Abstracts of Lectures

1. Symposia

S1

The Human SHOX Mutation Database

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The human SHOX gene (Short Stature Homeobox gene on the X-Chromosome) has been isolated by positional cloning and resides in the pseudoautosomal region 1 of the sex chromosomes (Rao et al. 1997, Elison et al. 1997). Heterozygousous SHOX mutations have been shown to cause idiopathic short stature (ISS), short stature phenotype in Turner Syndrome (TS) (Rao et al. 1997) and Lèri-Weill Dyschondrosteosis (LWD) (Belin et al. 1998, Shears et al. 1998). The homozygous loss of SHOX has been correlated with the Langer type of mesomelic dysplasia (Belin et al. 1998).

The Human SHOX Database has recently been established to provide clinicians and scientists access to a central source of information about all known SHOX mutations associated with short stature phenotypes. So far, the database contains 29 unique intragenic mutations of the SHOX gene. These mutations were detected in a total of 39 patients from different families. Fourteen of these mutations have been found from the SHOX research group at the Institute of Human Genetics in Heidelberg, Germany; 25 mutations are from data reported in the literature. Not included in this database are complete SHOX gene deletions which represent the majority of all detectable SHOX mutations (Rappold et al., 2002).

The database is accessible via the web site http://www.shox.uni-hd.de. It contains general information about the SHOX gene, allows remote users to search the data and to submit new mutations into the database. The gene structure is incorporated in order to facilitate data submission.

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FREQUENCIES AND ORIGINS OF COMMON DHCR7 MUTATIONS CAUSING THE SMITH-LEMLI-OPITZ SYNDROME

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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. Based on case frequency surveys the incidence of the SLOS has been estimated to range from approximately 1:20.000 to 60.000 in populations of European origin.

We are analysing the frequencies, origins, and ages of DHCR7 mutations in Europe (Italy, Spain, Poland, Austria, Germany, and Great Britain). Mutational spectra analysed in 81 SLOS patients were significantly different across populations with frequency maximum of the common mutations in East-Europe (W151X, V326L), North-West-Europe (IVS8-1G>C), and South-Europe (T93M).

Carrier frequency analysis of the IVS8-1G>C, W151X, T93M, and V326L mutations in 2250 healthy individuals from different European populations revealed much higher frequencies for these common mutations (e.g. 1:50 for the IVS8-1G>C in Austria, and 1:84 for the W151X in Poland) than expected from the reported prevalence of the SLOS. Based on these frequencies the expected incidence of SLOS patients with null mutations ranges from 1:2000 to 1:16.000. This discrepancy might be due to underdiagnosis of both severe and mild cases, but also to embryonic/fetal loss of homozygotes for frequent null alleles (W151X, IVS8-1G>C).

Using eight cSNPs in the DHCR7 gene haplotypes were constructed for 52 SLOS chromosomes. All chromosomes carrying the most common European mutation IVS8-1G>C shared the same haplotype suggesting a founder effect. Mutations T93M and R404C which involve CpG islands were found on 4 and 3 different haplotypes respectively suggesting that they are recurrent. The W151X mutation was present on different related haplotypes and is probably older than the SNPs used for haplotype construction indicating that it may be the oldest common SLOS causing mutation in Europeans. The data suggest an intriguing high frequency and heterogeneity of the ages and origins of common DHCR7 mutations in

S2

Genetic instability and darwinian selection in tumours

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Whether and how human tumours are genetically unstable has been debated for decades. There is now evidence that most cancers may indeed be genetically unstable, and that the instability exists at two distinct levels.

In a small subset of tumours, the instability is observed at the nucleotide level and results in base substitutions, deletions or insertions of a few nucleotides. This instability is most easily observed at short sequences of DNA repeats scattered throughout the genome, called microsatellites, thus generating the characteristic microsatellite instability (MIN) seen in these tumours. MIN tumours have nucleotide mutation rates two to three orders of magnitude higher than normal cells or mismatch-repair-proficient cancers of the same cell type. This form of instability arises from inactivation of DNA mismatch repair (MMR) genes such as MSH2 or MLH1. Strikingly, unlike the vast majority of solid tumours, MIN cancers have a normal complement of chromosomes. These cancers retain a diploid karyotype ¯ one pair of each of the chromosomes.

