS; All index cases had a positive family history of glaucoma. One large family originated from the UK and 3 smaller families were of Greek or Macedonian extraction. The median age of diagnosis was in the 4th decade and maximum recorded IOP was over 30mmHg in most diagnosed glaucoma cases. Half these cases required trabeculectomy. CONCLUSION;The Thr377Met mutation has been identified in two ethnic subgroups with a consistent glaucoma phenotype associated with elevated intraocular pressure with variable age of onset typically in early adulthood often requiring filtration surgery.

P1495. In search of mutations in ABCA4 underlying Stargardt disease (STGD) as a tool to charter the gene flow in southern Africa (SA)

A. V. September, *R. Ramesar, J. Greenberg*
University of Cape Town; Cape Town, South Africa
AS@ANAT.UCT.AC.ZA

Mutations in the ATP-binding cassette transporter gene (ABCA4) are responsible for a wide spectrum of inherited retinal disease phenotypes. These include STGD1, fundus flavimaculatus, recessive retinitis pigmentosa, recessive cone rod dystrophy and susceptibility to age-related macular degeneration. A panel of 80 unrelated individuals affected with STGD were selected. A comprehensive investigation of ABCA4 was undertaken using single stranded conformational polymorphism and heteroduplex analysis in conjunction with direct sequencing to identify mutations underlying disease in this cohort. The objectives of this study was to investigate possible correlations between (a) genotype and phenotype, and (b) genotype and ethnic origins of the subjects. The SA population is genetically interesting in that it is comprised of large groups of people, with well-defined ethnic origins. In many instances purity of certain groups has been maintained by political and legally entrenched policies of the apartheid era. This phenomenon facilitates a study of gene flow over many generations. Several novel mutations have thus far been identified in this study. More important, a number of compound heterozygote mutations have been identified which may facilitate determining the level of admixture in the cohort. The genetic findings from the population under investigation is extremely valuable in determining the effects of the various different mutations and the combinations of these mutations in ABCA4 on the wide spectrum of disease phenotypes and may aid the understanding the recurrence risk of STGD disease in families with known ABCA4 mutations.

P1496. A novel molecular basis for the development of Axenfeld-Rieger malformations

K. Kozlowski[1], *M. Priston*[2], *E. H on*[2,3], *M. A. Walter*[1]
[1]*University of Alberta; Edmonton, AB Canada;* [2]*Toronto Western Hospital; Toronto, ON Canada;* [3]*University of Toronto; Toronto, ON Canada*
kk1@ualberta.ca

Axenfeld-Rieger (AR) malformations are characterized by aberration of the anterior chamber of the eye and are associated with early-onset glaucoma. Mutation of the transcription factor PITX2 has been shown to underlie AR. Here we characterize a novel mutation of the PITX2 homeodomain. A V45L missense mutation of PITX2 found in a patient with AR malformation was introduced into the PITX2 cDNA by site-directed mutagenesis. Recombinant wildtype (wt) and mutant (mt) PITX2 proteins were expressed in COS-7 cells and subcellualr localization was examined by immunofluorescence. Electrophoretic mobility shift assays (EMSAs) were used to compare the DNA binding abilities of wt and mt PITX2 proteins. Activation of a luciferase reporter gene was used to test the transactivation activity of wt and mt PITX2 proteins in HeLa cells. The V45L protein correctly localized to the cell nucleus, but had a 2 to 7 fold decrease in DNA binding activity versus wt PITX2. Surprisingly, however, the V45L was able to transactivate a reporter gene at greater than 200% of wt levels. We have previously shown that a R46W missense mutation of PITX2 found in a patient retained 38% of wt PITX2 transactivation activity (Kozlowski and Walter, Hum Mol Genet 9; 2131-9, 2000). Our current results with the V45L mutation indicate that an alteration resulting in >200% of wt PITX2 transactivation activity is also associated with human disease. These findings demonstrate the existence of both upper and lower thresholds of PITX2 activity necessary for correct eye development and function.

P1497. Duchenne And Becker Muscular Distrophies In Chilean Patients; Frequency And Distribution Of Deletions In The Dystrophin Gene

P. Rocco[1], *L. Herrera*[2], *K. Kleinsteuber*[3], *M. A. Avaria*[3], *M. P. Carvallo*[1]
[1]*Departamento de Biologia Celular, Facultad de Ciencias Biologicas, P. Universidad Catolica de Chile; Santiago, Chile;* [2]*ICBM, Facultad de Medicina, Universidad de Chile; Santiago, Chile;* [3]*Servicio de Neurosiquiatria Infantil, Hospital Clinico San Borja Arriaran; Santiago, Chile*
procco@genes.bio.puc.cl

Duchenne and Becker muscular dystrophies (DMD/DMB) are X-linked disorders that result from heterogeneous mutations in the dystrophin gene. Several studies performed in groups of patients from different ethnic origins showed that the frequency of the DMD/DMB cases due to deletions of one or more exons varies between 0.23 and 0.86. The remaining cases are due to point mutations or duplications within the gene. DNA isolated from peripheral blood lymphocytes of 51 patients with DMD/DMB from Chile were analysed by multiplex PCR (Chamberlain and Beggs). Deletions were detected in 24 patients (47%), 22 with a diagnostic of DMD and 2 with

DMB phenotype. The deletions were mainly clustered in the central part of the gene (exons 43-52) in a 79 %. The proportion of deletions in our DMD/DMB patients differ from those reported for European and North American populations (55-65%), and is closer to the frequency of deletions found in the Mexican population (53 %). The status of the dystrophin protein was studied through Western blot in patients not showing deletions in the dystrophin gene. Monoclonal antibodies directed to the amino, rod and carboxi domains of the dystrophin were used. A total of 8 patients showed a complete lack of dystrophin, suggesting the presence of mutations leading into stop codons. Financed by Fundacion Andes, CHILE

P1498. DGGE-based mutation scan of the whole dystrophin gene in DMD/BMD patients

A. J. van Essen[1], *I. M. Mulder*[1], *R. H. A. Vossen*[2], *M. Kraak*[1], *M. Villerius*[2], *P. Elfferich*[1], *G. J. B. van Ommen*[2], *A. H. van der Hout*[1], *I. Ginjaar*[2], *C. H. C. Buys*[1], *R. M. W. Hofstra*[1], *J. T. den Dunnen*[2]

[1]*Department of Clinical Genetics, University Hospital Groningen; Groningen, The Netherlands;* [2]*Department of Human and Clinical Genetics, Leiden University Medical Center; Leiden, The Netherlands*

a.j.van.essen@medgen.azg.nl

Duchenne and Becker muscular dystrophy are caused by mutations in the dystrophin gene. Large deletions and duplications in the dystrophin gene in DMD and BMD are detected in about two thirds of patients. This enables accurate carrier detection and prenatal diagnosis in those families. When such large mutations are absent, carrier detection relies on linkage analysis and determination of creatine kinase activities in female family members. Still, detection of small mutations is very important for optimal carrier detection in all families. Previously used techniques for tracing microlesions focussed on exons in the two deletion hot spots of the dystrophin gene. However, microlesions do not cluster in hot spots. Therefore, we designed a comprehensive DGGE -based mutation scanning method of the dystrophin gene, with a set of 95 DGGE-amplicons covering all exons of the dystrophin gene including intron/exon boundaries. To reduce the workload, we used multiplex combinations of up to 6 exons. The system was validated by confirming 24 previously detected point mutations. We have screened 48 DMD patients, 34 BMD patients and 50 high-risk carriers. We identified 20 pathogenic mutations and 4 possible pathogenic mutations in the DMD group. In 34 BMD patients we found 4 pathogenic mutations and 2 possible pathogenic mutations. Reasons for the low mutation yield may be missed deep intronic mutations, inversions, small duplications, possible promoter mutations and wrong diagnosis. Especially in isolated BMD patients, where dystrophin analysis of muscle biopsies was not possible, misdiagnosis might play an important role.

P1499. Restoration of the mdx-mouse phenotype upon transfer of the entire human DMD-gene; analysis of the muscular expression profile using DNA-chips

J. T. den Dunnen, *E. M. de Meijer, R. Turk, E. Mank, E. Sterrenburg, J. M. Boer, G. J. B. van Ommen*

Human and Clinical Genetics, Leiden University Medical Center; Leiden, The Netherlands

ddunnen@lumc.nl

Starting from overlapping YACs, we have previously reconstructed the full 2.5 Mb human Duchenne Muscular Dystrophy (DMD) gene in yeast and subsequently set out to transfer it to murine ES cells. Given its Mb-size, we chose for yeast spheroplast / mammalian cell fusion. Of the several tens of DMD-gene-positive clones, two contained all DMD-exons without detectable gross mishaps in the gene. Transgenic mice containing the human DMD-gene (hDMD) were generated and subjected to detailed phenotypic and expression analysis. RT-PCR, Western blot analysis and immuno-histochemical studies, using human-specific vs. human/mouse crossreacting antibodiess showed that the human DMD-gene had the expected tissue specific expression of the various dystrophin isoforms. Moreover, the similar level of human and mouse muscle isoforms suggests that the transcription and translation levels are also properly regulated. To answer the question, whether the product(s) of the full human DMD gene can functionally complement a murine DMD mutation, the mice were crossed with mdx-mice. Phenotypical and immuno-histochemical analysis showed that the mdx-phenotype was restored to normal. Finally, analysis of global expression patterns using DNA-chip and micro-array technology was used to investigate the differences in muscle samples of normal, hDMD, mdx and human DMD-complemented mdx-animals. We will present data comparing normal and diseased mouse and human tissue which should give a wealth of novel insights into more global deleterious effects of DMD mutations. These data should help to understand the phenotypical differences of dystrophin deficiency in man and mouse and thus potentially lead to secundary interventions to reduce disease severity in patients.

P1500. Genotype - phenotype correlation in Becker muscular dystrophy patients with proximal dystrophin gene deletions

I. Novakovic[1], *S. Apostolski*[2], *S. Todorovic*[3], *L. Lukovic*[1], *V. Bunjevacki*[1], *D. Bojic*[4], *L. Mestroni*[5], *J. Milasin*[6]

[1]*Institute of Biology and Human Genetics, School of Medicine; Belgrade, Yugoslavia;* [2]*Institute of Neurology; Belgrade, Yugoslavia;* [3]*Clinic for Pediatric Neurology and Psychiatry; Belgrade, Yugoslavia;* [4]*Institute of Cardiovascular Diseases Dedinje ; Belgarde, Yugoslavia;* [5]*ICGEB; Trieste, Italy;* [6]*Institute of Biology and Human Genetics, School of Stomatology; Belgrade, Yugoslavia*

llukovic@eunet.yu

According to the frame shift hypothesis Becker muscular dystrophy is associated with in-frame mutations in the dystrophin gene. The most frequent type of mutations are intragenic deletions, mainly clustered in two regions; distal (exons 45-53), and proximal (exons 3-13). BMD phenotype, including skeletal myopathy and cardiac disorders, shows considerable clinical diversity. Generally, proximal deletions are associated with worse prognosis, while in distal deletions BMD has typical benign clinical course. The aim of this study is to analyze genotype - phenotype correlation in a group of six BMD patients with deletions affecting proximal part of dystrophin gene, encompassing exons 3-13. Dystrophin gene deletions were detected by standard multiplex PCR method. Clinical evaluation included detail neurological and cardiological examination. Four patients had deletions in the region from muscle promoter to exon 8, encoding for N-terminal distrophin domain. Early onset (9.4+/-4.8 y.) and faster progression of disease characterized this group. For example, one patient (del. of exons 6-8) was wheelchair bound since age 30, with severe cardiomyopathy, and another (del. of exons 3-6) at age 15 y. had severe muscular dystrophy. In two patients single exon 13 missing was detected, of dystrophin s rod domain. These patients had later presentation (16-20 y.) with moderate muscular dystrophy at middle-ages. Our study suggests that proximal gene deletions in BMD have various phenotypic effects depend on corresponding domain of protein dystrophin.

P1501. Electroretinogram Findings In Duchenne/becker Muscular Dystrophy And Correlation With Genotype

D. Er al[1], *A. Ulgenalp*[1], *H. Oner*[2], *M. Soylev*[2], *E. Bora*[1], *O. Giray*[3], *F. Afrashi*[4], *S. Kose*[4]

[1]*Dokuz Eylul University, Faculty of Medicine, Clinical Genetic Unit; Izmir, Turkey;* [2]*Dokuz Eylul University Faculty of Medicine Dept. of Opthalmology; Izmir, Turkey;* [3]*Dokuz Eylul University Facuty of Medicine; Izmir, Turkey;* [4]*Ege University Faculty of Medicine, Dept. of Ophthalmology; Izmir, Turkey*

ayferulg@yahoo.com

Purpose;To correlate the electroretinogram(ERG)findings in patients with Duchenne/Becker muscular dystropy (D/BMD) with the genotype and avaluate the ERG findings in obligate carriers. Materials and Methods;Fifteen deletion positive patients with DMD,two deletion positive patients with BMD and six obligate carriers in two families having positive DMD history were evaluated with DNA analysis,ophtalmologic and ERG findings. Findings;No pathology was detectedin the ophthalmic examination of the patients.In three out of 17 patients with D/BMD,ERG was normal(in two patients with DMD,and in one patient with BMD).In 14 patients ERG was electronegative in scotopic conditions.In these cases in photopic conditions b wave was recorded subnormal whereas a wave being magnified. B/A amplitude ratio was less than 2 in all DMD cases except two cases with normal ERG and in one patient with BMD.Mutational alterations observed in this three cases were exceptionally at 5 hot spot;not like the others within central region. In two patients Flicker ERG was found subnormal.In obligate carriers ERG findings were normal. Conclusion;ERG findings in D/BMD patients may show some correlations with molecular analysis,whereas it is not useful in obligate carriers.

P1502. A case report of a female carrier of Becker muscular dystrophy with manifesting disease

S. A. Kocheva[1], *A. J. Dimovski*[1], *S. Vlaski-Jekic*[2], *G. D. Efremov*[1]

[1]*Macedonian Academy of Sciences and Arts; Skopje, The former Yugoslav Republic of Macedonia;* [2]*Clinic for Neurology, Faculty of Medicine; Skopje, The former Yugoslav Republic of Macedonia*

svanko@yahoo.com

Duchene/Becker muscular dystrophy (DMD/BMD) is an X-linked recessive muscular dystrophy caused by mutations in the dystrophin gene located at Xp21. BMD is a milder form of the disease which is characterized with later onset and slower progression into adulthood. Most of the patients with this form of the disease have in frame deletions of part(s) of the dystrophin gene. Female carriers are rarely symptomatic and are mainly carriers of DMD associated mutations. On the other hand, only a few female carriers

of BMD with symptomatic disease have been described so far. In this report we present a female carrier of a typical BMD with a manifesting disease. The patient is a 45 years old female who has two sons with a mild form of Becker muscular dystrophy. She presented with pseudohypertrophy of the calves, mildly elevated serum CPK levels and myopathic electromyogram. DNA analysis using the multiplex PCR approach showed that the two sons have in-frame deletion of exons 46, 47 and 48. Quantitative PCR analysis using fluorescently labeled PCR products and automated fragment analysis on ABIPrism 310 Genetic Analyzer confirmed that the mother is also a carrier of this deletion. The reason for the expression of the disease in this patient is still unknown. The possibility of unequal X-chromosome lionization is currently under investigation.

P1503. Protein Analysis In Autosomal Recessive Limb-girdle Muscular Dystrophies (AR-lgmd)

M. Vainzof, *E. S. Moreira, F. de Paula, R. C. M. Pavanello, M. Passos-Bueno, M. Zatz*

Center for the Study of the Human Genome, IB- University of Sao Paulo; Sao Paulo, Brazil

mvainzof@usp.br

Nine forms of AR-LGMDs, a heterogeneous group of muscular progressive disorders, have been identified. Four genes, which code for the sarcoglycan glycoproteins from the DGC cause the more severe forms, the sarcoglicanopathies (LGMD2C, 2D, 2E and 2F). The five milder forms are LGMD2A, LGMD2B, LGMD2G, LGMD2H and LGMD2I and the known protein products are calpain 3 (2A), dysferlin (2B) and telethonin (2G). Through imunohistochemistry and western blot analysis, we studied the expression of the 7 known LGMD proteins in 218 muscle biopsies from patients with clinical diagnosis of LGMD (195 families). A total or partial deficiency of the following proteins was observed; sarcoglycan (a-, b-, g-, d-sarcoglycans) in 29 families, calpain 3 in 60 families, dysferlin in 25 families and telethonin, in 4 families. In patients with known pathogenic mutations it was possible to observe that; the primary deficiency of one of the SG protein leaded to the secondary deficiency of the whole complex in the majority of the SG patients, but SGs were normal in the 2A, 2B and 2G forms. Calpain 3 showed total or partial deficiency in LGMD2A patients but a secondary deficiency was also observed in two LGMD2B families. Patients with LGMD2B and 2G showed total deficiency of the respective proteins, but no secondary deficiencies of dysferlin or telethonin were observed in patients not linked to these genes. Through protein analysis it was possible to classify ~60% of the LGMD families, suggesting which gene should be primary screened for identification of mutations. Financial support; CEPID-FAPESP, PRONEX, CNPq, ABDIM

P1504. Recurrent Basque and Brazilian Mutation in LGMD2A (Calpainopathy) Brazilian Families.

F. Paula[1], *R. Viegas*[1], *A. Kai*[1], *E. Moreira*[1], *M. Passos-Bueno*[1], *M. Vainzof*[1], *V. Nigro*[2], *M. Zatz*[1]

[1]*Universidade de S o Paulo; S o Paulo, Brazil;* [2]*Universita degli studi di Napoli; Napoli, Italy*

flapvit@yahoo.com.br

The Limb-Girdle Muscular Dystrophy included an heterogeneous group of genetic disease characterized by weakness on proximal muscles. The most prevalent of them is the autosomal recessive form LGMD 2A, with a clinical course ranging from severe to mild. The LGMD 2A gene, which codes for calpain-3, has 24 exons. About 97 pathogenic distinct mutations have been identified along the gene; 70% of them are private mutations, but a few of them seem to be more prevalent in some populations. We are screening mutations in the CANP-3 gene in 24 LGMD 2A Brazilian families (including 68 patients), classified through linkage or with calpain deficiency in muscle. Up to now, pathogenic mutations were found in 21 families, 6 of them in just one allele. Four novel changes where identified; two nonsense (R748X and 509-510insA, exons 21 and 5) and two missense (K211E and M248R, exons 4 and 5) which were not found in 50 normal controls. Interestingly, although the Brazilian population is highly miscigenated, 2 recurrent mutations were found (corresponding to 45% of the sample); six families had the same 2362AG TCATCT mutation (prevalent in the Basque population) while five the same R110X change (identified only in Brazilian patients). Haplotype analyzes in 10 of these families suggest a common origin for these two mutations. No direct correlation was found between the type of mutation, calpain expression in muscle and severity of the clinical course, particularly due to high intrafamilial variability. Financial support; CEPID-FAPESP, PRONEX-CNPq, IAEA, Telethon-Italy.

P1505. Analysis of dysferlin mRNA in peripheral blood lymphocytes (PBL) of LGMD2B/Miyoshi Myopathy patients and controls; detection of mutations and polymorphisms. Evidence of physiological PBL specific exon skipping

J. Mela[1,2], *E. Ricci*[2], *F. Mangiola*[1], *A. Damiani*[1], *P. Tonali*[2], *L. Felicetti*[2], *G. Galluzzi*[1,2]

[1]*UILDM Center for Neuromuscular Diseases; Rome, Italy;* [2]*Institute of Neurology, Catholic University; Rome, Italy*

giuly@biocell.irmkant.rm.cnr.it

Mutations in dysferlin gene (DYSF) are responsible for both autosomal recessive LGMD2B and Miyoshi Myopathy (MM). DYSF gene maps to chromosome 2p13, spans more than 150 kb and contains 55 exons. The 230 kDa protein product is located on the muscle fiber membrane; its function is still unknown. Dysferlin RNA expression is strongly detected in skeletal muscle, hearth and placenta; lower expression is found in brain, kidney and lung. The entire coding sequence has been identified as a 6.9 kb mRNA. With the aim of investigating dysferlin expression in additional tissues, we analyzed dysferlin transcripts in peripheral blood lymphocytes (PBL) by RT-PCR in eight normal controls and in four families with one or more subjects showing a mild distal myopathy, compatible with MM. Using 14 overlapping nested sets of primers and PBL total RNA as template, we amplified the entire dysferlin coding sequence. In PBL mRNAs of both normal and MM individuals, we observed the same sequences as compared with muscle transcripts, except for the deletion of 42 bp corresponding to the skipping of exon 17. This finding suggests the presence of a PBL specific splice site. In order to identify small mutations, we performed Heteroduplex analysis of the amplified cDNA fragments; until now we found a patient homozygous for Arg959Trp missense mutation and detected several polymorphisms. Dysferlin transcripts analysis in PBL seems to be a useful method to screen patients for mutation detection and to study the organization of dysferlin gene and its expression in different tissues.

P1506. PFGE analysis of 4q35 rearrangements in Italian families with Facioscapulohumeral muscular dystrophy (FSHD); implications for the molecular mechanism of the disease

G. Galluzzi[1,2], *L. Colantoni*[1,2], *M. Rossi*[2], *B. Merico*[2], *F. Mangiola*[1], *P. Tonali*[2], *E. Ricci*[2], *L. Felicetti*[2,3]

[1]*UILDM Center for Neuromuscular Diseases; Rome, Italy;* [2]*Institute of Neurology, Catholic University; Rome, Italy;* [3]*Institute of Cell Biolgy, CNR; Rome, Italy*

giuly@biocell.irmkant.rm.cnr.it

In 95% of FSHD families the genetic defect is a deletion of a variable number of KpnI repeats in 4q35 region. Molecular diagnosis is based on the detection by probe p13E-11 of short EcoRI, BlnI-resistant fragments (10-35 kb). Sequence homology between 4qter and 10qter loci facilitates interchromosomal exchanges resulting in the reshuffling of 4q-type BlnI-resistant and 10q-type BlnI-sensitive repeats from one chromosome to the other. In order to verify whether the occurrence of interchromosomal exchanges could play a pathogenic role in association with the 4q35 specific rearrangement, we analyzed the segregation of BlnI-resistant and BlnI-sensitive alleles in 50 FSHD Italian families. DNA extraction and subsequent restriction steps with EcoRI, BlnI and Tru9I were performed directly in agarose blocks. The alleles were separated by PFGE and identified with p13E-11 and KpnI cloned sequences as probes. Both in affected and normal individuals, we observed all kinds of interchromosomal exchanges (trisomy, tetrasomy, monosomy, nullisomy and partial translocations). We were able to establish that; the small 4q35 fragment causing FSHD is rarely involved in interchromosomal exchanges; high MW alleles are more frequently implicated in subtelomeric translocations; in most monosomic patients, the translocated allele was inherited from the parent not transmitting the disease; in 4 out of 8 sporadic cases, both parents carried subtelomeric translocations. PFGE analysis of 4q35 rearrangements in FSHD greatly improves the accuracy of molecular diagnosis and reveals the extent of interchromosomal exchanges, suggesting that the instability of subtelomeric regions could play a significant role in the molecular mechanism of the disease.

P1507. Physical and transcriptional map of the Hereditary Inclusion Body Myopathy locus on chromosome 9p12-13

I. Eisenberg[1], *H. Hochner*[1], *M. Shemesh*[1], *T. Levi*[2], *T. Poticha*[1], *M. Sadeh*[3], *Z. Argov*[4], *C. Jackson*[5], *S. Mitrani-Rosenbaum*[1]

[1]*The Unit for Molecular Biology, Hadassah Hospital, The Hebrew University-Hadassah Medical School; Jerusalem, Israel;* [2]*Department of Genetics, Harvard Medical School; Boston, United States;* [3]*Department of Neurology, Wolfson Hospital; Holon, Israel;* [4]*Department of Neurology, Hadassah Hospital, The Hebrew University-Hadassah Medical School; Jerusalem, Israel;* [5]*Department of Pathology, Rhode Island Hospital and*

Brown University; Providence, United States
eisenb@md2.huji.ac.il
Hereditary Inclusion Body Myopathy (HIBM) is a group of neuromuscular disorders characterized by adult-onset, slowly progressive distal and proximal muscle weakness and typical muscle pathology. Previously, we have mapped the gene responsible for a recessive form of HIBM to chromosome 9p1 and narrowed the interval to one single YAC clone of 1Mb in size. As a further step towards the identification of the HIBM gene, we have constructed a detailed physical and transcriptional map of this region. A high resolution BAC contig that includes the HIBM critical region, flanked by marker D9S1804 and D9S1859, was constructed. This contig allowed the precise localization of twenty five genes and ESTs to the proximal region of chromosome 9. The expression pattern of those mapped genes and ESTs was established by Northern blot analysis. In the process of refining the HIBM interval, thirteen new polymorphic markers were identified, of which 11 are CA-repeats, and 2 are single nucleotide polymorphisms. Certainly, this map provides an important integration of physical and transcriptional information corresponding to chromosome 9p12-13, which is expected to facilitate the cloning and identification not only of the HIBM gene, but also other disease genes which map to this region.

P1508. Candidate gene characterization and exploration in the Xq28 critical interval toward the identification of the X linked Myopathy wih Excessive Autophagy (XMEA) gene

N. L vy[1], *P. Aubourg*[1], *D. Figarella-Branger*[2], *L. Villard*[1], *V. Des Portes*[3], *B. Chabrol*[4], *C. Navarro*[5], *B. Minassian*[6], *H. Kallimo*[7], *J. F. Pellissier*[2], *M. Fontes*[1], *.. and the ENMC European Consortium on XMEA*[8]
[1]*INSERM U491-Facult de M decine de la Timone; Marseille, France;* [2]*Laboratoire de Biopathologie nerveuse et musculaire; Marseille, France;* [3]*Service de Neurop diatrie-Hop. St Vincent de Paul; Paris, France;* [4]*Service de Neuropediatrie-Hopital Timone; Marseille, France;* [5]*Servicio deAnatomia patologica-Hospital de Meixoeiro; Vigo, Spain;* [6]*Division of Neurology, The Hospital for Sick Children; Toronto, Canada;* [7]*Department of pathology-Turku university Hospital; Turku, Finland;* [8]*European Neuromuscular Center; Nardeen, The Netherlands*
nicolevy@pacwan.fr
X-linked myopathy with excessive autophagy (XMEA) is an unusual neuromuscular disorder which we recently assigned to chromosome Xq28 by genetic linkage analysis. Biological and pathological studies evidence high levels of Creatine Kinase associated to an excessive number of autophagic vacuoles. By a collaborative network of neurologists, neuropathologists and geneticists, we have collected and phenotypically explored 12 XMEA families. According to the pathological homogeneity observed after muscle biopsy and immunohistochemistry experiments performed in affected males, this disorder appears to segregate as a monogenic trait. We thus embarked in a positional cloning project by choosing a candidate gene approach. To date, more than 20 genes have been either direcly evaluated for potential disease causing mutations or their structure and expression pattern has been first characterized before searching for mutations. In order to improve such approaches we now use large scale mutation screening apparatus as well as region specific expression microarray to characterize a specific muscle expression pattern correlated to this pathology. We will present phenotypic, immunohistochemical, and genetics data related to XMEA.

P1509. Functional importance of an A to G transition on position -4 of the acceptor splice site in intron 3 of the MTM1 gene

B. Halliger-Keller[1], *R. Bittner*[2], *W. Kress*[1]
[1]*Institute of Human Genetics; Wuerzburg, Germany;* [2]*Institute of Anatomy; Vienna, Austria*
Halliger-Keller@mail.uni-wuerzburg.de
In a boy suffering from severe congenital hypotonia and generalized muscle weakness the muscle histology favoured the diagnosis of a severe form of Myotubular Myopathy. The muscle biopsy showed small rounded muscle fibres with centrally located nuclei surrounded by a halo. Congenital Myotonic Dystrophy was excluded by detecting a CTG repeat number in the normal range on chromosome 19. All 15 exons of the Myotubularin (MTM1; MIM #310400) gene were checked by SSC analysis for sequence variations. Exon 4 showed an aberrant pattern of bands. Direct sequencing revealed a transition of A(194 - 4)G at the splice acceptor site of intron 3. We could not find this sequence variation in more than hundred control chromosomes. After isolation of RNA from the patient s fibroblasts a region between exon 3 and 5 was amplified by RT-PCR. A fragment of normal length containing exon 4 and a short one lacking exactly exon 4 could be produced in about equal amounts. This was confirmed by direct sequencing of the two bands. The RNA lacking exon 4 is out of frame and would lead to a non-functional protein. Although we do not have the exact amount of alternative splicing in muscle tissue, we postulate that the severe form of Myotubular Myopathy in this boy is caused by the described new unusual splice mutation in the Myotubularin gene.

P1510. The Molecular analysis of trinucleotide expansion in Myotonic Dystrophy in Iranian Patients

K. Kahrizi, *S. M. E. Mousavi, S. S. Hosseini Amini, H. Najmabadi*
University of Welfare and Rehabilitation; Tehran, Islamic Republic of Iran
maryamneishabury@yahoo.co.uk
Myotonic dystrophy (DM) is the most common form of adult muscle dystrophies with an estimated prevalence of about 1 in 8000. DM has polysystemic manifestations, including muscle weakness and myotonia. It has pattern of Trinucleotide (CTG) repeats, in 3 untranslated region of the serine-Theronine protein kinase gene located on chromosome 19q13.3. Due to similarity in clinical picture with the other dystrophic patients and the lack of diagnosis methods, we decided to establish moleculr analysis for DM gene. A combination of PCR and southern analysis technique was used. PCR products were visualised by silver staining. DNA probe was labeled by digoxigenin and southern analysis was performed. 60 cases were examined. DM gene mutation was detected in 30% of the cases.

P1511. Identification and characterization of candidate genes for the juvenile amyotrophic lateral sclerosis (ALS2) at human chromosome 2q33-q34

S. Hadano[1], *Y. Yanagisawa*[2], *N. Miyamoto*[2], *J. Showguchi-Miyata*[1], *R. Singaraja*[3], *S. W. Scherer*[4], *G. A. Rouleau*[5], *M. R. Hayden*[3], *J. Ikeda*[1]
[1]*The Institute of Medical Sciences, Tokai University; Kanagawa, Japan;* [2]*NeuroGenes, ICORP/JST; Kanagawa, Japan;* [3]*CMMT, University of British Columbia; Vancouver, BC Canada;* [4]*The Hospital of Sick Children; Toronto, ON Canada;* [5]*Centre for Research in Neuroscience, McGill University; Montreal, PQ Canada*
shinji@nga.med.u-tokai.ac.jp
Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that manifests as selective upper and lower motor neuron degeneration. The autosomal recessive form of juvenile ALS (ALS2) has previously been mapped to the 1.7 cM interval flanked by D2S116 and D2S2237 spanning approximately 3 Mb region on human chromosome 2q33-q34. To understand the molecular mechanism for the pathogenesis of ALS2, identification of the gene and mutation that links to ALS2 is essential. To identify the transcribed DNA sequences mapped within the candidate region, we searched the public databases in GDB, Gene map 99 of the Human genome and UniGene at NCBI. Further, genomic DNA sequences derived from public database were used as quarries to detect ESTs. So far, 42 putative non-overlapping transcriptional units were assigned to this interval. Those units contained 27 full-length transcriptional units. Fifteen of 27 genes were known (KIAA0005, CLK1, ORC2L, NDUFB3, CFLAR, CASP10, CASP8, FZD7, NOP5, UBL1, BMPR2, AIP-1, CD28, CTLA4, and AILIM) and 12 genes were novel (CALS-109, ALS2CR1, ALS2CR2, ALS2CR3, CALS-69, CALS-117, CALS-370, CALS-124, CALS-139, CALS-135, CALS-79, and CALS-156). Complete genomic organizations of 23 genes out of 27 full-length transcripts were defined by the computational analysis of cDNA and genomic DNA sequences. In addition, a total of 396 exons was detected in 42 transcriptional units. We analyzed the expression of transcripts by RT-PCR on total RNA extracted from normal human brain, spinal cord and lymphocyte. So far, 32 of 42 units have been analyzed for expression, and 31 of 32 units were expressed at least in the brain or spinal cord. Three single nucleotide polymorphisms (SNPs) that were associated with ALS2 were also identified in the intronic regions of CALS-139 and CALS-156. Further analyses of the candidate genes represented by these units in the ALS2 patients are being performed to explore the genetic defect of ALS2.

P1512. Molecular Investigations in Hereditary Spastic Paraplegias. An Italian study.

F. M. Santorelli[1], *C. Patrono*[1], *A. Tessa*[1], *A. Filla*[2], *A. Federico*[3], *A. Nuti*[4], *G. Siciliano*[5], *A. Malandrini*[3], *C. Casali*[6]
[1]*Bambino Ges ; Rome, Italy;* [2]*Inst. Neurology; Napoli, Italy;* [3]*Inst. Neurology; Siena, Italy;* [4]*Neurology; Viareggio, Italy;* [5]*Inst. Neurology; Pisa, Italy;* [6]*Inst. Neurology; Rome, Italy*
fms3@na.flashnet.it
Hereditary spastic paraplegias are characterized by progressive weakness and spasticity of the lower limbs due to degeneration of corticospinal axons. Genetic heterogeneity is suggested by different modes of transmission (autosomal dominant, recessive, and X-linked). Recently two genes, responsible for an autosomal recessive (SPG7) and a dominant (SPG4) form have been cloned. MtDNA deleteions have been found in one AD family. We investigated 14 AD and 9 AR HSP families. Clinical features,

neuroimaging findings and neurophysiological data have been reviewed. Molecular chatacterization included SPG4 and SPG7 genes, and mtDNA. Both pure and complicated forms have been recognized in our series. Optic neuropathy, retinopathy, dementia, ataxia, and deafness were found in the complicated forms. MRI disclosed a thin corpus callosum in two recessive patients who were not linked to chromosome 15q. We did not detect SPG7 mutations in AR-HSP families. Conversely, we found four SPG4 mutations in pure AD-HSP. Our data reinforce the notion of the the ample variability in the presentation of HSP and confirm the relative rarity of the SPG7-associated form. Also, we confirm that SPG4 mutations are frequent in pure dominant HSP but account for about 30% of the forms. Thorough clinical and molecular studies are needed to address genotype-phenotype correlations in HSP.

P1513. Dentatorubral-pallidoluysian Atrophy in Chinese

B. Soong[1], *I. Lee*[2]
[1]*National Yang-Ming University School of Medicine; Taipei, Taiwan Republic of China;* [2]*Taipei-Veterans General Hospital; Taipei, Taiwan Republic of China*
bwsoong@vghtpe.gov.tw

Dentatorubral-pallidoluysian atrophy (DRPLA) is a neurodegenerative disorder with characteristic neuropathologic findings of combined degeneration of both the dentatorubral and pallidoluysian systems of the central nervous system. The clinical symptoms are diverse with varying combinations of myoclonus, epilepsy, ataxia, choreoathetosis, and dementia. The prevalence of DRPLA in Japan was estimated as two to four per million, a rate that is similar to that of Huntington disease in Japan. Outside Japan, DRPLA remains rare with previous reports of only eight Caucasian kindreds, including one African American family with Haw River syndrome. The reason for different racial prevalence of DRPLA is unclear. A molecular defect in patients with DRPLA, identified in 1994, lies in the expansion of an unstable (CAG)n repeat in the DRPLA gene on chromosome 12p. Despite the demonstration of a common molecular defect in DRPLA patients, clinical and neuropathological differences have been reported among DRPLA patients, even within a family. There are also different ranges of normal CAG repeat lengths in different ethnic groups. Herein we describe the clinical, radiological and genetic features in the first reported Chinese DRPLA family, and compare the distribution of CAG repeat lengths at the DRPLA locus in normal Chinese with that of other ethnic groups.Our study indicated that although the DRPLA allele size is distributed similarly in Chinese and Japanese, DRPLA in Chinese is rare. So far, only one intermediate-sized allele containing more than 30 CAG repeats has been reported in Chinese. The ethnic prevalence of DRPLA seems to be better correlated with the prevalence of the intermediate-sized alleles in individual populations.

P1514. Genotype-Phenotype Correlation in SCA 1; Evidence for intermediate alleles

C. Z hlke[1], *A. Dalski*[1], *Y. Hellenbroich*[1], *K. B rk*[2], *E. Schwinger*[1]
[1]*Institut f r Humangenetik; L BECK, Germany;* [2]*Klinik f r Neurologie; T bingen, Germany*
zuehlke@medinf.mu-luebeck.de

Spinocerebellar ataxia type 1 (SCA 1 [OMIM 164400]) is an autosomal dominantly inherited progressive neurodegenerative disease that primerily affects cerebellar Purkinje cells, spinocerebellar tracts and pontine nuclei. The disease results from the expansion of a polyglutamine stretch within the SCA 1 protein (ataxin 1). The underlying mutation has been shown to be an unstable CAG trinucleotide repeat present within the coding region of the gene. Normal alleles range from 6 to 39 units. They are characterised by interruptions of 1 to 3 CAT trinucleotides that are thought to be involved in the stability of the trinucleotide stretch. Cerebeller ataxia has been reported in patients carrying 40 to 81 trinucleotides. Interestingly, these pathologic alleles miss any CAT interruptions. We analysed the repeat length and composition of 16 individuals with alleles between 36 and 41 triplets for genotype-phenotype correlations and have found the 39 trinucleotide-allele to be either interrupted by CAT repeats or formed by a pure CAG stretch. Preliminary correlation studies yield evidence for the existence of intermediate alleles in SCA 1.

P1515. Recruitment of Chaperones and Proteasome subunits into neuronal intranuclear inclusion bodies in SCA3

T. Schmidt[1], *K. S. Lindenberg*[2], *A. Krebs*[2], *L. Sch ls*[3], *F. Laccone*[4], *J. Herms*[5], *O. Riess*[1], *B. G. Landwehrmeyer*[2]
[1]*Medical Genetics, University Rostock; Rostock, Germany;* [2]*Department of Neurology, University Ulm; Ulm, Germany;* [3]*Department of Neurology, St. Josef s Hospital, Ruhr-University Bochum; Bochum, Germany;* [4]*Institute of Human Genetics, University G ttingen; G ttingen, Germany;* [5]*Department of Pathology, University G ttingen; G ttingen, Germany*
Schmidt.Thorsten@gmx.de

Spinocerebellar Ataxia Type 3 (SCA3) or Machado-Joseph-Disease (MJD) is an autosomal dominantly inherited neurodegenerative disorder cause by the expansion of a polyglutamine tract in the ataxin-3 protein. A neuropathological hallmark of SCA 3 are intracellular aggregates forming neuronal intranuclear inclusions (NII). To characterize cellular responses to nuclear aggregates in more detail, we analysed nuclear inclusions in human postmortem brain of patients affected by SCA3 and controls immunohistochemically using a panel of antibodies directed against chaperones and proteasome subunits. About 20% of the nuclear inclusions in pontine neurons stained positively for the chaperone Hsp90?, whereas no staining by antibodies to Hsp27, Hsp60, and Hsp/Hsc70 was observed. Most nuclear inclusions in SCA3 are ubiquitin-positive (75%) suggesting degradation by ubiquitin-dependent proteasome pathways. Surprisingly only a minority of the inclusions were immunopositive with antibodies directed against subunits of the 20S proteolytic core of proteasomes, whereas most inclusions were stained by antibodies directed against the 19S regulatory subunits thought to recognize, bind and unfold ubiquitinated proteins. In addition, most NII were immunoreactive with 11S subunits antibodies. A comparison with normal control brains indicates more intense cytoplasmatic staining and less intense nuclear signals in SCA3 neurons suggesting a redistribution of proteasomes. The high proportion of NII stained with proteosome subunits 11S and 19S stands in marked contrast to the small percentage of NII immunostained with the 20S antibodies used. These results suggest that most neurons containing NII recruit proteasome subunits to NII possible in an attempt to refold mutant ataxin-3 and to dissolve nuclear aggregates.

P1516. Molecular Analysis Of Iranian Patients With Huntington's Disease

F. Hormozian[1,2], *M. Houshmand*[1], *R. Ghiasvand*[3], *M. Sanati*[1]
[1]*National Research Centre of Genetic Engineering & Biotechnology; Tehran, Islamic Republic of Iran;* [2]*Azad University of Tehran; Tehran, Islamic Republic of Iran;* [3]*Organization for Welfare & Rehabilitation of Tehran; Tehran, Islamic Republic of Iran*
fabi_123@yahoo.com

HD is a progressive neurodegenerative disease of midlife onset,inherited in an autosomal dominant manner.The clinical picture is characterized by involuntary movement , psychiatric changes,intellectual and cognetive decline and weight loss. The symptoms apear to be caused by marked neuronal death, most notably in the caudate nucleus and putamen. The responsible mutation is an expansion of a CAG trinucleotide repeat,located at 4p16.3 gene named IT15. The repeat is polymorphic and varying between 8 and 36 units in normal chromosomes,but is expanded to at least 37 copies on HD. Here we have studied the number of CAG repeats in 25 individuals from 7 Iranian families affected with HD. The number of (CAG)n repeats in HD chromosomes varied from 41 to 58 ,while the range for the normal chromosomes was from 12 to 24 repeats.In three of these cases the transmission was paternal and anticipation was obsereved in one family.

P1517. Homozygous familial hypercholesterolaemia; Multiple founder mutations underlie phenotypic variation in the South African population

S. Jansen[1], *M. Callis*[1], *R. Thiart*[2], *M. J. Kotze*[2]
[1]*University of the Free State; Bloemfontein, South Africa;* [2]*University of Stellenbosch; Stellenbosch, South Africa*
gnmgmc@med.uovs.ac.za

Objective. Predominance of three low density lipoprotein receptor (LDLR) gene mutations in South African Afrikaner patients with familial hypercholesterolaemia (FH) significantly enhances the diagnostic prospects of the disease in this population. We studied the degree of genetic heterogeneity in Afrikaner FH homozygotes in relation to phenotypic expression of the disease. Methods. Polymerase chain reaction (PCR)-based methods were used to screen FH homozygotes without (or with only one copy of) the three founder-related mutations, D154N, D206E and V408M, for disease-related LDLR gene mutations. Afrikaner FH homozygotes with these mutations known to cause a receptor-defective or -negative phenotype, were grouped according to genotype for comparative analysis. Results. Mutations W66G, D200G (heterozygous state) and S285L (homozygous state) were identified in three of the four Afrikaner FH homozygotes subjected to mutation analysis. Significantly higher mean total- (P<0.008) and LDL-cholesterol (P<0.015) levels were demonstrated in 24 FH homozygotes with at least one copy of the receptor-negative mutation V408M, compared with 23 patients with receptor-defective LDLR gene mutations D154N and/or D206E. Conclusions. Mutations D200G and S258L may represent minor

founder events, since these mutations emanating from Europe have previously been detected in eight Afrikaner FH heterozygotes. Inclusion of these mutations in routine DNA screening would facilitate an improved diagnostic service for FH in the genetically homogeneous Afrikaner population.