In most other cancers, the instability is observed at the chromosome level, resulting in losses and gains of whole chromosomes or large portions thereof. Non-MIN tumours have a wide variation in chromosome number - their karyotypes are aneuploid. Such observations have led to the suggestion that cancers develop instability either at the sequence level (MIN) or at the chromosomal level, but not generally at both levels. In this view, the aneuploid karyotype is the readout of an underlying chromosomal instability (CIN). In a small proportion of cancers displaying CIN the loss of this checkpoint is associated with the mutational inactivation of a human homologue of the yeast BUB1 gene. BUB1 controls mitotic checkpoints and chromosome segregation in yeast.

Because the MIN and CIN forms of instability are rarely found to coexist in tumours, it would seem that one form of instability is sufficient to drive tumorigenesis.

Genetic instability appears early in tumorigenesis and is believed to play a critical role in the malignant process. Cells with CIN are found to activate or increase the number of copies of oncogenes and lose tumor-suppressor genes, whereas cells with MIN accomplish the same through mutations in repetitive DNA sequences. This relationship is well documented, and the effects of genetic instability in cancer development are straightforward in terms of Darwinian evolution: Genetic instability provides a repertoire of mutants from which the environment selects favorable variants.

Recent experiments have shown that CIN and MIN instabilities reflect resistance to different carcinogens and that genomic instability in cancers may mirror the mutagenic environments in which they evolve. It is therefore interesting to speculate that both MIN and CIN develop through selection pressures that can readily be understood in Darwinian terms rather than arising in a random and mysterious manner.

Functional dissection of signal transduction pathways in mammalian cells using RNA interference

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The RAS-RAF-MEK-ERK pathway is the major player in the regulation of oncogenic signalling in various types of cells. Most cells express different isoforms of the effector kinases downstream of RAS, however, the contribution of individual kinases is poorly understood. To elucidate the function of kinase isoforms in controlling cell growth and transformation, we have introduced siRNAs (small interfering RNA douplexes) specific for RAS, RAF, MEK and ERK-isoforms into HRAS-transformed rat (FE-8) and mouse (NIH-pEJ) fibroblasts and into human EJ bladder carcinoma cells harbouring endogenous activated HRAS. HRAS-specific siRNA strongly inhibited growth in all cells and morphological transformation in FE-8 and NIH-pEJ cells. KRAS-specific siRNA had no effect. Targeting kinases downstream of RAS in mouse NIH-pEJ by RNA interference revealed a differential contribution of MEK1 and MEK2 to growth control. Whereas the MEK1-specific siRNA inhibited cell growth only weakly (20%), interference with MEK2 expression resulted in 50% growth suppression. Interference with either MEK isoform had almost no effect on cell morphology. However, the combination of both siRNAs fully reverted the transformed phenotype of the cells and inhibited cell growth completely. This indicates a strong synergism between MEK1 and MEK2. In a similar way, full morphological reversion was achieved upon co-transfer of ERK1 and ERK2-specific siRNAs. Cell growth however, was inhibited up to 50% by either combining ERK1 and ERK2 siRNAs or after transfer of individual siRNAs. Knock-down of kinase isoform expression was confirmed by Western blot analysis. In-

hibition of ERK1 resulted in increased phosphorylation of ERK2 and vice versa. This suggests a cross-talk of the activated, phosphorylated forms of ERK1 and ERK2 kinases compensating inhibitory effects. Our data show that RNA interference is a powerful genetic tool for functionally dissecting the complex network of signalling pathways in mammalian cells.

High prevalence of BRCA2 mutations in pancreas cancer families and further evidence for a CDKN2A mutation associated pancreas cancer melanoma syndrome

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Familial pancreatic cancer (FPC) is a rare disease for which no major causative gene has been reported. Members of hereditary breast/ovarian cancer (HBOC) families with BRCA2 germline mutations, and of families with familial atypical multiple mole-melanoma (FAMMM) syndrome and with CDKN2A mutations have an increased pancreatic cancer (PC) risk. Provisionally, the latter have already been summarized under the pancreas cancer-melanoma syndrome (PCMS). The German National Case Collection for Familial Pancreas Cancer (FaPaCa) has been established for the clinical and genetic analysis of familial pancreas cancer (FPC) and of families suggestive of PCMS. Constitutional DNA of 14 index patients of FPC families was screened for sequence variants of the BRCA2 gene. Three different frame shift mutations were identified in three cases (21%) of which two have been described as oncogenic mutations and one as an unclassified variant in HBOC families. The latter was accompanied by an unclassified missense variant on the same allele. At the time of recruitment, none of the families with BRCA2 mutations presented with a tumor pattern suggestive of a HBOC family. Index patients of 18 FPC families and of 5 families with potential PCMS were screened for CDKN2A sequence variants. In none of the FPC but in 2 of the PCMS insinuating families CDKN2A frame shift mutations were identified. Thus, CDKN2A mutations seem to contribute to the familial occurrence of PC and MM in 2/5 of the cases. This provides further evidence for PCMS to represent a distinct hereditary cancer predisposition syndrome. Screening for BRCA2 and CDKN2A mutations in index patients of FPC and PCMS families may be feasible to allow a individualized PC risk assessment of family members by predictive genetic testing. However, penetrance data of the respective gene mutations and procedures sensitive enough for an early detection of PC are still lacking. The continuation and extension of FaPaCa may provide the logistic infrastructure for the prospective acqua in mutation carriers and for the evaluation of newly developed surveillance programs for early PC detection.

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Full-lenght cDNA and gene annotation

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Although a huge data of draft and finished sequences of the human genome are now available, it is still not a trivial task to identify genes from genome sequences. Thus, a full-length complementary DNA (cDNA), which is a complete DNA copy of mRNA, is required for the identification of a gene and the determination of its structure. We are now participating FLJ cDNA project to collect and sequence full-leng th cDNAA clones under support of
ministry of economy, industry and international trade. In this project, we extensively used cDNA libraries made by Oligo-capping method, a cap targeted selection procedure for full-length cDNAs. So far, we have accumulated more than 800,000 5' end sequences through random sequencing of cDNA clones from about 100 kinds of human cDNA libraries. Among them, 12340 clones have been fully sequenced. The accuracy of the sequences was more than 99.95%. The average length of cDNAs wwhose sequence were determined was about 2200bp, which distributed from 1kb to 5kb. Furthermore we could cluster 5' end of many mRNAs (more than 5000 genes), which could be mapped to onto the human draft genomic sequence. The detail of this project and its results will be presented.

Highly parallel sub-cellular localization and functional analysis of proteins encoded by novel full-length cDNAs

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cloning / localization: 200 open reading frames have been subcloned into expression vectors within the reported period, totaling over 300 ORFs. All ORFs have been cloned into YFP and CFP fusion vectors, the encoded proteins have been localized.Database: the clone/ORF tracking database has been established. This database is currently extended to be accessible through a web-interface. This will allow the submission of queries, but also the up-loading of data by the EMBL group to optimize the time between data generation and database integration.Assay development: Assays for the identification of proteins that are involved in the regulation of the regulatory pathway have been established and are routinely applied to all proteins localizing to the respective compartments (e.g. ER, Golgi, TGN, but also microtubules). Currently, assays to monitor protein involvement in cell proliferation and/or apoptosis are being developed. Automated 96well microscope: the high content screening microscope has been set up. Hard- and Software are operational and used for assays that are carried out in the project.