P1518. Genetic Studies of Oculodentodigital Dysplasia

S. A. Boyadjiev[1], *R. E. Shapiro*[2], *A. Wandstrat*[3], *A. B. Chowdry*[1], *J. W. Choi*[1], *M. LaBuda*[4], *M. Lovett*[5], *C. Burgess*[6], *M. Schalling*[7], *E. W. Jabs*[1]
[1]*Institute of Genetic Medicine, Johns Hopkins University; Baltimore, MD United States;* [2]*Department of Neurology, University of Vermont; Burlington, VT United States;* [3]*Department of Immunology, University of Texas, SW Med. Ctr.; Dallas, TX United States;* [4]*Department of Pediatrics, Johns Hopkins University; Baltimore, MD United States;* [5]*Washington Univ. School of Medicine; St. Louis, MO United States;* [6]*CuraGen Corporation; New Haven, CT United States;* [7]*Neurogenetics Unit, Karolinska Hospital; Stockholm, Sweden*
sboyd@mail.jhmi.edu

Oculodentodigital dysplasia (ODDD, MIM 164200) is an autosomal dominant condition of dysmorphogenesis and neurodegeneration. The physical characteristics include eye defects, craniofacial anomalies, dental anomalies, hand and foot malformations and neurologic deficits. The clinical and radiological findings appear to define a slowly progressive leukodystrophy and the possibility of genetic anticipation. We have refined the location of the ODDD gene on chromosome 6q22 between markers D6S266 and D6S1639, an interval of less than 3 cM. In an effort to clone the ODDD gene we have developed a YAC based physical map of the 6q22 region. We have characterized 37 YAC clones that form a complete contig of the candidate region with at least two-fold redundancy for each marker. We have identified the known genes assigned to the candidate region and have refined their map positions. Several genes were considered as possible ODDD candidates and were analyzed by sequencing and Southern Blot analysis. No mutations were identified in HSF2, HEY2, PLN, COL10A1 and GRIK2 genes. More genes are in process of being evaluated as candidates. Repeat extension detection analysis did not detect large CAG repeat expansions as a cause for ODDD. Direct cDNA selection was performed using eight YAC clones from the ODDD region and 800 non-repetitive clones were isolated. We have analyzed 270 clones by sequencing and computational methods and found many to have large open reading frames and no homologies to known genes and/or ESTs. We are in the process of further characterizing these clones that represent putative novel genes.

P1519. The origin of the Slovak alkaptonuria (AKU) mutations

A. Zatkova[1], *D. Beltran Valero de Bernabe*[2], *H. Polakova*[1], *E. Ferakova*[3], *V. Ferak*[3], *L. Kadasi*[1], *S. Rodriguez de Cordoba*[2]
[1]*Institute of Molecular Physiology and Genetics; Bratislava, Slovakia;* [2]*Unidad de Patologia Molecular and Unidad de Investigacion, Fundacion Jimenez Diaz; Madrid, Spain;* [3]*Department of Molecular Biology, Comenius University; Bratislava, Slovakia*
zatkova@fns.uniba.sk

Alkaptonuria (AKU) is an autosomal recessive disorder caused by the deficiency of homogentisate 1,2 dioxygenase (HGO) activity. The disease is characterized by homogentisic aciduria, ochronosis and ochronotic arthritis. AKU shows a very low prevalence (1;100,000-250,000) in most ethnic groups. In Slovakia, however, the incidence of this disorder raises up to 1;19000, that is difficult to explain by a classical founder effect since ten different AKU mutations have been identified in this relatively small country. We have determined the allelic associations of eleven HGO intragenic polymorphisms for 44 AKU chromosomes from 20 Slovak pedigrees. These data were compared to the HGO haplotypes for all AKU chromosomes carrying corresponding mutations characterized thus far from non-Slovak patients. The results show that the mutations P230S, V 300G, R58fs and IVS1-1G->A are shared by different populations and were probably introduced to Slovakia with the founder populations that spread throughout Europe. However, these mutations represent only 16% of the Slovak AKU chromosomes. On the other hand, six of the ten Slovak mutations, including the prevalent G152fs, G161R, G270R and P370fs, most likely originated at a single and very small geographical location in the northwestern part of Slovakia. Based on the evidence that all six Slovak AKU mutations are associated to HGO mutational hot spots, we suggest that an increased mutation rate at the HGO gene is responsible for the clustering of AKU mutations in such a small geographical region.

P1520. Corneal dystrophy and perceptive deafness (Harboyan syndrome) ; report of a new family and homozygosity mapping

M. J. Abramowicz[1], *J. Albuquerque-Silva*[2], *A. Zanen*[1]
[1]*Hopital Erasme, Free University of Brussels (ULB); Brussels, Belgium;* [2]*Free University of Brussels (ULB); Brussels, Belgium*
marcabra@ulb.ac.be

A syndrome of congenital corneal dystrophy and teenage-onset perceptive deafness has been reported in three sibships, one of which with consanguineous parents, consistent with autosomal recessive inheritance. It is referred to as Harboyan syndrome (MIM 217400). The corneal disorder in Harboyan syndrome is a congenital hereditary endothelial dystrophy (CHED). We report a previously undescribed family with Harboyan syndrome. The proband is the sixth of a sibship of nine, where four girls and one boy are affected. The parents are second cousins and unaffected. Genome-wide homozygosity mapping using 358 microsatellite markers showed 8 markers from 8 chromosome regions that were homozygous in DNA pooled from the patients while heterozygous in DNA pooled from the parents and unaffected siblings. Further study of individual DNA samples with closely spaced markers tentatively identified a segment at 20pter-p13 for which the affected sibling are homozygous by descent for a haplotype present in both parents. This preliminary result indicates the presence of a gene at 20pter-p13 whose mutation causes both corneal dystrophy and hearing loss. Of note, a gene for nonsyndromic autosomal recessive CHED, CHED2 (MIM 217700), was recently mapped to 20pter-p13. This suggests that Harboyan syndrome and CHED2 may be allelic, with some mutations associated with hearing loss. Interestingly, no locus was previously reported on 20p for recessive hearing loss, in spite of the unparalleled genetic heterogeneity thereof.

P1521. The elucidation of the molecular genetic causes of M bius syndrome.

B. van der Zwaag[1], *H. van Bokhoven*[1], *H. Verzijl*[1], *B. van den Helm*[1], *H. Kremer*[1], *A. Hellemons*[2], *P. Burbach*[2], *H. Brunner*[1], *G. Padberg*[1]
[1]*UMC Nijmegen; Nijmegen, The Netherlands;* [2]*Rudolf Magnus Institute; Utrecht, The Netherlands*
A.vanderzwaag@czzorlnm.azn.nl

M bius syndrome (MBS, MIM 157900) consists of a congenital paralysis of the VIIth (facial) cranial nerve, frequently accompanied by dysfunction of other cranial nerves. In addition, orofacial and limb malformations, defects of the musculoskeletal system, and mental retardation are seen in MBS patients. In our hospital two new loci were revealed for MBS, indicating genetic heterogeneity for this syndrome. The MBS2 locus was linked to chromosome 3q21-q22, between markers D3S1589 and ACPP, spanning 4.9 cM. The MBS3 locus was linked to chromosome 10q21.3-q22.1, between markers D10S581 and D10S502, spanning 3.7 cM. Several interesting candidate genes were selected, based on expression pattern and/or known function and analysed by SSCP or direct sequencing. CRBP1, SOX14, EGR-2 and hPGT have been screened, but no mutations were found in these genes that could account for the M bius phenotype. For a positional candidate gene approach (partial) YAC contigs have been constructed for both loci and several interesting EST s and genes have been mapped to these contigs, prior to further examination. RNA in situ hybridisation in mouse embryos was performed to determine the spatio-temporal expression pattern of positional candidate genes from the 3q21-q22 critical region. The in situ hybridisation for one paticularly promising candidate gene revealed expression in the ganglia as well as in other cell types. This expression pattern is compatible with MBS as the affected facial nerve emanates from the VIIth ganglion. Mutation analysis is being performed on this gene, as well as on several other candidates to proof their involvement in MBS.

P1522. Molecular analysis of the Duane Syndrome region on chromosome 8q

A. Pizzuti[1], *G. Calabrese*[2], *M. Bozzali*[3], *L. Telvi*[4], *A. Ion*[4], *L. Stuppia*[2], *G. Palka*[2], *B. Dallapiccola*[1]
[1]*Universit di Roma; Roma, Italy;* [2]*Universit di Chieti; Chieti, Italy;* [3]*Ospedale Policlinico; Milano, Italy;* [4]*Hopital S. Vincent De Paul; Paris, France*
antpizzuti@libero.it

Duane syndrome (DS) is characterized by primary strabism, globe retraction and narrowing of palpebral fissure, associated to variable sight impairment. DS is mostly sporadic. However, some familial cases have been reported with linkage to chromosome 2. DS has also been related to a contigous gene syndrome with deletions on chromosome 8q12-13. We previously identified a 3 cM smallest region of overlap (SRO) for DS between markers D8S533 and D8S1767 in a DS patient with a microdeletion. Then

we investigated a DS patient with gonadal dysgenesis, heterozygous for a translocation involving chromosome 8q13. STS and FISH analysis revealed the break-point position within the previously identified SRO, covered by YAC 820e6. Two cosmid subclones from YAC 820e6 were found spanning the breakpoint on chromosome 8q. In the breakpoint-surrounding region a combination of sequencing, data base search and hybridization experiments identified sequences similar to a few ESTs, none of which related to known genes. Most of them have been better characterized as well as the structure and position of the related genes. We positioned the chromosomal break in the second intron of one of these genes, which is therefore interrupted by the translocation. Expression of the interrupted gene is almost ubiquitous, with high levels in muscle, as defined by Northern blot analysis. However its real function and the possible mechanisms involved in the disease process remain unsettled

P1523. DNA Repair Foci in Fanconi anaemia cells

M. Digweed, *I. Demuth, K. Sperling*
Institut f r Humangenetik; Berlin, Germany
martin.digweed@charite.de

The characteristic sensitivity of Fanconi anaemia cells is towards crosslinking agents, however, there have been several reports of a deficiency in FA cells in the repair of double strand breaks (DSBs), lesions more usually associated with ionising radiation. Some reports have indicated subtle differences in DSB repair kinetics whilst other analyses suggest a compromise in the fidelity of DSB-repair. Similarly, Nijmegen breakage syndrome (NBS) is characterised by radiosensitivity but there are also numerous reports of increased sensitivity towards the lesions made by crosslinkers. These observations suggest an overlap in the repair mechanisms for crosslinks and for DSBs. The gene responsible for NBS was recently cloned and its protein product, nibrin, has been extensively analysed. Nibrin is found in the cell as a nuclear complex with the proteins hMre11 and hRad50. It is phosphorylated by ATM, the gene mutated in Ataxia teleangiectasia, in response to ionising radiation and mediates the relocalisation of the repair complex to discrete nuclear foci (ionising radiation induced foci, IRIF) visible by immunostaining with antibodies to hMre11, nibrin or hRad50. IRIF are the sites of DSB repair by non-homologous end joining which requires the nuclease activity of hMre11/hRad50. We have examined the occurrence of IRIF stained for Mre11, Rad50 and/or nibrin in FA primary fibroblasts and find no gross disturbances of this pathway in any of the 7 FA complementation groups. Transformation of primary fibroblasts by SV40 T-Antigen leads to a significant reduction in IRIF in all cells tested, possibly due to the interaction of T-antigen with one or more members of the complex. This effect is particularly strong in cells from one of the FA complementation groups, FA-D2, and leads to almost complete abrogation of IRIF formation. This suggests, that the hMre11/hRad50/nibrin pathway in these cells is far more readily disturbed by T-antigen than it is in control cells or cells from complementation groups FA-E and FA-G. Since nibrin is normally phosphorylated after irradiation in FA-D2 cells, the disturbance in foci formation must lie downstream of this event. These findings are discussed in reference to the cellular DSB repair machinery and its involvement in the processing of intermediates formed during crosslink repair.

P1524. OPA1 Gene Mutations Cluster in Functional Protein Domains in ADOA patients and Reveal a Founder Allele in the Danish Population.

D. L. Thiselton[1], *C. Alexander*[1], *S. Brooks*[1], *T. Rosenberg*[2], *D. Mackey*[3], *S. Andreasson*[4], *N. Van Regemorter*[5], *A. T. Moore*[6], *S. S. Bhattacharya*[1], *M. Votruba*[7]
[1]*Institute of Ophthalmology; London, United Kingdom;* [2]*National Eye Clinic; Hellerup, Denmark;* [3]*Royal Victorian Eye and Ear Hospital; Melbourne, Australia;* [4]*University Hospital; Lund, Sweden;* [5]*Hopital Erasme; Brussels, Belgium;* [6]*Addenbrooke s Hospital; Cambridge, United Kingdom;* [7]*Institute of Ophthalmology and Moorfields Eye Hospital; London, United Kingdom*
dthiselt@hgmp.mrc.ac.uk

Autosomal dominant optic atrophy (ADOA) is a hereditary optic neuropathy with estimated prevalence 1;12,000 (Denmark) to 1;50,000 (USA). The predominant genetic locus (OPA1; MIM 165500) resides at 3q28-29. The OPA1 gene has recently been shown to encode a dynamin-related GTPase targetted to mitochondria. We have screened a large panel of ADOA families to seek disease-related changes in OPA1 and genotype-phenotype correlations. Coding exons of OPA1 and splice sites were screened in >100 ADOA patients of diverse ethnic origin by SSCP, heteroduplex analysis and direct sequencing. Clinical data on visual acuity, colour vision, visual fields and optic disc appearance was collated. Putative mutations were tested for familial segregation by SSCP/restriction enzyme site alteration and in 100 control chromosomes. To date we have found 13 novel disease mutations and 19 new polymorphisms in OPA1. Mutations occur throughout the gene coding region, with three clusters emerging; in the mitochondrial leader, the conserved GTP-binding domain and the -COOH terminus. A frameshift in exon 28 resulting in 22 novel terminal amino acids is present in 14 unrelated Danish families and appears to represent a founder allele. Mutation clustering in the mitochondrial leader and GTPase domain of OPA1 corroborate their functional significance. No specific function has yet been ascribed to the -COOH terminus of OPA1, although bioinformatic analysis indicates a role in protein-protein interaction. A mutation cluster in this region highlights its physiological importance and may account for the higher Danish prevalence of ADOA. Preliminary data implicate other genetic or environmental factors in the ADOA phenotype.

P1525. Large fragile X premutation alleles may often contain two AGG interruptions

S. L. Nolin, *G. E. Houck, A. D. Gargano, W. T. Brown*
NYS Institute for Basic Research; Staten Island, NY United States
snolin@mindspring.com

Fragile X syndrome, a common cause of inherited mental retardation, is associated with a CGG repeat expansion within the FMR1 (fragile X mental retardation 1) gene. In the normal population, AGG triplets are usually present at regular intervals within the repeat region, most commonly at positions 10 and 20. AGG interruptions are thought to stabilize the region by preventing slippage during DNA replication. In families with fragile X, however, a different pattern of AGG interruptions has been observed. Several groups have used a variety of techniques to analyze the repeat organization in fragile X premutation males and observed either a single or no AGG interruption in the 5 end of the repeat and long tracts of uninterrupted CGG repeats in the 3 end. We have developed a procedure for sequencing the repeat that allowed us to examine the alleles in 8 premutation males with repeats ranging from 95 to 150. In contrast to the results of earlier studies, sequence analysis revealed that although one male had no AGG and another had one AGG, five had two AGG interruptions in the 5 end of the repeat. This suggests that two AGG interruptions may be a common finding in males with large premutation alleles. If this proves to be the case in males, two AGG interruptions may also be observed in some premutation females, particularly in those whose offspring seem less likely to undergo expansion to a full mutation.

P1526. Southern Blot Analysis Of Fragile X Syndrome In Chilean Families.

A. Alliende[1], *A. Valiente*[1], *B. Curotto*[1], *C. Rojas*[1], *T. Aravena*[2], *L. Santa Mar a*[1], *F. Cort s*[1]
[1]*INTA University of Chile; Santiago, Chile;* [2]*Clinical Hospital University of Chile; Santiago, Chile*
malliend@uec.inta.uchile.cl

Fragile X syndrome is the most common inheritable disease that accounts for mental retardation in 1;4000 males and 1;6000 females worldwide. The disease is caused by the expansion of a CGG repeat in the first exon of the FMR1 gene and the subsequent hypermethylation of the FMR1 promoter. By means of Southern blot analysis of EcoRI/EagI digested gDNA we determined the length of the CGG repeat segment and the methylation status of the FMR1 gene in 115 probands (105 males and 10 females) with mental retardation of unknown origin, 12 obligate carriers and 27 sibs. All 115 probands included in this analysis scored above 16 points according to Butler check list. 23 of them (20%) beared either full mutations (20), mosaic (2) or partially methylated full mutation (1). The high percentage of full mutations may result from an ascertainment bias, or an unusual high frequency of the disease in the Chilean population. In 12 carrier mothers with premutated allele (CGG)89-165 and full mutation, the recurrence risk of having an offspring with full mutation was 10/20 (50%) and 6/7 (86%), respectively. This is a relative lower risk of expansion even for large premutations. The absence of carriers with intermediate number of repeats is indicative of underdiagnosis and points out to the need for a fragile X screening program in schools for children with learning disabilities in Chile.

P1527. Identification and characterization of a candidate gene for cerebral cavernous malformation (CCM2)

J. S. Herbrick[1], *D. Verlaan*[2], *M. Li*[1], *J. Grover*[1], *G. Rouleau*[2], *S. W. Scherer*[1]
[1]*The Hospital for Sick Children; Toronto, ON Canada;* [2]*Montreal General Hospital Research Institute; Montreal, PQ Canada*
joanne@genet.sickkids.on.ca

Stroke is a leading cause of death and neurological disability in adults. Cerebral cavernous malformation (CCM) is a disease characterized by hemorrhaegic lesions which affect the brain leading to stroke. CCM, an autosomal dominant trait with incomplete penetrance, is relatively common

presenting in 0.5% of the general population. To date, three genetic loci for CCM have been identified. CCM1 is caused by mutations in the KRIT1 gene at 7q21 and it encodes a binding protein for Krev-1/rap1a, a putative tumor suppressor. CCM2 and CCM3 have been localized to chromosomes 7p13-p15 and 3q25.2-27, respectively, but the causative genes are still unknown. A CCM patient was identified having a familial inversion on chromosome 7 [46, XY, inv(7)(p15, q36)]. Using fluorescence in situ hybridization (FISH) mapping, the p15 breakpoint of the inversion could be localized within a yeast artificial chromosome clone that mapped to the critical region at 7p13-p15 defined by family studies. Further FISH mapping led to the identification of a bacterial artificial chromosome clone spanning the 7p15 inversion breakpoint. Through genomic DNA sequence analysis and cDNA characterization a gene was found that was directly interrupted by the inversion. The gene was determined to be expressed as a major transcript of 2.3 kb in all tissues examined and additional alternative spliced transcripts have also been characterized. We are currently screening DNA from CCM2 families as well as sporadic patients for mutations to prove this new gene is involved in the pathogenesis of cerebral cavernous malformation.

P1528. Pseudoxanthoma Elasticum ; evidence for the existence of a pseudogene highly homologous to the ABCC6 gene.

D. P. Germain
Hopital Europeen Georges Pompidou; Paris, France
dominique.germain@hop.egp.ap-hop-paris.fr

Pseudoxanthoma elasticum (PXE) is an inherited disorder of connective tissue, affecting essentially but not exclusively the elastic network, and resulting in skin lesions, decreased vision, and vascular symptoms, with highly variable phenotypic expression. We have recently identified mutations in the ABCC6 gene, which encodes a transmembrane protein member of the ATP binding cassette subfamily C, as the genetic defect responsible for PXE. The ABCC6 gene consists of 31 exons, spanning 75 kb of DNA on chromosome 16p13.1. Here, we report on the finding of a pseudogene that is highly homologous to ABCC6. During a mutational screening of the ABCC6 gene, we identified sequence changes which, although predicted to be truncating mutations, were detected not only in PXE patients but also in controls. Among them, a single nucleotide insertion inducing a frameshift, and a nonsense mutation were consistently detected at the heterozygous state in our seven PXE patients and all controls (n = 82). This indicates that the PCR products were being amplified from four rather than from two genomic copies. Molecular studies at the mRNA level demonstrated that these changes emanate from at least one non-expressed pseudogene, highly homologous to the ABCC6 gene. Our results emphasize that, due to these homologies with both exonic and intronic sequences of ABCC6, extreme care must be taken not to confuse variants in the pseudogene with mutations in the active gene, when genotyping patients.

P1529. Molecular pathology of EXT genes on patients with multiple hereditary exostoses and sporadic chondrosarcoma.

N. P. Bochkov[1], *M. Nemtsova*[2], *G. Chesnokova*[2], *V. Strelnikov*[2], *D. Zaletayev*[2]
[1]*Moscow Medical Academy; Moscow, Russian Federation;* [2]*Research Centre for Medical Genetics; Moscow, Russian Federation*
nik.bochkov@mtu-net.ru

Hereditary multiple exostoses (EXT) is an autosomal dominant disorder with an incidence of 1;50000. EXT is characterized by the presence of cartilage-capped exostoses located in the juxtaepiphyseal regions of the long bones. EXT is a genetically heterogeneous - genes were cloned on chromosomes 8q24.1 (EXT1) and 11p12 (EXT2). The aim was to investigate the rate and spectrum of EXT genes molecular anomalies on patients with EXT. Mutations and polymorphisms screened by combination of SSCP and heteroduplex analyses. Structural anomalies were analyzed by blot-hybridization with specific DNA-probes. Among 36 families studied, 25 had sporadic and 11 had familial forms of EXT. Point mutations and structural anomalies were detected in 28 cases (78%); 20 cases (56%) in EXT1 and 8 (22%) in EXT2; 23 cases (64%) are point mutations and 5 (14%) are structural anomalies, four of them in EXT2 . EXT1 mutations; C79T, 388delAG, 456delC, 742insTT, 876-877insT, C894A, 943delGA, G967A, G1022C, G1036A, T1037C, A1285G, 1633-39delC, 2055+5insT, 2056-30insC, C2056T. EXT1 polymorphisms; C1065T, A1296G, C2170G. EXT2 mutations; 75delC, C514T, C827T, G871A. Patients with G1022C, G1036A and A1285G had secondary chondrosarcoma and loss of heterozygosity (LOH) for markers mapped to chromosome 8 in tumors. Three patients from nine with sporadic chondrosarcoma have shown structural anomalies of EXT1 (1 case) and EXT2 (2 cases). LOH for chromosome 8 markers found in 4 tumors. Point mutations of EXT genes and LOH for chromosome 11 were not found in any sporadic tumors. Methylation pattern anomalies of the EXT genes promoter region were not found in exostoses and tumors.

P1530. Childhood-onset Spinal Muscular Atrophy (SMA) with homozygous deletion of SMNc gene

M. Mukherjee[1], *S. Srivastava*[1], *I. Panigrahi*[1], *S. Pradhan*[2], *B. Mittal*[1]
[1]*Dept. of Medical Genetics, Sanjay Gandhi Post Graduate Institute of Medical Sciences; Lucknow, India;* [2]*Dept. of Neurology, SGPGIMS; Lucknow, India*
monisha30@yahoo.com

Spinal muscular atrophy (SMA) is a group of autosomal recessive neuromuscular disorders characterized by degeneration of the anterior horn cells of the spinal cord, leading to symmetrical muscle weakness and atrophy. Deletions of the SMNt gene located at chromosome 5q13 have attributed in more than 90 % of the SMA patients. In the present study, we report an unusual case of childhood-onset SMA with a homozygous deletion of SMNc gene. The patient was a 5-year-old boy with delayed motor milestones, hypotonia, muscle wasting and weakness, clinically presenting a SMA phenotype. Molecular studies using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) and Single Stranded Conformation Polymorphism (SSCP) analysis with exons 7 and 8 of the SMN gene showed the presence of SMNt but complete absence of SMNc gene in the patient. The sequence analysis of the critical region (exon 7) of the SMN gene did not show any microdeletion, duplication or point mutation in SMNt and also confirmed the homozygous deletion of the SMNc gene copies. Densitometric analysis of exons 7 and 8 also revealed the presence of normal copies of the SMNt gene in the patient. The results of our study suggest that young onset SMA may result from centromeric deletion of the SMN gene in certain subtypes of SMA. This further adds to the clinical and genetic heterogeneity of spinal muscular atrophy.

P1531. Gene Conversion Events in Adult-Onset Spinal Muscular Atrophy

R. Mazzei[1], *A. Gambardella*[1,2], *F. L. Conforti*[1], *A. M. F. Patitucci*[1], *A. Magariello*[1], *A. L. Gabriele*[1], *D. Pirritano*[2], *A. Labate*[2], *F. Bono*[2], *M. Muglia*[1]
[1]*Institute of Experimental Medicine and Biotechnology, National Research Council; Mangone (CS), Italy;* [2]*Institute of Neurology, School of Medicine; Catanzaro, Italy*
mazzei@imseb.cs.cnr.it

Spinal muscular atrophy (SMA) is a frequent autosomal recessive neurodegenerative disorder leading to proximal paralysis with muscle atrophy. The gene involved in SMA, located on the chromosome 5q13, was cloned and named the survival motor neuron (SMN) gene. SMN gene is present in telomeric (SMNT) and centromeric (SMNC) copies. Most patients with SMA carry homozygous deletions of exons 7 and/or 8 of the SMN gene. Recent studies demonstrated that the loss of the SMNt occurs by two different mechanisms; deletion or conversion of SMNt to SMNc. Although two studies, found a roughly equal distribution of conversion event in all types of SMA, recently it has been reported that the conversion event usually occurs in patients with milder SMA phenotype. Until now, however, it still remains unknown if a sequence conversion event may also be associated with SMA type IV, which represents the mildest form within the spectrum of the SMA phenotype. We present the molecular analysis of three patients with adult onset SMA, who carried an apparent homozygous deletion of SMNt exon 7 but not of exon 8. By a simple PCR-based assay, we demonstrated that in each case the apparent isolated deletion of SMNt exon 7 was a gene conversion event of SMNt to SMNc. In conclusion, our results provide first evidence that a conversion event may be also associated with adult-onset SMA phenotype, and further support the notion that a gene conversion event may be correlated with a milder SMA phenotype and a later onset of disease.

P1532. Deletion analysis of the SMN and NAIP genes in spinal muscular atrophy (SMA) patients from Ukraine.

O. Ekshyyan, *L. A. Livshits*
Institute of Molecular Biology and Genetics, National Academy of Science of Ukraine; Kiev, Ukraine
ekshyyan@excite.com

We have studied the deletions of the SMN (exons 7, 8) and NAIP (exon 5) genes in 61 SMA patients. Homozygous deletions of either one or both exons 7 and 8 of SMN1 have been demonstrated in 98,4% of SMA patients. Of 61 patients, 17 showed a homozygous deletion of NAIP exon 5 and SMN1 exons 7 and 8; 36 showed a homozygous deletion of SMN1 exons 7 and 8; and 8 have chimeric SMN2/SMN1 gene. Homozygous deletions of exon 5 of NAIP gene have been demonstrated in 53,3% of SMA type I and 5% of SMA type II patients. Chimeric SMN2/SMN1 gene

has been demonstrated in 3,3% of SMA type I, 10% of SMA type II and 45,5% of SMA type III patients. Prenatal diagnosis has been performed in 20 SMA families by means of analysis of deletion of SMN1 and NAIP genes and STR-polymorphisms. SMN1/SMN2 ratio was analyzed by the densitometry analysis (ALFexpress /Amersham-Pharmacia-Biotech/ and Ultroscan XL /LKB/) of PCR products of exon 7 of SMN genes digested by DraI. This ratio ranged from 0.4 to 2.7 for SMA carriers (n=59) and from 0.9 to 3.8 for control individuals (n=97). For all ratios from 0.4 to 1 and from 1.5 to 3.8 the significant differences between SMA carriers and individuals from control group have been observed. We suggest that such approach will be useful for population screening of SMA carriers.

P1533. A simple and rapid assay of gene-conversion in 8th exon of SMN gene

***I. Shagina**, A. Polyakov, O. Evgrafov*
Research Center for Medical Genetics; Moscow, Russian Federation
stver@online.ru

Spinal muscular atrophy (SMA) is a frequent autosomal recessive neuromuscular disorder leading to weakness and atrophy of voluntary muscles.The survival motor-neuron gene (SMN), a SMA-determining gene, is present in two highly homologous copies (telSMN and cenSMN). The mutations in telSMN are responsible for SMA. The large majority of SMA patients show homozygous deletions of either exon 7 or both exons 7 and 8 in this gene. The homozygous deletion of exons 7 and 8 of telSMN gene was detected in 85 % of the Russian SMA patients. 37 (12 %) SMA patients show absence of exon 7 telSMN gene but retention of exon 8. This picture is caused by either large deletion up to exon 8 or gene-conversion event, resulting in existence of chimeric gene. We suggest a simple and rapid assay of identification chimeric gene. This method allows to carry out analysis using one-step PCR with telSMN/cenSMN specific primers. Chimeric gene was revealed in patients with mild form of the desease.

P1534. Role of SMN and NAIP deletions in phenotypical expression of spinal muscular atrophy in Hungarian families

***V. Karcagi**[1], V. Tarnawa[1], L. Timar[1], A. Herczegfalvi[2]*
[1]National Center for Public Health; Budapest, Hungary; [2]Heim P. Children s Hospital; Budapest, Hungary
karcagi.okk@antsz.hu

Proximal spinal muscular atrophy (SMA) is a neuromuscular disorder with three major clinical phenotypes (SMA type I, II and III). The disease is caused by deletion, conversion or in rare cases, by point mutations in the telomeric survival motor neuron (SMN1) gene. There is a centromeric copy of the SMN gene, SMN2 whose copy number influences clinical severity, indicating that SMN2 gene is the main modulator in the clinical phenotype. Another important neighbouring gene coding for neuronal apoptosis inhibitory protein (NAIP) might also play a role in influencing the phenotype. In this study, we present the genetic analysis of 108 SMA families aimed to find genotype-phenotype correlations. In addition to the 122 index patients, carrier parents and sibs, 195 individuals, were also included in the study. Homozygous deletions in exons 7 and 8 of SMN1 and in exon 5 of NAIPt genes were recorded and correlated with the type of clinical severity. In addition, lack of SMN2 gene was also recorded, although the exact copy number could not be established. Results were compared with healthy controls. In our data deletion of exon 5 of NAIPt occured more frequently in SMA I (63%), than in SMA II (9.3%) and III (4,5%) patients. The same findings were reported in other SMA populations, however the difference between clinical types was much higher in our patients. In conclusion, the size of the deletion in 5q13 might be in closer correlation with the severity of the disease in the Hungarian SMA patients.

P1535. Quantitative Analysis Of Smn Gene Copies In Spanish Sma Patients; Identification Of Compound Heterozygous And Genotype-phenotype Correlation

***Y. Martin**[1], A. Valero[1], I. Cusco[2], E. F. Tizzano[2], C. Hernandez-Chico[1]*
[1]Hospital Ramon y Cajal; Madrid, Spain; [2]Hospital Sant Pau; Barcelona, Spain
ymsantodomingo@yahoo.es

Spinal muscular atrophy (SMA) is a common autosomal recessive neuromuscular disorder with a variable phenotype caused by mutations in the survival motor neuron gene (SMN1) and phenotypically modulated by SMN2 copies. In our sample the SMN1 gene is absent in 84.2% of SMA patients so according to Hardy-Weinberg equilibrium 99.3 % of all SMA cases must carry at least one SMN1 deleted chromosome. In order to identify the compound heterozygous in our group of nondeleted SMA patients and to study the correlation between the SMN2 copy number and the severity of SMA disease, we have developed a PCR-quantitative assay using fluorescent primers. This assay based on the method described by McAndrew has enabled us to determine the SMN1 and SMN2 copy number. From 35 non-deletion SMA patients analysed, we have found six individuals with only one copy of SMN1. Moreover, we have studied several patients suffering from different type of SMA who were compound heterozygous carrying the same mutation in the SMN1 gene. We have ascertained the SMN1 and SMN2 copy number in six independent patients carrying the 430del4 mutation and in a type II patient carrying the 800ins11 mutation in their SMN1 gene. The type I patient retained two copies of SMN2 gene, while type II and III patients had three or four SMN2 copies. Our results corroborate the influence of SMN2 gene on the clinical phenotype.

P1536. Deletion analysis of SMN 1, SMN 2 and NAIP genes in SMA patients from North-West region of Russia.

***A. S. Glotov**, T. E. Ivaschenko, A. V. Kiselev, V. S. Baranov*
Ott s Institute of Obstetrics&Gynecology; St.-Petersburg, Russian Federation
anglotov@yahoo.com

Spinal muscular atrophy (SMA) is the second most common fatal autosomal recessive disease after cystic fibrosis, with the frequency 1;10000 newborn. The results of deletion analysis in 23 SMA families from the North —West region of Russia are reported. The deletions in the SMN 1 gene were the major mutations in these families. Different types of deletions were found in 22 of 23 patients with SMA (96%). Homozygous deletions of exon 7 and 8 in SMN 1 were identified in 65 % of our patients, while the NAIP gene (exon 5) was deleted in 22% (5/23) of them. Homozygous deletion of exon 7 of SMN 1 was detected in one SMA patient (4.3%) only. Analysis of deletions of SMN 1, SMN 2 and NAIP genes have shown that patients from the North-West region of Russia have 7 types of deletion damage. The most frequent deletion in SMA patients was the deletion exons 7 and 8 in SMN 1 gene.

P1537. Preimplantation genetic diagnosis (PGD) for spinal muscular atrophy

***E. H. Cho**[1], S. Y. Park[1], C. K. Lim[2], H. M. Ryu[3], I. S. Kang[3]*
[1]Laboratory of Medical Genetics, Samsung Cheil Hospital; Seoul, Republic of Korea; [2]Reproductive Biology and Infertility, Samsung Cheil Hospital; Seoul, Republic of Korea; [3]Department of Obstetrics and Gynecology, Samsung Cheil Hospital; Seoul, Republic of Korea
genelab@samsung.co.kr

Spinal muscular atrophy (SMA) is a common severe autosomal recessive disease. Homozygous deletion of exon 7 in the telSMN gene is found in 90 - 98% of SMA patients. Couples having children with SMA face a 25% risk of having affected offspring with spontaneous conception. PGD is an alternative method to prenatal diagnosis for couples who have a high risk of an inherited disease in their offspring. Two couples with SMA affected children were enrolled in PGD-IVF program. We performed PCR using a mismatch primer to detect exon 7 deletion of telSMN. To determine the efficiency of PCR at single cell level, we tested 55 blastomeres. The PCR efficiency was 69%. In PGD-IVF cycle, 67% (4/6) and 64% (7/11) of blastomeres were found to be unaffected and four embryos were replaced back to the uterus in each patient. One patient failed to get pregnant and the other is now at 34 weeks of uneventful ongoing pregnancy. Genetic status of the fetus was confirmed by prenatal diagnosis with amniotic fluid cells performed at 16 weeks of gestation. This study shows that PGD is feasible for couples having a child with a deletion of homozygous exon 7 in telSMN.

P1538. Update on the myotubularin gene family and MTM1 mutations in X-linked myotubular myopathy.

***V. Biancalana**[1], J. Laporte[2], O. Caron[1], F. Blondeau[2], A. Buj-Bello[2], N. Romero[3], J. L. Mandel[2,1]*
[1]Faculty of Medicine; Strasbourg, France; [2]IGBMC; Illkirch, France; [3]Hopit l Piti -Salp tri re; Paris, France
bianca@faust.u-strasbg.fr

X-linked myotubular myopathy is characterized by neonatal hypotonia, muscle weakness and respiratory distress in affected males, leading often to early death. It is caused by mutations in the MTM1 gene (myotubularin). The myotubularin family counts at least 10 members in humans, 6 in drosophila and a single one in yeast. It can be subdivided in four subgroups, already present in C. elegans, and three groups have predicted phosphoinositide binding domains. MTMR2, a close homologue of MTM1, was recently found mutated in a recessive form of Charcot-Marie-Tooth neuropathy. These proteins share the consensus active site of tyrosine and dual-specificity protein phosphatases, and of the lipid phosphatase PTEN. Recent results indicate that myotubularin is primarily a lipid phosphatase, acting on phosphatidylinositol 3-monophosphate (PI3P), and may be involved in the regulation of PI3-kinase pathway. We have recently found

mutations in 14 patients, including 7 novel ones. We identified the most distal point mutation, a nonsense associated to a very severe phenotype. Missense N180K was found in a 65 years old grandfather (the oldest known patient with an MTM1 mutation), previously suspected to have autosomal centronuclear myopathy, and in his two grandsons also mildly affected. P226T was found in a 12 years old boy, who had respiratory distress at birth, but attends now regular school and walks unaided. The later two cases confirms that some missense mutations are associated with mild muscular phenotype and very prolonged survival. We present a summary of the 140 different mutations identified up to now in 211 families.

P1539. Frataxin deficiency enhances apoptosis in cells differentiating into neuroectoderm

M. Pandolfo[1], *K. Ohshima*[2], *M. Santos*[1]
[1]*Centre Hospitalier de l Universit de Montr al; Montr al, PQ Canada;* [2]*National Cancer Center Research Institute; Tokio, Japan*
massimo.pandolfo@umontreal.ca

Deficiency of the mitochondrial matrix protein frataxin is the cause of Friedreich ataxia, an autosomal recessive disease characterized by neurodegeneration and cardiomyopathy. Frataxin is thought to prevent toxic iron accumulation in mitochondria. Accordingly, progressive atrophy of neurons and cardiomyocytes in Friedreich ataxia may result from mitochondrial dysfunction caused by iron-mediated free radical damage. However, the disease is likely to involve a specific developmental cell loss, particularly affecting sensory neurons, in addition to the more obvious degenerative process. The early embryonic lethality of frataxin -/- mice also suggests a role of this protein during development. To study the role of frataxin during differentiation, we utilized the multipotent P19 mouse embryonic carcinoma cells. Frataxin expression is induced two to three-fold when P19 cells differentiate into neurons, cardiomyocytes or endodermal cells, an increase that can only be partially explained by an increase in mitochondrial mass, particularly in the case of neural differentiation. Stable transfection of undifferentiated P19 cells with a frataxin antisense construct does not inhibit their proliferation as precursor cells, but severely and specifically compromises neuronal differentiation. Apoptotic cell death, that normally occurs in a few cells when this differentiation pathway is induced, is greatly enhanced by frataxin deficiency. Induction of cardiomyocyte or endodermal differentiation, which is not normally accompanied by detectable apoptosis, is not affected by low frataxin. Hence, frataxin deficiency does not lead to apoptosis by itself, but appears to increase the vulnerability of differentiating cells to normally occurring apoptosis. This effect may result from an enhanced production of reactive oxygen species, as indicated by increased levels of mitochondrial superoxide dismutase in frataxin deficient cells. Our results raise the possibility that the early lethality of frataxin knockout mice and sensory neuronal loss in Friedreich ataxia may be due, at least in part, to higher sensitivity to apoptosis of frataxin-deficient embryonic cells. Such vulnerability may be due to interference with signaling pathways rather than to a direct damaging effect of free radicals.

P1540. First experiences on molecular diagnosis of facioscapulohumeral muscular dystrophy(FSHD)

I. Nagy[1], *H. Piko*[1], *J. Balog*[1], *A. Herczegfalvi*[2], *V. Karcagi*[1]
[1]*National Center for Public Health; Budapest, Hungary;* [2]*Heim P. Children s Hospital Dept. of Neurology; Budapest, Hungary*
ildi10@freemail.hu

FSHD is the third most common hereditary disease of muscle. It is a higly variable disorder considering the age of onset and the clinical severity, even within the same family. The disease is transmitted in autosomal dominant fashion, the estimated incidence is 1 in 20000, although new sporadic patients have been reported frequently. The FSHD locus has been mapped to 4q35 (FSHD1A) and is closely linked to the highly polymorfic locus D4F1104S1. No gene has been detected in this region but a number of short repetitive sequences (D4Z4) were found. Deletion of D4Z4 leads to FSHD. A marker p13E-11, that mapped to 4q35, revealed shorter fragments in genomic DNA digested with EcoRI in patients with FSHD compared to control individuals. The fragment in patients is smaller than 38 kb. We introduced FSHD molecular diagnosis in our laboratory at the beginning of 2000 using p13E-11 probe. Since than seven families including 18 patients have been examined. Asymptomatic family members have been included into the study as well. Out of these cases three patients were identified as familiar and two as sporadic FSHD cases. Two additional families are under evaluations. The genetic analysis was based on EcoRI/BlnI digestion, Southern-blotting, and hybridisation with p13E-11 probe. This method enabled us to distinguish between the FSHD locus on 4q35 and the highly homologous region of 10q26. The analysis was extended by dosage test using BglII/BlnI double digestion and the same probe in order to identify individuals carrying translocated alleles from the homologous region of 10q26.

P1541. The genetic study of nine Portuguese patients with Oculopharyngeal Muscular Dystrophy (OPMD).

L. F. Lopes[1,2], *E. M. Vieira*[1], *T. Evangelista*[3], *P. M. Jorge*[1], *M. R. Santos*[1]
[1]*Instituto de Gen tica M dica Jacinto Magalh es; Porto, Portugal;* [2]*Universidade da Madeira; Funchal, Portugal;* [3]*Servi o de Neurologia do Hospital de Santa Maria; Lisboa, Portugal*
ondagen@portugalmail.pt

Oculopharyngeal Muscular Dystrophy (OPMD) is a late onset autosomal dominant (AD) disease, characterized by dysphagia, ptosis and proximal limb weakness. OPMD is caused by an expansion of a GCG trinucleotide stretch in the first exon of the PABP2 gene, which usually contains six GCG copies ($[GCG]_6$), whereas the mutated gene contains 8 to 13 copies. The $[GCG]_7$ allele, found in 2% of the population, may act as a recessive mutation or as a modifier of the dominant genotype.
Nine Portuguese patients with OPMD (comprising six families) and 110 randomly selected controls were studied by DNA analysis. Fluorochrome-tagged primers were designed to amplify a fragment encompassing the GCG repeats. Amplicons were subjected to capillary electrophoresis and sized with the ABI GeneScan program (Applied Biosystems). Allele sizes were confirmed by sequencing several samples from homozygous and heterozygous individuals.
The diagnosis of OPMD was confirmed in the five families, all of which presented mutations in heterozygosity in accordance with the observed AD pattern of inheritance. The $[GCG]_9$ expansion was found in four familiesand $[GCG]_{10}$ in the fifth. The $[GCG]_9$ allele was previously found to be the most prevalent amongst French-Canadian patients. In order to test the one founder theory, studies with linked markers are in progress to determine the haplotype background of the $[GCG]_9$ allele. Two individuals heterozygous for $[GCG]_6$ and $[GCG]_7$ alleles were also detected in the control population, corroborating the described polymorphic nature of the latter in this allelic combination.

P1542. Analysis of FRDA patients with interrupted GAA expansions in the frataxin gene by fluorescent Triplet Primed PCR.

M. Coss e[1], *M. Schmitt*[1], *E. Meikatt*[1], *J. L. Mandel*[2], *M. Koenig*[2]
[1]*Faculty of Medicine; Strasbourg, France;* [2]*IGBMC; Strasbourg, France*
cossee@igbmc.u-strasbg.fr

Friedreich ataxia (FRDA), the most frequent inherited ataxia, is caused by loss of function of frataxin. In Caucasian population, the most frequent mutation is a large GAA repeat expansion in the first intron. In some rare cases, the expansion is interrupted by other repeat sequences. The presence and size of the GAA expansions can be determined by Southern-blot analysis which is reliable but time consuming, or more commonly by long range PCR which is simpler but not reliable for the detection of heterozygotes. We are now currently analysing the FRDA (GAA) repeat by the fluorescent triplet primed PCR (TP-PCR) method initially developped by Warner et al. for myotonic dystrophy (1996). This method is simple and reliable to detect all the cases with at least one expansion, which is not the case for the long range PCR. However artefacts and pitfalls may cause difficulties to distinguish homozygous from heterozygous expansions, the main exemple being when the GAA expansion is interrupted by other repeat sequences. We present a study of patients from Reunion Island who present interruption of the GAA expansion by a (GAAAGAA) repeat sequence, and some other patients from different origins who present another type of interruption, a (GAGGAA) hexanucleotide repeat. The small size of these expansions, and their stability observed during transmissions, may suggest that interruptions of the GAA repeat confer meiotic stability, as it has been observed for other disease causing expansions such as the fragile X (CGG) repeat.

P1543. Polymorphisms distribution of Int13, Int22 and St14 VNTRs in Mexican population and their application in gene diagnosis of haemophilia A.

R. G. Mart nez[1], *D. Arenas*[1], *E. Benitez*[2], *F. Salamanca*[2]
[1]*Iztacala UNAM, IMSS; Mexico City, Mexico;* [2]*IMSS; Mexico City, Mexico*
rafamg@yahoo.com

The variable number of tandem repeats (VNTR) Int13, Int22 and St14 was analysed to find out the polymorphism distribution in normal individuals in the central region of Mexico and evaluate the efficacy of this markers for carrier detection of hemophilia A. The polymerase chain reaction (PCR) was done in 162 X-chromosomes from unrelated Mexicans and the same method was applied to carrier detection in hemophilia A families. Screening revealed the existence of at least eight different alleles for Int13, four

for Int22 and ten for St14. Their heterozygous rate were 41.3%, 52.6% and 83% respectively. Compared to Caucasians, the Mexican population showed a markedly low heterozygous rate for Int13 marker. However Int22 showed a relatively higher heterozygosity similarity to Turkish and Chinese populations. The St14 marker was the most informative and a new 680 pb allele were detected. Gene diagnosis was performed in 30 suspected haemophilia A carriers from 6 families. 22 females (73%) were heterozygous for at least one of the markers used, only 8 females (26%) were homozygous. Determination of polymorphisms in Int13, Int22 and St14 VNTRs should prove to be a useful tool in the genetic diagnosis of haemophilia A in Mexico.

P1544. Molecular Analysis in a Boy with Progeroid Syndrome and Signs of Ehlers-Danlos Syndrome

G. Carakushansky[1], *T. Okajima*[2], *K. Furukawa*[2], *M. Carakushansky*[3]
[1]*Federal University of Rio de Janeiro; Rio de Janeiro, Brazil;* [2]*Nagoya University School of Medicine; Nagoya, Japan;* [3]*Johns Hopkins School of Medicine; Baltimore, MD United States*
gercar@ruralrj.com.br

A distinct nosologic entity that shows manifestations of progeroid syndrome combined with Ehlers-Danlos (E-D) features (OMIM 130070) has been reported in about a half-dozen cases. We have recently studied a 4 year-old brazilian boy with features consistent with this diagnosis. The patient exhibited developmental delay, short stature, progeroid facies, craniofacial disproportion with small face, prominent ears, scanty eyelashes, late closure of fontanels, bilateral cataracts, loose skin and prominent veins on both hands, bruisability, surgically repaired bilateral inguinal hernia , pectus excavatus, joint hypermobility in digits, bilateral hip dislocation and waddling gait.
The molecular basis of this rare progeroid syndrome with affected proteoglycan biosynthesis has been searched by some investigators. The cDNA of the galactosyltransferase I (XgalT-1) was isolated by two of us (Okajima, T and Furakawa, K) and after that we were able to identify and characterize two mutations in the galactosyltransferase I gene of one patient (J Biol Chem 274 (41);28841, 1999). The results indicated that mutations in XGalT-1 were at least one of main molecular basis for progeroid form of E-D syndrome.
In the boy here reported and in his unaffected parents the genomic sequence of galactosyltransferase 1 (b4GalT7) was analysed by direct sequencing of PCR products obtained from amplification of exon segments with primers located in flanking intron or UTR sequences. However, no mutations could be detected except for a single silent heterozygous mutation (R73R, CGC to CGT) in exon 2. This finding may imply that other related glycosysltransferases are probably mutated in this patient and that further study of its pathogenic role in this disease should be undertaken.

P1545. Frequency of CFTR mutations and polymprphisms in Czech partners with severe reproductive disorders and in the control population

A. Krebsova[1], *M. Macek*[1], *M. Machatkova*[1], *I. Horka*[1], *M. Koudova*[1], *A. Sobek*[2], *P. Potuznikova*[1], *S. Vilimova*[1], *I. Kurucova*[1], *R. Alanova*[1], *M. Macek Jr.*[1]
[1]*Institute of biology and Medical Genetics; Prague, Czech Republic;* [2]*FERTIMED Olomouc; Olomouc, Czech Republic*
pavel.roubic@lfmotol.cuni.cz

In 104 couples with reproductive disorders (RD) and in randomly selected controls (109 males/105 females) the 19 most common Czech CFTR mutations - delta F508, delta I507, G551D, G542X, N1303K, 1717-1G->A, W1282X, R553X, R347P, R334W, 3849+10kbC->T, R117H, 621+1G->T, A455E, S549N, R506T, 1898+1G->A, 2143delT, CFTRdele2,3(21kb) and polymorphisms 1540A/G, IVS8(TG)n, IVS8(T)n were examined. Only the males with non-obstructive azoospermia were included in this study. The Y chromosomal microdeletions, chromosomal aberrations and other infertility factors were evaluated. Analyzed males with RD were divided into 3 groups according to their sperm counts (I.; 0-1x106/ml, II.; 1-20x106/ml, III.; >20x106/ml). Significant differences to controls were observed mainly in Group I., where the frequency of delta F508 mutation was raised to 11.4% (p=0.015) in 100% association with the TG10/T9 haplotype. Moreover these patients had an increased prevalence of TG12/T7 (15.9%;p=0.015) and a decrease of TG11/T7 (27.3%,p=0.0002) haplotypes. Increased frequency of 1540 A/G AA genotypes was observed in Groups I.-III., with the highest significance (p=0.0004) observed in Group I. The Y chromosome microdeletions were found in 9.1% of Group I. males only. In females from RD couples the tendency towards a decrease of IVS8(T)9 (4.3%,p=0.05), increase of both the IVS8(TG)12 (15.4%,p=0.03) and (TG)12/(T)7 haplotypes (14.9%,p=0.04) were disclosed. The patogenetic impact of these findings has to be clarified by further studies. These results support the hypothesis, that CFTR gene mutations and polymorphism might also affect spermatogenesis/sperm viability and point out the importance of CFTR mutation/polymorphism screening in couples with severe RD. Supported by grants #6250-3, 6411-3,000000064203;111300003,ME258,OK192 and LN00A079.

P1546. CFTR Mutation Spectrum Identified By DGGE In Dutch Patients With CF, CBAVD, Or Other CFTR Associated Disorders

H. Scheffer[1], *D. de Jong*[1], *G. J. J. Mol*[1], *J. P. H. Tuerlings*[2], *P. M. Hartman*[3], *J. P. H. Drenth*[2], *C. H. C. Buys*[1]
[1]*University of Groningen; Groningen, The Netherlands;* [2]*Univ. Hospital Nijmegen; Nijmegen, The Netherlands;* [3]*University Hospital Groningen; Groningen, The Netherlands*
h.scheffer@medgen.azg.nl

Cystic fibrosis transmembrane conductance regulator (CFTR) mutation scanning using an optimized denaturing gradient gel electrophoresis (DGGE) system has been performed in clinically well defined cohorts of patients of Dutch origin with cystic fibrosis (CF), congenital bilateral absence of the vas deferens (CBAVD), oligospermia, or pancreatitis. In over 98% of (>800) fully analysed, independent alleles of CF patients a mutation has been identified in the coding sequence of the CFTR gene or in a flanking intronic sequence, including six novel mutations. In addition, in two patients a hemizygous genomic deletion involving part of a CFTR allele has been identified. In approximately 90% of (50) fully analysed, independent alleles of CBAVD patients a mutation has been identified, including four novel mutations. Particular mutations including A455E and 3272-26A>G that are associated with mild CF were also detected in CBAVD. Homozygosity for the A455E mutation was shown in a CBAVD patient. The relative frequency of the IVS8 5T allele appears to be hardly increased amongst Dutch CBAVD patients (4/50 alleles). In a cohort of clinically strictly defined oligospermia patients (160 alleles studied), an increased frequency of CFTR missense or (potential) splicing mutations was identified, in addition to two deltaF508 alleles, and an IVS8 5T allele. Some mutations have been reported as (potentially) pathogenic, including 296+9A>T (once; opposite to the non-pathogenic mutation R668H), A120T (once), G576A and R668C on a single allele, P750L (once), L997F (twice), S1235R (twice). This results in a frequency of 11/80 oligospermia patients carrying a pathogenic CFTR mutation. Other detected variants most likely do not, or hardly alter the function of the CFTR protein. These mutations include V11I, R75Q (8 times), R297Q, and 1341+12T>A. No truncating mutations have been identified. A similar analysis in pancreatitis patients is currently ongoing. A complete overview of the mutations identified in all categories of patients studied will be presented.

P1547. Molecular Diagnosis Of Cystic Fibrosis In Ukraine

L. Livshits[1], *M. Nechyporenko*[2], *V. Pampuha*[2]
[1]*Institute of Molecular Biology & Genetics; Kiev, Ukraine;* [2]*Institute of Molecular Biology & Genetics National Academy of Science of Ukraine; Kiev, Ukraine*
livshits@imbg.org.ua

Fifteen common mutations in the cystic fibrosis transmembrane conductance regulator gene (CFTR) were studied in 305 CF patients from Ukraine. The most frequent mutation found according to our expectation was delF508 (40.8%). Ten further mutations were identified in our sample; N1303K (2,65%), CFTRdele2,3(21kb) (1.4%), G542X (0,6%), W1282X (0,5%), R334W (0,3%), R553X (0,3%), 1677delTA (0,3%), R347P (0,3%), 1717-1GtoA (0,3%), G551D (0,16%). Mutations R117H, 621+1GtoT, 1154insTC were not found of patients. We conclude that the 21kb deletion is a frequent mutation in Ukranian population as well as in populations of Eastern- and Western-Slavic descent. Screening for this deletion will significantly improve the molecular genetic diagnosis of cystic fibrosis in families from Ukraine. Pilot carrier screening of these mutations was performed in 126 patients from IVF clinic involved in IVF program. We have detected CF carrier status in 3 case (delF508). The same mutation analysis program was performed in 98 non fertile (non CBAVD) men involved in ICSI program. We have detected CF carrier status in 2 case (delF508). The CFTR gene mutation test for CF carrier partners was recommended. Early prenatal diagnosis CVS have been performed by means of direct analysis of mutations in 59 cases. In families, with non identified mutations prenatal diagnosis has been performed by analysis of RFLP markers .(KM19(PstI), CS.7/Hin6I, Xv-2c/TaqI, MetH(MspI), M6D9(MspI), 4bp tandem repeats (3'-end of intron 6 of CFTR gene) and polymorphic STR systems - IVS8/GT, IVS17(b)/CA (ALFexpress /Amersham-Pharmacia-Biotech).

P1548. Quantitative transcript analysis as indicator of disease progression in Cystic Fibrosis Patients

B. Steiner[1], S. Keller[1], M. Schneider[1], R. Kraemer[2], S. Gallati[1]
[1]Molecular Human Genetics; Bern, Switzerland; [2]Department of Paediatrics; Bern, Switzerland
bsteiner@dkf2.unibe.ch

The genotype-phenotype relation in Cystic Fibrosis (CF) is known to be very complex. Disease severity even varies among CF patients carrying the same CFTR genotype. Factors that contribute to this variability are topics of intense study. The rate of exon 9 skipping from CFTR mRNA is believed to be one of these contributing factors. Variations in the TG(m) and T(n) polymorphic repeats at the 3 end of intron 8 of the CFTR gene are associated with the alternative splicing of exon 9. The most common mutation delta F508 is linked to the TG10/T9 polymorphism, which was previously shown to lead only to negotiable amounts of exon 9 skipping. Using the LightCycler system we developed a new sensitive one-step RT-PCR method with high accuracy. In a clinically well-described group (n=10) of delta F508 homozygous CF Patients (TG10T9/TG10T9), we performed quantitative real-time RT-PCR to determine the ratio of the aberrantly spliced transcripts (Range between 2 and 23 %) in nasal epithelium and buccal cells. In addition, we determined the total number of normal and aberrantly spliced transcripts using ribosomal 28S rRNA for standardization. We found that a decrease of CFTR transcripts (up to 70 fold) and an increase in aberrantly spliced transcripts (up to 10 fold) in early childhood is associated with a severe course of CF disease. Therefore quantitative transcript analysis is a valuable indicator of disease progression.

P1549. A simple and rapid assay of the 11 more frequent mutations in CFTR gene

A. Chuhrova, I. Shagina, N. Petrova, A. Polyakov
Research Center for Medical Genetics; Moscow, Russian Federation
stver@online.ru

Cystic fibrosis transmembrane conductance regulator (CFTR) functions as a chloride channel and controls the regulation of other transport pathways. Mutations in the CFTR gene are the cause of cystic fibrosis. Nowadays it a lot of mutations with different frequency in different populations are known. We have developed two multiplex PCR systems for simple and rapid detection of the 11 more frequent mutations in CFTR gene in Russian population patients. The first one includes 8 pairs of primers which allowed reviled the follow mutations; del 21 kb, ˇF508, DI507, 1677delTA, 2143delT, 2184insA, 394delTT, 3821delT. The second one allows to detect G542X, W1282X and N1303K mutations by PCR/restriction analysis. We constructed pairs of primers which create the Bse NI recognition site in normal allele of each PCR product. The common informativity of both systems are 71%.

P1550. Analysis Of CFTR Gene In Children With Chronic Respiratory Symptoms

A. Bassotti[1], R. Padoan[1], M. Seia[2], A. Ambrosioni[2], D. A. Coviello[2]
[1]CF Centre, Istituti Clinici di Perfezionamento; Milan, Italy; [2]Molecular Genetic Laboratory, Istituti Clinici di Perfezionamento; Milan, Italy
coviello@unige.it

Role of CFTR gene is well recognised in the pathogenesis of several lung diseases in adulthood, but few data are available in the paediatric population with respect to some lung diseases suggestive of CF with normal or borderline sweat test. In order to verify the pathogenetic role of mutations or polymorphism in the CFTR gene, we performed the DNA analysis in a cohort of 80 patients (aged 4mo-17yr), not related and with no familial history for CF. Patients were referred to our Paediatric Department because the presence of chronic (over 3 months) respiratory symptoms (cough, wheezing, or sputum production). The screening of 11/27 exons of the CFTR gene and their intronic flanking regions was performed by Denaturing Gradient Gel Electrophoresis (DGGE) analysis and automatic sequencing was performed on the anomalous DGGE patterns. Ten different known mutations (F508del, G542X, I148T, 1717-1G->A, R553X, R1066C, S1235R, R117H, 3667ins4 and D110H) were identified at heterozygous state in 14/80 unrelated patients (17.5%). Seven compound heterozygotes (M1V/4382delA, F508del/R352Q, F508del/S1252N, F598del/3849+10kbC->T (3), A120T/F1052V) leading to CF diagnosis were identified in 7/80 (8.75%). In 21/80 (26%) of paediatric patients, with chronic respiratory symptoms, at least one CFTR gene mutation was present, showing a significant increase over the expected carrier frequency of 1/28 (3,5%) in the same population. The clinical, laboratory and radiological evaluations identify several causes of respiratory symptoms and some subjects were afterwards diagnosed as cystic fibrosis patients (CF). We observed nasal polyposis in 7 patients (1 CF), chronic sinusitis in 6, allergic bronchopulmonary aspergillosis in 5, (3 CF), disseminated bronchiectasies in 8 (2 CF), lobar atelectasia in 5, BPCO in 28, asthma in 14, bronchiolitis in 7 (1 CF). All patients presented sweat test in the normal or borderline ranges (<60mEq/l), and also their pancreatic function was normal. In conclusion, in our paediatric cohort, CFTR gene plays a relevant role in determining lung disease together with behavioural factors. It is noticeable the relevant number of Cystic Fibrosis diagnosis among this cohort of patients with sweat test in the borderline or normal range. We suggest performing a molecular study of CFTR gene in paediatric patients with chronic respiratory symptoms also with a normal sweat test.

P1551. Clinical and Molecular Genetics preliminary studies of Cystic Fibrosis in the 5th Region, Chile.

G. Molina[1], R. Cave[2], M. Cornejo[2], S. Navarro[2], M. Deglin[3], A. Milinarsky[3], P. Carvallo[4], F. Gonz lez[1]
[1]Laboratorio de Biolog a y Gen tica Molecular. Centro de Investigaciones M dicas. Escuela de Medicina. Universidad de Valpara so; Valpara so, Chile; [2]Laboratorio de Inmunolog a. Escuela de Medicina. Universidad de Valpara so; Valpara so, Chile; [3]Departamento de Pediatr a. Escuela de Medicina. Universidad de Valpara so; Valpara so, Chile; [4]Laboratorio de Gen tica Molecular Humana. Facultad de Ciencias Biol gicas. Pontificia Universidad Cat lica de Chile; Santiago, Chile
Graciela.Molina@uv.cl

Background; Cystic fibrosis (CF) is the most common autosomal recessive disease in the Caucasian population, caused by several mutations in the cystic fibrosis transmembrane regulator (CFTR) gene. Mutation frequencies vary among ethnic groups, being the most common a Phenylalanine 508 deletion (˜F508). Aim; Detect the presence of the mutations ˇF508, G542X, N1303K, G551D, R553X and S549N and other mutations in exon 10, 11 and 21 of CFTR gene, in 5th region patients with a previous clinical diagnosis of CF. Patients and methods; 14 non-related patients were studied, 13 had sweat tests chloride concentrations higher than 60 mEq/L and frequent respiratory tract infections. 9 were pancreatic insufficient. In order to discard the presence of an immune-deficiency, we determined sera immunoglobulins (IgG, IgA, IgM), and total, CD3+ and B lymphocytes. The mutations and M470V polymorphism were analysed by PCR amplification, restriction enzyme digestion and electrophoresis and single strand conformation analysis (SSCA). Results; Patient s immunological parameters were between normal ranges. 5 patients presented ˇF508 mutation and 2 patients had G542X mutation, both found in one of their chromosomes. In two patients, we detected a different patern in SSCA that could be due by a second mutation in the exon 10. No association between M470V polymorphism and the presence of the mutations were found. Conclusions; For the first time G542X mutation had been detected in Chilean population. This mutation is prevalent in Spaniards and we can hypothezise that it could be the origin of the mutation found in chilean CF chromosomes.Apertus, Laboratorios Andr maco

P1552. Novel, rare splice site variants of IVS8 in the cystic fibrosis gene

T. D rk[1], D. Gl ser[2], M. Stuhrmann[1], E. Buratti[3], F. Pagani[3], F. Baralle[3]
[1]Medizinische Hochschule; Hannover, Germany; [2]Laborpraxis Drs. Mehnert; Neu-Ulm, Germany; [3]International Center of Genetic Engineering and Biotechnology; Trieste, Italy
doerk.thilo@mh-hannover.de

A unique feature of the cystic fibrosis gene (CFTR, ABCC7) is the presence of a polymorphic polypyrimidine tract within the splice acceptor site of intron 8 which genetically determines alternative exon 9 splicing. Whereas most individuals carry either 7T or 9T alleles which allow for over 80% normal splicing, the 5T allele induces exon skipping in over 70% of CFTR mRNA transcripts and is commonly associated with partial symptoms of cystic fibrosis. We here report further allelic variants (3T, 6T and 8T) of the IVS8 polythymidine tract. While the 6T and 8T alleles were observed in single carriers with no known clinical phenotypes, the 3T allele was identified in a male adult who had a pancreatic-sufficient form of cystic fibrosis and was a compound heterozygote for the DeltaF508 mutation and the TG13T3 haplotype. Transcript analyses in lymphoblastoid cells from this patient confirmed the loss of exon 9 in about 95% of non-deltaF508 CFTR mRNA. Introduction of the TG13T3 sequence into a CFTR minigene construct with subsequent transfection of Hep3B cells and characterisation of the resulting splicing patterns also revealed exon 9 skipping in the range of 85-98 %, depending on the addition of distinct splicing factors. We conclude that the allelic heterogeneity of IVS8 of the CFTR gene is higher than initially thought and the possibility of additional variants, though rare, should be considered in the development of tools for the diagnosis of CFTR-associated diseases.

P1553. The results of molecular studies in the group Polish patients suspected to have Angelman syndrome

A. Szpecht-Potocka, *K. Borg, P. Stankiewicz, E. Obersztyn, J. Bal, T. Mazurczak*
National Research Institute of Mother and Child; Warsaw, Poland
aspotocka@hotmail.com

We present results of studies carried out in a group of 70 patients with tentative clinical diagnosis of Angelman syndrome (AS). Most of the patients (66%) were referred from other pediatric centers in Poland. In 38 (54%) cases probant DNA was only available to limit molecular analysis. Analyses of; DNA methylation, microsatellite polymorphism for loci within the AS/PWS region, gene dosage of SNRPN and UBE3A mutation analysis performed by SSCP and DNA sequencing are included in our molecular diagnostic procedure. Cytogenetic analysis and FISH were performed for 4 patients. The clinical diagnosis of AS was confirmed only in 7 cases. AS diagnosis was excluded in 2 patients (one case of FRAX syndrome & one of Cohen syndrome). The very low ratio of confirmed clinical diagnosis of AS is absolutely surprising. We cannot exclude the possibility that we missed a molecular defect. However, the greatest probability is that in tested patients the clinical diagnosis of AS was mistaken. It points to how much still needs to be done to increase practical knowledge of AS among pediatricians and clinical geneticists in Poland.

P1554. Parkinson s disease; Mutation analysis of the synphilin-1 gene in German patients

C. Holzmann[1], *L. Li*[2], *R. Krueger*[3], *O. Riess*[1]
[1]*University Rostock, Dept. of Medical Genetics; Rostock, Germany;* [2]*Tongji Medical University, Dept. of Tratitional Chinese Medizine; Wuhan, China;* [3]*Ruhr-University Bochum, Dept. of Neurology; Bochum, Germany*
carsten.holzmann@med.uni-rostock.de

Parkinson s disease is one of the most common neurodegenerative disorders affecting about 1% of the population over the age of 50. Recently, mutations in the alpha-synuclein gene have been found to be responsible for some rare forms of autosomal dominantly inherited PD. Subsequently, alpha-synuclein has been identified as a major component of Lewy bodies also in sporadic patients. The search for interacting proteins of alpha-synuclein revealed the identification of a protein called synphilin-1, which was shown to be another component of the Lewy bodies. Therefore we analyzed the synphilin-1 gene as a candidate for PD. We screened 416 sporadic and familiar German Parkinson s disease patients for mutations in the coding region of the human synphilin-1 gene (SNCAIP). We identified no disease-causing mutation in the coding region of the SNCAIP gene, but direct sequencing revealed 1 intronic (A to G at position -69 5 of the first translated exon) and 5 exonic basepairsubstitutions (C729T in exon 3, C1603T in exon 7, G1896C, C1953T and G2218C in exon 9). Whereas the first three exonic substitutions are silent polymorphisms, the C1953T leads to an Arg652Cys exchange and G2218C to an Glu740Gln substitution. As the G2218C substitution is present in several individuals of our unsffected control group, we conclude, that the G2218C transversion is rather a polymorphism than a common cause for PD. The functional implication of the G2218C transversion is currently being investigated.

P1555. Mutation analysis of phenylketonutia (PKU) in Kuwait

E. Samilchuk, *I. Al-Suleiman, S. Al-Awadi*
Kuwait Medical Genetics Center; Ministry of Health, Kuwait
samilchuk@hotmail.com

Mutations underlying PKU in Kuwait (as well as in other Gulf countries) were unknown. To identify these mutations we studied DNA samples from 13 unrelated PKU families comprising all PKU cases diagnosed in Kuwait. Six families were of Kuwaiti origin, 5 Bedouins, 1 Egyptian and 1 Lebanese. Most families were consanguineous. DNA samples were tested for several common PAH gene mutations, including IVS10nt546 (IVS10nt-11g->a). Negative samples were studied by heteroduplex analysis in MDE gel followed by sequencing. Six PAH mutations were identified; IVS10nt546 (34.6%), K363fsdelG (15.38%), IVS2nt5 g->c (11.5%), G352fsdelG (7.6%), P281L (3.8%) and delE2/IVS2nt1 (3.8%). IVS10nt546 was found in Kuwaiti, Bedouin and Egyptian, K363fsdelG and IVS2nt5g->c in Kuwaiti and Bedouin, G352fsdelG in Lebanese, delE2/IVS2nt1 in Kuwaiti and P281L in Egyptian probands. All IVS10nt546 chromosomes had an identical haplotype as judged by PCR-RFLP and VNTR markers. The same was true for K363fsdelG and IVS2nt5g->c (the latter mutation was accompanied by IVS2nt19t->c polymorphism). The methodology used allowed detection of PAH mutations on 20 of 26 PKU chromosomes analyzed. More sensitive technique such as SSCP or DDGE has to be used for analysis of the remaining six PKU chromosomes (their haplotypes allow to anticipate four other PAH mutations).

P1556. Rare mutations in PKU patients from Lithuania

J. Kasnauskiene, *L. Cimbalistiene, V. Kucinskas*
Human Genetics Center, Vilnius University; Vilnius, Lithuania
Jurate.Kasnauskiene@mf.vu.lt

Phenylketonuria (PKU)is highly heterogeneous at the molecular level. More than 380 different mutations in PAH gene have been described to date. They are associated with different geographical and ethnic origin of individuals with PKU. Six PAH gene mutations identified in PKU patients from Lithuania represent 80% mutant PAH alleles in PKU patients from Lithuania. The most common mutations R408W (67%), R158Q (8%) and R261Q (5%) have been detected in previous investigations (Kucinskas et al., 1997). The present study included PKU patients from Lithuania with PAH gene alleles not yet identified. DGGE-based screening for PAH gene mutations and subsequent identification of found changes in nucleotide sequence by direct DNA sequencing were performed. On this basis, five PAH gene mutations rare in our PKU patients (frequency of a corresponding mutation is shown in parenthesis) were identified; G272X (4.04%), E280K (2.02%), A403V (2.02%), R261X (1.01%) and L311X (1.01%). Out of them, four (E280K, A403V, R261X, and L311P) were new in Lithuania. Estimation of PKU phenotype (clinical manifestation and biochemical phenotype) dependence on PAH locus genotype was based on the predict level of phenylalanine hydroxylase activity and the pre-treatment serum level of phenylalanine. Our data confirm PAH locus genotype correlation with PKU phenotype revealed by other investigators; R408W/G272X - severe PKU, R408W/E280K - severe PKU, R408W/R261X - severe PKU, R408W/L311K - severe PKU, R408W/A403V - mild PKU.

P1557. Mutations and Polymorphisms Analysis of the Phenylalanine Hydroxylase Gene in High Risk PKU Families from Ukraine

M. Nechyporenko, *L. A. Livshits*
Institute of Molecular Biology and Genetics; Kyiv, Ukraine
livshits@imbg.org.ua

We carried out genetic investigation of 90 patients with clinically diagnosed phenylketonuria in order to identify the following mutations; R408W, R158Q, Y414C, Ivs10nt546, Ivs12nt1. The most frequent mutation found according to our expectation was R408W (56%). The frequency of other mutations were; R158Q (2,8%), Y414C (1,68%), Ivs10nt546 (1,1%). Mutation Ivs12nt1 was not found. The allelic variations of the minisatellite VNTR-polymorphism in 380 chromosomes were analysed. 7 allelic variants and 15 haplotypes were identified. The VNTR-system showed heterozygosity of 72% in Ukraine. We present an analysis of microsatellite STR-polymorphism by ALFexpress (Amersham-Pharmacia-Biotech). The STR-alleles in 380 chromosomes ranged continuously from 226-254 bp, we identified 8 allelic variants and 24 haplotypes. The heterozygosity of STR system in Ukraine was 74%. The linkage disequilibrium between mutation R408W and minihaplotype VNTR3/SRT238 in Ukraine was found. Strong linkage disequilibrium between mutation and minihaplotype has substantiated the hypothesis of a single origin of this mutation. Early prenatal diagnosis based on examination of CVS has been performed in 20 PKU families by means of direct analysis of mutations and by STR - , VNTR- polymorphisms analysis.

P1558. Molecular genetic study of phenylketonuria in Bashkortostan of Russia

V. L. Akhmetova[1], *T. V. Viktorova*[2], *E. K. Khusnutdinova*[1]
[1]*Biochemistry and Genetics; Ufa, Russian Federation;* [2]*Institute of Biochemistry and Genetics; Ufa, Russian Federation*
ildus@ufacom.ru

The autosomal recessive disease phenylketonuria (PKU) is the result of a deficiency in phenylalanine hydroxylase (PAH) enzymatic activity due to mutations in the PAH gene. The average frequency of classical PKU in Bashkortostan is 1;10707 newborns. We have carried mutational analysis in 57 families with PKU from the Bashkortostan. Using SSCP analysis followed by sequencing of 7 exon of the PAH gene we have identified 4 mutations; R261Q, R252W, P281L and R252P, and one polymorphism V245V. The mutation R261Q was observed in 8,2% of the PKU chromosomes. Two mutations R252W and P281L were detected in 2,9 and 2,5% of the PKU chromosomes respectively. The new mutation R252P in codon 252 of exon 7 was found in one PKU family for the first time. The most of the patients investigated are compound heterozygotes for the missense mutation R408W and other identified or unknown mutation. The results of the molecular genetics study of PKU can be used for direct DNA diagnostics of this disease in Bashkortostan.

P1559. Mutation analysis of thyroid peroxidase gene in Taiwanese patients with total iodide organification defect

F. Tsai[1], *J. Wu*[1], *S. Shu*[2], *C. Yang*[1], *C. Tsai*[1]
[1]*China Medical College Hospital; Taichung, Taiwan Republic of China;* [2]*Taichung Veteran General Hospital; Taichung, Taiwan Republic of China*
d0704@hpd.cmch.org.tw

Total iodide organification defect (TIOD), where the iodide in the thyroid gland can not be oxidized and/or bound to the protein is caused by the defect in the thyroid peroxidase (TPO) gene. Single strand conformation polymorphism (SSCP) analysis was used to screen for mutations in the TPO gene from five unrelated TIOD patients in Taiwan and five novel mutations were detected. Three frameshift mutations were found; a single T insertion between nucleotide position 2268 and 2269 (c.2268-2269insT) in exon 13 and two single C deletions at nucleotide positions 843 (c.843delC) and 2413 (c.2413delC) in exon 8 and 14, respectively. In addition, two single nucleotide substitutions (c.G1477>A and c.G2386>T) located in exon 9 and 13, respectively, were identified. The former is a single nucleotide transition, resulting in amino acid substitution (Gly493Ser) in the highly conserved region of the TPO polypeptide. The latter is a single nucleotide transversion, resulting in either amino acid substitution (Asp796Tyr) or alternative splicing. Of those identified TPO mutations, c.2268-2269insT was most prevalent and was detected as heterozygous in all but one TIOD patients. All five Taiwanese TIOD patients investigated were compound heterozygous in the TPO gene. The method presented in this study could be used for carrier assessment and mutation analysis of newly identified TIOD patients.

P1560. Towards molecular genetics cystinuria diagnosis; results of SLC7A9 and SLC3A1 mutation testing in unclassified patients

C. Schmidt[1], *A. Albers*[2], *J. Tomiuk*[3], *C. Wagner*[2], *S. Lahme*[4], *A. Hesse*[5], *F. Lang*[2], *K. Zerres*[1], *T. Eggermann*[1]
[1]*Institute of Human Genetics; Aachen, Germany;* [2]*Institute of Physiology; T bingen, Germany;* [3]*Institute of Anthropology and Human Genetics; T bingen, Germany;* [4]*Department of Urology; T bingen, Germany;* [5]*Department of Urology; Bonn, Germany*
cschmidt@post.klinikum.rwth-aachen.de

Cystinuria is a common inherited disorder of defective renal reabsorption of cystine and dibasic amino acids. Mutations in the SLC3A1 gene encoding the glycoprotein rBAT cause cystinuria type I. Secondly, variants in the SLC7A9 gene have been demonstrated in non-type I cystinuria; its gene product b0,+AT is a subunit of rBAT. To estimate the role of both genes in the aetiology of cystinuria, we searched for sequence alterations in SLC7A9 and SLC3A1. 30 unclassified were investigated. In 15 unclassified patients, we found point mutations in SLC3A1/rBAT (50%). Screening of the SLC7A9 gene revealed 10 mutations in 8 unclassified patients (27%). In addition to previously published mutations in the SLC7A9/b0,+ AT gene, we detected two new mutations (F140S, c747delG). Furthermore, 5 new polymorphic sites were identified in SLC7A9. Three out of these, localised in exons 2, 5 and 6, showed statistically significant differences between patients and controls. These variants might be markers of a functional variant in the SLC7A9 gene or in other genes related to cystinuria. An overall detection rate of 73% (22/30) in unclassified patients is delineated for mutations in both genes. Evidently, mutations in further genes and modulating factors might influence the phenotype in patients lacking mutations in SLC3A1 and SLC7A9. However, the detection rate does not reach 100% owing to the limitation of the applied methods. The presented data show that testing for mutations in the two currently known cystinuria genes is a meaningful molecular diagnostic approach as a first step towards a well-directed individual therapy. Acknowledgement; We thank the International Cystinuria Consortium for providing us with information on SLC7A9.

P1561. Characterization of a Novel Rat Genetic Model; A Presumed Transgene Insertion Mutant Associated with Postnatal Male Lethality and Female Growth Retardation.

J. Slade, *T. Wells, D. A. Carter*
University of Wales, Cardiff; Cardiff, United Kingdom
sladejp@cardiff.ac.uk

The production of transgenic rodents through the illegitimate recombination of pronuclear-injected DNA can result in insertional disruption of host genes. Insertional mutants have formed both important models of mammalian development, and routes to the molecular cloning of obscure loci. Of five rat (Sprague-Dawley) transgenic lines generated in our laboratory using a promoter-reporter (egr-1/NGFI-A- d4EGFP) construct, only one line, of intermediate copy-number, displayed an overt phenotype. The stably-inherited mutation is characterized by post-natal male lethality occurring at post-natal (PN) days 5-11, which follows apparently normal littering, peri-natal nurture and weight gain. A sub-group of (transgenic[tg]) females within each litter exhibit an intermediate phenotype manifested through general growth retardation (PN8; tg 13.2–0.3g, n=10; non-tg 17.0–0.6g, n=6; PN41; tg 104.8–3.9g, n=4; non-tg 137.7–1.7g, n=8 [mean–sem]), fur growth retardation associated with (possibly secondary) skin chafing from PN8/9, and early eyelid-opening (PN11/12 v PN 14/15 in wild-type animals). In 3 of 14 mutant rats a curling/ kinking of the tail is seen. The severity of this is variable, even within the same litter. Parental care of the mutant female offspring is maintained normally until weaning, and the transgenic mutants proceed to exhibit normal sexual maturity and fecundity. Preliminary observations have excluded a change in pituitary growth hormone expression as the underlying cause of growth retardation. Current research is aimed at determining the genomic location of transgene insertion.

P1562. Mutations Of The Androgen Receptor In Two Subjects With Gynecomastia Due To Androgen Insensitivity Syndrome (AIS)

L. Machuca Tzili[1], *J. Zenteno*[1], *F. Vilchis*[2], *B. Chavez*[2], *S. Kofman-Alfaro*[1]
[1]*Hospital General de Mexico. Facultad de Medicina UNAM; Mexico City, Mexico;* [2]*Department of Reproduction Biology. INCMNSZ.; Mexico City, Mexico*
ltzili@yahoo.com

Mutations in the androgen receptor (AR) gene result in an X-linked form of male pseudohermaphroditism known as AIS. These patients present virilization defects that varies from sligthly undervirilized men with or without impaired spermatogenesis (partial AIS) to subjects with aparently female external genitalia (complete AIS). We report the molecular analysis of the AR in two males with gynecomastia and normal male external genitalia in which two mutations in the steroid binding domain (SBD) of the AR were detected. In subject #1, a point mutation was identified in exon 8; a C to T (GCG to GTG) substitution originating an Ala870Val change. In subject #2, a previously undescribed mutation was observed in exon 6; an A to G transition (CAG to CGG) originating a Gln802Arg change. Mutations affecting amino acid alanine at position 870 of the AR have been previously described in four unrelated patients, three of them (mutants for valine instead of alanine) exhibiting a partial form of AIS characterized by severe hypospadias and the other one (alanine to glycine) with a predominatly female phenotype. In this report we describe a new case of an AR gene mutation altering codon 870 which, in contrast with previous reports, causes only gynecomastia in a subject with normal male external genitalia and normal sperm counts, indicating the phenotypic diversity associated to identical mutations in the AIS. Our findings are in accordance with previous reports indicating that most cases of subjects with gynecomastia due to AIS have mutations within the SBD of the AR.

P1563. Molecular analysis of the androgen receptor gene in patients with androgen insensitivity syndrome in Russia.

A. Dergacheva[1,2], *G. Osipova*[1], *A. Polyakov*[2], *O. Evgrafov*[2]
[1]*Russian Medical Academy of postgraduate education; Moscow, Russian Federation;* [2]*Research Center for Medical Genetics; Moscow, Russian Federation*
dnalab@online.ru

The androgen-dependent development of male phenotype and genital structures depends on the presence of an intact human androgen receptor (AR) that mediates androgen action. The human androgen receptor gene is located on X-chromosome Xq11-12. Mutations in the AR gene results in the X-linked androgen insensitivity syndrome (AIS). AIS is characterized by complete or partial absence of hormone action on target cells and results in spectrum of disorders in individuals with karyotype 46XY ranging from normal female phenotype (complete AIS) to undervirilized or infertile males (partial AIS). We analyzed 7 out of 8 exons (2 - 8) of AR gene in 42 patients with diagnosis complete or partial forms of AIS and their mothers from 33 families. AR gene have been investigated by PCR-SSCP analysis, direct sequencing and restriction analysis. Nine missense mutations and one mutation in splice site have been identified in 16 individuals with complete and partial forms AIS. In three families mothers of affected individuals were heterozygous carriers of mutations. Three of the mutations are novel. Two out of these three novel mutations (C559S (TGC->TCC), IVS 6 DS + 2 T->C) were found in patients with complete form of AIS, one of the mutations (G568R (GGG->AGG)) was found in patients with partial form of AIS. Mutation C559S creates a new Mnl 1 restriction site. This mutation has been confirmed by restriction analysis. Seven mutations were previously reported. They were identified both in patients with complete form of AIS (V866M, A765T, L722F, G724D), and patients with partial form of AIS (R855H, M780I, D695N). Two indentical missens mutation (R855H) were found in 2 unrelated families in patients with partial form of AIS.