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Molecular identification of genes and pathways involved in skeletogenesis by EST sequence analysis and microarray expression profiling of human mesenchymal stem cell differentiation

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The differentiation of mesenchymal stem cells into the chondrogenic or osteogenic lineage is an integral part of multistep processes important in pattern formation, ossification, postnatal growth and development of the skeleton. Our project aims at the identification of genes and pathways involved in these complex processes. Candidate genes are expected to be of value for diagnosis and treatment of monogenic and multigenic heritable disorders of the skeleton.
So far, 5´end sequencing of randomly selected clones from an unique human fetal cartilage cDNA library (provided to us exclusively by Dr. B. Lee, BCM, Houston) has lead to the generation of 5.000 ESTs. 4.000 of these sequences have been run through ESTsweep, an EST identification task which we initiated in close collaboration with the HUSAR bioinformatics team (Dr. Hotz-Wagenblatt, Dr. Glatting, DKFZ/Heidelberg). Most of the ESTs (69,6%) show significant similarity to known genes of the human RefSeq collection (NM_#), and another 4,8% correspond to human model RNAs (XM_#). About 23% do not show any similarity to defined mRNAs or proteins, but to genomic clones or anonymous ESTs. A number of these genes of unknown function are currently under detailed functional investigation. Preliminary data revealed putative transcription factors as well as genes probably involved in RNA transcription/processing. Already in the present stage the data provide an interesting insight into the gene expression pattern of the developing skeleton. All EST sequences will be stored in a dedicated database, and will be used to generate cDNA microarrays for the expression profiling of human mesenchymal stem cells (hMSC) which can be differentiated into osteoblasts/osteocytes and chondroblasts/chondrocytes. This enables us to select candidate genes involved in bone/cartilage development for further experimental analysis, and to characterize the pattern of gene expression during stem cell differentiation.

Comparative methylation analysis of CpG islands in human Xq28 and mouse

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Xq28, the distal end of the long arm of the human X-chromosome, is one of the most gene dense regions of the human genome. To date more than 30 human disease genes have been mapped to this region. We use comparative mapping and sequencing of 6 Mb syntenic region between Ids and Dmd of the mouse X chromosome to investigate the organization/evolu-

tion of Xq28 and as a tool for detection of novel regulatory elements.
Methylation of CpG islands in higher eukaryotic insulates sequences from the interaction with DNA binding proteins. Only unmethylated promoter regions are accessible targets for binding and interaction.
We have analyzed more than 600 kb syntenic sequences in mouse and man for the occurrences of CpG islands: In the regions ZFP92 to CDM (375 kb) and FLN to NEMO (240 kb) we detected 28 CpG islands in man, 23 of them were also present in mouse. Most of the CpG islands (19 human and 18 murin) are associated with the 5' end of known genes. Four CpG islands are located within genes in both species. The additional CpG islands (5 human and 1 murin) are not close to any known gene. We try to elucidate, whether the se CpG islands represent elements of alternative promoters or are clues for yet unknown genes.
Therefore we have examined the methylation status of 13 selected CpG islands conserved in both species using the bisufite genome sequencing technology. Two of the analyzed internal CpG islands were unmethylated and represent elements of alternative promoters. Four of the human CpG islands that are not associated with known genes are unmethylated. Here we detected at least two new genes and a new, yet unidentified 5'UTR of the PMCA3 gene.
Furthermore, we present detailed comparison of methylated versus unmethylated CpG islands to improve the prediction of functional CpG islands in man and mouse.

Genomic sequencing of the rat major histocompatibility complex (RT1) region
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The human major histocompatibility complex (MHC) plays a major role in human disease, most of them being of autoimmune or infectious nature, for instance insulin dependent diabetes mellitus (IDDM), rheumathoid arthritis, and multiple sclerosis. Situated on human chromosome 6 and occupying 3.6 Mb of genomic DNA, the MHC is one of the most gene-dense regions described so far in the human genome. Of the 224 identified human genes, an estimated 40% have functions associated with immune response and participate in diverse pathways, e.g. antigen processing, antigen presentation and T-cell interaction. To shed further light onto evolutionary, biological and biomedical aspects of the MHC, we have set out to sequence the rat MHC (RT1 complex) on rat chromosome 20. Towards this end, we have generated a BAC/PAC contig across the RT1 complex region and have established a minimal tiling path that consisted of 39 clones. As of May 2002, HTGS data have been submitted to Genbank for 24 clones (For updates see http://seq.molgen.mpg.de/hgs/chrom_rat.html). The entire region is actively being worked on, by completing draft sequence and by annotation of finished clones. Annotation is carried out using the program Genotator, upgraded by us by implementing additional sequence analysis tools for efficient comparisons between mRNA and genomic sequence and between genomic sequences from different organisms. We will describe the present status of our RT1 sequencing effort and will present a preliminary comparative analysis with the human and the currently available mouse MHC sequence.
Acknowledgement of funding: RT1 sequencing is supported by the German National Genome Research Network (NGFN).