P1564. Male infertility; molecular analysis of Y-chromosome microdeletions in a non-selected population

G. Massazza, *M. Poma, M. R. Iascone, U. Giussani, D. Chinaglia, A. Parma*
Ospedali Riuniti; Bergamo, Italy
sanatomi@ospedaliriuniti.bergamo.it

Among couples with fertility problems, male factors are the aetiology in about 50% of cases. In addition to chromosomal anomalies, microdeletions in the azospermic factor region (AZF) in the long arm of Y-chromosome have been detected in men with azoospermia or severe oligospermia. Every year in our Department about 1750 semen analysis are performed, 30% of which interests patients affected by varicocele and the remaining regarding men with fertility impairment. The aims of this study were; 1) to evaluate oligo-azoospermia incidence on the total number of semen analysed 2) to assess if the only sperm count < 5x106 spermatozoa /ml in oligospermia could be a parameter sufficient to justify the chromosome-Y microdeletion screening 3) to correlate the clinical phenotype of infertile men with specific chromosome-Y microdeletions In 11 months we have performed 1615 semen analysis according to WHO guidelines (1992). The patients mean age was 33 years (range 15-62). We found 55 azoospermic (3,4%), 282 oligospermic (17,5%) and 1278 normospermic subjects (79,1%). Oligospermic and azoospermic patients showed all the morphological and functional semen parameters altered compared to mean values of normospermic population. Genomic DNA was extracted from 176 semen samples (45 azoospermic, 131 oligospermic with sperm count < 5x 106/ml) and from lymphocytes of 16 oligospermic men enrolled in the protocol for assisted reproduction. The samples were analysed for chromosome -Y microdeletion by multiplex-PCR. Initially we amplified a panel of 6 Y-specific STS that can identify 90% of possible microdeletions in AZF a, b, c regions (Simoni et al 1998). ZFY and SRY were used as internal controls of PCR reactions. Among patients selected according to their sperm count we found 1 azoospermic subject deleted in AZFc locus. The successive histological examination of a testicular biopsy revealed a Sertoli-cell only syndrome. Among the 16 men, enrolled to assisted reproduction protocol, the cariotype was 46,XY in 15 subjects and XX, in 1 subject. Four out fifteen oligospermic men were deleted in AZFb regions (26,6%). Although the number of patients analysed is still low, our findings support that the research for Y- microdeletions could be indicated in patients with idiopathic infertility and sperm concentration < 5x 106/ml. The high frequency of deletion (26,6%) found in our patients candidate for assisted reproduction suggests the recommendation to perform accurate genetic and molecular screening and genetic counselling before their enrolment in an intracytoplasmic injection/ in vitro fertilization programme.

P1565. Transthyretin Ser-44 Mutation in a Case with Vitreous Amyloidosis

A. Murakami, *K. Fujiki, S. Hasegawa, H. Kawano, A. Kanai*
Juntendo University School of Medicine; Tokyo, Japan
amurak@med.juntendo.ac.jp

Purpose; To report a case of vitreous amyloidosis with a transthyretin (TTR) variant Ser44. Case; A 44-year-old Japanese female had a 2-month-history of visual disturbance of the both eyes. No history for ocular and neurological disease existed among relatives. Corrected visual acuity was RE; 0.02 and LE; 0.9. Both pupils did not react to light and were about 6 mm in diameters. The vitreous in the both eyes were cloudy, in which fine fibrilar opacities were observed. Pars plana vitrectomy and biopsy of conjunctiva of the right eye was performed. Methods; Pathological and molecular genetic studies were performed with the patient s informed consent. The vitreous and conjunctiva specimens were subjected for histopathological examination. DNA was isolated from peripheral blood cells of the patient and sequencing of the TTR gene was performed. Results; The vitreous and conjunctiva specimens showed typical light microscopic and ultrastructural features of amyloidosis. Direct sequencing of exon 2 of the TTR gene revealed a single base-pair substitution, which results in an amino acid substitution at position 44, phenylalanine to serine (TTR Ser-44). TTR Ser-44 variant had been found only in an American patient with amyloidal peripheral neuropathy, however no ocular symptoms were described. Conclusion; Our studies indicate TTR Ser-44 variant could cause vitreous amyloidosis. TTR-related amyloidosis should be considered as a possible cause of vitreous opacities even in the absence of the family history.

P1566. NPC1; Complete genomic sequence, mutation analysis and identification of a susceptibility haplotype in Caucasian NP-C Patients.

P. Bauer[1], *R. Knoblich*[1], *C. Bauer*[1], *A. Hufen*[1], *J. Kropp*[1], *S. Braun*[1], *K. Harzer*[2], *A. Rolfs*[1]
[1]*University of Rostock; Rostock, Germany;* [2]*University of T bingen; T bingen, Germany*
peter.bauer@med.uni-rostock.de

Niemann-Pick type C (NP-C) disease is a rare, autosomal recessive lipid storage disorder. Its phenotype is clinically characterized by progressive neurological deterioration with ataxia, epilepsy and supranuclear gaze palsy. Biochemically a block in the translocation of LDL-derived cholesterol from the lysosomes and Golgi apparatus is considered as the cause of the phenotypic alteration. NPC1 could be established as candidate gene in about 95% of all NP-C patients, and its cDNA sequence and exon-intron boundaries have been characterized. We describe for the first time the complete genomic sequence of human NPC1 covering 51kb. Mutation analysis in 12 Caucasian NP-C patients revealed 6 novel and 3 known disease-causing alterations. Furthermore, several common polymorphisms in exonic and intronic parts of the gene were identified. To assess the transmission of NPC1 alleles, 12 Caucasian NP-C patients have been haplotyped for their mutational patterns including the polymorphic exonic loci H215R (644 a>g), I858V (2572 a>g) and N931N (2793 c>t). Additionally, the latter SNPs have been haplotyped in 154 alleles of healthy Caucasian control subjects. While 71,8% of our NP-C alleles and especially two NP-C patients (4 alleles), lacking a second mutated allele, shared the haplotype (644g, 2572g, 2793t), this haplotype accounts for only 46,3 % in the control group. Therefore we consider this haplotype to be associated with a non-coding, disease-causing mutation. Whenever complete sequencing of the open reading frame of NPC1 reveals only one potentially disease-related heterozygosity in NP-C patients it is recommended to haplotype these SNPs.

P1567. A novel 7.9 kb deletion causing alpha+-thalassemia in two independent families from Surinam.

C. L. Harteveld[1], *P. van Delft*[1], *P. W. Wijermans*[2], *M. C. Kappers-Klunne*[3], *P. C. Giordano*[1]
[1]*Leiden University Medical Center; Leiden, The Netherlands;* [2]*Community Hospital Leyenburg; The Hague, The Netherlands;* [3]*Academic Hospital Rotterdam Dijkzigt; Rotterdam, The Netherlands*
c.l.harteveld@lumc.nl

Alpha-Thalassemia is most frequently the result of deletions involving one or both alpha-globin genes on the short arm of chromosome 16. These deletions involve homologous or non-homologous recombinations based upon the presence or absence, respectively, of homology between parental sequences at the site of recombination. One of the most common mechanisms leading to alpha+-thalassemia involves misalignment between the two highly homologous 4-kb duplicated units in which the alpha-globin genes are embedded. Crossing-over between the misaligned homology boxes (X, Y and Z) gives rise to the frequently occurring -alpha 3.7 (Rightward) and -alpha 4.2 (Leftward) deletion. However, several other alpha+-thalassemia determinants have been described for which the mechanisms are either still unknown or involve a non-homologous recombination event. We report the characterization of a novel 7.9 kb deletion taking away one of the duplicated alpha-globin genes and causing an alpha+-thalassemia phenotype in two independent carriers of Surinam-Indian origin. The molecular characterization of the deletion breakpoint fragment revealed neither involvement of Alu-repeat sequences nor the presence of homologous regions prone to recombination, suggesting a non-homologous recombination event. This alpha+-thalassemia deletion was found to give rise to an atypical HbH-disease characterized by a non-transfusion dependent moderate microcytic hypochromic anemia, in combination with a poly-adenylation signal mutation of the alpha-globin gene (alpha2 -AA). Characterization of alpha+-thalassemia defects is, in spite of the mild phenotype, important for preventing obsolete and deleterious iron therapy in the carrier and severe HbH disease in the progeny.

P1568. Rapid detection of §-thalassemia mutations by the PCR based methods

R. Basalic[1], *L. Neagu*[2], *L. Gavrila*[1]
[1]*University of Bucharest; Bucharest, Romania;* [2]*Instutite A. Rusescu for Mother and Chil Care; Bucharest, Romania*
rbasalic@botanic.unibuc.ro

The thalassemias are a group of inherited recessive disorders in with a defect in synthesis of globin polipeptide chain of hemoglobin is present. Reliable diagnosis of the thalassemia syndromes can be achieved only by using modern methods of molecular genetics. New procedures to detect

beta-thalassemia mutations have been developed. All of them are based on PCR methodology. their main advantages are simplicity and rapidity. The analysis of genomic DNA isolated from the blood of the patient affected with beta-thalassemia is carried out by the Amplification Refractory Mutation System (ARMS) method. The ARMS-PCR is a simple and rapid method of detecting point mutations, restriction fragment length polymorphisms (RFLPs) and small nucleotide insertion or deletion. The IVS1-110 and IVS2-745 point mutations were identified in beta-thalassemia patients. These are the most common mutations in Romanian beta-thalassemic populations.

P1569. Double heterozygosity for hemoglobins C and Lepore-Boston-Washington in a patient from Croatia

K. G. Bardarova[1], *D. Plaseska-Karanfilska*[1], *V. Preden-Kerekovic*[2], *G. D. Efremov*[1]
[1]*Macedonian Academy of Sciences and Arts; Skopje, The former Yugoslav Republic of Macedonia;* [2]*Clinical hospital Merkur ; Zagreb, Croatia*
dijana@manu.edu.mk

Double heterozygosity for hemoglobin (Hb) C and Hb Lepore is a rare condition which has been described only in a few cases. Hb C is predominantly found in Blacks with the highest frequency in West Africa, while Hb Lepore-Boston-Washington is the most common hybrid Hb found mainly in the Balkan peninsula. Here we present a 25-year old patient from Croatia with this condition. The presence of two abnormal hemoglobins was observed on cellulose acetate gel electrophoresis during routine hematological and hemoglobin investigation. Hb C was characterized by DNA sequencing, while the presence of Hb Lepore-Boston-Washington was confirmed by PCR amplification of the Lepore hybrid gene using 5 delta- and 3 beta-globin gene specific primers, followed by Pvu II restriction enzyme digestion. Family studies showed that Hb Lepore was inherited from the patient s father who has a Croatian ancestry, while Hb C was inherited from his mother who originates from Hungary. Since Hb C has been found very rarely in this region, haplotype analysis were performed to trace its origin in this family. It is known that Hb C occurs almost exclusively on one haplotype, with a few exceptions due to recombination in the cluster. Hb C in this family was found on haplotype which differs at two polymorphic sites from the usual Hb C haplotype. Additional two informative polymorphisms (Taq I/inter-gamma and Hpa I/3 beta) confirmed that the beta-C chromosome in this family was not derived from African Black chromosomes, but represents a new mutation.

P1570. A new Pro->Ser amino acid substitution at position 119 of the Alpha-1 globin chain induces a mild a-thalassemia phenotype in a Moroccan family

P. J. M. Kok[1],*C. L. Harteveld*[2], *P. van Delft*[2], *R. Plug*[2], *F. G. A. Versteegh*[1], *P. C. Giordano*[2]
[1]*Groene Hart Hospital; Gouda, The Netherlands;* [2]*Leiden University Medical Center; Leiden, The Netherlands*
P.C.Giordano@lumc.nl

Alpha-thalassemia is generally considered to be an expression defect caused mostly by deletions silencing the expression of one or more alpha globin genes. Although non-deletion mutation alpha-thalassemia is considered rare, we frequently observe in our laboratory alpha-thalassemia phenotypes induced by points mutation by various mechanisms. A family of recent Moroccan origin living in The Netherlands was studied because of the persisting microcytic hypochromic anemia observed in a non-iron depleted young son. No abnormal hemoglobin components were visible on electrophoresis or HPLC but the expression unbalance of beta/alpha = 1.27 measured in absence of HbH inclusion bodies, indicating the depletion of one alpha gene. No deletion defects were detected by Southern Blot analysis or by brake point PCR. Amplification and direct sequencing revealed a normal sequence of both the alpha2 genes and the heterozygous pattern for a C->T transition at position 119 of the alpha1 genes. The CCT triplet at position 119 of the alpha gene codes for a proline which is located at the beginning the H helix of the alpha chain and is therefore a fundamental residue in the formation of the secondary structure of the alpha-globin chain. Over 200 single amino acid substitutions have been described affecting the homologous alpha1 and alpha2 globin chains, mainly inducing detectable abnormal hemoglobin tetramers. The CCT->TCT mutation, changing proline 119 to a serine, is the first mutant ever described at this particular position, suggesting that residue 119 might be fundamental for dimers and tetramers formation. Indeed no abnormal hemoglobin fraction was detectable in the lysate probably indicating the absence of tetramers containing the anomalous chain and an early proteolytic degradation of the monomer resulting in the unbalanced beta/alpha ratio and in the mild alpha-thalassemia phenotype observed in the carriers of this mutation.In spite of the mild character, this mutation could generate an intermediate hemolytic anemia (HbH disease) in the progeny of parents with a combination of this point mutation with one of the frequent alpha zero deletion defects.

P1571. Using multiplex PCR to detect beta-thalassemia mutations in a Vietnamese patients

N. H. Phuc, *N. H. Giang*
Center of Biotechnology, Vietnam National University; Hanoi, Viet Nam
nhgiang@netnam.org.vn

Beta-thalassemia is a prevalent genetic disease in Vietnam. From people in all over the world, more than 200 different thalassemia mutations in beta-globin gene have been reported so far. The aim of our research is getting distribution of beta-thalassemia mutations in a Vietnamese population. Further more, those data will be served for prenatal diagnosis of thalassemia mutations soon. Using multiplex PCR with control and specific primers for 6 mutations; frameshift codons 41-42(-CTTT), codons 71-72(+A), nonsense codon 17(A-T), codon 28(A-G), codon 29(A-G) and IVS II-654(C-T) to screening 195 Vietnamese patients. The result shows that the mutation at codons 41-42 is the most common i Vietnam (42,6%). Whereas mutations 28, 71-72 and IVS II-654 are less frequent (3,6 - 3,1 and 2,0%). 49 beta-thalassemia families were performed for those mutations, as well;

- 18 cases a child and mother/father had mutation at codons 41-42
- 7 cases had mutation at codon 17
- 3 cases had mutation at codon 29
- 1 case hadmutation at codon 28
- 3 cases had mutation at codons 71-72
- 2 cases had mutation at IVS II-654.
- One patient had 2 mutations (71-72 from his mother and IVS II-654 from his father).

P1572. Genotype-phenotype correlation in ataxia-telangiectasia

R. Bendix[1], *A. Osmers*[1], *M. Stuhrmann*[1], *D. Schindler*[2], *M. Stumm*[3], *R. Wegner*[4], *S. Scott*[5], *M. Lavin*[5], *T. D rk*[1]
[1]*Medizinische Hochschule; Hannover, Germany;* [2]*Biozentrum, Universit t; W rzburg, Germany;* [3]*Otto-von-Guericke-Universit t; Magdeburg, Germany;* [4]*Humboldt-Universit t; Berlin, Germany;* [5]*Royal Brisbane Hospital; Brisbane, Australia*
doerk.thilo@mh-hannover.de

Ataxia-telangiectasia (A-T) is an autosomal recessive multi-organ disease characterised by cerebellar ataxia and the variable presence of other neurological symptoms, immunological deficiencies, telangiectasia and cancer proneness. In most cases, A-T is caused by truncating mutations of the ATM gene, whose product is involved in the regulation of cellular responses to radiation injury. We have been investigating the correlation between type of ATM gene mutations, function of the gene product and clinical phenotype in 40 unrelated A-T patients. The median age of the patients was 11 years; four of them had been deceased between age 5-20 years from cancer or infections. Ataxia usually started in early childhood but a milder course and, thus, delayed diagnosis of A-T was apparent for a few patients with splice mutations, amino acid substitutions or in-frame-deletions. Of note, one 58 year-old-patient was found to be a compound heterozygote for a double missense mutation (D2625E&A2626P) and a new splice mutation (IVS7+5G>A). Our functional studies in cultured lymphoblasts from A-T patients assessed ATM protein levels as well as radiation-induced phosphorylation of p53 and nibrin. Residual ATM protein was found in a few cell lines with splice, missense or unknown mutations. Residual kinase activity was demonstrated in five A-T cell lines established from patients carrying splice or missense mutations but not in those harbouring two truncating mutations. These results indicate that the clinical variability of A-T is at least in part determined by the type of mutation and, in exceptional cases, the disease course can extend to late adulthood.

P1573. Identification of SURF1 gene mutations in Polish patients with Leigh syndrome

D. Abramczuk-Piekutowska, *E. Popowska, E. Karczmarewicz, M. Pronicki, M. Krajewska-Walasek, E. Pronicka*
Children s Memorial Health Institute; Warsaw, Poland
dorck@czd.waw.pl

Leigh syndrome (LS) is the most common mitochondrial cytopathy of early age (infancy and childhood) and it may result from several defects of mitochondrial enzyme complexes. One of the most frequent form of LS (classical form) is associated with cytochrome c oxidase (COX, complex IV) deficiency and is inherited as an autosomal recessive trait. No mutations in any of 13 polypeptide subunits of human COX have been detected in LS patients. Recently, LS-COX- locus has been mapped to chromosome 9q34

and mutated SURF1 gene has been identified in LS patients. We present the identification of SURF1 gene mutation in 18 Polish patients with classical form of Leigh syndrome. Sequence analysis revealed the presence of novel 704T>C transversion (Met235Thr), and two different recurrent dinucleotide deletions (758delCA, 845delCT). 845delCT was identified in 75% of all our patients in homozygote or heterozygote state. Besides, one nucleotide 573C>G transversion resulted in silent mutation (Thr191Thr) was detected. Our study confirms the recent observations that SURF1 gene is consistently involved in disorders of mitochondrial respiratory chain in patients with typical Leigh syndrome. The study was partially supported by KBN-CMHI Project No.S76/2000.

P1574. Screening of Bruton s tyrosine kinase gene in Russian patients with X-linked agammaglobulinemia.

S. Tverskaya[1], *N. Vasserman*[1], *I. Resnick*[2], *A. Polyakov*[1], *O. Evgrafov*[1]
[1]*Research Center for Medical Genetics; Moscow, Russian Federation;* [2]*Research Institute for Paediatric Haematology; Moscow, Russian Federation*
stver@online.ru

X-linked agammaglobulinemia (XLA) is an immunodeficiency caused by lymphocyte differentiation block. XLA is caused by mutations in all five domains of the gene encoding Bruton s tyrosine kinase (BTK). The gene is localised at Xq21.3-Xq22 and consists of 19 exons. There are no frequent mutations in this gene. Point mutations are the most common. We have developed multiplex systems for simultaneous amplification and SSCP-analysis of several exons of the BTK gene. DNA samples from 26 unrelated patients with XLA were analysed by this method. We have found 15 mutations, only two of them are described in BTK database. The novel rearrangements mutations from large deletions of some exons up to different missence mutations were detected in different domains of BTK gene. Three of them were found in 6th exon. The correlation between genotype and clinical features are rather interesting.X-linked agammaglobulinemia (XLA) is an immunodeficiency caused by lymphocyte differentiation block. XLA is caused by mutations in all five domains of the gene encoding Bruton s tyrosine kinase (BTK). The gene is localised at Xq21.3-Xq22 and consists of 19 exons. There are no frequent mutations in this gene. Point mutations are the most common. We have developed multiplex systems for simultaneous amplification and SSCP-analysis of several exons of the BTK gene. DNA samples from 26 unrelated patients with XLA were analysed by this method. We have found 15 mutations, only two of them are described in BTK database. The novel rearrangements mutations from large deletions of some exons up to different missence mutations were detected in different domains of BTK gene. Three of them were found in 6th exon. The correlation between genotype and clinical features are rather interesting.

P1575. Mutations in steroid 21-hydroxylase (CYP21) in Russia.

N. S. Osinovskaya[1], *T. E. Ivaschenko*[2], *V. S. Baranov*[2], *V. V. Potin*[2]
[1]*Inst.of Obst.& Gynecol.; St.Petersburg, Russian Federation;* [2]*Inst.of Obst.&Gynecol.; St.Petersburg, Russian Federation*
natosinovskaya@mail.ru

The spectrum of mutations in the steroid 21-hydroxylase gene (CYP21B) and the frequency of 11 mutations among 70 patients with different forms of congenital adrenal hyperplasia (CAH) were analyzed by means of PCR analysis. Each of the CAH forms was characterized by specific pattern of diagnostically important mutations. The salt-wasting (SW) form of the disease was more frequently associated with gene deletion (39%) and the 668-13C-G mutation in the second intron (23.5%) of CYP21 gene, whereas the majority of simple virilizing (SV) CAH cases were associated with the 1172N mutation in exon 4 (23.5%), gene deletion (16.5%), and the 668-13C-G mutation (16.5%). Mutations in the steroid 21-hydroxylase gene were detected in 70% of the chromosomes from the patients with the SW and SV forms of CAH respectivly. We investigated 78 chromosomes from the patients with nonclassic form (NC) of CAH; only in 1.3% mutations (gene deletion) were found. Neither V281L nor P30L mutations common for the NC CAH patients from other populations were detected in this study. This result can be explained either by the fact that NC CAH cases in Russia are associated with other major mutations, or with difficulties in clinical diagnostics of nonclassic form in our patients.

P1576. Genetic testing in Czech patients with idiopathic and hereditary chronic pancreatitis

M. Koudova[1], *R. Keil*[2], *J. Spicak*[3], *J. Martinek*[3], *J. Nevoral*[4], *E. Michkov* [4], *V. Vavrova*[5], *M. Macek*[1], *A. Krebsov* [1], *M. Macek Jr.*[1]
[1]*CF Center-Institute of Biology and Medical Genetics; Prague, Czech Republic;* [2]*Clinic of Internal Medicine; Prague, Czech Republic;* [3]*Clinic of Gastroenterology-IKEM; Prague, Czech Republic;* [4]*1st Clinic of Pediatrics, Charles Univ. Prague; Prague, Czech Republic;* [5]*2nd Clinic of Pediatrics, Charles Univ. Prague; Prague, Czech Republic*
koudova@lfmotol.cuni.cz,

We have analyzed the entire CFTR coding region by DGGE and screened for common PRSS 1 (R122H, N29I), SPINK 1 (N34S) mutations in a total of 65 chronic pancreatitis (CP) cases (idiopathic CP-iCP /incl. criteria; N Engl J Med 1998, 339;653; 22 children, 32 adults/; hereditary CP- hCP /EUROPAC incl. criteria; Med Clin North Am 2000, 84;575; 7 children, 4 adults/ and compared their frequency to 100 random controls /50F, 50M; age range 18-45 yrs./). In iCP the frequency of CFTR mutations deltaF508, R117H, L997F was increased only in adults (p=0.01) and was associated with an increase of the IVS8(T)9/(TG)10 haplotype (p=0.001) and of the 125G/C polymorphism (p=0.01), compared to controls. The frequency of R122H and N29I PRSS 1 mutations was significantly increased in both children/adults (3/22) with hCP (p<0.01), but not in all iCP cases (2/108) compared to controls. However, the SPINK 1 N34S mutation was found at a considerable frequency also in controls (4/200 chromosomes) - an observation challenging its previously reported pathogenic potential. Our data indicate that mutations in CFTR, PRSS 1 genes are associated with iCP/hCP, with PRSS 1 alleles being more often observed in early onset CP. In cases with positive screening results genetic counseling and long-term monitoring for the event. development of other cystic fibrosis-related symptoms (in iCP) and the adenocarcinoma of pancreas (in hCP) should be provided. Supported by grants;#6250-3, 6411-3, 000000064203; #111300003, ME258, OK192, LN00A079.

P1577. Linkage disequilibrium reduces the locus for venous malformations with glomus cells (VMGLOM) to a single YAC of 1.48 Mbp.

A. Irrthum[1], *P. Brouillard*[2], *L. M. Boon*[3], *B. R. Olsen*[4], *J. B. Mulliken*[4], *O. Enjolras*[5], *M. Vikkula*[1]
[1]*Christian de Duve Institute of Cellular Pathology; Brussels, Belgium;* [2]*Christian de Duve Institute of Cellular Pathologygoy; Brussels, Belgium;* [3]*Universit catholique de Louvain; Brussels, Belgium;* [4]*Harvard Medical School; Boston, MA United States;* [5]*H pital Lariboisi re; Paris, France*
vikkula@bchm.ucl.ac.be

Venous malformations with glomus cells (glomuvenous malformations) are localized cutaneous lesions of vascular dysmorphogenesis. They are usually sporadic, but sometimes familial. Using five families, we have mapped the locus, VMGLOM, to chromosome 1p21-p22. In order to refine this locus, spanning 4-6 Mbp, we now studied seven additional families. They exhibited linkage to VMGLOM and the combined LOD score for all twelve families was 18.41 at q = 0.0 for marker D1S188. We found a distinct haplotype shared by seven families, comprising seven alleles that are rare in the general population (P < 0.01). This indicated that the haplotype is identical by descent in all seven families, and hence the locus could be refined by inferring ancestral crossovers. Using this approach, we positioned the causative gene between two markers on the same non-chimeric YAC of 1.48 Mbp. Positional cloning recently lead to the identification of the mutated gene, a novel regulator of vascular morphogenesis that we named glomulin. Mutational analyses verified the common origin of the identified haplotype. (vikkula@bchm.ucl.ac.be)

P1578. Comparison of whole genome amplifications for genetic diagnosis

J. Kim[1], *J. Kim*[1], *B. Lee*[1], *Y. Kim*[1], *H. Ryu*[2]
[1]*Laboratory of Medical Genetics, Samsung Cheil Hospital; Seoul, Republic of Korea;* [2]*Department of Obstetrics and Gynecology, Samsung Cheil Hospital; Seoul, Republic of Korea*
genelab@samsung.co.kr

The purpose of this study was to evaluate the utility of DNA preamplification for the molecular analyses of a single or few cells. We therefore compared the efficiency of original primer extension preamplification (PEP), degenerate oligonucleotide primer-PCR (DOP), and modified PEP (M-PEP) using each of the 5 amniocytes obtained from 50 pregnant women. As a preliminary experiment for the preimplantation genetic diagnosis (PGD) in order to avoid the X-linked disorders, DMD and SMA, we have amplified X-, Y-centromeric sequences, dystrophin gene exon 46 and SMN gene exon 7 from a small aliquot of each whole gemone amplification (WGA) products. Comfirmed with chromosome analysis, we determined that 25 fetuses had the Y chromosome and the detection rates of PEP, DOP and M-PEP were 4, 68, and 96% in Y-specific amplification respectively. The amplification rates of X-specific region were 66, 96, 100 % in PEP, DOP, M-PEP respectively, and 14, 52, 66 % of dystrophin gene, and 4, 46, 74 % of SMN gene. Contaminated DNA sequences and false posi-

tive for female cells in Y-specific PCR were not detected. This study indicates that the efficiency of M-PEP was significantly greater than the other methods, and that M-PEP should be used for the diagnosis of fetal inherited diseases.

P1579. DHCR7 gene mutations — post- and prenatal diagnosis of Smith-Lemli-Opitz syndrome in Polish families

E. Ciara, *E. Popowska, M. Borucka-Mankiewicz, D. Abramczuk-Piekutowska, M. Krajewska-Walasek*
Children Memorial Health Institute; Warsaw, Poland
dorcik@czd.waw.pl

Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive disorder of cholesterol biosynthesis, characterized by facial dysmorphisms, mental retardation and multiple congenital anomalies. The birth prevalence of SLOS is estimated to be 1;20 000 to 1;40 000, possibly the second most common recessive metabolic disorder after phenylketonuria. The carrier frequency is calculated to be 1;100 to 1;50. SLOS is caused by mutations of the D7-sterol reductase (DHCR7) gene. Up to now about forty different mutations, in all translated exons 4-9, have been described. The majority (50%) of the mutations are clustered in exon 9. We present mutational analysis of the DHCR7 gene investigated in a new group of Polish patients with SLOS and in two pregnant women at risk. Five different point mutations of a nucleotide substitution type, W151X in exon 6 (on one allele), L157P in exon 6 (on two alleles), V326L in exon 9 (on six alleles), V338M in exon 9 (on one allele) and IVS8-1;g>c in splice acceptor site (on one allele) were identified. Mutation V338M is a novel one, the other substitutions are recurrent in Polish and European populations and have been described previously. Three mutations were found in one patient. Analysis of patient s parents revealed the presence of two different mutations on mother s allele. In two families, molecular diagnosis of chorionic villi excluded SLOS in fetuses. These studies were supported in part by KBN, Project No.4P05E09118 and the Subsidies for Scientists 2000 Programme of the Foundation for Polish Science.

P1580. Mutations in Smith-Lemli-Opitz syndrome Russian patients.

N. Vasserman, *N. Demina, S. Tverskaya, A. Polyakov*
Research Center for Medical Genetics; Moscow, Russian Federation
stver@online.ru

The autosomal recessive Smith-Lemli-Opitz syndrome (SLOS) is an inborn disorder of sterol metabolism with characteristic congenital malformations and dimorphisms. All patients suffer from mental retardation. The gene of 7-sterol reductase (DHCR7) responsible for the de novo biosynthesis of cholesterol have been identified as a SLOS gene by Fitzky B.U. et al (1998). DHCR7 gene is localised on 11q13 and consists of 9 exons. DNA from 14 unrelated patients with dubious diagnosis SLOS were analyzed for mutation in DHCR7 gene for different diagnostics. Four mutations in 2 families were revealed. The prenatal diagnostics without proband DNA was performed in one family with SLOS. Fetus received chromosome with mutation in 9th exon of DHCR7 gene from his mother and chromosome without mutation in 6th exon from father. Prognosis for fetus was favourable. Among these 4 mutations one was described by Fitzky B.U. et al (1998), three other were novel.

P1581. The human LDL receptor gene (LDLR) database; Molecular analysis of 625 mutations

L. Villeger, *M. Abi-Fadel, S. Rochaud, A. Robin, J. Rabes, C. Junien, C. Beroud, C. Boileau, M. Varret*
INSERM UR-383, Hopital Necker-Enfants Malades; Paris, France
villeger@necker.fr

To date, 711 mutations have been identified in the LDLR gene, encoding the low-density lipoprotein receptor, in subjects with Familial Hypercholesterolemia. Although genotype/structure-function correlations have been substantially investigated, genotype/phenotype correlations have not been explored. Thus, we have compiled a database containing standardized data for each LDLR mutation, and developed the software that provides sorting tools and allows optimized multicriteria research [http;//www.umd.necker.fr]. The analysis of the 625 point mutations in the UMD-LDLR database gives the following information; [1] 58% of the mutations are missense, and 17% occur in CpG dinucleotides known to be mutational hot spots; [2] although widely distributed throughout the gene, there is an excess of mutations in exons 4 and 6 (ligand-binding repeats), 7 (EGF-like repeat), and 9 (EGF-precursor-like); [3] there is a deficit of mutations in exons 10 and 13 (EGF-precursor-like), 15 (O-linked-sugar), 16 (transmembrane), 17 and 18 (cytoplasmic); [4] 47% of the small deletions occur between repeated sequences and can be explained by the slipped-mispairing model described by Krawczak and Cooper; [5] 68% of the mutations in the ligand-binding domain affect conserved amino-acids involved in LDL binding; [6] the functional data available for 183 (29%) mutations indicate 38% of class 2B (transport defective) and 33% of class 1 mutations (null alleles); [7] finally, the investigation of genotype/phenotype correlations is difficult since the clinical data is usually incomplete in mutation reports. Direct access to the database through the web site should facilitate the input of high quality clinical information and should overcome this shortage.

P1582. Analysis of CANP-3 gene by PCR and hybridization with oligonucleotide microchips in Russian Families with LGMD patients.

I. Krakhmaleva[1], *S. Zakharov*[1], *S. Shishkin*[1], *S. Limborska*[2], *T. Pogoda*[2], *N. Shakhovskaya*[3], *S. Artemieva*[3], *I. Lunga*[1], *T. Nasedkina*[4], *D. Gryadunov*[4], *A. Mirzabekov*[4], *N. Gerasimova*[5]
[1]*Institute of Human Genetics, Research Center for Medical Genetics RAMS; Moscow, Russian Federation;* [2]*Institute of Molecular Genetics RAS; Moscow, Russian Federation;* [3]*Children s Psycho-Neurological Hospital of Moscow Region; Moscow, Russian Federation;* [4]*Engelhardt Institute of Molecular Biology RAS; Moscow, Russian Federation;* [5]*Interregional Association of assistance to people suffering neuromuscular diseases The Hope ; Moscow, Russian Federation*
socrates@orc.ru

CANP-3 gene consisted of 24 exons is one of 11 different genes in which mutations cause Limb-girdle muscular dystrophy (LGMD2A) but just defects of this gene determine considerable part of recessive LGMD cases. Many missense mutations and microdeletions, detected in CANP-3 gene, make difficulties for DNA-diagnosis of LGMD. The combination of PCR-analysis and hybridization of PCR products with oligonucleotide microarrays may permit to resolve this problem.

Information about 326 LGMD patients is collected in computer database MYODYS-2.2 that we created by use common diagnostic criteria and medical documentation about 3900 Russian families with hereditary neuromuscular disorders. DNA samples of 43 individuals from LGMD families were obtained and PCR products of several parts of CANP-3 gene (Pm, exons 1, 2, 3, 4, 5 , 6, 19, 20, 21 22) were analyzed. 17 and 3 patients with LGMD are showed polymorphism (SSCP) in exon 4 and exon 21, accordingly. By subsequent sequencing two mutations in exon 4 - deletion of 1 bp (550˘A) and deletion of 15 bp (˘598-612) were detected. SSCP analysis and allele-specific PCR revealed 550˘A of CANP-3 as common mutation in our group of Russian families with recessive LGMD (57% homo- and heterozygous both). In control group (donors from healthy families, n=34) one person turned out heterozygous for 550DA. Using computer analysis of exon 4 sequence we synthesized special set of six pairs of oligonucleotides to detect 6 mutations, identified in exon 4 of CANP-3 gene earlier. The microarray was prepared by immobilization of oligonucleotide probes in gel pads using the previously described technology. Hybridization of DNA samples with this microarray revealed the distinctive discrimination between 550DA homo- and heterozygous. This approach seems to be successful for the fast detection of the main part of point mutations in CANP-3 gene.

P1583. Male Infertility; Chromosome And Genome Analysis

C. Curcio[1], *M. Travi*[1], *P. Primignani*[1], *F. Sbrocca*[1], *A. Biasi*[1], *M. Seia*[1], *A. Ambrosioni*[1], *S. Guarneri*[2], *P. Ilardi*[2], *D. Diaferia*[3], *G. Ragni*[3], *A. Cant Rajnoldi*[1], *D. A. Coviello*[1]
[1]*Molecular Genetic Laboratory, Istituti Clinici di Perfezionamento; Milan, Italy;* [2]*Cytogenetic Laboratory, Istituti Clinici di Perfezionamento; Milan, Italy;* [3]*Infertility Center, Istituti Clinici di Perfezionamento; Milan, Italy*
coviello@unige.it

Connection between chromosome abnormalities and male infertility are well documented. Recent studies have indicated that microdeletions on at least 3 regions (AZFa, AZFb, AZFc) on the Y chromosome code for spermatogenesis factors. Moreover, the involvement of CFTR gene defects have been demonstrated in obstructive azoospermia and, in general terms, in male infertility. The purpose of this study is to evaluate the connections between chromosome analysis, Y chromosome microdeletions, CFTR gene and different male infertility phenotypes. Phenotypes discrimination criteria; Azoospermia, Criptozoospermia (<1x106/ml), severe oligospermia (<5x106 ml). DNA microdeletions analysis . Molecular study of AZFa, b, c regions has been performed in 274 infertile men coming to our Centre from different Italian regions or from other Countries; in particular 82 subjects with azoospermia, 13 with criptozoospermia and 179 with severe oligospermia. The PCR study screened for 18 STS markers spanning the euchromatic Yq region. Chromosome study . Kariotype analysis was carried out on 169 infertile men. The remaining cases were tested in other laboratories. CFTR Gene analysis-TGm and Tn (T5, T7, T9) locus.

The Cystic Fibrosis gene mutations study was at first carried out on 139 azo-oligospermic subjects and subsequently in a second more selected group of 41 Congenital Bilateral Aplasia of Vas Deferent (CBAVD) patients. CFTR gene mutation was carried out by Polymerase Chain Reaction-Oligonucleotide Ligation Assay (31 CF mutations simultaneously), DGGE analysis and polymophism analysis of IVS 8-(TG)m, Tn tracts. We found Yq microdeletions in 4 infertile patients (deletion frequency of 1.46%); the first deletion involve the AZFb region, the second involves the AZFb and AZFc regions, the third and fourth involves AZFc region. Patients with AZFb or AZFb,c deletions were azoospermic, patients with AZFc deletion were criptozoospermic and azoospermic respectively. Constitutional chromosome abnormalities were identified in 12 subjects (frequency 4,38%); 4 cases in azoospermic patients, 3 in criptozoospermic pts and 3 in oligospermic pts. CFTR gene analysis; 41 CBAVD subjects ; 7/41 (17%) homozygous/ heterozygous compound; 5/41 (12%) heterozygous + 5T carrier; 12/41(29%) single mutation heterozygous; 7/41 (17%) 5T carrier. The frequency of locus Tn in 34 pts CBAVD is;5T=14,7%, 7T=58;8%, 9T=26.5%. CFTR gene analysis; 139 azo-oligospermic subjects; 9/139 (6.5%) single mutation heterozygous; 130/139 (93,5%) no known mutations. The frequency of locus Tn in 139 azo-oligospermic pts is; 5T=9%, 7T=79,5%, 9T=11

P1584. Primary ciliary dyskinesia, an heterogeneous genetic pathology; evaluation of candidate genes

G. Pennarun[1], *A. M. Bridoux*[1], *E. Escudier*[2], *S. Amselem*[1], *B. Duriez*[1]
[1]*Inserm U468; Creteil, France;* [2]*Service d Histologie, Pitie-Salpetriere; Paris, France*
pennarun@im3.inserm.fr

Primary ciliary dyskinesia (PCD) is a group of heterogeneous inherited disorders, usually transmitted as an autosomal recessive trait. The disease phenotype is characterized by various axonemal abnormalities of respiratory cilia and sperm tails, leading to bronchiectasis and sinusitis, sometimes associated with situs inversus (Kartagener s syndrome) and male sterility. We have isolated the first gene involved in PCD, DNAI1, using a candidate-gene approach. To date, DNAI1 is the only gene known to be involved in PCD; it is highly likely that numerous PCD-causing genes remain to be identified. We have evaluated the possible involvement of two candidate genes in PCD; HP28 and HFH4. Mutation of the Chlamydomonas reinhardtii p28 gene, which encodes a dynein light chain, leads to an absence of inner dynein arms in flagellar axonemes of this unicellular alga. In a mouse model, the targeted disruption of the transcription factor hfh4 results in complete absence of cilia and randomization of left-right asymmetry. We, therefore, considered the human orthologs of p28 and hfh4 as good candidates for the PCD phenotypes characterised by similar defects. Four PCD independent patients presenting with an absence of inner dynein arms, two independent patients having no cilia, and one family with three affected brothers having few cilia, were investigated. Molecular screening of the complete coding regions of the two candidate genes, HP28 and HFH4, in our PCD population revealed no mutation. However, given the genetic heterogeneity in PCD, this result does not exclude their possible involvement in other PCD patients.

P1585. The DNAH11 (axonemal heavy chain dynein type 11) gene is mutated in one form of Primary Ciliary Dyskinesia (PCD)

L. Bartoloni[1], *Y. Pan*[2], *J. L. Blouin*[1], *A. K. Maiti*[1], *C. Gehrig*[1], *C. Rossier*[1], *N. Scamuffa*[1], *G. Sail-Duriaux*[1], *H. Mitchison*[3], *M. Meeks*[3], *C. D. DeLozier-Blanchet*[1], *R. M. Gardiner*[3], *W. J. Craigen*[2], *S. E. Antonarakis*[1]
[1]*Division of Medical Genetics, University of Geneva Medical School; Geneva, Switzerland;* [2]*Department of Molecular and Human Genetics, Baylor College of Medicine; Houston, TX United States;* [3]*Department of Pediatrics, Royal Free and University College Medical School; London, United Kingdom*
lucia.bartoloni@medecine.unige.ch

PCD is a genetically heterogeneous autosomal recessive disease. To define genes responsible for PCD we analysed the DNA of a patient with immotile cilia, situs inversus and respiratory distress. This remarkable patient presents uniparental (paternal) disomy of chromosome 7 (patUPD7) and homozygosity for CFTR- F508del. We performed mutation analysis in 67 of the exons of the heavy chain dynein DNAH11 gene mapping on chromosome 7 and homologue to Lrd (mutated in the mouse iv/iv model of situs inversus). A nonsense mutation (R to X), homozygous because of UPD7, was identified in one of the exons coding for the motor domain of DNAH11. The normal ciliary ultrastructure in this patient is compatible with the nonsense mutation. The truncated protein can be incorporated in the dynein arm even if inactive since it lacks the microtubule-binding domain. This is the first report of a mutation in an axonemal heavy chain dynein gene causing PCD. A microsatellite internal to DNAH11 was used in 31 families with at least 2 affecteds, to select 6 families with co-segregation of the same DNAH11 alleles in affecteds. The mutation search in these 6 families showed 52 variants, 30 intronic and 22 exonic (ten of which are silent polymorphisms and 8 are common variants). Studies to differentiate potential disease-causing mutations from polymorphisms are in progress, as well as the cloning of the few remaining exons of DNAH11 and the completion of the mutation analysis.

P1586. A sensitive molecular test for Marfan syndrome

I. Kaitila[1], *J. K rkk* [2], *L. L nnqvist*[3], *L. Ala-Kokko*[2]
[1]*Department of Clinical Genetics, Helsinki University Hospital; Helsinki, Finland;* [2]*Center for Gene Therapy, Tulane University Medical Center; New Orleans, LA United States;* [3]*Department of Human Molecular Genetics, National Public Health Institute; Helsinki, Finland*
ilkka.kaitila@hus.fi

Molecular diagnostic confirmation/exclusion of Marfan syndrome (MFS) through FBN1 gene analysis by single stranded confirmation polymorphism (SSCP) is insensitive, time consuming, and expensive. Yet, it would be valuable for atypical cases and possibly affected family members. After sequencing the exon boundaries of the human FBN1 we designed PCR primers to amplify all FBN1 exons and exon boundaries, and screened two sets of patients and control individuals for FBN1 mutations by conformation sensitive gel electrophoresis (CSGE) followed by sequencing. The first set consisted of 46 DNA samples from 12 sporadic and 12 familial cases with MFS, 3 sporadic and 2 familial cases with annuloaortic ectasia (AAE), 1 case with familial ectopia lentis, and from 16 control individuals. The diagnosis was based on clinical evaluation and use of the diagnostic criteria for MFS (AmJMedGenet, 1996, 62;417-). The molecular analysis was performed blindly first with CSGE. Sequence variations observed by CSGE were identified by sequencing. The results showed FBN1 mutations in all patients with MFS, and in the patient with ectopia lentis but no mutations in the patients with AAE or in control individuals. The second set consisted of 18 patients with MFS and previously detected FBN1 mutations. CSGE followed by sequencing allowed us to characterize all but one of the previously detected mutations. Thus, the sensitivity and specificity in these sets were 100 per cent. Thus, CSGE followed by sequencing can be offered as a diagnostic test with high detection rate for confirmation/exclusion of MFS diagnosis.

P1587. A pathogenetic mechanism in classical Ehlers-Danlos syndrome and resolution of the fibril diameter paradox.

R. J. Wenstrup[1], *J. B. Florer*[1], *A. Xue*[1], *W. G. Cole*[2], *M. C. Willing*[3], *D. E. Birk*[4]
[1]*Children s Hospital Research Foundation; Cincinnati, OH United States;* [2]*Hospital for Sick Children; Toronto, ON Canada;* [3]*University of Iowa; Iowa City, IA United States;* [4]*Thomas Jefferson University; Philadelphia, PA United States*
wensr0@chmcc.org

Approximately one-third to one-half of individuals with classical Ehlers-Danlos syndrome (types I/II) have detectable abnormalities in the genes encoding the proa1(V) (COL5A1) or proa2(V) (COL5A2) chains of type V collagen, a quantitatively minor component of type I collagen-rich fibrils. Electron micrographs of skin from some patients with EDS were reported to have larger diameter collagen fibrils than control. In general, larger diameter collagen fibrils correlate directly with structural properties (stiffness and maximum force) and material properties (modulus and maximum stress) of tissues, yet dermal tissues of classical EDS patients are friable and dermal scars have apparent reduced mechanical strength. To investigate this apparent paradox, we examined matrix deposition and collagen fibril morphology in long-term cultures of fibroblast cell strains that have a fourfold difference in the ratio of type V to type I collagen gene expression. Compared to control cells, fibroblasts from 2 patients with OI type I due to COL1A1 haploinsufficiency deposited 46% of hydroxyproline per mg protein and EDS cells from 2 patients with COL5A1 haploinsufficiency deposited 38%. However, the EDS cells synthesized approximately the same amount of total collagen as controls and had similar secreted ratios of type I/type III collagen as control cells. Average collagen fibril diameter from EDS cells was 39.6– 12.5 nanometers compared to 32.6–6.9 nm for control and 25.1– 5.2 nm for OI cells. These data indicate that type V collagen may serve a dual function in dermal tissues. In addition to its posited role as a negative regulator of collagen fibril diameter, type V collagen may serve as a rate limiting nucleator of collagen fibrillogenesis in dermis. A reduced rate of collagen fibril formation in the dermis of classical EDS patients with COL5A1 haploinsufficiency may override any mechanical advantage conferred by increased fibril diameter.

P1588. Identification of the COL4A5 gene mutations in Slovenian Alport syndrome families

M. Slajpah[1], *A. Vizjak*[1], *A. Hvala*[1], *M. Koselj*[2], *M. Bidovec*[3], *B. Gorinsek*[1], *M. Ravnik-Glavac*[1], *D. Ferluga*[1], *D. Glavac*[1]
[1]*Laboratory of Molecular Genetics, Institute of Pathology, Medical Faculty; Ljubljana, Slovenia;* [2]*Department of Nephrology, University Medical Center,; Ljubljana, Slovenia;* [3]*Department of Pediatrics, University Medical Center,; Ljubljana, Slovenia*
maja.slajpah@mf.uni-lj.si

Alport syndrome (AS) is an important hereditary disorder characterized by nephritis, sometimes accompanied by impairment or loss of vision and hearing. Alport syndrome can be caused by mutations in COL4A5, one of the six type IV collagen genes. The most common form of the Alport syndrome is an X-linked dominant trait that has been associated with the gene of the alpha5 chain of type IV collagen (COL4A5). More than 300 different mutations have been identified in COL4A5 gene and they appear randomly along the whole gene. We report the first systematic mutation screening of all 51 exons with boundary intronic sequences of COL4A5 gene by single stranded conformation analysis (SSCA) after amplification of each exon by the polymerase chain reaction (PCR) method in forty-two patients from 16 randomly collected AS suspected families in slovenian population. The nucleotide sequence of all PCR products revealed by the abnormal mobility shift on PCR-SSCA was determined by the direct sequencing method using dsDNA cycle sequencing system (Perkin Elmer). PCR-SSCA of the COL4A5 gene of eight unrelated Alport families showed the presence of seven different mutations. Seven different mutations were identified in 8 families by cycle sequencing. Six of them are to the best of our knowledge new; G669R (GGT>CGT), G325R (GGA>CGA), R266X (CGA>TGA), G811R (GGA>AGA), G319D (GGT>GAT) and 1234+17 T del. Intronic mutation 1234+17 T del most likely influences the splicing of mRNA. As previously described (5) mutation G624D(GGT>GAT) has been identified in two families.

P1589. Large family with MODY diabetes and a novel mutation in HNF-4a

C. T. Monney[1], *P. Cousin*[1], *J. Ruiz*[2], *C. Bonny*[1], *D. F. Schorderet*[1]
[1]*Division of Medical Genetics; Lausanne, Switzerland;* [2]*Division of Endocrinology; Lausanne, Switzerland*
Carole.Monney@chuv.hospvd.ch

Maturity-onset diabetes of the young (MODY) is a subtype of non-insulin-dependent diabetes characterized by autosomal dominant inheritance and early age of onset. Several genes are known to or supposed to induce MODY ; HNF-4a/MODY1, glucokinase/MODY2, HNF-1a/MODY3, IPF1/MODY4, TCF2/MODY5, IB1, IB2 and NeuroD genes. We studied a Swiss family with 12 non-insulin-dependent diabetic patients over 3 generations. The average age of diagnosis was 36 – 15 years and the average BMI 25 – 3 kg/m2. In addition, 1 individual had an abnormal oral glucose tolerance test and 2 had gestational diabetes only. All candidate genes but HNF-4a were excluded by linkage analysis, using 2 to 4 flanking markers. Direct sequencing of the 14 exons of HNF-4a in 3 patients revealed the missense mutation gtc to atc in exon 4, resulting in V121I. This mutation is located in the DNA binding domain of HNF-4a, a strongly conserved region across almost all species. This mutation cosegregates in all the MODY affected patients and was never observed in 100 control chromosomes. Identification of this missense mutation allowed for the presymptomatic diagnosis in the young generation. This should improve the medical follow-up of the predisposed individuals.

P1590. Clinical and Molecular Study of an Iranian Kindred with Leber Congenital Amaurosis

M. Karimi-Nejad[1], *T. Rezaie*[2], *M. Meshkat*[3], *S. Sohbati*[1], *R. Karimi-Nejad*[1], *H. Najmabadi*[1], *M. Sarfarazi*[2]
[1]*Karimi-Nejad Pathology & Genetics Center; Tehran, Islamic Republic of Iran;* [2]*Molecular Ophthalmic Genetics Laboratory, University of Connecticut Health Center; Farmington, CT United States;* [3]*Department of Ophthalmology, University of Medical Sciences; Kerman, Islamic Republic of Iran*
mhkarimi@mavara.com

A large Iranian family with 102 members (8 deceased) from the Lore Tribe of Sirjan, Kerman was identified and studied. This kindred composed of 25 affected subjects in 16 nuclear families that spans over 11 interconnecting generations. Clinical examination of 12 subjects aged between 8-46 years revealed initial presentation of congenital blindness as early as 1-3 months of life. Infantile nystagmus, keratoconus, leukoma and cataracts developed later in life. Clinical presentation in this kindred is compatible with the diagnosis of Leber Congenital Amaurosis (LCA). DNA extracted from 41 subjects (including 12 affected) and cDNA synthesized for 6 affected and normal members. Highly polymorphic DNA markers from 5 different chromosomes (1, 6, 14, 17 and 19) previously linked to various LCA loci was genotyped in this family. Hint of linkage was identified with the 17p13.1 region containing the two known loci of LCA1 and LCA4. Homozygosity in affected subjects was only observed with D17S1881, D17S578, D17S960 and D17S1353 that are tightly linked to the LCA1 locus thus indicating that the phenotype in our family is also linked to the same locus. All affected subjects shared two identical haplotypes for these markers while healthy individuals inherited either two normal chromosomes or one of the two affected haplotypes. At least 4 different mutations in the Retinal Guanylyl Cyclase (retGC or GUCY2D) have been reported in different LCA1-linked families. This gene is 1,103 amino acids long and encodes for an extracellular, transmembrane and a cytoplasmic domain. Mutation screening of GUCY2D is currently in progress.

P1591. Two novel mutations of the TSH b subunit gene in Greek patients with low TSH hypothyroidism.

A. Sertedaki[1], *A. Papadimitriou*[2], *A. Voutetakis*[1], *M. Dracopoulou*[1], *C. Dacou-Voutetakis*[1]
[1]*A Department of Paediatrics, Athens University; Athens, Greece;* [2]*Department of Paediatrics, Penteli Children s Hospital; Athens, Greece*
aserted@cc.uoa.gr

Isolated CH caused by low TSH synthesis is rarely reported. To date, five different mutations of the TSH § subunit gene have been identified worldwide. The G29R mutation in the CAGYC region of exon 2 of the gene was identified in three different Japanese families. The C105T mutation was identified in two related Brazilian and two unrelated German families, as well as in one sporadic case from Belgium. Although this mutation appears to be common in western populations, has not so far been detected in any Greek patient with low TSH hypothyroidism. In our population we have identified three different mutations. The first mutation, a G to T transversion at nucleotide 94 in exon 2 changing the codon for the 11th aminoacid from glycine to a premature stop codon resulting in a truncated protein, was previously reported. Thus far three different Greek families with CH have been identified carrying this mutation. Two novel mutations were recently identified in exon 3 of the TSH § subunit gene in two siblings and in one sporadic case. The two siblings were diagnosed at 3 weeks. They both carry a previously undescribed homozygous mutation in exon 3 of the TSH § subunit gene, namely a C to T transition, resulting in a premature stop at codon 49, leading to the formation of a truncated protein. More than half of the mature peptide (aminoacids 50 to 118), including the bL3 loop, most part of the bsheet, the seat belt and the carboxyl-terminal are absent. It can therefore be postulated that since functionally important residues for the TSH heterodimer formation are missing, no TSH is secreted. The two siblings had different hormonal and clinical profile. The reason for this differential effect of an identical mutation is not apparent. The parents (both heterozygous for the same mutation) denied consaguinity. Nevertheless, they both originate from a small village. The second mutation is a T to C transition resulting in a change at codon 85 from cysteine to arginine. The cysteine residues of all glycoproteins are highly conserved, it is therefore speculated that this mutation results in conformational changes of the § subunit rendering it incapable to form a functional heterodimer with the a subunit. The rarity of reports on defects of the TSH § subunit gene is unexplained. Is it really a rare genetic defect or does it escape detection? As expected, this form of CH is not detected by neonatal screening using TSH methodology.

P1592. Molecular and functional analysis of human L1CAM missense mutations

U. Finckh, *P. Michelson, C. Hartwig, A. Veske*
Institute of Human Genetics, University Hospital Eppendorf, University Hamburg; Hamburg, Germany
finckh@uke.uni-hamburg.de

Mutations in *L1CAM* lead to X-linked disorders including the symptoms hydrocephalus, adducted thumbs, agenesis or hypoplasia of corpus callosum, spastic paraplegia and mental retardation (L1-disease). Approximately 35% of the more than 140 known pathogenic mutations in *L1CAM* are missense mutations and show some variability in phenotype expression which may depend on type and site of the mutation. Rare coding variants of unknown biological significance are also known. We established an experimental model for quantitative and qualitative analysis of the effects of *L1CAM* missense mutations by transfecting NIH-3T3 cells either with full length L1 encoding wild type (wt) or mutant constructs. Stable expression on the cytoplasmic membrane was confirmed by immunocytochemistry and FACS analysis. Western blot analysis revealed bands corresponding to fully processed L1 protein from the wt construct and in addition or predominantly aberrant bands in the mutants. Transfected cells were used as

feeder cells for primary neurons prepared from cerebellum of P5 mice. All mutations introduced so far were associated with a significant reduction of neurite outgrowth measured after 23 hrs of co-culturing. The common phenotypic outcome (reduced neurite outgrowth) seems to be a combined effect of (1) amount of expression of L1 on feeder cell surface, (2) altered L1 protein glycosylation or processing, and (3) altered $L1_{neurite}$-$L1_{feeder}$ homophilic interaction due to structural alterations in mutated L1. We conclude that measurement and statistical analysis of neurite outgrowth in vitro may be a very sensitive and non-invasive tool in order to predict pathogenic effects of *L1CAM*missense mutations. [Supported by DFG, SFB 444, C3]; correspondence; finckh@uke.uni-hamburg.de

P1593. Functional studies on AIRE protein and gene defective in a monogenic autoimmune disease

T. Meriluoto[1], *P. Eskelin*[1], *M. Halonen*[1], *J. Korhonen*[1], *M. Kolmer*[1], *H. Kangas*[1], *J. Palvimo*[2], *M. Pelto-Huikko*[3], *L. Peltonen*[1], *I. Ulmanen*[1]
[1]*National Public Health Institute; Helsinki, Finland;* [2]*University of Helsinki; Helsinki, Finland;* [3]*University of Tampere; Tampere, Finland*
Tanja.Meriluoto@ktl.fi

Autoimmune polyendocrinopathy - candidiasis - ectodermal dystrophy [APECED; OMIM 240300] is a monogenic autoimmune disease with autosomal recessive inheritance and no association with the HLA region. We have cloned the defective gene AIRE and identified 20 mutations in APECED patients. Currently we are searching for patient mutations in the promoter region of AIRE gene. In human and mouse tissues AIRE protein displays mostly in nuclei, while cytoplasmic and nuclear localization can be observed in transfected tissue culture cells. Further, we aim to study the distribution of AIRE in differentiated neurons. In human AIRE protein is expressed in immunologically relevant tissues. Our results indicate that mouse Aire protein is expressed in specific thymal cells as well as in several other immunological and nonimmunological tissues. We have also shown that AIRE forms homodimers through HSR domain and acts as a powerful transcriptional coactivator. These functions are defective in certain patient mutations found in this domain. Our goal is to identify the genes regulated by AIRE and to characterize the transcriptional regulation of the AIRE gene expression. Interestingly, we have identified putative transcription factors binding to the Aire promoter.

P1594. Identification of Two Novel Missense Mutations in the CSX Gene encoding a Cardiac Specific Transcription Factor

I. Gutierrez[1], *T. Sluysmans*[2], *K. Devriendt*[3], *M. Vikkula*[1]
[1]*Christian de Duve Institute of Cellular Pathology; Brussels, Belgium;* [2]*Universit catholique de Louvain; Brussels, Belgium;* [3]*Katholieke Universiteit Leuven; Leuven, Belgium*
vikkula@bchm.ucl.ac.be

Non-syndromic cardiac septation defects are common, yet the responsible genetic changes remain uncharacterised. However mutations in the CSX/Nkx2-5 gene encoding a cardiac specific homeobox transcription factor have been reported to cause atrial septal defects (ASD) associated with atrioventricular (AV) conduction block. The CSX gene is expressed in cardiac muscle during embryonic, fetal and adult life, and its Drosophila ortholog tinman, is essential for the formation of the dorsal vessel. Targeted homozygous disruption of Nkx2-5 in mice causes lethality due to failure of heart looping. In man, eleven mutations in the CSX gene have been described in individuals with variable cardiac phenotypes. The majority has ASD and/or atrioventricular conduction defects. In addition, ventricular septal defects (VSD), tetralogy of Fallot (TOF), subvalvular aortic stenosis, ventricular hypertrophy, pulmonary atresia, mitral valve abnormalities and Ebstein s anomaly are observed. Along our studies on non-syndromic familial cardiopathies, we have collected two families with some members presenting ASD and AV-block. In both families we identified a novel CSX mutation in the homeodomain Variable expressivity in the phenotype was observed in one of the families. In the first generation, pacemakers have been implanted, in the second, two unaffected carriers and two members with ASD secundum (ASDII) and TOF were identified. Finally, in the third generation, members with ASDII, VSD, pulmonary stenosis, atrioventricular septal defect and patent ductus arteriosus (PDA) were observed. Thus the phenotypes caused by CSX mutations vary from non-expression, severe arrhythmia, to TOF and PDA. Importantly, mutation carriers do not necessarily present AV-block at young age. (vikkula@bchm.ucl.ac.be)

P1595. Molecular genetic analysis of autosomal dominant polycystic kidney disease in Czech families

J. Stekrova[1], *J. Reiterova*[2], *M. Kohoutova*[2], *J. Zidovska*[1], *J. Kapras*[2], *M. Merta*[2]
[1]*General Teaching Hospital of 1st Faculty of Medicine, Charles University; Prague, Czech Republic;* [2]*1st Faculty of Medicine, Charles University; Prague, Czech Republic*
jstek@lf1.cuni.cz

Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary renal disease. The disease is caused by mutations of PKD1 and PKD2 genes, though in several ADPKD families the PKD1 and/or PKD2 linkage was not found. DNA presymptomatic diagnosis is performed in our referential laboratory, using highly polymorphic microsatellite markers for DNA linkage analysis. According to European recommendations CW2 (D16S663), 16AC2.5 (D16S291), CW3D (16S664) and KG8 are used for linkage to PKD1 gene and D4S231, D4S414, D4S1534 and D4S1563 for linkage to PKD2 gene. Presymptomatic DNA diagnosis was performed in 160 unrelated ADPKD families. Linkage analysis gave strong evidence in favor of linkage to PKD1 in 60 (38%) families and linkage to PKD2 in 8 (5%) families. In 92 (58%) families mutation in either tested genes could not be excluded by linkage analysis. The direct detection of PKD2 mutation is performed in proved PKD2 families using heteroduplex analysis (HA) and sequencing. Five mutations were identified; four of them have been novel; 1) missense mutation in exon 4, 964 C>T (R320W) 2) frameshift mutation in exon 6, 1339-1345 ins gCAACAg 3) frameshift mutation in exon 10, 2046 - 2053 del TACT 4) missense mutation in exon 13,sub 2398 A>C (M800L). Establishment of localisation and type of mutations and their genotype -phenotype correlation in ADPKD families will improve DNA diagnosis and could help to assess the clinical prognosis of ADPKD patients.

Supported by the grant projects IGA MZ ?R NE/5996-3 and GAUK 203 469

P1596. Reciprocal translocation t(4;12)(q26;p12) in a patient with Andersen syndrome (periodic paralysis and long QT syndrome)

S. Pereira[1], *D. Depetris*[1], *A. Massacrier*[1], *M. Matt i*[1], *N. Levy*[1], *P. Cau*[1], *J. Pouget*[2], *P. Szepetowski*[1]
[1]*INSERM U491; Marseille, France;* [2]*CHU la Timone; Marseille, France*
pereira@medecine.univ-mrs.fr

A large number of paroxysmal neuromuscular and cardiac disorders are now known to be channelopathies. Of particular interest are the syndromes in which cardiac and neuromuscular symptoms concur, as they represent powerful tools to identify common pathways involved in cardiac and neuromuscular functioning. Andersen syndrome is such a condition in which cardiac arrythmias (long QT) and periodic paralysis are associated. Sporadic as well as familial cases have been reported but no gene has been identified so far. Several long QT syndromes (LQT) have been defined on a genetic basis. To date, six long QT genes have been localized; five of them (LQT1-3, 5-6) are now identified and encode potassium or sodium channels. The LQT4 gene has been mapped to chromosome 4q25-q27 but the critical region extends over 18 cM and the gene remains unknown. One patient with Andersen syndrome was detected. Cytogenetic studies revealed the existence of a de novo reciprocal translocation t(4;12)(q26;p12). Co-localization of the breakpoint at 4q26 with the critical area defined for LQT4 syndrome at 4q25-q27 strongly suggested that both syndromes could be caused by different alterations of the same gene. The translocation breakpoint thus represented a very powerful tool to identify the disease gene. FISH experiments with YACs and PACs showed that the translocation breakpoint at 4q26 lies in a 120-kb area entirely comprised within the LQT4 critical region. Work is in progress in order to identify junction fragments from cosmid clones encompassing the breakpoint. Molecular cloning of the breakpoint should lead to identification of a gene for both the Andersen syndrome and the LQT4 syndrome. Identification of this gene will be an important step towards the understanding of both periodic paralysis and long QT syndromes. As the gene is likely to contribute the QT-interval variability between normal individuals, it could also play a major role in normal cardiac functioning. Once the gene is identified, large-scale genetic studies could be performed to test the involvement of the gene in various cardiac arrythmias. Moreover, functional studies and the establishment of cellular and animal models will represent powerful tools to test various pharmacological and genetic therapeutic approaches, as well as to better understand normal cardiac and muscular functioning.

P1597. Alstrom syndrome; report of a family and exclusion of rab-1 and rhotekin as candidate genes

L. Titomanlio[1], *M. Sperandeo*[2,1], *D. De Brasi*[1], *M. Sibilio*[1], *A. Pepe*[1], *G. Andria*[1], *G. Sebastio*[1]
[1]*Department of Pediatrics, Federico II University; Naples, Italy;* [2]*Telethon Foundation; Rome, Italy*
ltitomanlio@libero.it

Main features of Alstrom syndrome (MIM 203800; AS) include pigmentary retinal dystrophy, obesity, non-insulin dependent diabetes mellitus and

sensorineural hearing loss. The AS locus was assigned to a 14.9 cM region on 2p13-p14 by linkage analysis, further restricted to a 6.1-cM interval between markers D2S327 and D2S286. We observed two AS brothers, born to non-consanguineous, healthy parents. Pregnancy and delivery were uneventful in both cases. Patient 1 developed photophobia and bilateral nystagmus at the age of 2 months. From the age of 3 months he suffers from bronchial asthma poorly responsive to conventional therapy. At the age of 3 years a diffuse pigmentary retinopathy was evident in both eyes. Visual Evoked Potentials (VEPs) showed a conductive delay. The electroretinogram (ERG) was strongly altered. Patient 2 presented with photophobia, bilateral nystagmus and dilated cardiomyopathy at the age of 2 years. A diffuse pigmentary retinopathy was found at the ophtalmological examination. At present, both patients are obese. Neither alterations in glucose metabolism nor hearing loss are present. Mitochondrial DNA analysis for common mutations underlying NARP and Leber s hereditary optic neuropathy was negative. We tested two genes, both located in the AS critical region, Rab-1 and rhotekin, respectively, which appear good candidates for AS on the basis of their physiological functions. By direct sequencing of the coding region of Rab-1 and of the coding region and exon-intron junctions of rhotekin, no mutations were found except for one polymorphic change in Rab-1 gene. This change is located downstream the termination codon of the gene.

P1598. Homologues of the yeast SNM1 (PSO2) gene and DNA crosslink sensitivity in mammalian cells

I. Demuth, *M. Digweed*
Humboldt-Universit t, Charit , Campus Virchow, Institut f r Humangenetik; Berlin, Germany
ilja.demuth@charite.de

DNA interstrand crosslinks are critical lesions for the mammalian cell since they affect both strands and block both transcription and replication; their repair is essential for survival. Although the involvement of nucleotide excision repair (NER) proteins has been implicated, little is known about the mechanisms of crosslink repair in human cells. One approach to understanding this cellular process is the identification of crosslink repair genes by exploiting mutant mammalian cells with particular sensitivity towards agents which cause DNA crosslinks such as Mitomycin C and photoactivated psoralens. Cells from patients with the autosomal recessive disorder Fanconi anaemia (FA) are particularly sensitive to these agents and are presumed to have defects in the crosslink repair pathway. Similarly, a range of hamster cells is available selected for crosslink sensitivity. These cells can be assigned to complementation groups on the basis of cell fusion and thus define separate genes in the crosslink repair pathway. Disruption of the yeast gene SNM1 (PSO2) results in hypersensitivity towards DNA crosslinking agents and homologues of this gene are presumably involved in crosslink repair in mammalian cells too. We have examined the ability of the yeast SNM1 gene to complement the defect in 4 crosslink sensitive hamster cell lines and in human FA-D2 cells in terms of survival and chromosome damage. One hamster cell mutant was partially but significantly corrected after gene transfer, suggesting that the hamster SNM1 gene is mutated in these cells. Three human SNM1 homologues have been identified and are being examined for complementation of these cells. Mutation analysis in FA cells from complementation groups B and D1 has so far excluded the involvement of human SNM1 in this disorder.

P1599. Founder effect in Unverricht-Lundborg disease in La Reunion island

B. MOULARD[1], *F. Darcel*[2], *C. Buresi*[1], *A. Roubertie*[3], *A. Biraben*[4], *A. Malafosse*[1]
[1]*HUG; Chene-Bourg, Switzerland;* [2]*CH St-Pierre; St-Pierre, Reunion;* [3]*CHU Montpellier; Montpellier, France;* [4]*CHU Rennes; Rennes, France*
bruno.moulard@hcuge.ch

We present the genetic study of 11 patients affected by the Unverricht-Lundborg disease (ULD) and 3 relatives with isolated seizures, extracted from 8 families and originating from La Reunion island. Ten subjects harbour the typical ULD mutation, including one with isolated seizures, a homozygous expansion of a dodecamer repeat in the cystatin B (CSTB) gene promoter beyond 40 repetitions. Clinically, those cases are very similar, with the typical photosensible myoclonus, associated with generalised tonic-clonic seizures (GTCS), cerebellar involvement and mild mental deterioration. The mean age at onset is 9.6 years (range 5 to 21), with a mean disease duration of 19.7 years (all patients are alive, range 4 to 39). We observed a founder effect, as the 10 patients with the homozygous expansion present the same haplotype with homozygous alleles for 7 markers spanning less than 1 cM of genomic DNA (D21S1890, D21S1885, D21S2040, D21S1259, D21S1912, PFKL, D21S171, from centromere to telomere), bounded by GT10 and D21S1446. We also confirmed the genetic instability, since the expansion size was variable (mean 55.3, range 48 to 63), despite a probable single common ancestor.

P1600. Linkage of benign familial infantile convulsions (BFIC) to chromosome 16p12-q12 suggests allelism to the ICCA syndrome

P. Szepetowski[1], *R. Caraballo*[2], *S. Pavek*[3], *A. Lemainque*[3], *M. Gastaldi*[1], *B. Echenne*[4], *J. Motte*[5], *P. Genton*[6], *R. Cersosimo*[2], *V. Humbertclaude*[4], *N. Fejerman*[2], *A. P. Monaco*[7], *J. Rochette*[8], *M. G. Lathrop*[3]
[1]*INSERM U491; Marseille, France;* [2]*Pediatric Hospital Juan Garrahan; Buenos Aires, Argentina;* [3]*Centre National de G notypage; Evry, France;* [4]*Hopital Saint Eloi; Montpellier, France;* [5]*American Memorial Hospital; Reims, France;* [6]*Hopital Henri Gastaud; Marseille, France;* [7]*Wellcome Trust Centre; Oxford, United Kingdom;* [8]*G n tique M dicale; Amiens, France*
szepetow@medecine.univ-mrs.fr

Benign familial infantile convulsions (BFIC) is an autosomal dominant epileptic disorder characterized by convulsions with onset at age 3-12 months and a favorable outcome. BFIC had been linked to chromosome 19q, while the ICCA syndrome, associating BFIC with paroxysmal dyskinesias, had been linked to chromosome 16p12-q12 in several families from France. Association of BFIC with paroxysmal dyskinesias seems quite frequent, as many additional families of diverse ethnic background with similar syndrome, have been linked to the chromosome 16 ICCA region. Moreover, one large pedigree with paroxysmal kinesigenic dyskinesias only, has also been linked to the same genomic area. This raised the possibility that families with pure BFIC could be linked to chromosome 16 as well. Seven families with BFIC inherited as an autosomal dominant trait were collected in Argentina and France. Genotyping was performed with markers at chromosome 19q and 16p12-q12. While chromosome 19q could be excluded, evidence for linkage in the ICCA region was found, with a maximum two-point LOD score at 3.32 for markers D16S3131 and SPN. This result definitely proves that human chromosome 16p12-q12 is a major genetic locus underlying BFIC and/or paroxysmal dyskinesias in affected patients and families. The peculiar phenotype displayed by one homozygous patient suggests that the great intra-familial variability of the ICCA syndrome could be sustained by modifying genetic factors.

P1601. Nuclear Membrane Delamination With Bleb Formation In Dilated Cardiomyopathy With Atrio-ventricular Block Caused By Novel Lamin A/c Gene Mutations.

E. Arbustini[1], *M. Grasso*[1], *A. Pilotto*[1], *M. Diegoli*[2], *M. Concardi*[1], *C. Campana*[3], *A. Negri*[1], *A. Repetto*[3], *C. Serio*[3], *E. Porcu*[1], *L. Tavazzi*[3]
[1]*Cardiovascular Pathology, Molecular Diagnostic and Research Transplantation Laboratories, IRCCS Policlinico San Matteo; Pavia, Italy;* [2]*Department of Pathology, University of Pavia; Pavia, Italy;* [3]*Cardiology Division, IRCCS Policlinico San Matteo; Pavia, Italy*
e.arbustini@smatteo.pv.it

Lamin A/C (LMNA) gene defects have been recently reported as a major cause of the dilated cardiomyopathy (DCM) associated with conduction system disease. To assess whether the DCM with conduction system disease is associated with LMNA gene defects, we screened the 12 exon of the gene in 5 familial DCMs with atrio-ventricular (A-V) block. We identified 5 novel LMNA mutations in the five DCM families, and first documented the corresponding ultrastructural morphologic defect in the nuclear membrane of the cardiac myocytes. Three mutations are missense; A423G in the exon 1 of the cDNA (K97E), C702T in the exon 3 (R190W) and G1083A in the exon 6 (E327K). One is a non-sense mutation G465T of the cDNA, E111X, predicting a truncated protein of 110 aminoacids, and one is a 4 bp insertion duplicating CTGC at 2869 in the exon 9. In all probands and one affected relative, the ultrastructural study of the endomyocardial biopies showed structural defects of the nuclear membranes consisting of focal delamination of the inner and outer membranes with bleb formation. These features were absent in 50 EMB from DCM patients without conduction system disease proven by molecular analysis as non-related with LMNA gene defects. The ultrastructural changes of the nuclear membrane are specifically associated with LMNA defects and, combined with the conduction system disease, constitute useful markers for successful identification of LMNA gene defects.

P1602. An overview of a clinical and molecular study of holoprosencephaly

V. David[1], *M. Blayau*[1], *L. Lazaro*[2], *T. Atti -Bittach*[3], *L. Pasquier*[2], *C. Dubourg*[1], *B. Le Marec*[1], ***S. Odent***[2]
[1]*G n tique Mol culaire; Rennes, France;* [2]*G n tique M dicale; Rennes, France;* [3]*D partement de G n tique; Paris, France*
sylvie.odent@chu-rennes.fr

Holoprosencephaly (HPE; 1/16.000 live births; 1/250 conceptuses) is a common development defect affecting both the forebrain and the face. Clinical expressivity is variable, ranging from a single cerebral ventricule and cyclopia to clinically unaffected obligated carriers in familial HPE. The disease is genetically heterogeneous but additional environmental agents also contribute to the aetiology of HPE. This study includes 59 unrelated HPE cases (19 familial and 40 clinically sporadic cases). For inclusion in the study, the proband must have had a typical documented HPE (alobar, semilobar or lobar) or a typical face (proboscis, premaxillary agenesis and absence of nose) and normal chromosomes. We included also in the molecular study 9 polymalformed cases and 21 atypical cases. We provide clinical data and report 13 novel heterozygous mutations, 7 in Sonic hedgehog (SHH), 4 in ZIC2, and 2 in SIX3. One SHH mutation was associated with colobomatous microphtalmia, another with only a single upper incisor and nasal pyriform aperture stenosis. One SIX3 mutation was associated with atelencephaly. We found 6 mutations in familial cases whereas only 7 mutations were identified in apparently sporadic cases. SHH mutations were all maternally inherited whereas the ZIC2 and SIX3 mutations were either paternally or maternally inherited. Patients will be now tested for TGIF. This study confirms the extremely variable phenotypes in HPE families and the genetic heterogeneity of the disease.

P1603. Molecular Mechanism Accounting For Syndromic Combined Pituitary Hormone Deficiency In A Patient Carrying A Lhx3 Gene Defect

M. Sobrier[1], *I. Netchine*[1], *H. Krude*[2], *A. Gr ters*[2], *M. Goossens*[1], *S. Amselem*[1]
[1]*U468 INSERM; CRETEIL, France;* [2]*Charit hospital; Berlin, Germany*
marie-laure sobrier@im3.inserm.fr

In humans, combined pituitary hormone deficiency (CPHD) has been linked with rare abnormalities in genes encoding pituitary-specific transcription factors. Very recently, we have described the first molecular defects of LHX3, a gene encoding a member of the LIM-homeodomain proteins, the murine counterpart of which having been shown to be necessary for anterior pituitary development. LHX3 consists of seven exons, with exons 2, 3, and 4 and 5 encoding the LIM1, LIM2 and homeo domains, respectively. One of the patients, born to a consanguineous union, displayed a complete deficit in all but one (adrenocorticotropin) anterior pituitary hormones and a rigid cervical spine associated with a secondary enlargement of the anterior pituitary, as documented by MRI. Screening of LHX3 had revealed a homozygous 23-bp deletion involving the last 3 bases of exon 3 and the adjacent splice-donor site. To evaluate the functional consequences of this small deletion we have studied expression LHX3 transcripts of the mutated allele by means of in vitro transcription. This analysis demonstrated the shift of the normal LHX3 splicing pathway towards an exon-3 deleted transcript. If translated, the exon skipping event would result in a frameshift leading to a premature stop codon. The mutant LHX3 protein would lack both the LIM2 and the homeo domains. In vitro expression of the recombinant mutant protein in CHO cells showed that it was unable to transactivate the bTSH promoter. These functional assays, which are consistent with loss of function of the mutant LHX3 protein, raise the question of the mechanism underlying the secondary occurrence of the enlarged anterior pituitary documented in the patient.

P1604. A novel missense FGFR3 mutation associated with achondroplasia

M. Friez*, A. Toburen, K. Clarkson, J. Longshore*
Greenwood Genetic Center; Greenwood, SC United States
friez@ggc.org

Greater than 99% of individuals clinically diagnosed with achondroplasia have been shown to have the same amino acid substitution (G380R) in the transmembrane region of the FGFR3 gene. We present a newborn male with physical and radiographic studies consistent with achondroplasia. The patient experienced severe episodes of apnea in the perinatal period that continued until 10 months of age. Standard diagnostic techniques did not detect either reported point mutation responsible for causing the G380R substitution. Fluorescent sequencing of exon 10 encompassing the transmembrane region of FGFR3 failed to show any variations from normal. Additional regions known to have point mutations in other FGFR3 related skeletal dysplasias were also analyzed. Analysis demonstrated a novel A to T transversion at nucleotide 835 in exon 7 of the FGFR3 gene in this patient. This missense mutation substitutes a cysteine for serine at amino acid 279 (S279C) in the extracellular region of the FGFR3 protein. Two other mutations (R248C and S249C) that create cysteine residues in this region have been previously associated with cases of Thanatophoric dysplasia type I. To date, only one other mutation (Y278C) affecting the extracellular domain of FGFR3 has been reported to be associated with an achondroplasia phenotype. These two patients demonstrate that exon 7 in FGFR3 is a strong candidate for mutational analysis in individuals with the clinical diagnosis of achondroplasia that have been shown to have a normal FGFR3 transmembrane domain.

P1605. Mutations in the TWIST gene without phenotypic significance?

W. Kress[1], *B. Halliger-Keller*[1], *G. Lieb*[2], *H. Collmann*[3]
[1]*Institute of Human Genetics; Wuerzburg, Germany;* [2]*Children s Hospital; Wuerzburg, Germany;* [3]*Department of Paediatric Neurosurgery; Wuerzburg, Germany*
wkress@biozentrum.uni-wuerzburg.de

In many cases of a classical Saethre-Chotzen syndrome (acrocephalosyndactyly type III; MIM #101400) the TWIST gene encoding a basic helix-loop-helix transcription factor is the mutational target. In addition to the craniofacial features of this autosomal dominant syndrome, there are (often subtle) limb anormalities like cutaneous syndactyly of hands and feet, clinodactyly and/or broad halluces with valgus deviation. World-wide and in our own cohort of more than 30 patients many private TWIST mutations were found. About 66% were stop, frameshift or large deletion mutations. Haploinsufficiency appears to be be the pathogenic mechanism involved. In a group of patients with no clear diagnosis, we found three probands not giving satisfaction to the clinical criteria but harbouring a TWIST sequence variation. There was the mother of a boy with trigonocephaly, being not affected herself, who carried an in-frame deletion 261del21; her son with trigonocephaly do not have the mutation. In a boy with an isolated sagittal craniosynostosis a Ser201Tyr missense mutation was detected; the unaffected father carried the same mutation. Both sequence variants were not found in more than three hundred control chromosomes. We conclude that there are rare silent sequence variations in the TWST gene defining functional less important domains.

P1606. Phenotype analysis of monozygotic twins with Holt-Oram syndrome

T. Huang[1], *J. G. Seidman*[2], *C. E. Seidman*[3]
[1]*Children s Hospital; Boston, MA United States;* [2]*Howard Hughes Medical Institute, Harvard Medical School; Boston, MA United States;* [3]*Howard Hughes Medical Institute,Brigham and Women s Hospital, Harvard Medical School; Boston, MA United States*
thuang@rascal.med.harvard.edu

ABSTRACT; Holt-Oram syndrome is characterized by congenital cardiac defects and upper limb anomalies. Mutations of the TBX5 gene cause this condition. Intrafamilial phenotypic variability is well documented, but etiology is clear. Here, we report on a set of monozygotic (MZ) twins with Holt—Oram syndrome. Monozygosity was confirmed by DNA microsatellite analysis. Both twins carried a one-basepair de novo deletion mutation in TBX5, which leads to a frameshift and predicts a premature termination of TBX5 protein. The twins shared concordant and complex cardiac defects including secundum ASD, a large membranous VSD, multiple muscular defects, pulmonic narrowing and mild tricuspid regurgitation. However, their limb defects are discordant. Skeletal X-rays show one twin has absence of thumbs bilaterally, hypoplastic radial bones and hooked clavicles and a bifid rib. The other twin has similar anomalies on her right, but a thumb is present on the left, though distal metacarpal and phalanx are hypoplastic. This genotype-phenotype disparity suggests that the genetic background might play a important role in the intrafamilial cardiac phenotypic variability observed in Holt-Oram syndrome. Factors other than genetic background such as blood supply or the position of fetus in uterus may also contribute to limb anomalies. Here we propose a monoallelic expression model to explain the discordance.

P1607. Holt-Oram syndrome; Report of a new mutation in the TBX5-Gene in 2 German families

C. Gruenauer-Kloevekorn*, U. G. Froster*
Institut f r Humangenetik, Leipzig University; Leipzig, Germany
patrick.gruenauerkloevekorn@planet-interkom.de

Introduction; In Holt-Oram syndrome (HOS), a developmental defect involving upper limb malformalities and cardiac defects, mutations in the TBX5 gene were demonstrated as the underlying molecular defect. We report on two unrelated families with HOS presenting with the same stop mutation in exon 5 of the TBX5 gene wich had not been reported before. Case reports; Three affected and all available unaffected family members of each family were examined. Methods; Primers for amplification and for direct sequencing of exon 2 to exon 9 of the TBX5 gene were designed and exons of genomic DNA from affected and unaffected family members were analysed by direct sequencing. To confirm the results PCR products were restriction digested with CSP6I. Results; In all affected family mem-

bers a heterozygous single base pair substitution (408C->A) was detected converting a tyrosine codon to a stop codon(Tyr136stop).This mutation is predicted to result in a premature termination of the TBX5 protein. None of 100 unaffected DNA samples had the 408C->A change in the TBX5 gene. Discussion; With respect to earlier reported truncation mutations in TBX5 the identified stop mutation presumably has led to a truncated protein unable to bind and activate target DNA. The spectrum of phenotypes reported in patients with stop mutations ranges wide with respect to limb anomalies as well as cardiac malformalities. In all affected patients reported here, however the cardiac malformation is an ASD while upper limb malformalities ranged wide. The reported families might help to further define genotype-phenotype correlation in HOS.

P1608. Y440X, a SOX9 mutational hotspot in campomelic dysplasia

G. Scherer[1], *C. Zeschnigk*[1], *E. Levy-Lahad*[2], *J. Paterson*[3], *R. Santos*[4], *E. Seemanov* [5], *N. Tommerup*[6]
[1]*Institute of Human Genetics and Anthropology; Freiburg, Germany;* [2]*Shaare Zedek Medical Center; Jerusalem, Israel;* [3]*Kennedy-Galton Centre; Harrow, United Kingdom;* [4]*Istituto de Gen tica M dica; Porto, Portugal;* [5]*Institute of Biology and Medical Genetics; Prag, Czech Republic;* [6]*IMBG, Panum Institute; Copenhagen, Denmark*
scherer@humangenetik.ukl.uni-freiburg.de

Campomelic dysplasia (CD), a skeletal malformation syndrome with XY sex reversal, usually leads to death in the neonatal period (MIM 114290). CD is caused by heterozygous de novo mutations in and around the developmental regulatory gene SOX9 that encodes a transcription factor of 509 amino acids with a central DNA-binding domain and a C-terminal transactivation domain. Some 29 missense, nonsense, frameshift and splice mutations in SOX9 have been described that scatter over the entire coding region. No significant mutational hotspot has emerged but for the nonsense mutation Y440X present in three independent cases. We have identified the Y440X mutation in 5 out of 38 additional, unpublished, CD cases with proven SOX9 mutations. This mutation thus accounts for about 10% of SOX9 coding region mutations in CD. The TAC tyrosine codon at position 440 is mutated to either TAG (5 cases) or TAA (3 cases), resulting in loss of an RsaI site allowing for simple diagnosis. As previously shown, the truncated SOX9 protein resulting from the Y440X mutation retains some transactivation potential, and may be regarded as a milder mutant allele, as 5 of the 8 patients survived the neonatal period.

P1609. Distinct Mutations in the Receptor Tyrosine Kinase ROR2 Cause Brachydactyly Type B

G. Schwabe[1], *S. Tinschert*[2], *C. Buschow*[1], *P. Meinecke*[3], *G. Wolff*[4], *G. Gilessen-Kaesbach*[5], *M. Oldridge*[6], *A. O. M. Wilkie*[6], *R. K mec*[7], *F. Majewski*[8], *S. Mundlos*[2]
[1]*Max-Planck-Institute Molecular Genetics; Berlin, Germany;* [2]*Institute for Medical Genetics, Charite; Berlin, Germany;* [3]*Altonaer Kinderkrankenhaus; Hamburg, Germany;* [4]*Institute for Human Genetics; Freiburg, Germany;* [5]*Institute for Human Genetics; Essen, Germany;* [6]*Institute of Molecular Medicine, John Radcliffe Hospital; Oxford, United Kingdom;* [7]*Kinderklinik Universit tsklinikum; Mannheim, Germany;* [8]*Institute for Human Genetics; D sseldorf, Germany*
schwabe@molgen.mpg.de

Brachydactyly type B (BDB) is an autosomal dominant skeletal disorder, characterized by hypoplasia/aplasia of distal phalanges and nails. Recently heterozygous mutations of the orphan receptor tyrosine kinase ROR2 within a distinct segment directly after the tyrosine kinase (TK) domain have been shown to cause BDB. We report four novel and one previously described mutation in ROR2 (three frame-shifts, one splice mutation, one nonsense mutation) in six BDB families. The mutations predict truncation of the protein immediately before and after the TK. Patients affected with distal mutations exhibit a more severe phenotype than those with proximal mutations. Our analysis includes an individual with homozygous BDB with a mutation before the TK showing extensive hypoplasia of phalanges, metacarpals/metatarsals and anonychia. Moreover vertebral anomalies, brachymelia of arms, and a ventricular septal defect are present in this patient and reminiscent of Robinow syndrome which is also caused by ROR2 mutations. The BDB phenotype and the localization of BDB mutations suggest a mutational effect that cannot be explained by simple haploinsufficiency and is distinct from that in Robinow syndrome.

P1610. Two novel mutations and one recurrent polymorphism in the JAG1 gene in Polish patients with Alagille syndrome.

D. Jurkiewicz, *E. Popowska, J. Rujner, M. Krajewska-Walasek*
The Children s Memorial Health Institute; Warsaw, Poland
dorcik@czd.waw.pl

Alagille syndrome (AGS) is an autosomal dominant disorder characterized by developmental abnormalities of liver, heart, eyes, vertebrae and facial features. The minimal estimated frequency is 1; 70 000 live births. AGS phenotype is caused by mutations of the JAG1 gene located on chromosome 20p12. The JAG1 gene spans 36 kb and consists of 26 exons, encoding a 5,5 kb transcript. The product of the gene is a ligand in the Notch signaling pathway. The purpose of our study was to identify JAG1 gene mutations in the next group of Polish patients with AGS. We are currently studying seven patients. We have detected five DNA-alternations by agarose electrophoresis, SSCP and HA analyses. DNA sequencing revealed presence of two novel mutations in the JAG1 gene. One of them was a 7 nucleotide deletion in exon 2 (codons 58 - 60). The other mutation was a substitution G to A in exon 4 (Cys187Tyr). In both cases identified mutations appeared de novo. Besides, a transversion C to G in exon 22 resulting in an amino acid substitution (Pro871Arg), known polymorphism, was identified in a proband and his mother . The study was supported by KBN - CMHI Project No. S73/99.

P1611. JAGGED1 RNA analysis in patients with Alagille syndrome

C. Crosnier[1], *C. Driancourt*[1], *N. Raynaud*[1], *M. Hadchouel*[2], *M. Meunier-Rotival*[1]
[1]*INSERM U347; Le Kremlin-Bicetre, France;* [2]*INSERM U347 and Service Hepatologie Pediatrique; Le Kremlin-Bicetre, France*
crosnier@infobiogen.fr

Mutations in the JAGGED1 gene, encoding a ligand of the NOTCH receptors, are responsible for Alagille syndrome (AGS), a polymalformative disorder. SSCP and heteroduplex analysis have identified JAGGED1 mutations in 65% of patients with AGS. More than 200 such mutations have been described. We used RT-PCR and sequencing to study JAGGED1 transcripts in lymphoblastoid cell lines from 9 patients with AGS without known mutations. Mutations were identified in 3 of them, resulting in 2 nonsense mutations and a deletion at an exon-intron boundary. Thus, analysis of JAGGED1 transcripts can identify mutations not detected by SSCP. For 5 other patients, complete sequencing of the RT-products revealed no mutation, but the presence of heterozygous polymorphisms suggest that both alleles were equally transcribed. For 2 patients with previously identified splice mutations, transcript analysis revealed the effects of these mutations at the RNA level; mutations in the donor sites of introns 21 and 25 are responsible for deletion of exons 20 and 24, respectively. These results demonstrate that RNA analysis is a very useful tool for identifying mutations which SSCP cannot. It is informative about the stability and the splicing of the transcripts. However, it cannot be used as a routine procedure because lymphoblastoid cell lines are not available for every patient with AGS. crosnier@infobiogen.fr

P1612. Prevalence of MYBPC3 gene defects in hypertrophic cardiomyopathy.

N. Banchieri[1], *A. Pilotto*[1], *E. Porcu*[1], *L. Scelsi*[2], *F. Cecchi*[3], *I. Olivotto*[3], *D. Vargiu*[3], *A. Negri*[1], *L. Tavazzi*[2], *E. Arbustini*[1]
[1]*Cardiovascular Pathology, Molecular Diagnostic and Research Transplantation Laboratories, IRCCS Policlinico San Matteo; Pavia, Italy;* [2]*Cardiology Division, IRCCS Policlinico San Matteo; Pavia, Italy;* [3]*Cardiomyopathy Center Careggi Hospital ; Firenze, Italy*
diagnostica.molecolare@smatteo.pv.it

Hypertrophic cardiomyopathy is an autosomal dominant disorder characterized by hypertrophy of the left ventricular walls, particularly of the interventricular septum. The golden standard for the clinical diagnosis is echocardiography which measures septal and free wall thickness. HCM is genetically heterogeneous and 9 disease genes have been identified to data. Of these genes, bMHC had been originally reported as that most frequently linked to the disease while defects of the MYBPC3 gene (chr 11p11.2) seemed originally less frequent. We screened for mutations the 35 exons of MYBPC3 using Polymerase Chain Reaction, Denaturing High Performance Liquid Chromatography (DHPLC, Transgenomic Wave Systems TMHA) and automated sequencing. The aim of the study was to assess the prevalence of MYBPC3 mutations in unrelated 85 probands affected by echocardiographically proven HCM. We identified 10 different mutations in 20 of 85 patients (24%). Of these mutations, 8 are novel; Val158Met, Glu165Asp, Glu223Lys, Ser236Gly, Arg723Trp, Gly800Arg, donor splice site G-A (+1, 16638, intron 28), C insertion in exon 30 at 18348 nt position; while 2 had been previously reported in HCM families; Glu258Lys, Arg502Gln. MYBPC3 gene defects are associated with HCM in one of 4 patients. The DHPLC method allows fast screening of large patient populations.

P1613. DHPLC Analysis of the Three Major Genes (MYH7 e MYBPC3 AND TNNT2) Causing Familial Hypertrophic Cadiomyopathy

S. Nasti[1,2], *E. Blair*[2], *M. Oliveira*[2], *A. Caleffi*[3], *R. Bertorelli*[3], *C. Autore*[4], *P. Spirito*[5], *H. Watkins*[2], *D. A. Coviello*[1]

[1]*Molecular Genetic Laboratory, Istituti Clinici di Perfezionamento; Milan, Italy;* [2]*Department of Cardiovascular Medicine, University of Oxford; Oxford, United Kingdom;* [3]*Servizio di Genetica Medica, Policlinico di Modena, Modena; Modena, Italy;* [4]*Dipartimento di Scienze Cardiovascolari e Respiratorie, Universit la Sapienza Roma; Roma, Italy;* [5]*Divisione di Cardiologia, E.O. Ospedali Galliera; Genoa, Italy*

coviello@unige.it

Familial Hypertrophic Cardiomyopathy (FHC) is a condition with a prevalence in the general population of about 1/500 and transmitted as autosomal dominant trait. The disease is a primary abnormality of the myocardium and is morphologically characterised by a hypertrophied and non-dilated left ventricle. The clinical course of the disease is heterogeneous; patients may remain asymptomatic, some develop severe symptoms of heart failure, others die suddenly and unexpectedly often in the absence of previous symptoms. Molecular investigations have identified FHC causing mutations in nine cardiac contractile proteins; b-myosin heavy chain (b-MHC), myosin binding protein-C (MyBP-C), cardiac troponin T (TNNT2), a-tropomyosin (a-TM), the two myosin light chain genes, cardiac troponin I (TNNI3), a-cardiac actin (ACTC) and cardiac Titin (TTN). Genotype-phenotype correlation is crucial to understand the natural history of the disease and to assess the potential impact of a genetic diagnosis on the identification and management of patients at high risk of sudden death. Due to the large size of the major genes involved in the disease, our previous work has been done mainly on part of one gene, the b-MHC. The recent availablility of the DHPLC technology has inproved the screening of mutations that is now more efficient and faster. We analysed the coding sequence of the three major genes (b-MHC, MyBP-C and TNNT2) in twelve unrelated Italian patients with FHC. PCR reactions were performed using specific primers and conditions. Subsequently, DHPLC analysis (denaturing high-pressure liquid chromatography) was carried out on the WaveTM DNA Fragment Analysis System (Transgenomic, Cheshire, UK). DNA fragment elution profiles were displayed using the Transgenomic WAVEMAKER-TM software. Chromatograms were analysed and amplified fragments showing alterations were confirmed and identified by automated bi-directional sequencing (MWG Biotech, Germany). Sequence traces were analysed using the Chromas program. We found three already known mutations ; Glu258Lys, Arg502Gln, Gln969Stop in myosin binding protein-C (MyBPC) and we also found four novel nucleotidic changes leading to four aminoacid changes; Arg17Cys, Val158Met in myosin binding protein-C and Asp1208His, Ala100Thr in b-myosin heavy chain (b-MHC). We are checking the segregation of these variants in other affected members of the families to know if they are proteic polymorfisms or novel mutations. Our results confirms that DHPLC analysis is a highly sensitive and specific tool for DNA sequence variant detection.

P1614. Molecular Investigation Lqt-syndromes In Russian Families.

E. Zaklyazminskaya[1], *T. Kovalevskaya*[1], *S. Chuprova*[2], *A. Polyakov*[1], *O. Evgrafov*[1]

[1]*Research Center for Medical Genetics; Moscow, Russian Federation;* [2]*2-Federal Child Center of Arrhythmia s; Moscow, Russian Federation*

dnalab@online.ru

Congenital long QT syndrome (LQTS) is inherited cardiac disorder characterized by a prolonged QT interval and high risk of sudden death. At least five genes, when mutated, produce this phenotype; KCNQ1 (LQT1), HERG (LQT2), SCN5A (LQT3), KCNE1 (LQT5), KCNE2 (LQT6). Patients from 60 unrelated Russian families were available for DNA analysis (122 patients with Romano-Ward syndrome and 3 patients with Jervell and lange-Nielsen syndrome). All the patients were observed in Federal Child Center of Arrhythmia s, Moscow. Diagnoses were confirmed using criteria according to Schwartz et al. (1993). DNA from all the patients were tested for mutations in 4 genes; KCNQ1 (exons 2, 6, 7), HERG (exons 6, 7), KCNE1 and KCNE2 using PCR-SSCP analysis. We identified the abnormal bands in 8 DNA samples by SSCP-analysis of KCNQ1. One patient has R174H mutation, another two have G314S and in 3 families A341V was revealed. All mutations were reported. PCR-SSCP analysis of exon 7 HERG reveals abnormal conformers in 16 cases, 11 of them are the same. This anomalous band was observed in control healthy individuals too. PCR-SSCP analysis of KCNE1 gene revealed abnormal conformers in 35 DNA samples; moreover 26 of them were equal. We suggested that it was A141G, frequent polymorphism, which was found in control healthy individuals DNA samples too. The identical abnormal conformers were found in the KCNE2 gene in 6 samples. This conformer was not found in the control group.

P1615. Novel compound heterozygous mutations in the KvLQT1 gene associated with autosomal recessive Long QT syndrome (Jervell Lange-Nielsen syndrome)

M. Qi[1,2], *L. Ning*[1,2], *A. Moss*[3], *W. Zareba*[3], *J. Robinson*[3], *D. Ryan*[1]

[1]*Dept. of Pathology & Laboratory Medicine, University of Rochester; Rochester, NY United States;* [2]*Center for Cardiovascular Research; Rochester, NY United States;* [3]*Dept. of Medicine, University of Rochester; Rochester, NY United States*

Ming_Qi@urmc.rochester.edu

The hereditary long QT (LQT) syndrome is a familial cardiac disorder that causes syncope, seizures, and sudden death from ventricular arrhythmias, specifically torsade de pointes. Both autosomal dominants LQT (Romano-Ward syndrome) and autosomal recessive LQT (Jervell and Lange-Nielsen syndrome, JLNS) have been defined. Multiple heterozygous mutations in several ion channel genes (KvLQT1, HERG, SCN5A, KCNE1, and, KCNE2) have been shown to cause autosomal dominant LQT. Autosomal recessive LQT, which is associated with deafness, has been shown to occur with homozygous mutations in KvLQT1 or KCNE1 in JLNS families in which QTc prolongation was inherited as a dominant trait. Recently, an Amish JLNS family has been reported with QTc prolongation inherited as an incomplete dominant and deafness inherited as a recessive trait. In vitro biochemical and functional studies indicate that the KvLQT1 C-terminus functions as an assembly domain for KvLQT1 subunits. In this study, a family with clinical evidence of JLNS was analyzed for mutations by use of single-strand conformation polymorphism and DNA sequencing analyses for mutation screening in KvLQT1 gene. Novel compound heterozygous nonsense mutations R423X / Q435X in the C-terminus of KvLQT1 were identified in both affected dizygotic twins; both the parents and a sibling each carried only one of the mutant alleles and were asymptomatic with modestly prolonged QTc intervals (.46, .50 and .45 s., respectively). These two nonsense mutations lead to premature termination C-terminus with truncation of the postulated assembly domain. Our results show that novel compound heterozygous nonsense mutations in C-terminus of KvLQT1 cause JLNS.

P1616. A deletion in plakoglobin is the cause of arrhythmogenic right ventricular cardiomyopathy with palmoplantar keratoderma and woolly hair (Naxos disease).

S. Jeffery[1], *G. McKoy*[1], *A. Crosby*[1], *A. Tsatsopoulou*[2], *A. Anastasakis*[3], *M. Norman*[1], *A. Coonar*[1], *N. Protonatarios*[2], *W. J. McKenna*[1]

[1]*St George s Medical School; London, United Kingdom;* [2]*Yannis Protonotarios Medical Centre of Naxos; Naxos, Greece;* [3]*University of Athens; Athens, Greece*

s.jeffery@sghms.ac.uk

Naxos disease (ND) is an autosomal recessive form of arrhythmogenic right ventricular cardiomyopathy (ARVC) which co-segregates with palmoplantar keratoderma and woolly hair. We mapped the locus for ND to 17q21, where the gene for plakoglobin is located. Plakoglobin is a key component of desmosomes and adherens junctions, including those in heart and skin. The human gene sequence was ascertained by RT-PCR, and analysis in affected individuals showed that all carried a homozygous 2 base pair deletion. This mutation produces a frameshift which terminates the protein prematurely, altering the last 5 amino acids in the 13th armadillo repeat, and truncating the C-terminal domain by 56 residues. The finding that desmosomal molecules are involved in the pathogenesis of Naxos disease suggests that defects in cell junction integrity may be important in ARVC. The recent discovery that recessive mutations in desmoplakin also cause cardiomyopathy (Norgett et al, Hum Mol Genet 200;9;2761-2766) supports this hypothesis. Since the cardiac phenotypes in recessive and dominant ARVC are very similar, it could be that other proteins in the cell adhesion complex present themselves as good candidates for the more common form of the disorder.

P1617. Sex specific alleles disequilibrium between the ahr(b) and ahr(d) alleles in mice

A. L. Pawlak[1], *E. Strauss*[1], *E. Florek*[2]

[1]*Institute of Human Genetics, Polish Academy of Sciences; Poznan, Poland;* [2]*Medical University; Poznan, Poland*

pawlakal@man.poznan.pl

Alleles ahrb and ahrd occur in inbred strains of mice C57BL and DBA/2, respectively. They are linked with differences in susceptibility to the toxic and carcinogenic effects of polycyclic aromatic hydrocarbons (PAHs). The C57BL ahrd ahrd mice strain congenic with the C57BL ahrb ahrb has been generated in our laboratory after 10 backcrosses, each followed by the

selection. Here we describe the transgeneration transmission of the studied alleles in cross-breeding between the parent C57BL ahrb ahrb and the congenic C57BL ahrd ahrd strains. The in vivo exposure of animals to the toxic concentrations of cigarette smoke during the period of pairing and the early pregnancy was shown by us to affect the fertility of ahrd males to greater extend than that of the ahrb males. The transmission of the ahrb and ahrd alleles was assessed by genotyping the STR polymorphism at D12Mit2 locus linked with the ahr gene (the length of PCR products of the D12Mit2 alleles is 132 and 149 nucleotides respectively). It was found that, depending on the sex of the progeny, the linkage disequilibrium may occur in favor of the either allele. In the progeny of the heterozygous mothers (ahrb ahrd) the sex ratio was distorted in favor of the males and decreased frequency of ahrb allele was noted among the females. Following the cigarette smoke exposure the allele specific toxic effects on the fertility combine with the effects on the alleles disequilibrium in progeny.

P1618. Carrier diagnosis of haemophilia A in a family with partial deletion of the FVIII gene using quantitative real-time duplex PCR.

J. Lavergne[1], *R. d Oiron*[2], *E. Gautier*[3], *J. Costa*[3]
[1]*H pital Bic tre Inserm U143; Le Kremlin Bic tre, France;* [2]*H pital Bic tre D partement d H matologie; Le Kremlin Bic tre, France;* [3]*H pital Am ricain de Paris; Neuilly, France*
lavergne@infobiogen.fr

In a family without prior haemophilia history, a patient was diagnosed as having severe haemophilia A (FVIII ;C < 1U/ml). The intron 22 inversion was negative. Mutation screening of the entire coding and flanking intronic sequences of FVIII gene was performed. Results only revealed lack of amplification of exon 26, indicating that partial deletion of the FVIII gene was the molecular defect in this family. His sister had a low FVIIIC level, strongly suggesting she was carrier for haemophilia . However, FVIIIC was normal in other female members and their carrier status could not be easily determined. A specific FVIII gene dosage by duplex real-time PCR was developed in order to determine wether one or two copies of exon 26 were present. Relative quantitation was expressed as a ratio between the copies number of FVIII exon 26 sequences and copies of a reference autosomal gene copies number. Twelve samples from non haemophilia families were tested as controls. Ratios study indicates that two clear non overlapping populations can be identified ; a ratio of 1.06 +/- 0.06 (n=6) was found for female individuals while it was 0.51 +/- 0.01 (n=6) for males, demonstrating the efficiency of the method. Four independent analysis of relative female members revealed a mean ratio of 0.46 and 0.57 for the patient s mother and sister respectively. Therefore, they were both carrier for the FVIII exon 26 deletion. Using the same approach, other female members of the same family were tested and their status easily determined.

P1619. The disease correlates in a case of the doubly mutated allele of MTHFR 677T; 1298C

E. Strauss[1], *A. L. Pawlak*[1], *A. Plawski*[1], *P. Rogala*[2], *R. Slomski*[1]
[1]*Institute of Human Genetics, Polish Academy of Sciences; Poznan, Poland;* [2]*Medical University; Poznan, Poland*
ewazm75@poczta.onet.pl

Two variants of the MTHFR gene produced by transitions 677(C>T) and 1298 (A>C) occur at high frequencies (0.1-0.37 and 033, respectively) in the studied populations. In spite of this no case of combination of these two mutations on the same chromosome was found by authors studying this subject (van der Put et al., 1998). It may be presumed that the recombination between the polymorphic sites separated by 620 bp distance will not only be a rare event, but also should increase the pathogenicity of the new allele, with consequent elimination of the cases. Both polymorphic transitions were studied by PCR-RFLP. The PCR products encompassing 677(C>T) mutation was 198bp and that including 1298(A>C) - 163bp. The products were cleaved with HinfI and MboII, respectively. The doubly mutated allele MTHFR cis 677T;1298C found in Polish population, seems to confirm that the disruption of the function associated with this recombination exceeds the additivity of the defects. In the studied person the doubly mutated allele of MTHFR is paired with MTHFR 1298C on the second chromosome and associated with the spina bifida at the level between L4 and S1. The antigen HLA B27, considered as a factor possibly predisposing to spinal defect, was not present in the probant. Among the first degree relatives of the proband hypertension and stroke were reported to occur at young age.

P1620. Lysinuric protein intolerance; mutational analysis reveals strong allelic variability.

M. P. Sperandeo[1,2], *V. Fiorito*[2], *A. Pietrosanto*[2], *A. Pepe*[2], *M. T. Bassi*[3], *G. Borsani*[3], *P. Augoustides-Savvopoulou*[4], *P. Robinson*[5], *G. Andria*[2], *G. Sebastio*[2]
[1]*Telethon Foundation; Naples, Italy;* [2]*Department of Pediatrics, Federico II University; Naples, Italy;* [3]*Telethon Institute of Genetics and Medicine (TIGEM); Naples, Italy;* [4]*Aristotele University of Thessaloniki; Thessaloniki, Greece;* [5]*Royal Hospital for Sick Children; Glasgow, United Kingdom*
sebastio@unina.it

Twenty-seven patients from 23 families (17 Italian, 2 Japan, 1 Moroccan, 1 Greek, 1 Iraqui and 1 Pakistan; 37 independent alleles) affected by lysinuric protein intolerance (MIM 222700, LPI) were studied for SLC7A7 gene mutations. Thirty-two of 37 independent alleles (86.5 %) were characterized. Fourteen mutations were identified by SSCP analysis and direct sequencing of all SLC7A7 exons. Nine mutations (M1L, M50K, T188I, Y457X, 197del543, 455delCTCT, 786insT, IVS6G->T, and del ex4) were found in single families. Only 1625insATCA, W242X, 1425delCTCT, IVS3+1G->A, and S386R mutations were identified in more than one independent family. Most mutations are located in the SLC7A7 coding region, except for two splicing mutations. Genotypes were completely defined in 30 patients; a single mutant allele was characterized in two patients. For two patients, who received the LPI diagnosis on the basis of clinical and laboratory criteria, no mutation was found in the SLC7A7 coding region (after sequencing of exons and intron-exon junctions). Conclusions; 1) strong allelic variability is observed in non-Finnish LPI patients if compared to Finnish LPI patients where a single mutation was found; 2) this allelic variability is more evident in Southern Italy where 9 different mutations were detected; 3) further studies (e.g. characterization of the promoter) are needed to understand the molecular bases of the LPI-like phenotype seen in patients without mutations of the SLC7A7 coding region. Aknowledgements; Financial supports of PRIN 1998 and Telethon E.652 to G.S.; M.P.S. is supported by Telethon- Italy (grant n.29cp) and is an Assistant Telethon Scientist

P1621. Absence of IVS4 + 4A ->T Mutation In Two Brazilian Fanconi Anaemia Cases With VACTERL-H Phenotype.

J. C. Coelho[1], *S. Gonzalez*[1], *L. C. G. Fonseca*[1], *J. C. Paix o*[1], *A. A. P. Santa-Rosa*[1], *J. C. Cabral de Almeida*[1,2], *J. C. Llerena Jr*[1,2]
[1]*Instituto Fernandes Figueira/FIOCRUZ; Rio de Janeiro, Brazil;* [2]*Unidade de Citogenetica Humana (IBCCF/UFRJ); Rio de Janeiro, Brazil*
llerena@iff.fiocruz.br

Fanconi anaemia (FA) is an autosomal recessive disease characterised by diverse clinic symptoms.This extreme phenotypic heterogeneity is also associated to a genetic heterogeneity. At least seven complementation groups (A to F) have been identified and their relative prevalence varies widely among the ethnical backgrounds.The association of vertebral defects,anal atresia,tracheo-esophageal fistula,renal malformation,radial ray defects,cardiovascular defects,other limb abnormalities,genital malformations and hydrocephalus (VACTERL-H) has emerged as a unique syndrome frequently associated to FA,particularly to the mutation IVS4 + 4A->T in the FANCC gene.A regional FA register in the state of Rio de Janeiro, Brazil,was started since 1991 comprising 48 confirmed FA patients.Two FA cases were selected based on the severe phenotype described as VACTERL-H. Both patients had a conspicuous increased chromosomal instability in lymphocyte cultures exposed to DEB.Genomic DNA was extracted and a 131bp fragment across the intronic region of the IVS4 + 4A->T mutation was amplified by PCR and then sequenced. Neither patients carried this mutation.The clinical overlap of FA with other malformation syndromes points to the wide clinical spectrum of FA to which the clinician should be aware and promptly confirm the diagnosis by the cytogenetic diagnostic DEB-test or using a known FA mutation panel.However, the genetic heterogeneity of FA may impose several obstacles in establishing a diagnostic molecular protocol, since different phenotypes in FA from different ethnical backgrounds have been described carrying the same mutation.We demonstrated that the severe clinic phenotype of FA characterised as VACTERL-H does not correlate with the IVS4 + 4A->T mutation in two Brazilian FA cases.(Finantial support FAPERJ)

P1622. Rna Studies In Wilson Disease Patients Carrying Consensus Sequence Splicing Mutations

G. Loudianos[1], *M. Lovicu*[2], *V. Dessi*[3], *M. Tzetis*[4], *E. Kannavakis*[4], *L. Zancan*[5], *L. Zelante*[6], *C. Galv z-Galv z*[7], *A. Cao*[2]
[1]*Ospedale Regionale per le Microcitemie ASL 8, Cagliari; Cagliari, Italy;* [2]*IRTAM-CNR; Cagliari, Italy;* [3]*Dipartimento di Scienze Biomediche e Biotecnologie, Universit Studi di Cagliari; Cagliari, Italy;* [4]*First Department of Pediatrics, University of Athens, Greece; Athens, Greece;* [5]*Dipartimen-*

to di Pediatria-Clinica Pediatrica I, Universit Studi di Padova; Padova, Italy; [6]Servizio di Genetica Medica, IRCCS-Ospedale S. Giovanni Rotondo; S. Giovanni Rotondo, Italy; [7]Servicio de Neurologia, Hospital Militar de Sevilla; Sevilla, Spain
gloudian@mcweb.unica.it
Wilson disease (WD) is an autosomal recessive disorder characterized by toxic accumulation of copper in the liver and subsequently in the brain and other organs. To date more than 150 WD causing mutations have been identified. They are mostly missense mutations but also nonsense, insertion/deletion, and splice site mutations. Herein we report the RNA analysis for four WD mutations that occur in the consensus sequence of the donor or the acceptor splice in exons 4, 8, 13 and 14. RT-PCR was performed on patient s total RNA extracted from peripheral lymphocytes and on normal control s RNA extracted from peripheral lymphocytes or liver biopsies, using appropriate primers for a first round PCR reaction followed from nested PCR. The 1707+3 insT found in compound heterozygosity with an unknown mutation in a patient of Greek origin resulted to skipping of exon 4 that contains Cu5 and created a downstream stop codon at aa position 609. The 2866-6 T->G also found in compound heterozygous state with an unknown mutation in a patient of Italian origin resulted to defective RNA processing i.e. in frame exon skipping by removing exon 13 that codes for Tm6 the predicted ion channel and exon skipping of both 13 and 14 exon that codes for part of the large ATP loop where different critical functionally, protein regions reside. The 2122-8 T->G was detected in homozygosity in an Italian WD patient. The alterated RNA processing due this substitution resulted to removal exon 8 by exon skipping and activation of a cryptic splice site thus adding 23 nucleotides to exon 8 that creates a frameshift and a downstream stop codon at position 722. The 3061-12 T->A detected in a patient of Spanish origin in compound heterozygosity with a missense mutation results in an in frame skipping of exon 14 removing part of the large ATP loop where different critical regions for protein function reside.

P1623. Identification Of New Genetic Variants In The Gene Responsible For Gitelman s Syndrome

L. M. Syr n[1], *L. Cesareo*[2], *S. Tedeschi*[2], *A. Cant Rajnoldi*[2], *D. A. Coviello*[2], *G. Colussi*[3], *R. Bellantuono*[4], *M. Procaccio*[1], *A. Edefonti*[1], *A. Bettinelli*[1], *G. Casari*[5]
[1]*Pediatric Clinic I, University of Milan; Milan, Italy;* [2]*Molecular Genetic Laboratory, Istituti Clinici di Perfezionamento; Milan, Italy;* [3]*Niguarda-C Granda Hospital; Milan, Italy;* [4]*Bari Hospital; Bari, Italy;* [5]*Telethon Institute of Genetics and Medicine; Milan, Italy*
coviello@unige.it
The SLC12A3 gene encodes the thiazide-sensitive NaCl-cotransporter (NCCT) expressed in the apical membrane of the distal convoluted tubule of the kidneys. NCCT consists of 1021 aminoacids organized in 12 transmembrane domains with the amino- and carboxy-terminals located intracellularly. Inactivating mutations of this gene give rise to Gitelman s Syndrome (GS), inherited as an autosomal recessive trait. We have searched for mutations in the SLC12A3 gene in a group of italian patients with typical clinical features of GS (hypokalemia, metabolic alkalosis, hypomagnesemia, and hypocalciuria). All coding regions (26 exons) with their intron-exon boundaries were analysed using the PCR and SSCP techniques. Samples with abnormal migration pattern were sequenced bidirectionally. In 21 patients we identified 19 different mutations that were evenly distributed along the gene without any hot-spot for mutations. Sixteen of these are novel variants, including missense- and splice site-mutations, one deletion, and one deletion-insertion; R158Q, T163M, A166E, W172R, G374V, G463E, A464T, R642G, S615W, S615L, R852S, R958G, C985Y, 531-2A->G, fs 697->X724, fs 707->X735. It remains to be determined whether some of these missense variants (as S615L and S615W) are responsible for a loss-of-function of NCCT.

P1624. Characterization Of Glucocerebrosidase Recombinant Alleles Causing Gaucher Disease

A. Diaz[1], *B. Cormand*[1], *L. Vilageliu*[1], *A. Chab s*[2], *D. Grinberg*[1]
[1]*Department of Genetics, University of Barcelona; Barcelona, Spain;* [2]*Institut de Bioqu mica Clinica; Barcelona, Spain*
adiaz@porthos.bio.ub.es
Gaucher Disease (GD), the most prevalent sphingolipid storage disorder in humans, is caused by a recessively inherited deficiency of the enzyme glucocerebrosidase. More than 100 mutations have been described in the glucocerebrosidase gene causing GD. Some of them are complex alleles, bearing several mutations present in the highly homologous pseudogene, due to crossover or gene conversion events between the gene and the pseudogene. The most frequent complex allele in GD is RecNciI, defined by the presence of mutations L444P, A456P and V460V. We analysed patients who carried the L444P mutation and checked for the presence of the other two mutations. In total we studied DNA samples from 24 unrelated Spanish and 37 unrelated Argentinian patients by Southern blot, PCR and sequencing. The molecular analysis showed three distinct Southern patterns for RecNciI alleles. Two of these patterns correspond to alleles generated by either a crossover or a gene conversion event. The third pattern, which involves a new genomic rearrangement, is currently being characterised. Other recombinant alleles were also studied. A particular case of a patient who bears a new complex allele due to a recombination in intron 2 of the glucocerebrosidase gene was analysed and the recombination site was narrowed down to 18 nucleotides.

P1625. Rare Glucocerebrosidase Gene Mutations Causing Gaucher Disease; Expression And Characterization

L. Vilageliu[1], *M. Montfort*[1], *A. Chab s*[2], *D. Grinberg*[1]
[1]*Department of Genetics, University of Barcelona; Barcelona, Spain;* [2]*Institut de Bioqu mica Cl nica; Barcelona, Spain*
lluisa@porthos.bio.ub.es
The mutational screening of over 50 Spanish Gaucher disease patients allowed the identification of 95% of the mutant alleles, some of which were new. In order to characterize the resulting enzyme, these mutant alleles were expressed in an improved baculovirus system. The new method generates recombinant baculovirus relaying on site specific transposition in modified E.coli cells and allows selection of recombinant baculovirus. In particular, mutations P391L, N392I, and I402T were studied. Mutation P391L was found in a type 1 patient, in heterozygosity with mutation N370S. Mutation N392I was identified in a type 2 patient in heterozygosity with mutation L444P. And mutation I402T was found in homozygosity in type 1 patient. Expression of wild type cDNA results in an overexpression of acid beta-glucosidase. Enzyme activity (measured with 4MU-beta-glucoside) was about 5 to 6-fold the value of control fibroblasts. Beta-xylosidase was also detected with activity increased over 4-fold. In the studies with mutant cDNAs, the mutant proteins were characterised by enzyme activity and western blot. Preliminary studies with mutant cDNAs show reduced residual beta-glucosidase activity and recognition by policlonal acid beta-glucosidase antibodies, consistent with the expected results on the basis of the type of the amino acid change.

P1626. Funtional Stuides of Batten Disease via Identifying Two Novel Battenin-Interactive Proteins.

N. Zhong
New York State Institute for Basic Research; Staten Island, NY United States
omrddzhong@aol.com
Neuronal ceroid lipofuscinoses (NCLs) is a group of neurodegenerative disorders characterized by pathological finding of lysosomal storage of autofluorescent lipofuscins. Five genes designated CLN1, CLN2, CLN3, CLN5, and CLN8 associating with infantile (INCL), classical late-infantile (LINCL), juvenile (JNCL), Finnish LINCL (fLINCL), and progressive epilepsy with mental retardation (EPMR), respectively, have been cloned and characterized although genes CLN4, CLN6, and CLN7 associating with ANCL (adult NCL), pLINCL (Potugal variant form of LINCL), and tLINCL (Turkish variant form of LINCL) have not been identified. CLN1 and CLN2 are characterized to encode lysosomal enzymes. CLN3, CLN5, and CLN8 encode membrane proteins with unknown function. Presently, pathogenic mechanism underlying NCLs is yet unknown. We previously hypothesized that a common pathogenic pathway exists, which consists of various CLN gene-encoded proteins and is involved in the development of each individual NCL. We recently identified two novel proteins, the slow and fast Battenin-interactive proteins (BIPs and BIPf) which interact with CLN3-encoded protein Battenin. Both BIPs and BIPf are highly hydrophobic with unknown function. We have characterized that BIPs ang BIPf interact with each other, as well as with CLN8-encoded protein. More interestingly, BIPf showed a stronger interaction with Battenim, the most common mutant protein resulted from a 1.02-kb deletion in CLN3 gene, compared to with Battenin. In addition, BIPf strongly interacts with mitochondrial ATPase subunit C that pathologically accumulates in all NCLs except infantile NCL and BIPs interacts weakly with CLN1 and CLN2 encoded proteins. Our data have suggested a novel trans-membrane protein complex in which BIP proteins appear to play important roles involved in the pathogenesis of NCLs.

P1627. The First Study In The Italian Population Of Mutations In Clcnkb Gene Responsible For Bartters Syndrome Type Iii

L. Cesareo[1], *M. Syr n*[1], *S. Tedeschi*[1], *A. Cant Rajnoldi*[1], *D. A. Coviello*[1], *M. Procaccio*[2], *A. Edefonti*[2], *G. Casari*[3], *A. Bettinelli*[2]
[1]*Molecular Genetic Laboratory, Istituti Clinici di Perfezionamento; Milan, Italy;* [2]*Pediatric ClinicII, University of Milan; Milan, Italy;* [3]*Telethon Institute*

of Genetics and Medicine; Milan, Italy
coviello@unige.it
Bartters Syndrome (BS) is clinically characterized by hypokalemic metabolic alkalosis and renal salt wasting. It is divided into three clinical subtypes, I, II, and III, where nephrocalcinosis is present in types I and II, but typically absent in the third type. Subtype III results from a defective reabsorption of chloride across the basolateral membrane in the thick ascending limb of Henle in the renal tubules; it is caused by mutations in the CLC-NKB gene which encodes a protein of 687 aminoacids and contains 12 transmembrane domains, like other members of the chloride channel family. We screened a group of 10 italian patients for mutations of CLCNKB gene. The coding regions (19 exons) were analysed using PCR-SSCP technique, followed by bidirectional sequencing when an abnormal migration pattern was observed. Five patients, presenting a severe hypokalemia (S-K; 2.4-2.9 mM) and early onset of the clinical manifestation (<5 years), were found to carry variant alleles. The genetic variants included; one 3 splice site mutation in IVS 7, one insertion of 5 bp in exon 12, two deletions of exon 6 (breakpoints are underway), and one alteration of an aminoacid in exon 12 (I419V). This latter alteration has been reported as a polymorphism. These are the first genetic alterations described of the CLCNKB gene in the Italian population.

P1628. DGGE Analysis of the Tissue Non-Specific Alkaline Phosphatase Gene in 60 Patients/Carriers with Hypophosphatasia

S. R. Mumm[1,2], *J. Jones*[1], *P. Finnegan*[1], *P. S. Henthorn*[3], *M. C. Eddy*[2], *M. N. Podgornik*[2], *M. P. Whyte*[1,2]
[1]*Washington University School of Medicine; St. Louis, MO United States;* [2]*Shriners Hospital for Children; St. Louis, MO United States;* [3]*University of Pennsylvania; Philadelphia, PA United States*
smumm@im.wustl.edu
Hypophosphatasia is a heritable form of rickets/osteomalacia for which there is no established medical treatment. This inborn error of metabolism manifests an extraordinary range of clinical severity spanning death in utero to premature loss of teeth. To delineate the molecular pathology causing the extreme clinical variability and various patterns of inheritance of hypophosphatasia, we initiated comprehensive mutational analysis of the tissue non-specific isoenzyme of alkaline phosphatase (ALPL) gene in our large patient population. Genomic DNA from more than 120 probands, spanning the entire clinical spectrum, is being studied. Using a subset of 10 patients and single-strand conformational polymorphism (SSCP), we detected approximately 70% of the potential mutations (assuming two mutations for each case of autosomal recessive inheritance and one mutation for rare cases of dominant inheritance). This success rate is in accord with published efficiencies for SSCP. To increase the efficiency of mutation detection, we have developed the technique of denaturing gradient gel electrophoresis (DGGE) for the ALPL gene. DGGE primers and conditions have been developed for all the coding exons (2-12) and adjacent splice sites; the amplified products incorporate a GC clamp on one end. For the same subset of 10 patients, we have detected 100% of expected mutations using DGGE. Hence, our preliminary results demonstrate that DGGE analysis will be significantly more efficient than SSCP. To date, at least 65 different mutations (in about 70 patients) have been identified as causative for hypophosphatasia. For our group of 60 individuals studied thus far, including patients and carriers, we have identified approximately 20 previously unreported mutations, further demonstrating the vast genotypic variability of the disease. Characterization of the ALPL mutations in our large patient population will elucidate the molecular pathology and inheritance patterns, and should improve prognostication for hypophosphatasia.

P1629. Genetic lesions of UGT1A1 causing Crigler-Najjar type II syndrome

P. Labrune[1], *J. Francoual*[1], *P. Trioche*[1], *A. Myara*[2], *L. Capel*[1], *A. Lindenbaum*[1]
[1]*hopital Antoine B cl re; Clamart, France;* [2]*h pital Saint Joseph; paris, France*
philippe.labrune@abc.ap-hop-paris.fr
Patient and methods; this 5-year-old girl, born to second-cousins parents was referred for isolated unconjugated hyperbilirubinemia appeared since birth. Except jaundice, physical examination proved normal. Bilrubin serum concentration was 176 mol/l, entirely unconjugated. Hepatic bilirubin UGT activity was 15% of controls and serum HPLC of bilirubin conjugates suggested decreased glucuronidation. After 3 weeks of treatment with phenobarbital, serum bilirubin concentration had decreased to 60 mol/l.Genomic DNA was extracted and the promoter and the 5 exons of the BGT gene were PCR-amplified then directly sequenced. Results; the child was homozygous for a TA8 polymorphism within the promoter and for a missense mutation in exon 4 (N400D).Conclusions; Crigler Najjar type II syndrome may be due to a combination of both polymorphism within the promoter and missense mutation in the coding sequence of the BGT GENE.

P1630. FSHD-diagnosis;EcoRI-fragments shortened by about 20 kb after additional cleavage with BlnI

S. Jakubiczka[1], *S. Vielhaber*[2], *V. Hackel*[3], *P. Wieacker*[1], *T. Bettecken*[4]
[1]*Institut f r Humangenetik; Magdeburg, Germany;* [2]*Klinik f r Neurologie II; Magdeburg, Germany;* [3]*Neurologische Klinik und Poliklinik; Halle/Saale, Germany;* [4]*Institut f r Humangenetik, GSF Forschungszentrum; Neuherberg, Germany*
sibylle.jakubiczka@medizin.uni-magdeburg.de
Facioscapulohumeral muscular dystrophy (FSHD, MIM 158900) is one of the most frequent forms of muscular dystrophies. In about 98% of families autosomal-dominant FSHD is mapping to 4q35 and associated with a deletion of a variable number of 3.3 kb KpnI tandem repeat units (D4Z4). This deletion can normally be detected on Southern-blots hybridized with probe p13E-11 (D4S104S) through EcoRI-fragments smaller than 38 kb. Cross-hybridization of a homologous locus on 10q26 may complicate the interpretation of results. This problem can be overcome in most cases by additional cleavage with BlnI, which will shorten 4q-type EcoRI-fragments by about 3 kb while 10q-type EcoRI-fragments, containing an internal BlnI-site, are becoming very small. It has been reported that a subtelomeric interchange between 4q35 and 10q26 occurs in 21% of normal individuals. In about 8% of individuals, the interchange occurs within the repeat regions, resulting in hybrid repeat arrays being composed of both 4q-type and 10q-type-units. These data were ascertained by pulsed field experiments. Here, we report on two patients were probably such an interchange had occurred and became evident after routine continuous electrophoresis. In both unrelated individuals probe p13E-11 (D4F104S1) is detecting large EcoRI-fragments (> 40 kb) which are shortened, after additional cleavage with BlnI, by more than 20 kb instead of the expected 3 kb. Currently, we interpret these findings as hybrid arrays resulting from an 4q-10q interchromosomal exchange with breakpoints in the tandem repeat region

P1631. Detection of C1 Inhibitor Gene Mutations in Czech Patients with Hereditary Angioedema by Denaturing Gradient Gel Electrophoresis

T. Freiberger[1], *L. Kolarova*[1], *P. Kuklinek*[2], *J. Litzman*[2], *M. Vyskocilova*[1]
[1]*Department of Immunogenetics, Research Institute of Child Health; Brno, Czech Republic;* [2]*Department of Clinical Immunology and Allergology, University Hospital at St. Ann; Brno, Czech Republic*
tfreiber@med.muni.cz
Introduction; Hereditary angioedema (HAE)(McKusick 106100) is characterised by recurrent attacks of skin and mucous membrane oedemas. The most serious manifestation is the oedema of larynx mucosa, which endangers the patient s life. The genetic basis of HAE is the deficiency of C1 inhibitor (C1 INH). HAE is an autosomal dominant trait, its frequency is 1;10000 - 1;50000 in the general population. Mutations can be detected in all regions of the gene. Material and methods; DNA was isolated from 22 unrelated patients with HAE. A denaturing gradient gel electrophoresis (DGGE) was introduced to detect mutations in the C1 INH gene. The heteroduplexes were visualized in the UV light in ethidiumbromide stained polyacrylamide gels. The exons with proved presence of heteroduplexes underwent sequencing analysis to identify the particular mutations. The method of DNA sequencing with radioactively labeled 33P-dATP was used. If restriction enzyme specific for mutated, resp. non-mutated allele existed, the mutation was verified by restriction analysis.
Results; Novel mutations S48X, 8493-8494delCC, 16720delA and V432G were identified in Czech patients with HAE. All of them are supposed to be pathogenic, the former three mutations result in truncated protein and the latter one is located to the same codon as V432E, which was proved to cause HAE of type II. Previously described mutations R444C and R444H were found in patients with clinical type II of HAE.
Conclusion; DGGE is an efficient method for screening of mutations in C1 inhibitor gene. Additional analyses of the individual mutations may allow more detailed structure-function relationships to be ascertained.
This study was supported by grant No. 5558-3 of IGA MZ CR.

P1632. Evaluation of Real-Time PCR for Rapid Detection of Deletions or Duplications Associated with Human Disease

D. J. Cockburn[1], *P. Maguire*[1], *C. Greno* [1], *D. E. Barton*[1,2]
[1]*National Centre for Medical Genetics; Dublin, Ireland;* [2]*Dept of Medical Genetics, University College Dublin; Dublin, Ireland*
djcockburn@netscape.net
Deletion or duplication within human genes is associated with a number of diseases. Most notable from the perspective of the diagnostic laboratory

are dystrophin gene rearrangements in Duchenne and Becker muscular dystrophy and PMP22 deletions or duplications seen in hereditary neuropathy with liability to pressure palsies and hereditary motor and sensory neuropathy respectively. We are evaluating the application of real-time PCR to the detection of such rearrangements. The technique employs dual-labelled probes, such as Scorpions, which monitor the accumulation of PCR products. By using probes with differently labelled fluorophores, amplification profiles from two amplicons in multiplex reactions can be compared, revealing any quantitative differences that exist. We have demonstrated the effectiveness of this approach in a test system comparing dystrophin exons 8 and 45. This could be extended to multiple dystrophin exons in a cost-effective manner by use of generic probes. We have also examined PMP22 gene dosage and preliminary results are encouraging. We conclude that real-time PCR offers the potential for rapid gel-free analysis, suitable for diagnostic quantitative applications.

P1633. Screening Mutations in Angelman Syndrome Gene

C. Camprub *[1], M. D. Coll[1], I. Lorente[2], E. Gabau[2], J. Artigas[2], C. Brun[2], M. Guitart[2]*

[1]Universitat Aut noma de Barcelona; Bellaterra, Spain; [2]Corporaci Parc Taul ; Sabadell, Spain

Cristina.Camprubi@uab.es

Introduction; Angelman syndrome (AS) is a neurobehavioral disorder characterized by mental retardation, absence of speech, seizures, motor disfunction and a happy disposition. This syndrome is caused by genetic abnormalities affecting the maternal copy of chromosome region 15q11-q13. A deletion of this region is found in about 70% of patients, paternal uniparental disomy (2-3%), imprinting defects (3-5%), and mutations in UBE3A gene (4-8%). Most of the reported mutations produce truncating protein defects and the long exon 9 or exon 16 that contains the catalytic site are involved. Subjects and methods; We have studied 23 patients with a clinical diagnosis of AS with a biparental inheritance and a normal methylation pattern. Exons 15 and 16 were analyzed by SSCP technique. PCR products from exon 16 were cleaved with MspI enzyme before electrophoresis. Products were separated on 1XMDE gel solution at room temperature without glycerol and visualized by silver nitrate (0 2%) staining. Exon 9 has been sequenced directly. PCR products were sequenced on an ABI373A automated DNA sequencer. Results and Discusion; We have found two mutations in exon 16. The first mutation is a 4 nucleotide deletion (3093del4) reported previously in one case by Fang et al. (1999). The second mutation (not described before)is a 14 nucleotide deletion (3167del14) that predicted to affect the polyA addition. This one is present in a patient affected by a severe clinical; spatic tetraplegia (no prehension and unable to sit) and a severe hypoplasia of corpus callosum. As reported in other cases, mutation of the UBE3A gene are associated with typical features of AS, therefore in addition to the mutation the hypoplasia of corpus callosum could explain this severe phenotype.

P1634. A New Mutation Of Ed1 Gene In X-linked Anhidrotic Ectodermal Dysplasia

R. Bertorelli[1]*, A. Caleffi[1], S. Mauro[2], S. Manieri[2], A. Forabosco[1], D. A. Coviello[1,3]*

[1]Servizio di Genetica Medica, A.O. Policlinico di Modena; Modena, Italy; [2]Divisione di Pediatria, P.O. Nard , AUSL Lecce 1; Lecce, Italy; [3]Molecular Genetic Laboratory, Istituti Clinici di Perfezionamento; Milan, Italy

coviello@unige.it

Ectodermal Dysplasias (EDs) are a group of more then 50 distinguishable hereditary disorders with impaired development of ectodermal structure. The molecular basis for EDs is largely unknown. One of the most common types of ED is X-linked anhidrotic ectodermal dysplasia (EDA) and the identification of the gene ED1, mutated in this condition, is one of the entry point to study the pathogenesis of the disease. EDA is a clinical syndrome characterised by loss of hair, sweat glands, and teeth. ED1 gene encodes a protein product named ectodysplasin. EDA gene undergoes extensive alternative splicing and produces several transcripts; the original shortest EDA-O form encodes a 135 amino acid product that localise to the outer cell membrane. The longest ED1 transcript encodes a 391 amino acid form, EDA-A that includes a positively charged domain, 19 repeats of Gly-X-Y, similar to the group of membrane-associated collagenous type II proteins. In addition to the collagen domain, the C-terminal domain of the EDA-A protein contains a domain of homology to the member of the tumor necrosis factor (TNT) ligand family. More than 36 mutations of the ED1 gene have been identified so far. In the present study we characterise a new ED1 mutation in a patient with clinical manifestation of EDA; sparse and dry hair, incomplete teething and pointed incisors teeth, dry skin, short stature (10¡-25¡ percentile), and learning disability. We amplified and sequenced all the exons of ED1 gene using the ABI-377 sequencer and we identified a transition T->C at position 884 causing the amino acid change Val295Ala. The mutation was present in one allele of the probands mother but not in the grandmother indicating a de novo mutation.

P1635. Novel retinoic acid-sensitive genes isolated by an induction gene trap approach

M. Schlicker[1]*, D. Barthelmes[1], K. Recker[1], W. Wurst[2], I. Hansmann[1]*

[1]Institut f r Humangenetik und Medizinische Biologie, Martin-Luther-Universit t; Halle, Germany; [2]Molekulare Neurogenetik, GSF; Neuherberg, Germany

mike.schlicker@medizin.uni-halle.de

In man and mammals prenatal exposure to the teratogens retinoic acid (RA) and/or retinoids (RX) results in characteristic congenital anomalies including cardiac and craniofacial malformations. Incidence and pattern of the malformations apparently depends on dose and stage of exposure. Based on a retinoic acid-induced gene trap approach (Forrester et al., 1996) we set up a strategy to identify RA/RX downstream genes of mouse and human development that are possible candidate genes involved in the generation of congenital malformations. By RACE- PCR of trapped ES clones we identified two novel genes that are expressed in early brain and/or heart. In northern analyses a cDNA fragment of the 1st gene (EScD-1) identifies a brain-specific 6 kb transcript in embryonic head (10d p.c.) and adult brain. The 2nd gene (EScD-2) is expressed in fetal brain (2.5 kb) and heart (1.4 kb). By screening of mouse and human Marathon-cDNA libraries and analyzing homologous ESTs we identified about 2 kb of the respective cDNA sequences including the polyA-tails. The mouse and human sequences share homologies of 96 % (EScD-1) and 98 % (EScD-2). Searches for human homology revealed that the human gene EScD-2 is represented within human BAC and cosmid sequences mapping to chromosome 16p13.3. Breakpoints within that chromosome segment are known to be associated with cardiac anomalies. Using EscD-1 cDNA primers we isolated a human BAC clone and localized the respective gene on chromosome segment 7q32 by FISH. The detailed analysis of the expression patterns of EScD-1 and EScD-2 by whole mount in situ hybridization and the study of the mouse gene trap phenotypes will give information about the involvement of the two genes in early human development and specific disorders of brain and/or heart. (Supported by DFG).

P1636. Simultaneous analysis of >80 loci for deletions/duplications; an alternative to quantitative Southern blotting.

S. J. White*, M. Kalf, M. P. Villerius, G. J. van Ommen, M. H. Breuning, J. T. den Dunnen*

Leiden University Medical Center; Leiden, The Netherlands

s.white@lumc.nl

Currently most genetic diagnostic protocols are PCR-based and do not readily yield quantitative data. As a consequence, potential deletions and duplications of the regions analyzed often go undetected unless specific methods are applied. Southern blotting is the most commonly used method but is time consuming and laborious, and the results are not always easy to interpret. We have applied a new method, called Multiplex Amplifiable Probe Hybridization (MAPH). This technique allows the analysis of many regions simultaneously using a series of probes constructed in such a way that all can be PCR amplified with only one primer pair. Following hybridization to immobilized DNA the probes are reamplified and separated by gel electrophoresis. Alternatively, using fluorescently labeled primers it is possible to separate the different products on the ABI3700, a 96-capillary sequencer. This allows up to 96 samples to be analyzed concurrently. By comparing peak areas of products between controls and patients we are able to detect both deletions and duplications. Microarray analysis has also been performed, circumventing the size limitations imposed by electrophoretic separation. We have applied this technique to the diagnosis of several genetic disorders, including Duchenne/Becker Muscular Dystrophy, Limb Girdle Muscular Dystrophy and mental retardation of unknown etiology. In the muscular dystrophies individual exons were examined, whereas in the mentally retarded cases probes specific for the subtelomeric regions of each chromosome were prepared. We are investigating the possibility of combining different probe sets and detecting products using different fluorescent labels, further increasing the resolving power of the technique.

P1637. Developing of non-viral system for transfer foreign DNA

M. A. Bogdanova[1]*, N. Sinogeeva[1], B. N. Akifiev[1], E. Zamiralova[1], A. Perevozchikov[1], V. Baranov[2], M. Shavlovsky[1]*

[1]Experimental Medicine; St-Petersburg, Russian Federation; [2]Gynaecology and Midwifery; St-Petersburg, Russian Federation

sinonat@mail.ru

For non-viral gene therapy we managed to optimize the synthesis of tissue specificity protein conjugates combining capacity of internalization and a high level of expression of the gene. Selection of the chemical method of modification of the components has a significant effect on the success of the gene therapy experiment for it influences on the activity of the incoming components and size complex of DNA-conjugate. Proteins with high affinity for hepatocyte receptors (asyaloorosomucoid), hepatocyte and myocyte receptors (lactoferrin(Lf)), cancer cells (transferrin(Tf), a-fetoprotein (a-Ft)) etc. were isolated and purified. We mixed plasmid DNA with native Lf and with conjugates which contained protein ligands mentioned above and compounds tightly bound to DNA. At the same time, we developed a new method of modification of hydrocortisone 21-hemisuccinate for carrying foreign DNA. This carrier was chosen due to its ability to penetrate easily into target-cells and into their nuclei, very low background concentration in the blood plasma, high rate of absorption it from the bloodstream. We tested synthetic conjugates and complexes with DNA by laser spectroscopy, immunochemical, physicochemical and enzymatic methods. The efficiency of gene delivery (human apolipoprotein A-I (apo A-1), human distrofin cDNA (pDMD1) and reporter genes; lacZ, gfp) was tested in vitro and in vivo on laboratory animals. Some conjugates we tested using a model mouse line (mdx) with Duchenne-type muscular dystrophy. The tissue specificity of gene delivery was demonstrated. The dynamics of gene expression (7-21 days after injection) was evaluated in accord with the protocols of the synthesis of protein conjugates.

P1638. Expression of dystrophin, beta-galactosidase and luciferase genes in the organs of transfected mice.

M. Shokenova, *L. Lukasheva*
Research Centre for Medical Genetics; Moscow, Russian Federation
shokenova@mail.ru

Duchenne muscular dystrophy (DMD) is a severe sub-lethal disease which results in early lost of ability to walk at the age of 12 years approximately, and premature death at the age of 20-25 years. In spite of some progress in therapy of this disease, it is still impossible to improve quality and duration of life considerably. The most promising approach is gene therapy. We are studying optimization of a transfection of mouse skeletal muscles (mdx mice — a biological model of DMD) by looking for effective vectors and ways for plasmid DNA delivery. For this purpose we have compared an gene expression following intramuscular injection of these genes in the complex with different vectors. (1) Synthetic olygopeptide K8 and JTS-1 — number of transfected myofibres was increased compared to naked plasmid DNA from 1.1% up to 12% for Lac-Z gene and from 1.2% up to 7% for dystrophin gene. (2) Using of an adenovirus EDS-76 and polylysine reliable has not increased an expression of a luciferase gene in muscles of mice. A receptor-mediated transfection using insulin ligand associated with polylysine (3), bird s adenovirus EDS-76 (4) or adenovirus Ad5neo (5) have not resulted in increase of luciferase gene expression compared to naked plasmid pCMV-Luc. Following intravenous injection of naked plasmid pCMV-Lac-Z the expression of beta-galactosidase was studied in a different mouse organs (skeletal muscles, heart, lung, liver and spleen). In addition, the optimum concentration of plasmid and duration of marker gene expression were determined.

P1639. Gene therapy approach for GM2-Gangliosidosis B1 variant

M. G. R. Ribeiro[1], *C. A. F. Teixeira*[2], *M. Sena-Esteves*[3], *L. Lopes*[1], *M. C. S. Miranda*[1]
[1]*Unidade de Neurobiologia Gen tica, IBMC; Unidade de Enzimologia, IGMJM; Porto, Portugal;* [2]*Unidade de Neurobiologia Gen tica, IBMC; Porto, Portugal;* [3]*Molecular Neurogenetics Unit, Massachusetts General Hospital; Boston, MA United States*
gribeiro@ibmc.up.pt

The hexosaminidase deficiency leads to autosomal recessively inherited lysosomal storage disorders characterised by the accumulation of ganglioside GM2. Mutations in the alpha-chain of lysosomal hexosaminidase underlay two distinct biochemical phenotypes known as variant B and B1 of GM2 gangliosidosis. The work reports the transduction of human B1-type fibroblasts (producing catalytically inactive alpha-chains) with a retroviral vector encoding the human hexosaminidase alpha-chain cDNA. In transduced cells the HexA (alpha-beta dimer) activity with both synthetic and physiological substrates was comparable to control enzyme activity and the newly synthesised enzyme was correctly processed and targeted to the lysosomes. The high levels of recombinant enzyme corrected the metabolic defect, enabling the cells to efficiently degrade the accumulated product in lysosomes. Transfer of the human transgene product to B1-type deficient fibroblasts led to an increase of activity against the alpha-chain specific synthetic substrate that might be sufficient to restore the normal ganglioside GM2 metabolism in recipient cells. The data obtained demonstrate that B1-type phenotype can be efficiently correct in cultured cells by retroviral-mediated gene transfer. This work was supported by grants PRAXIS/PSAU/C/SAU/60/96 and PRAXIS XXI/BIC/14717/97 from Fun- da o para a Ci ncia e Tecnologia, Portugal.

P1640. In vitro correction of CF epithelial cells using SFHR techniques

F. Sangiuolo[1], *E. Bruscia*[1], *E. Bonifazi*[1], *D. Gruenert*[2], *B. Dallapiccola*[3], *G. Novelli*[1]
[1]*Tor Vergata University; Rome, Italy;* [2]*University of Vermont; Burlington, PA United States;* [3]*La Sapienza University; Rome, Italy*
novelli@med.uniroma2.it

Targeting of genomic sequences by replacement with homologous DNA segments can be used to modify specific genes within chromosomal DNA. We used the small fragment homologous replacement (SFHR) strategy to correct transformed human airway epithelial cells from cystic fibrosis (CF) individuals homozygous for the ˘F508 mutation (CFBE41o-). The transfection was made using liposomes (GenePorter, Gene Therapy Systems, USA) combined with different concentrations of purified double strand DNA fragments. Lipoplexes were then added to cells and incubated for 5 hours a 37¡C. Replacement at the appropriate genomic locus (exon 10) by exogenous DNA and expression of the modified CFTR mRNA were assayed using polymerase chain reaction (PCR) amplification. Different conditions that modulate SFHR-mediated correction were evaluated, including the type modification introduced into the genomic DNA (ie, deletion or insertion), the fragment to lipid charge ratio and stability of the modification as a function of time after transfection. PCR analysis was performed on exon 10 genomic CFTR DNA and on mRNA extracted from transfected cells after 5 days of transfection duration. A corrected genotype (˘F508/wild-type) was obtained as demonstrated by genomic and RNA analysis, transfecting 5x106 CF cells with a liposome to DNA fragment charge ratio equal to 6;1. To confirm the results, we transfected wild-type cell lines (16HBE14o-) with a DNA fragment carrying the ˘F508 mutation. Heterozygous mutant cells were obtained when a charge ratio of 2.2;1 was used, corresponding to 12000 ng of DNA fragment. Kinetics of transfection experiment was also performed in order to optimize the transfection protocol of correction. Corrected alleles are efficiently transcribed up to 120 hours from transfection, as demonstrated by genomic DNA amplification. To gain further insights about the pathway(s) involved in the delivery of therapeutic DNA fragments, transmission electron microscopy (TEM) was used to monitor lipoplexes within the cells after transfection of gold-labelled DNA fragment. At 72 hrs after transfection, large endosomes containing lipoplexes were detected perinuclear. This study demonstrate that SFHR technique is able to target and modify specific CFTR alleles in normal and ˘F508 human airway epithelial cells, by deletion and insertion of 3-bp, respectively. Acknowlegments; This work was supported by Ministero della Sanit and Regione Lazio Fondo Sanitario Nazionale per la Prevenzione e la Cura della Fibrosi Cistica (Legge 23 dicembre 1993, n.548).

P1641. Myoblast transplantation for cell-mediated gene therapy (Protocol single muscle treatment) to DMD patients in Russia.

S. Shishkin[1], *S. Terekhov*[1], *T. Krokhina*[1], *A. Podobedova*[2], *N. Shakhovskaya*[2], *V. Tarasov*[3], *G. Lyannaya*[3], *I. Krakhmaleva*[1], *S. Zakharov*[1], *S. Limborska*[4], *T. Pogoda*[4], *P. Kutyina*[5], *E. Zotikov*[5], *N. Gerasimova*[6]
[1]*Institute of Human Genetics, Research Center for Medical Genetics RAMS; Moscow, Russian Federation;* [2]*Children s Psycho-Neurological Hospital of Moscow Region; Moscow, Russian Federation;* [3]*Children s Orthopedic-Surgical Hospital of Moscow Region; Moscow, Russian Federation;* [4]*Moscow Institute of Molecular Genetics RAS; Moscow, Russian Federation;* [5]*Research Center for Hematology RAMS; Moscow, Russian Federation;* [6]*Interregional Association of assistance to people suffering neuromuscular diseases The Hope ; Moscow, Russian Federation*
socrates@orc.ru

Duchenne muscular dystrophy (DMD) with lack of dystrophin causes a progressive degeneration of skeletal muscles. Several years ago special approach for DMD gene correction using transplantation of myoblast cell cultures into patient s muscles is developed. These cultures are prepared from muscular biopsy samples of healthy donors in which contained mononuclear satellite cells. Satellite cells are myogenic precursor cells that are activated during muscle growth and regeneration. There are some data that donor s myoblast can fuse with recipient s myofibrils and expression of donor s genome contained normal DMD gene repair hereditary gene defect.

In Russia we begin clinical trail of myoblast transplantation to volunteers - DMD patients by protocol single muscle treatment and using own tech-

nique for preparation of myoblast cell cultures. All donors were previously screened for human immunodeficiency virus, hepatitis B, C and herpes infection. For the selection of donor - recipient pairs it was analyzed several HLA antigen, blood groups and Rh, Every recipient received cyclosporine (3-5 mg/kg body weight per day) two weeks before transplantation and one month after transplantation for immunosuppresion. Suspension (10 ml) contained 50-90 millions of myoblast cells was transplanted (10-12 intramuscular injections) into m. tibialis anterior of one leg. Injection of equal volume of physiological salt solution into other leg (m. tibialis anterior, control) was administered in a similar manner. There were no any side effects in all recipients during 6 months - 1 year clinical trail. Biopsy specimens of four DMD boys (age 6 - 10) 6 months after myoblast transplantation were examined to detect donor s DNA and dystrophin expression. It was revealed donor s DNA in two cases and dystrophin expression (immunohistochemical labeling with Novocastra lab. dystrophin antibodies kit) in one case. Dystrophin and donor s DNA were absent in the sham-injected muscles.

P1642. Effects of nonsense suppressor tRNAs genes transfection in vitro and in vivo.

A. V. Kiselev[1], *O. V. Ostapenko*[1], *A. N. Baranov*[1], *E. A. Lesina*[1], *L. L. Kisselev*[2], *V. S. Baranov*[1]
[1]*Ott s Institute of Obstetrics&Gynecology; St.-Petersburg, Russian Federation;* [2]*Engelhardt s Institute of Molecular Biology; Moscow, Russian Federation*
kisanet@mail.ru

It is estimated that as many as 20% of Duchenne muscular dystrophy patients have nonsense mutations in dystrophin gene. Nonsense suppressor tRNAs have been suggested as potential agents for human somatic gene therapy. To investigate effects of suppressor tRNA gene transfection in vivo we used mdx mice which have an ochre mutation in 23 exon of dystrophin gene. After in vivo transfection of genetic construction containing suppressor tRNA gene (pcDNA3 sup tRNA ochre) by means of lactoferrine the number of dystrophin positive (NDPM) myofibers was 2,5 % which is greater than background level of revertant myofibers (1%). NDPM after control transfection of genetic construction containing dystrophin gene full-length cDNA (pTG 11025) was 3,8%. In order to study possible toxic effects of suppressor tRNA in vitro we have made a co-transfection of HeLa cells using two plasmids - pcDNA3 sup tRNA (ochre or amber) and pCMV LacZ by means of non-viral vehicle VSST-525. High concentration of suppressor tRNA construction led to tenfold decrease of number transfected cells in comparison with control transfection of pCMV LacZ only. Further increase in concentration resulted in complete absence of transfected cells. On the contrary decreasing concentration led to augmentation of the number beta-galactosidase positive cells. Thus over-dose of suppressor tRNAs in the cell have clear toxic effects in vitro. Our results suggest existence of concentration threshold for the level of suppressor tRNA in the cell to gain therapeutic effect of suppression without toxicity.

P1643. In vivo gene tranfer in the GM2 gangliosidosis variant 0 (Sandhoff disease) animal model using AAV vectors

C. Bourgoin[1], *P. Montanucci*[2], *C. Emiliani*[2], *L. Poenaru*[1], *C. Caillaud*[1]
[1]*INSERM U129, Laboratoire de G n tique; Paris, France;* [2]*Department of Cellular and Molecular Biology; Perugia, Italy*
bourgoin@cochin.inserm.fr

Sandhoff disease is an autosomal recessive neurodegenerative disease characterized by the intralysosomal accumulation of GM2 ganglioside. It is due to mutations in the hexosaminidases beta-chain gene, resulting in a Hex A and B deficiency. This disease predominantly affects the central nervous system. Its evolution is dramatic and it still does not have any efficient treatment. In order to test the feasibility of gene transfer methods in this model, specific AAV vectors were constructed. They contain the HEXA and HEXB cDNAs under the control of the CAG promoter. HeLa cells infected with AAV-HEXA or AAV-HEXB showed a high level of hexosaminidases activity, demonstrating the functionality of both recombinant vectors. AAV-HEXB was then injected in the murine model of Sandhoff disease. Intramuscular injections led to a huge restoration of Hex A and B activities in the injected muscle, but no secretion was observed. AAV-HEXB was also injected intracerebrally in hexb -/- neonates. Histological staining showed a high enzyme activity in widely diffuse areas and total hexosaminidases activity in the whole brain was restored to about the normal level. However, when hexosaminidases activity was assayed after Hex A and B separation by DEAE cellulose chromatography, a massive restoration of Hex B activity was obtained, even though Hex A level only reached 20% of normal. The coadministration of HEXA and HEXB vectors will probably permit to reach therapeutic levels of both hexosaminidases A and B, especially in the central nervous system, which is the mainly affected organ in this pathology.

P1644. Chimeric Rna/dna Oligonucleotide-based Gene Therapy For Fabry Disease.

L. Lai, *Y. H. Lien*
University of Arizona; Tucson, AZ United States
llai@u.arizona.edu

Fabry disease is an X-linked recessive disease, caused by a defective lysosomal enzyme, a-galactosidase A (a-GalA), characterized by skin lesions and involvement of heart, central nerve system, blood vessels, and kidneys. We have identified a family of patients with Fabry disease. RT-PCR sequencing of the peripheral lymphocyte RNA from all female carriers and a male patient reveals a C to T mutation, resulting in an amino acid substitution of Pro-40 by Ser. The serum a-GalA activity from the male patient was undetectable and the lymphocyte a-GalA activity was less than 10 % of the normal value. Recently, a novel strategy to correct a point mutation using chimeric RNA/DNA oligonucleotides has been reported. To test whether it is feasible to correct this mutation by chimeric RNA/DNA oligonucleotides, we transfected the chimeric RNA/DNA oligonucleotides with the correct sequence into cultured peripheral lymphocytes and EB Virus transformed lymphoblasts from the male patient. The cells were transfected with a mixture of chimeric RNA/DNA oligonucleotides and Effectene (Qiagen) and harvested 5-7 days later for preparation of genomic DNA and cell extract. The conversion of mutant T to C was confirmed by allele specific PCR assay on the genomic DNA. The treatment increased the a-GalA enzymatic activity from baseline 9.2–0.7% and 8.5–1.6% (of the normal value) to 15.0–2.1% and 20.9–4.9%, in cultured peripheral lymphocytes and EB Virus transformed lymphoblasts, respectively. In conclusion, a point mutation in the a-GalA gene was identified in a family with Fabry disease. This point mutation can be corrected with chimeric RNA/DNA oligonucleotides, as demonstrated by allele specific PCR assay and increased level of a-GalA enzymatic activity in the treated cells. Potentially, this gene therapy strategy can be used to treat Fabry disease in this family.

P1645. A potential treatment for spinal muscular atrophy

J. G. Chang[1], *H. M. Hsieh-Li*[2], *Y. J. Jong*[3], *N. M. Wang*[1], *C. H. Tsai*[1], *H. Li*[2]
[1]*China Medical College Hospital; Taichung, Taiwan Republic of China;* [2]*Academia Sinica; Taipei, Taiwan Republic of China;* [3]*Kaohsiung Medical University; Kaohsiung, Taiwan Republic of China*
d6781@www.cmch.org.tw

Spinal muscular atrophy (SMA) is an autosomal recessive disease characterized by degeneration of the anterior horn cells of the spinal cord leading to muscular paralysis with muscular atrophy. No effective treatment of this disorder is presently available. Studies of the correlation between disease severity and the amount of SMN protein have indicated an inverse relationship. We report here that compound A effectively increases the amount of exon 7-containing SMN protein in SMA lymphoid cell lines by changing the alternating splicing pattern of exon 7 in the SMN2 gene. In vivo, compound A treatment of SMA-like mice resulted in increased expression of SMN protein in motor neurons of the spinal cord. Furthermore, SMA clinical symptoms of the mice significantly improved. Oral administration of compound A to intercrosses of heterozygous pregnant knockout-transgenic SMA-like mice decreased the birth rate of severe types SMA-like mice, and SMA symptoms were ameliorated for all three types of SMA-like mice. These results suggest that compound A may be an effective drug for treatment of human SMA patients.

P1646. In vitro assessment of endogenous and therapeutic inhibitors of urinary cystine precipitation

P. R. Goodyer, *P. Parvex*
McGill University; Montreal, PQ Canada
Paul.Goodyer@muhc.mcgill.ca

Children with fully recessive (Type I/I) cystinuria have a high risk of stone formation in the first decade of life. However, among 9 such children with proven rBAT mutations followed from birth, onset of nephrolithiasis did not occur until age 7-10 years, despite maximal urinary cystine excretion in the first years. To assess inhibitors of urinary cystine precipitation, we developed an assay in which radiolabelled cystine (4mM) was dissolved in urine at 37 degrees C. after alkalinization to pH10 with NaOH. Samples were then brought to pH5 with HCl, centrifuged and the %cpm precipitating was calculated. The assay was also used to compare inhibition of urinary cystine precipitation by endogenous macromolecules and by several standard and novel therapeutic agents. Percent cystine precipitation varied widely among normal children (74+/-34%), whereas variability of repeated determinations on a single individual was modest (64+/-3%). Cystine precipitation (%control) was strongly inhibited by D-penicillamine (8+/-9%) and DMSA (6+/-3%)at concentrations corresponding to predicted urinary levels following oral doses of 2g/day and 2.1g/day, respectively. Thiola was mod-

erately effective (37+/-21%)whereas captopril was a weak inhibitor (77+/-21%). To assess the ontogeny of endogenous inhibitors, urine samples were dialyzed against PBS to normalize osmolarity. Cystine precipitation was greater in urine from children from children 6-10 years (86+/-5%), than in children 0-5 years (58+/-11%) p<0.025. In conclusion, non-dialyzable inhibitors of urinary cystine precipitation probably account for protection from nephrolithiasis in very young cystinuria children. DMSA is comparable to D-penicillamine as an in vitro inhibitor of cystine precipitation and may potentially be a therapeutic agent for cystinuria.

P1647. Neural tube defects (NTD) in human embryos; expression of the SHH, GLI3 and JAG1 genes

I. Kirillova[1,2], *I. Novikova*[2], *L. Galaganova*[2], *J. Auge*[1], *S. Audollent*[1], *D. Esnault*[1], *C. Crosnier*[1], *F. Encha-Razavi*[1], *T. Attie-Bitach*[1], *M. Vekemans*[1]
[1]*Hospital Necker and INSERM U397; Paris, France;* [2]*Institute for Hereditary Diseases; Minsk, Belarus*
ikiril@bcsmi.minsk.by

Five human embryos with NTD — craniorachischisis (CRS) and spina bifida (SB)- were revealed among 40,582 first trimester abortuses screened for malformations. Expression of SHH, GLI3 and JAG1 genes and their relations to one another in the central nervous system were analyzed and compared to the age-control pattern. Expression of the genes of both the SHH and NOTCH signaling pathways was disorganized in NTD embryos. Broad, double or multiplied SHH signal was revealed in the floor plate in subtotal CRS and SB, a decreased floor plate SHH expression was noted in total CRS. Two signals were revealed in the site of the split cervical portion of the notochord (primary site of neural tube closure). Additional domains of GLI3 expression were noticed between the two floor plates in total CRS and SB. GLI3 was abnormally expressed in the floor plate in subtotal CRS. In the completely flat portions of the open neural tube pattern of GLI3 expression characteristic of the neural plate persisted, but in the concave segments where the elevation of neural folds has already occurred GLI3 domain was located in the lateral parts corresponding to the dorsal side of the neural tube. Additional domains of JAG1 expression were revealed in the neural tube of NTD embryos. Abnormal pattern of genes expression may reflect partial or complete duplication of the axial structures in NTD.

P1648. Expression and Role of p27kip1 in Neuronal Differentiation of Embryonal Carcinoma Cells

J. Furuyama[1], *S. A. Tamura*[2], *K. Sasaki*[3], *J. Senoh*[1], *T. Hashimoto-Tamaoki*[1]
[1]*Dept. Genet., Hyogo Coll. Med.; Nishinomiya, Japan;* [2]*2nd Dept. Int. Med., Hyogo Coll. Med.; Nishinomiya, Japan;* [3]*5th Dept. Int. Med., Hyogo Coll. Med.; Nishinomiya, Japan*
tomokots@hyo-med.ac.jp

We examined the expression and the regulation of the p27kip1 cdk inhibitor in P19 mouse embryonal carcinoma (EC) cells following treatment with all-trans retinoic acid (ATRA) to induce neuronal differentiation. The levels of p27 mRNA and protein increased within 24 h of treatment with ATRA, reaching a plateau 4-5 days later prior to neurite formation. Induction of muscle differentiation from P19 cells by treatment with dimethyl sulfoxide caused only transient increases in p27 levels. In a mutant P19 cell line, RAC65, treatment with ATRA induced neither p27 accumulation nor neuronal differentiation. In contrast, treatment of RAC65 cells with 9-cis retinoic acid induced both p27 expression and neuronal differentiation. Correlation between p27 expression and neuronal differentiation was also observed in NT2/D1 human EC cells. Luciferase reporter assays showed that p27 promoter activity increased in ATRA-treated cells, consistent with the elevation of p27 mRNA levels. Inhibition of p27 expression by antisense p27 oligonucleotides resulted in blockade of neuronal differentiation. These results strongly suggest that the expression of p27 is indispensable for neuronal differentiation of EC cells.

P1649. Functional Characterization of Etr2, a Novel Gene Encoding for an RNA-binding Protein, in Neural Development

M. Meins[1], *K. Kiemann*[1], *M. S Iter*[2], *J. Missbach*[1], *T. Pieler*[2], *F. Laccone*[1]
[1]*Institute of Human Genetics, University of Goettingen; Goettingen, Germany;* [2]*Department of Developmental Biochemistry, Center of Biochemistry and Molecular Cell Biology, University of Goettingen; Goettingen, Germany*
mmeins@gwdg.de

Post-transcriptional processes like mRNA stabilization, post-transcriptional modification and mRNA transport influence mRNA translation and protein levels and have been suggested to play an important role in developmental gene regulation. RNA-binding proteins are involved in all these mechanisms, but their contribution to cell differentiation is still poorly understood. Our aim is to elucidate the function of genes encoding for RNA-binding proteins in normal and abnormal development of neuronal structures in mammals. We have recently characterized a novel mouse cDNA which we called Etr2 and which is closely related to the elav-type gene family. We demonstrate that Etr2 is expressed in the developing central nervous system in the mouse (telencephalon, rhombencephalon, spinal cord). With ongoing differentiation Etr2 expression becomes restricted to the cerebellum where it persists in the adult organism. To further analyze the possible function of Etr2 in neural development, we applied transient expression of Etr2 in a heterologous system by microinjection of synthetic Etr2 mRNA into Xenopus embryos at the two-cell stage. Our results suggest that overexpression/misexpression of Etr2 in Xenopus alters the expression profile of neuronal markers and/or developmental fate of neuronal precursor cells along the closing neural tube. To support these findings we are studying the effect of Etr2 transfection on neural differentiation in rat PC12 cells. We are also on the way to isolate the Xenopus orthologue of Etr2 for structural and functional comparison. We predict Etr2 and the respective human homologue as candidate genes for developmental defects of the central nervous system.

P1650. Microarray Analysis of Temporal Gene Expression in the Developing Retina.

C. J. Zeiss
Yale University School of Medicine; New Haven, United States
caroline.zeiss@yale.edu

Numerous mutations which initiate abnormal retinal development have been identified, however subsequent cellular events causing retinal pathology are poorly understood. PURPOSE; To identify groups of co-ordinately expressed genes during normal retinal development in the mouse. METHODS; Retinas were collected from normal mice at post-natal (PN) days 5, 8, 12 and 15. For each time-point, Cy-3 labeled probes were prepared by RT-PCR of 1 g of total retinal RNA. Probes were hybridized to microarrays containing 4600 murine genes. Each gene is arrayed in duplicate, creating a chip with 9200 spots. The ratio of expression for each gene between subsequent time points was compared. For each time point, genes which were expressed beyond 2 standard deviations from the mean on both spots of the array were selected for further analysis. This subset was subjected to cluster and k-means analysis so that the expression pattern for each gene over the four time points could be assessed. RESULTS; Over the four time points, 369 genes had significant changes in expression. At each time point, gene expression could be divided into 4-6 general patterns using k-means analysis. CONCLUSIONS; Alteration in gene expression during retinal development is complex and dynamic. Patterning and cell cycle genes are predominantly expressed between PN5-8. Thereafter, they are superseded by genes controlling housekeeping and neuron-specific metabolic processes. Confirmation of the expression pattern of selected genes by northern blotting and in-situ hybridization is in progress.

P1651. Cholesterogenic enzymes are expressed in neural crest derivatives and limb buds; implications for the pathogenesis of CHILD-syndrome and Conradi-H nermann-Happle syndrome

D. Laubner, *R. Breitling, J. Adamski*
GSF-National Research Center for Environment and Health; Neuherberg, Germany
laubner@gsf.de

Disruption of post-squalene cholesterol biosynthesis by specific inhibitors (AY9944, BM15,776) or mutations in cholesterogenic enzymes leads to characteristic embryonic malformations including craniofacial dysmorphisms, postaxial polydactyly, shortened limbs, chondrodysplasia, and sensorimotor impairment. Mutations in the following enzymes have been associated with human syndromes; 7-dehydrocholesterol reductase = Smith-Lemli-Opitz syndrome, C3-sterol dehydrogenase = CHILD syndrome, sterol-delta8-delta7 isomerase = Conradi-H nermann-Happle syndrome. Mouse models for all three syndromes have been described recently (bare patches and tattered mutants, and 7DHCR-k.o. mice). The pathogenic mechanisms are not known, but some symptoms significantly resemble the phenotype of Hedgehog signaling mutants. The Hedgehog family of proteins plays a key role in embryonic morphogenesis of both vertebrates and invertebrates. The family members are post-translationally modified by covalent attachment of a cholesterol moiety and their function can be inhibited by certain plant alkaloids (cyclopamine, jervine) which show a structural similarity to cholesterol. To elucidate the disruptive mechanism we investigated the expression of cholesterogenic enzymes in the mouse at various developmental stages by mRNA in situ hybridisation. Both proximal (7-DHCR, bare patches, tattered) and distal enzymes (HMG-CoA reductase, IPP isomerase) share a characteristic pattern of

expression; In addition to a weak ubiquitous expression, a strong well-defined staining occurs in dorsal root ganglia. Further foci of cholesterogenesis are observed in cranial ganglia, pharyngeal pouches and limb buds. These results corroborate a specific function of cholesterol biosynthesis in developmental pathways.

P1652. Characterization of the limb phenotype in the Ulnaless mutant, a mouse model of HoxD gene deregulation

***D. Corzo**, C. Tabin*
Harvard Medical School; Boston, MA United States
corzo_d@a1.tch.harvard.edu

Background. Hox genes control patterning of many different embryonic structures including the axial and appendicular skeleton. In the developing limbs Hox genes are expressed within specific bone elements. Loss-of-function mutations indicate that mice with targeted deletions of specific Hox genes are missing the bone element in which that Hox gene would be normally expressed. Ulnaless is a semi-dominant mutation and a model for deregulation of posterior HoxD gene expression. The Ulnaless limb phenotype in the zeugopod is allelic to the phenotype in Hoxa-11; Hoxd-11 double mutant mice. This phenotype is characterized by severe reduction in the size of ulna and radius. Methods. Wild type and mutant Ulnaless embryos at different development stages were obtained and their limb phenotype compared. Limbs were processed to obtained skeletal preparations and histological sections. mRNA expression of several genes was studied by in situ hybridization. Results. On skeletal preparations, a reduction in the size of the mutant humerus, radius and ulna, as well as an abnormal shape of the radius and ulna is evident, as early as 13.5 dpc. Histology sections showed a delay in the differentiation of the mutant humerus, radius, and ulna of 3, 5 , and 7 days of delay, respectively, when compared to wild type littermates. Delay in differentiation in Ulnaless mutant radius and ulna was confirmed by in situ hybridization using Ihh (expressed by prehypertrophic chondrocytes) and Collagen X (expressed by hypertrophic chondrocytes) as markers. Expression of genes of the Ihh feedback loop, like PTHrP and PTH/PTHrP receptor are delayed, in accordance with delayed Ihh expression. Additional in situ hybridizations for a limited number of genes, among them TGFb1, TGFb2, and several BMPs showed either a normal temporal expression or delay. Conclusions. Reduction in size and abnormal shape of the Ulnaless zeugopod is evident as early as stage 13.5 dpc. The Ulnaless limb shows skeletal and histological evidence of delay in development that affects more severely the ulna. Several genetic markers confirmed this delay in development. Although our gene expression analysis has been extremely informative, none of the candidate regulators of bone morphogenesis is expressed in an altered manner suggestive of being a direct Hox gene target. Additional studies are needed to identify the network of Hox gene transcriptional targets involved in bone development in vertebrates.

P1653. Molecular characterization of the WT1-gene in the pathogenesis of congenital nephrotic syndrome

***G. Pitschke**, S. Sonner, B. Royer-Pokora, V. Schumacher*
Institute for human genetics, Heinrich-Heine University; Duesseldorf, Germany
gpitschke @gmx.de

The Wilms« tumor suppressor gene (WT1) encodes a transcription factor that is responsible for normal kidney and gonadal development. It is well demonstrated that mutations in the WT1 gene (11p13) are leading to Denys-Drash-Syndrome (DDS). DDS is characterised by nephrotic syndrome, Wilms tumor and pseudohermaphroditism masculinus. Most of the genes known to be regulated by WT1 (e.g. IGF-II, TGF§1, PAX-2 and Bcl-2) were identified in in-vitro assays. However, little is known about the in-vivo situation. To identify the whole spectrum of genes that are physiologically regulated by WT1 and genes that are misregulated in DDS we performed expression analyses from glomerular cells of 3 DDS patients and 2 normal controls. The process was as followed; first we isolated mRNA from frozen tissue by Laser-Micro-Dissection. The extracted RNA was amplified with the SMART-Technology, labeled with 33P and hybridized on Unigene and Atlas-Arrays. Whereas the Unigene-filters set represents more than 35.000 known and unknown human EST«s, the Atlas-Array human 3.6 consists of 3.600 known cDNA«s. The evaluation using the AIDA software revealed some important and interesting differences in the expression patterns of the control and the DDS glomerular cells. The differences have now to be confirmed by RT-PCR and immunhistochemistry analysis in cases where antibodies are available.

P1654. Wt1 Gene Expression In Cd34+lin- Hematopoietic Cells From Patients With Acute Myeloid Leukemia And Its In Vitro Modulation By Cytokines.

***M. Hern ndez**[1], J. Montesinos[2], P. Flores[2], D. Arenas[2], H. Mayani[2], F. Salamanca[2], R. Pe aloza[2]*
[1]IMSS, UNAM; M xico City, Mexico; [2]IMSS; M xico City, Mexico
ehdezc@yahoo.com

The WT1 protein has been implicated in the control of proliferation and differentiation of hematopoietic cells. An inverse correlation has been observed between WT1 expression and the cell maturation stage, thus, a higt WT1 expression has been found in CD34+ cells, whereas mature blood cells show no WT1 activity. Acute myeloid leukemia (AML) is a clonal hematologic neoplasia that arises from the transformation of a hematopoietic stem cell, resulting in altered proliferation and differentiation. High levels of WT1 have been reported in bone marrow cells from AML patients. To date, however, WT1 expression in cultures of AML CD34+ cells supplemented with growth factors has not been assessed. In the present study, we have determined the expression of WT1 in CD34+Lin- cells from normal and AML origin and evaluated its expression in cultures supplemented with recombinant cytokines (G-CSF and GM-CSF). CD34+Lin- cells, both from umbilical cord blood (normal control) and leukemic marrow were obtained by immune-based negative selection; WT1 transcripts were determined by RT-PCR and confirmed by DNA sequencing; hematopoietic cell maturation was determined by morphologic analysis. Our results show higher levels of WT1 in leukemic CD34+Lin- cells than in their normal counterparts. When leukemic cells were cultured with G-CSF and GM-CSF for 10-20 days, large numbers of mature blood cells were produced; interestingly, WT1 expression remained higt. These findings suggest that WT1 expression does not interfere with cytokine-induced myeloid terminal maturation of AML cells in vitro.

P1655. Analysis of differential gene expression during development of cardiac hypertrophy

*T. Hahn, I. Hansmann, **M. Schlicker***
Institut f r Humangenetik und Medizinische Biologie; Halle/ S., Germany
torsten.hahn@student.uni-halle.de

The spontaneously hypertensive rat (SHR) is a well-known model for the study of hypertension and heart failure. Diverse quantitative trait loci (QTLs) for hypertension and cardiac hypertrophy indicate that different chromosomal areas account for the development of the two diseases. Within the development of the clinical symptoms the SHR passes through distinct stages. Up to four weeks after birth the animals are normotensive, and then males firstly evolve hypertension (12 weeks) and afteron hypertrophy of the left heart ventricle (26 weeks). To identify candidate genes that contribute to cardiac hypertrophy we screened the hearts of these SHRs for differentially expressed genes. For having a high-resolution method we established a subtractive hybridization system based on cDNA selection and suppression PCR. The subtractive hybridizations from the 4- to 12- and to 26-weeks stages resulted in 144 different cDNA clones. The following differential screening suggested 48 cDNAs to be candidates for differentially expressed genes. Northern blot analyses of meanwhile 18 cDNAs identified 4 genes which are upregulated at their expression level in the 26-weeks stage in comparison to the wild type Wistar-Kyoto rat. According to homology searches in electronic databases we identified 4 novel genes that are differentially expressed in hypertrophic growing SHR hearts. Next, we will check the chromosomal localization of these genes in respect to known QTLs for cardiac hypertrophy by FISH analysis and analyze the expression and integrity of these genes in other hypertrophic animal models.
(Supported by BMBF)

P1656. Characterization of growth and differential gene expression of CNTF-expressing immortalized embryonic stem cell (ST14A) for the application in the animal model of Parkinson s disease

***R. Knoblich**[1], S. Weinelt[1], P. Bauer[1], T. Boettcher[1], E. Mix[1], S. Peters[1], D. Koczan[2], A. Wree[3], E. Cattaneo[4], A. Rolfs[1]*
[1]Department of Neurology; Rostock, Germany; [2]Institute of Immunology; Rostock, Germany; [3]Institute of Anatomy; Rostock, Germany; [4]Institute of Pharmacology; Milano, Italy
rupert.knoblich@gmx.de

Embryonic stem cells (ES) are the origin of mature cells of the central nervous system during fetal development. They provide the ability of plasticity and reconstitution to the brain. There are experimental data that in neurodegenerative disorders, e.g. Parkinson s disease, the transplantation of ES may reduce the progressive loss of cells and their functional deterioration. Conditionally immortalized striatal ES (ST14A) of the rat were

transfected with the rat ciliary neurotrophic factor (CNTF) cDNA in order to improve the functional efficiency of the grafted cells by secreting the neurotrophic factor. The extent of CNTF expression was determined on the transcription level by using real-time PCR (FRET-technology) and on the protein level by Western blottting and flow cytometry. Cells were characterized with regard to proliferation (H3-thymidine assay, WST-1 test) and apoptosis (TUNEL-assay). Differential RNA expression patterns were investigated by using a microarray system. Transfected cell clones with considerably different expression levels of CNTF were isolated. They had an increased proliferative activity compared to the native cells. Switching from non-permissive (33¡C) to permissive (39¡C) culturing conditions led to decrease of proliferation. Proportions of apoptotic cells were not significantly different between CNTF transfected and native cells. About 250 differentially expressed genes were analyzed. Concerning the CNTF transfected cells the microarray data suggest a tendency towards a neuronal differentiation and a decreased level of pro-apoptotic factors under permissive culturing conditions. Further efforts have to be done in order to characterize the neuronal differentiation of CNTF transfected ST14A cells on the protein level. The transfected cells will be characterized regarding their differentiation and migration in the in vivo transplantation

P1657. Neuronal death during development in control and SMA spinal cords.

E. F. Tizzano[1], *C. Soler*[1], *I. Ferrer*[2], *I. Gich*[1], *M. Baiget*[1]
[1]*Hospital Sant Pau; Barcelona, Spain;* [2]*Hospital de Bellvitge; Barcelona, Spain*
etizzano@hsp.santpau.es

The degeneration and loss of the motor neurons of the anterior horn is the major neuropathological finding in childhood spinal muscular atrophy (SMA). Neuronal death naturally occurs during development of the spinal cord but the mechanism and timing of motor neuron death in SMA remain unknown. The objectives of this work were to compare the neuronal death in control and SMA spinal cord and to determine whether the neurodegenerative process and the loss of motor neurons in SMA spinal cord are due to an enhanced cell death through an apoptotic mechanism. A quantitative study was carried out comparing neuronal death in 57 control fetuses and 12 SMA fetuses with homozygous absence of the SMN1 gene. Between 12-15 weeks of gestational age, a significant increase in nuclear DNA vulnerability was detected in SMA fetuses and was reflected by a decrease in the number of neurons of the anterior horn. Neurons with nuclear DNA vulnerability are no longer detected at the end of the fetal period and the postnatal period. The study by immunohistochemistry of two proteins involved in apoptosis such as Bcl-2 and Bax did not show any differential expression in control and SMA spinal cords. Our findings indicate that in type I SMA, the absence or dysfunction of the SMN1 gene is reflected by an enhanced neuronal death. This is associated with a progressive loss of motor neurons towards the neonatal period. Supported by Fundaci Marat TV3 and FIS 00-481.

P1658. Molecular characterization and promoter analysis of the murine and human fork head genes Foxq1 and FOXQ1

B. Pasche, *A. Bieller, K. Witt, A. Janssen, B. Zoll*
Institut f r Humangenetik; G ttingen, Germany
bpasche@gwdg.de

Murine Foxq1 is a fork head transcription factor mainly expressed in kidney and stomach. The 2.7 kb transcript was found in all tested embryonic stages as well as in embryonic stem cells. In order to analyze the transcriptional regulation we isolated the genomic clones of the mouse Foxq1 and human FOXQ1 genes including the 5 region. The DNA sequences of both genes are 80,5% identical and show high homology to the rat gene FoxQ1 (former HFH-1). The genes contain no intron and no TATA-motif within the region of 25-35 bp 5 of the putative transcription start was detected by sequence analysis. The predicted proteins contain an identical DNA binding domain and two putative activation domains, which are conserved between the 3 species. Reporter gene assays reveal that important regulatory elements are located within a 200 bp sequence which is highly conserved between mouse and man and is located around 500 bp upstream of ATG. Analyzing the region we identified three binding sites of members of the Sp1 family of transcription factors, band shift assays with these sites showed two specific DNA/protein complexes. Using antibodies against Sp1 and the related transcription factor Sp3 both complexes disappeared. Reporter gene assays with the Drosophila Schneider cell line SL2 together with Sp1 and/or Sp3 expression vectors showed, that both proteins are able to activate the reporter gene. A Yeast One Hybrid screen should help to identify other factors which could explain the tissue specificity of Foxq1.

P1659. The Role of runx2/cbfa1 in Chondrocyte Differentiation

S. Stricker, *R. Fundele, S. Mundlos*
Max Planck Institut fuer molekulare Genetik; Berlin, Germany
strick_s@molgen.mpg.de

The transcription factor runx2/cbfa1 belongs to the family of runt-related genes and is an essential factor for bone development. In humans, mutations in runx2/cbfa1 are responsible for cleidocranial dysplasia (CCD). Mice homozygous for a targeted deletion of runx2/cbfa1 die immediately after birth and exhibit a complete lack of bone formation due to an early block in osteoblast development. Recently it has been shown that runx2/cbfa1 is also involved in chondrocyte development. To further investigate this, a transgenic mouse model was created in which the transcriptional activity of runx2/cbfa1 is downregulated specifically in chondrocytes. A dominant-negative construct consisting only of the DNA-binding domain of runx2/cbfa1 under control of the Collagen2a1-promoter was used. This promoter is active only in resting and proliferating chondrocytes. The transgenic mice exhibit a markedly reduced size of the scapula and the stylopod while zeugoppod and autopod are less affected. This phenotype can be increased by crossing the mice to homozygousity. Using in-situ hybridisation it is shown that downregulation of runx2/cbfa1-signalling results in a marked delay in chondrocyte differentiation. Thus, runx2/cbfa1 acts as a positive regulator during chondrocyte differentiation contributing to the correct pace of bone growth.

P1660. Fibrillin-1 antisense affects on the developing chick embryo

S. Plaza, *S. V. Vinogradov, D. Johnson, S. Beiraghi, A. V. Kabanov, M. Godfrey*
University of Nebraska Medical Center; Omaha, NE United States
mgodfrey@unmc.edu

Mutations in fibrillin-1 a microfibrillar glycoprotein with a ubiquitous distribution, are known to cause the Marfan syndrome (MFS). Given the pathophysiology of MFS, it has been hypothesized that fibrillin-1 plays primarily a load bearing function in the extracellular matrix. However, it has been shown that fibrillin-1 is expressed as early as the primitive streak in the developing chick embryo. Since load bearing is not a critical function at that stage of development, we used an antisense approach to target fibrillin-1 during early chick development. Our initial studies were focused on getting antisense oligonucleotides into the embryo. Using fluorescence tagged oligonucleotides, we observed a greater accumulation of oligonucleotide in the embryo if the oligonucleotides were bound to cationic block copolymers of polyethyleneglycol and polyethylenimine. Block copolymer bound phosphorothioate antisense oligonucleotides were inoculated into eggs starting at four hours post fertilization. Control eggs were inoculated with equivalent volumes of PBS, copolymer alone, antisense oligonucleotides without copolymer, and sense oligonucleotide with copolymer. At both 3 and 6 days, all control embryos were at normal stages. Embryos inoculated with antisense to fibrillin-1 with the block copolymer showed a range of abnormalities from delay in development to gross morphological abnormalities. Immunohistochemical analysis was performed on fibrillin-1 antisense targeted embryos using antibodies to chick fibrillin-1 and fibrillin-2. While significant immunostaining was observed in control and mock-targeted embryos, we observed specific decrease in fibrillin-1 staining in embryos targeted with antisense to fibrillin-1. Fibrillin-2 immunostaining was unaffected in these fibrillin-1 targeted embryos. These studies point to the critical role fibrillin- 1 plays in early embryogenesis and the greater efficiency and efficacy of block copolymer bound antisense oligonucleotides.

P1661. Identification of murine sex-specific genes by differential screening

P. A. Bernard, *P. Dewing, S. T. Ching, E. Vilain*
UCLA; Los Angeles, CA United States
bernardp@ucla.edu

The molecular mechanisms of mammalian sex determination are poorly understood. To date, only a minority of sex-reversal phenotypes in humans can be explained by mutations in known sex-determining genes, suggesting the existence of other intermediates in the sex determination pathway. We hypothesize that developmentally regulated genes differentially expressed between the developing male and female gonad may control sex determination. The purpose of our experiments was to compare genital ridges from males and females at E11.5, immediately prior to the differentiation of seminiferous cords in the male gonad. The suppressive subtractive hybridization (SSH) technique was used to compare cDNA populations between males and females at E11.5. The time point and tissues chosen provide the possibility of monitoring the SSH efficiency by examining genes such as SRY and DAX1, known to be differentially expressed

between male and female gonads. We have identified 150 candidate sex-determining genes that are expressed only in the male developing gonad and 200 other candidate genes expressed only in the developing ovary. The most promising candidates are being further analyzed using a virtual slot blot assay for their expression profile during development and by in situ hybridization to confirm their tissue specific expression.

P1662. Evidence For The Exclusion Of Dmrt1 As The Testis Determining Factor In Ellobius Lutescens

A. Baumstark[1], *W. Vogel*[1], *M. Akhverdyan*[2], *W. Just*[1]
[1]*University of Ulm; Ulm, Germany;* [2]*Uppsala University; Uppsala, Sweden*
annette.baumstark@medizin.uni-ulm.de

In male mammals the Y-chromosomal gene SRY is usually required to initiate testis determination. Nevertheless there are some species with a testis determination that is independent of SRY. In the mole vole Ellobius lutescens both sexes have the same karyotype 17,X. On the cytogenetic level there are no sex-specific differences and the absence of Sry has been shown by molecular methods. It is now speculated that one of the sex determining genes acting usually downstream of Sry is mutated and has taken over testis determining function. The only candidate gene for testis determination without SRY is SOX9. In humans and mice it has been shown that an elevated dosage of the gene can lead to XX sex reversal. Recently we were able to exclude a testis inducing function of Sox9 in Ellobius lutescens by linkage analysis in an E. lutescens pedigree. Another candidate gene that could possibly induce testis development is DMRT1. The DMRT1 gene shows a testis-specific expression during embryonic development and in the adult organism in all vertebrates tested to date. Because of its localization on the bird Z chromosome it is considered a candidate gene for the postulated dosage dependent sex determination in birds. We hypothesize that a comparable mechanism - based on the existence of two Dmrt1 alleles differing in expression levels - could also work in Ellobius lutescens. To test this hypothesis we started searching for polymorphic markers in the Ellobius Dmrt1 gene in order to define different alleles of the gene. We isolated and sequenced a phage clone including exon 3 and parts of intron 2 and 3. The intronic sequences were analyzed by an enzymatic mutation detection system (EMD) and a polymorphism was identified in the second intron. The allele distribution of this marker was defined in 23 animals from an Iranian subpopulation but there was no association of marker alleles with the sex of the animals. Because of the population structure in Ellobius lutescens that is postulated to be permissive for the establishment of an extensive linkage disequilibrium and the close linkage between the tested marker and the Dmrt1 gene we assume that marker alleles and Dmrt1 alleles are also associated. On the basis of this assumption Dmrt1 is excluded from being the testis determining factor in Ellobius lutescens. This study was supported by grant Re 413/6-1 from the Deutsche Forschungsgemeinschaft.

P1663. Hidden Gonadal SRY Mosaicism In 46,XX True Hermaphroditism

G. Queipo[1], *A. Jimenez*[1], *R. Pe a*[2], *J. Zenteno*[1], *S. Kofman-Alfaro*[1]
[1]*Genetics Department, Hospital General de Mexico-Facultad de Medicina, UNAM; Mexico City, Mexico;* [2]*Department of Pathology, Hospital Infantil F. Gomez ; Mexico City, Mexico*
gqueipo99@yahoo.com

True hermaphroditism (TH) is a sex reversal condition characterized by development of testicular and ovarian tissue in a single individual. TH is a genetically heterogeneous condition as 60% of the cases are 46,XX while the remainder are 46,XY or mosaics with normal or abnormal Y chromosome. Molecular studies have shown that 10% of 46,XX TH present SRY sequences and the mechanisms leading to testicular development in Y negative 46,XX TH remain unknown. We report the results of molecular studies performed in 4 TH patients, presenting a 46,XX karyotype. Patients 1 and 2 were SRY negative in both leukocytic and gonadal DNA. PCR analysis in DNA obtained from blood leukocytes of patients 3 and 4 demonstrated the absence of SRY, but SRY was positive in DNA obtained from their gonads. In patient 3, SRY presented a deletion at its 5 region and in patient 4, a normal SRY sequence was found. The molecular findings in patients 3 and 4 indicate unusual mechanisms leading to 46,XX TH; in patient 3, SRY was absent in leukocytes but it was present and partially deleted in DNA from gonads. in patient 4 SRY was absent in leukocytic DNA but present and confined to the gonad; We propose that this is an unusual mechanism for XX TH and that the early presence of SRY was sufficient to partially differentiate testicular tissue with subsequent loss (vanishing mosaicism) of this genetic material in most tissues.

P1664. Deletions of 9p and the Quest for a Conserved Mechanism of Sex Determination

M. Fellous, *C. Ottolenghi, F. Vialard, K. McElreavey*
Institut Pasteur; Paris, France
mfellous@pasteur.fr

Distal chromosome 9p contains a locus that, when deleted, is a cause of 46,XY gonadal dysgenesis in the absence of extragenital anomalies. This locus might account for the frequently observed cases of 46, XY pure gonadal dysgenesis who do not harbor mutations in SRY, the sex master regulator gene found in mammalian species. The genomic organization of 9p positional candidate genes is currently being studied and mutational screens are ongoing. Among pthers positional candidates, including two additional doublesex-related genes, the evidence to support a role for the gene DMRT1 in vertebrate male sexual development is accumulating. Al though formal proof of the requirement of DMRT1 in gonadal sex fate choice has not been obtained so far, the particular interest in this gene and perhaps other doublesex-related genes identified in vertebrates lies in that they may provide an entry point to a conserved mechanism of sex determination across animal phyla. We discuss recent results and emerging views on the genetics of sex determination, while stressing that the majority of cases of 46,XY gonadal dysgenesis remain unexplained. The latter is likely to be efficiently addressed by positional cloning efforts, particularly by considering the wealth of sequence data provided by the Human Genome Project.

P1665. Heterochromatic der(15) in a male infertility patient; Case report and review of the literature

G. Bujdos [1], *G. Schwanitz*[2], *E. Koltai*[1], *A. Ehrbrecht*[2], *R. Raff*[2]
[1]*Institute of Forensic Medicine of Semmelweis University, Research Unit; Budapest, Hungary;* [2]*Institute of Human Genetics; Bonn, Germany*
dunay@igaz.sote.hu

Chromosome analyses have been performed in a sample of infertility patients. In the case reported, a supernumerary marker chromosome was found by Q- and G-banding after lymphocyte culture. The male patient showed no clinical abnormalities besides abnormal spermatogenesis/azoospermia/. The relatives in the ascending and collateral line of the index patient were included in the cytogenetic investigation. Three of them, all females, were also carriers of the der(15). They showed neither an increased abortion rate nor decreased fertility. Molecular analysis of the AZF gene in the index patient gave normal results. The marker chromosome was identified by FISH as a heterochromatic deviate of chromosome 15. The role of heterochromatic marker chromosomes in male sterility will be discussed.

Supported by the PPP-program of DAAD/M B and the Hungarian Academy of Science.

P1666. Germline mutations at human STR in spontaneously aborted embryos

S. A. Nazarenko, *T. V. Nikitina*
Institution of Medical Genetics; Tomsk, Russian Federation
snaz@img.tsu.ru

Recently, Spandidos et al. (1998) have reported a high level of microsatellite mutation in spontaneously aborted embryos. We have analyzed STR mismatches at 55 families with a history of early embryonic failure (missed abortions and anembryonic pregnancies) and normal karyotype. DNA was extracted from fetal membranes or chorionic villi and peripheral blood of abortuses parents. We used polyacrylamide gel electrophoresis following PCR amplification for 21 tetranucleotide high-polymorphous loci located on seven autosomes. In 1222 parent/abortus allelic transfers, 10 isolated STR mismatches in six loci were observed. The mutation rate of was 8.3 x 10-3 per locus per gamete per generation with the prevalence of the male germline mutations. The study of alleles segregation of other DNA markers permitted exclusion with high probability non-paternity and uniparental disomy as the possible reasons for parent/abortus mismatches. Together with two additional cases of somatic microsatellite mutations which we detected in spontaneous abortuses the mean mutation rate of studied tetranucleotide loci was 9.8 x 10-3 per locus per gamete per generation. This is about five times higher then the spontaneous mutation rate of this type STR loci reported by other authors in normal families. It can be suggested that genome instability detected at the level of repeated DNA sequences can involve not only genetically neutral loci but also active genomic regions crucial for embryonic viability. However, because the mutation rates of different loci can differ to a marked degree an additional analysis of spontaneous mutation rate of studied loci in normal population is needed as well as the necessity of further investigation of microsatellite mutation in a larger sample of families with miscarriages.

P1667. Human intrachromosomal telomerik-like repeats; relics of ancestral double-strand break repair

E. Giulotto[1], C. M. Azzalin[1], S. G. Nergadze[1], N. Archidiacono[2], M. Teti[2]
[1]University of Pavia; Pavia, Italy; [2]University of Bari; Bari, Italy
giulotto@ipvgen.unipv.it

Intrachromosomal location of (T2AG3)n telomeric sequences was reported in different species and several authors proposed that interstitial telomeres (ITs) are relics of ancestral telomeric fusions. Sequence analysis of about 70 ITs allowed us to identify three classes; (i) short ITs, composed by few essentially exact T2AG3 units; (ii) subtelomeric ITs, composed by larger arrays (several hundred bp) including degenerate units within subtelomeric domains; (iii) fusion ITs, in which two extended telomeric stretches are oriented head-to-head. Surprisingly, only one IT, mapping at 2q13, belongs to the fusion class, indicating that mechanisms other than the telomeric fusion generated most human ITs. In particular, several short ITs interrupt precisely repetitive elements or are flanked by direct repeats of 10-43 bp. This sequence organization suggested to us that these ITs were inserted during the repair of double-strand breaks (DSBs) that occurred in the germ-line. We sequenced the region homologous to one short IT from different primate species. In humans, this IT extends for 67 bp and is flanked by a 43 bp direct repeat. The telomeric stretch and the direct repeats are conserved in humans, chimpanzee and gorilla. Interestingly, in orango and in gibbon, the IT is missing and the 43 bp repeat is present only in one copy. These data unambiguously confirm that the IT was inserted during evolution through the repair of a staggered DSB that produced 43 bp protruding ends. The events leading to the insertion of this IT occurred 7-15 m.y.a., between the radiation of the genera Pongo and Gorilla.

P1668. Evolutionary Breakpoints on Human Chromosome 21 in comparison with mouse

M. T. Davisson[1], E. C. Akeson[1], L. J. Bechtel[1], D. Slavov[2], K. Gardiner[2]
[1]The Jackson Laboratory; Bar Harbor, ME United States; [2]The Eleanor Roosevelt Institute; Denver, CO United States
mtd@jax.org

Human Chromosome 21 is conserved in mouse Chromosomes 10, 16, and 17. To facilitate the construction of appropriate mouse models of Down syndrome, the evolutionary breakpoints between the three conserved segments on human chromosome (Chr) 21 were defined. The annotation of the complete genomic sequence of Chr 21 was used to identify all genes in the relevant breakpoint regions, followed by database searches to identify the homologous mouse genes. The mouse T-31 radiation hybrid (RH) panel and PCR primers were used to map these genes to mouse chromosomes. This directly demonstrated the inclusion of two zinc finger genes, an ankyrin/kinase gene, and an anonymous ORF in the telomeric region of mouse Chr 16 and places the Chr 16 - Chr 17 breakpoint between genes ZNF295 and ABCG1. By similar means the Chr 17 - Chr 10 breakpoint was located between genes KIAA0179 and PDXK. These data show that the mouse Chr 17 component is ~1.5 Mb and contains possibly as few as 25 genes. These data provide additional information on the genetic content of the segmental trisomy Ts65Dn model for Down syndrome. In addition, FISH was used to show that the mouse homologue of NCAM2 (Ocam) is not contained in Ts65Dn, while the homologues of APP and GABPA are in the trisomic chromosome.

P1669. Molecular definition of pericentric inversion breakpoints on the chimpanzee and orangutan equivalents of human chromosome 17

H. Kehrer-Sawatzki, H. Hameister
University of Ulm, Department of Human Genetics; Ulm, Germany
hildegard.kehrer-sawatzki@medizin.uni-ulm.de

Sequence analysis has revealed pronounced conservation among the genomes of higher primates. For example, the genome of the chimpanzee (Ptr) is supposed to be more than 98% identical to the human genome. However, genetic disparities are assumed to account for the reproductive isolation and phenotypic separation of the species. The most conspicuous differences between the karyotypes of members of the family of Hominidae are the fusion of great ape chromosomes 12 and 13, changes in heterochromatin distribution and pericentric inversions. Comparing the G-banded chromosomes of human and chimpanzee, nine pericentric inversions and one insertion, but no paracentric inversions or further translocations are observed. In the case of 5 of these chromosomes with pericentric inversions, the inverted regions are large enough and contain several Mb of euchromatin to foster the suspicion that these pericentric inversions had an effect on reproductive isolation. The molecular definition of pericentric inversions is necessary to address their contribution to human evolution and to investigate whether the characterization of evolutionary breakpoints provides insights into general mechanisms of chromosomal rearrangements. To isolate the genomic regions harbouring pericentric inversion breakpoints we compared the FISH pattern of BAC contigs in the respective regions on human chromosome 17 between chimpanzee, orangutan and human. We identified BAC clones closely adjacent to and spanning the inversion breakpoints on the equivalents of chromosome 17 in chimpanzee and orangutan. BAC subclones were generated by PCR or restriction digestion and localized by FISH to further narrow the breakpoints to more restricted regions. Preliminary results suggest that based on the corresponding human sequence at 17q21.3 a gene should be affected by the inversion resulting in altered genomic organization of that locus comparing human and chimpanzee.

P1670. Nucleotide Diversity In § Globin For Non-human Primates

D. Podpecnik[1], U. Potocnik[1], M. Ravnik-Glavac[1], J. Wine[2], A. Novak[1], T. Krasovec[1], D. Glavac[1]
[1]Medical Faculty; Ljubljana, Slovenia; [2]Stanford University; Stanford, CA United States
dpodpecnik@mf.uni-lj.si

Animal models of genetic diseases are important in order to understand etiological and pathophysiological processes also in humans. Hemoglobinopathies and thalassemias are the most common single gene diseases in humans. Their distribution, which is geographically localised, overlaps the distribution of monkey populations. We therefore screened 6 monkey species for nucleotide diversity at the § globin locus. Adult hemoglobin A comprises two ? and two § chains. The ? chain locus has two equivalent genes, and ? thalassemias typically result from large deletions. Because there are two ? genes and because our methods are optimized for detecting point mutations, we chose to focus on the § globin locus. Results show that we can amplify § globin with human intronic primers in all 6 primate species we examined. We defined 6 different allels, including one normal, in each of three exons in § globin gene. There were 3 missense, 8 silent and 10 intronic alternations close to exon/intron boundary (Figure 1), together 21 alternations. One of the intronic alternations was frame shift alternation due to T insertion. M. nemestrina is the most diverse of the species we studied. For this species 12 alternations were seen across all 3 exons.

P1671. Comparative mapping of human chromosome 20q13 segment and identification of new genes as candidate for imprinting

S. Worch, S. Ku§mann, A. Hehr, I. Hansmann, D. Schlote
Institut f r Humangenetik und Medizinische Biologie; Halle, Germany
dietmar.schlote@medizin.uni-halle.de

The human chromosome 20q13 (HSA20q13) segment has been shown to be syntenic to distal mouse chromosome 2 (MMU2) containing imprinted genes. In order to refine and expand the syntenic map between MMU2 and HSA20q13 we have generated a human-BAC-contig of approximately 1000 kb in size spanning the area around the anchor markers GNAS1 and EDN3 which is the basis for the identification of novel genes within the region of interest as candidates for imprinting. Several genes have been isolated and characterized in terms of structure and expression pattern but their function remains unknown since they do not show homology to known genes nor proteins. Herewith we introduce two of such candidate genes which contain the human EST-markers stSG10922 and A005Z17, respectively. Starting out from stSG10922 a complete cDNA containing a 921 bp ORF has been isolated consisting of 9 exons. Northern-hybridisation revealed a transcript of approximately 1,8 kb in size expressed in all tissues analyzed. In case of the second gene a 3,2 kb transcript was detected in brain only. The corresponding cDNA has been isolated containing a 1683 bp ORF and deriving from at least 13 exons. For both of these novel transcripts homologous cDNA clones of the mouse have been isolated. They will serve to verify the physical localisation using a YAC-contig of distal MMU2. The characterization of homologous mouse genes will be the basis to investigate monoallelic expression using a translocation mouse model to identify novel candidate genes for imprinting in mouse and man. Supported by DHGP.

P1672. Inheritance Patterns Of Rhythm Parameters; Exposure To Exogenous Signals

Y. Weigl[1], L. Peleg[2], A. Dotan[1], I. E. Ashkenazi[1]
[1]Unit of Chronobiology, Department of Human Genetics and Molecular Medicine, Sackler School of Medicine, Tel Aviv University; Tel Aviv, Israel; [2]Danek Gertner Institute of Human Genetics, Sheba Medical Center; Tel Hashomer, Israel
yweigl@hotmail.com

To elucidate the inheritance patterns of rhythms patterns we used three

month old BALB/c and C57BL/6J male mice and their F1 progenies. The mice were exposed to 12;12 L;D illumination for three weeks. Cis-platinum (0.04 mg/mouse) or saline was injected i.p. at (22 HALO) for 10 consecutive days. On the 11th day, 3 mice of each group were sacrificed at equidistant times (during 30 hours). Plasma WBC count, and kidney Creatine phosphokinase(CPK), Alkaline phosphatase(AP) and Urea Nitrogen were determined. Rhythm parameters were determined and were compared among the groups. Results; Prior to injection all group exhibited the same circadian pattern and phase in WBC count. After injection two of the groups exhibited a semicircadian rhythm and another group exhibited a phase change. Pre and post injection different changes of the individual rhythm parameters were observed in the two parental strains and their offspring with regard to AP, CPK activity and Urea Nitrogen concentration. . Conclusions; 1. Strain dependent variations in time structure were found in two mice strains and in the F1 progeny, albeit the identical entraining conditions. 2. The stress induced by the drug, affected differently the rhythms of the examined variables in the three mice groups. 3. Rhythms parameters were segregated independently from each other in the F1 progenies, a fact that suggests the existence of an independent genetic control for each.

P1673. Comparative genomic sequencing of the human and mouse RPGR gene identifies tissue-specific coding sequences that are mutated in patients with X-linked retinitis pigmentosa

R. Kirschner[1], *D. Erturk*[1], *C. Zeitz*[1], *S. Sahin*[1], *J. Ramser*[1], *T. Rosenberg*[2], *A. A. B. Bergen*[3], *F. P. M. Cremers*[4], *H. H. Ropers*[1], *W. Berger*[1]
[1]*Max-Planck-Institute for Molecular Genetics; Berlin, Germany;* [2]*National Eye Clinic for the Visually Impaired; Hellerup, Denmark;* [3]*The Netherlands Ophthalmic Research Institute; Amsterdam, The Netherlands;* [4]*Department of Human Genetics of the University Medical Centre Nijmegen; Nijmegen, The Netherlands*
kirschne@molgen.mpg.de

X-linked retinitis pigmentosa 3 (XLRP3), a progressive retinal degeneration, is caused by mutations in the retintis pigmentosa GTPase regulator (*RPGR*) gene residing in Xp21.1. *RPGR* transcription studies in man and mouse revealed several alternatively spliced isoforms in different tissues, including retina. To further elucidate the genomic structure of this gene and to understand the regulation of tissue-specific expression and splicing, we performed comparative genomic sequencing of *RPGR* and flanking regions in mouse and man. Both genes span nearly 59 kb. All previously identified exons are conserved between the 2 species. In addition, more than 25 conserved regions were found in introns. One of them is identical to a novel *RPGR* exon (ORF15), that was reported recently as mutation hotspot. 30% of all our XLRP patients showed frameshift mutations in this sequence. Northern blot analysis with an ORF15-probe revealed high molecular weight *RPGR* transcripts (>15 kb) in retina and brain. In contrast, the ubiquitously expressed consensus cDNA is only 2.8 kb in length and the discrepancy can not be explained by the ORF15 sequence. Further transcription studies are underway in order to isolate additional expressed sequences. Our results demonstrate that comparative genomic sequencing enables the discovery of tissue-specific exons when ESTs are not available or in those cases where exon prediction programs fail. Moreover, conserved sequences in *RPGR* introns represent potential regulatory elements which are important for alternative splicing or tissue-specific gene expression.

P1674. Potential importance of the Glucocorticoid Receptor Promoter Binding Protein (GRF1) in an inherited retinal disease pathway.

B. Zangerl, *Q. Zhang, G. Aguirre*
J.A. Baker Institute, Cornell University; Ithaca, NY United States
bz24@cornell.edu

Human retinitis pigmentosa (RP) is the phenotypic equivalent to canine progressive rod-cone degeneration (*prcd*), an autosomal recessive retinal disease mapped to CFA9. Synteny of the region to the human chromosome 17q suggests that *prcd* is the canine homolog of the human RP17 locus, neither of which have been molecularly identified. Once the responsible mutations are found, knowledge of genes involved in the disease pathway is necessary to understand the pathogenesis of these disorders. We have used a subtraction approach to detect potential novel candidate genes with modified expression caused by *prcd* which may be causally associated with the disease, or, alternatively, be involved in the molecular mechanisms leading to the disease phenotype. This resulted in identification of a 5.6 kb cDNA for an unknown canine gene, containing 4,503 nt of coding sequence and predicted to encode a protein of 1,500 amino acids. The gene shows about 90% homology to the human *GRF1* and human and rat *p190* genes at both the nucleotide and protein levels. Gene transcripts were detected in several different tissues, including retina. The canine *GRF1* maps to CFA1 close to *CRX*, a region synteny to human chromosome 19q13 and therefore has been excluded as a candidate gene for prcd. However, recent studies have revealed the importance of glucocorticoids in the retina, possibly to maintain a protective function. Thus, the expression pattern of *GRF1* in retina and its gene regulating function displayed in human and rat supports its potential role in the disease pathology.

P1675. Comparative genomics of the SOX9 region in human and Fugu; conservation of short sequence elements within large intergenic regions

S. Bagheri-Fam[1], *D. Pfeifer*[1], *C. Ferraz*[2], *J. Demaille*[2], *G. Scherer*[1]
[1]*Institute of Human Genetics and Anthropology; Freiburg, Germany;* [2]*Institut de G n tique Humaine; Montpellier, France*
bagherifam@aol.com

Campomelic dysplasia (CD), a skeletal malformation syndrome with XY sex reversal (MIM 114290), is caused by heterozygous mutations in and around the SOX9 gene on distal 17q. SOX9 has an extended 5« control region as indicated by CD translocation breakpoints scattering over 1 Mb proximal to SOX9, and by expression data from mice transgenic for human SOX9-spanning YACs. We have previously generated ~1 Mb of genomic sequence from the SOX9 5«-flanking region that could now be extended to an almost uninterrupted sequence contig of ~3.7 Mb around SOX9. Seven protein-coding genes, two pseudogenes and three non-coding transcripts were found in this region. Analysis of ~195 kb of genomic sequence from the SOX9 region in the pufferfish Fugu rubripes revealed a similar low gene content of only five genes. Four of these genes have been mapped in human, and all reside on chromosome 17, revealing extensive intrachromosomal gene shuffling when compared to the gene order in Fugu. In both species, very large intergenic distances separate SOX9 from ist flanking genes; 2 Mb and 500 kb either side of SOX9 in human, and 68 kb and 94 kb either side of SOX9 in Fugu. Comparative sequence analysis of the intergenic regions revealed five conserved potential regulatory elements, E1-E5, up to 290 kb 5« to human SOX9 and up to 18 kb 5« to Fugu SOX9, and three such elements, E6-E8, 3« to SOX9. From the YAC transgenic data, elements E3-E5 are candidate enhancers for SOX9 expression in limb and vertebral column, and eight of ten CD translocation breakpoints separate these elements from SOX9. Work is in progress to test the identified elements in transgenic mice.

P1676. Human chromosome 21;genomic sequence annotation, gene expression and evolutioary conservation

K. J. Gardiner, *D. Slavov, A. Fortna*
Eleanor Roosevelt Institute; Denver, CO United States
gardiner@eri.uchsc.edu

Analysis of the recently reported sequence of human chromosome 21 identified 225 genes and gene models. To aid in studies relevant to Down syndrome, we have directed efforts towards refinement of the genomic annotation, development of gene expression patterns, and identification of evolutionarily conserved genes. Genomic sequence annotation includes structures of genes and models, the identification of mouse, rat and zebrafish ESTs, pufferfish conservation (Exofish), promoter predictions (PromoterInspector) and mouse genomic sequence conservation. Expression analysis uses genomic annotation followed by RT-PCR from 30 tissues and cell lines. This defines tissue specific and ubiquitously expressed genes, and refines models, sometimes combining two gene models into one or splitting a single model into two. Complexities include large genes, ambiguous open reading frames, and overlapping genes. Rat/mouse ESTs add support to weak models and define rarely expressed genes. Searches of the complete databases of yeast, C.elegans and Drosophila, and the growing zebrafish ESTs define sets of evolutionarily conserved genes showing strong similarities throughout entire proteins or well beyond known functional domains. For the majority of these there is as yet no mutation information in any organism. Lastly, genes highly similar to those on chromosome 21 are identified elsewhere in the human genome; expression data do not suggest divergent tissue specificities. These data also show that thorough and complete annotation of human genomic sequence remains a time consuming and gene-specific process, yet essential for understanding the basis of the Down syndrome phenotype.

P1677. Characterization of 600 canine (Canis familiaris) cDNA clones

H. Murua Escobar[1], *L. Borrmann*[1], *I. Nolte*[2], *J. Bullerdiek*[1]
[1]*Center for Human Genetics; Bremen, Germany;* [2]*Clinic for Small Animals, Veterinary School; Hannover, Germany*
escobar@uni-bremen.de

Kuska (1999) has recently predicted that geneticists will go to the dog in the next millennium and it goes almost without saying that for several reasons the dog provides a suitable model for novel therapies. The dog is well suited as a model system for a variety of human diseases as e.g. the spinal muscular atrophy, arthritis progressive, retinal atrophies, or von Willebrand disease. Furthermore, dogs can produce a huge offspring during their life providing geneticists with large well documented pedigrees impossible to obtain in human studies. Even several examples of gene therapy of canine diseases have already been reported. On the other hand, molecular genetic tools allowing for a more advanced knowledge of canine molecular genetics are far behind of what we know for humans. We established a cDNA library from canine testis tissue and picked random clones for analysis. By sequencing and characterizing 600 clones we could show that the homology between man and dog is much higher as the so far estimated 80%. The characterized clones showed an homology of 85% - 100% to human genes or ESTs. In addition we compared sequence homologies of open reading frames and 3 UTRs separately. Combining these data with gene mapping will be a powerful tool to analyse human diseases by using the dog as model system.