S5

Fetal Determinants of Adult Health
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After organogenesis has been established and is proceeding, a number of important developmental processes occur in the human fetus. Recognition of these processes and their importance for adult health will undoubtedly impact research related to complex genes. Fetal maternal microchimerism, thrombophilias, fetal movement, cytokines and steroids, maternal nutrition and intrauterine growth retardation all interact with each other and have individual long-term affects. Fetal maternal microchimerism plays an important role in autoimmune and connective tissue disorders; fetal movement and use are essential to the development of most organs; cytokines and steroids play a role in the maturation and growth of various fetal structures; maternal nutrition is important to intrauterine growth and may also have transgenerational affects; and intrauterine growth retardation is being recognized as having long term effects producing diabetes, hypertension and heart disease in adulthood.

Phenotypic Spectrum of Noonan Syndrome Patients with Mutations in the PTPN11 Gene
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Noonan syndrome (NS, MIM 163950) is a well-known autosomal dominant disorder characterized by short stature, facial dysmorphism and cardiac defects (i.e. pulmonary stenosis and hypertrophic cardiomyopathy). Recently mutations in the PTPN11 gene have been described by Tartaglia et al. (Nat. Genet. 29: 465-468, 2001). We have screened the PTPN11 gene for mutations in 92 familial and sporadic NS cases as well as 5 patients with cardio-facio-cutaneous syndrome (CFC), that has been suggested to be allelic to NS. Mutations were identified in 32 patients with NS (5 families and 18 unrelated individuals). 86% of patients with mutations had a cardiac defect, with pulmonary stenosis (76%) and/or septal defects (14%) being the most common. Catheter intervention or surgery were necessary in nearly half of them. Mild mental retardation or developmental delay were observed in 42% of patients. 75% of boys had maldescended testes. Apart from the absence of hypertrophic cardiomyopathy, patients with mutations could not be distinguished clinically from patients without mutations. We did not find any mutation in patients with CFC, suggesting genetic heterogeneity.

Clinical Variability of Opitz BBB/G syndrome: a challange for clinicians and biochemists
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Opitz BBB/G is a malformation syndrome of the ventral midline, which is characterized by a large spectrum of symptoms including hypertelorism and hypospadias, cleft lip and palate, laryngotracheoesophageal fistulas, congenital heart defects, developmental delay and imperforate anus. Autosomal dominant (chromosome 22) as well as X-linked recessiv inheritance have been shown in families with Opitz BBB/G syndrome. While a gene has been identified on the X-chromosome (called MID1 gene), the underlying genetic defect on chromosome 22 remains unknown. There is an ongoing discussion among clinicians wheather or not particular symptoms can be defined eather for the X-linked or for the autosomal form of OS. We have now summerized the phenotypes of all patients in which we have identified mutations in the MID1 gene and compared them to patients with the autosomal form. Interestingly, the whole spectrum of OS features can be found in both groups. Moreover, the variability of the phenotypes even within one family is enormous. The MID1 protein function, e.g. the regulation of phosphatase 2A, a central serin / threonin phosphatase, pave the way for speculations regarding the molecular basis of the observed variability.

S6

Predictive Testing for Late-Onset Neurodegenerative Diseases
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The recent identification of numerous gene defects causing neurodegeneration in humans promises widespread genetic predictive testing also for late manifesting disorders. Due to its current limitations (lack of treatment, disease progression, and for some diseases inadequate test sensitivity and specificity), however, genetic testing is not generally recommended. Therefore, it is anticipated that predictive analysis of late manifesting diseases should be accompanied by testing programs. The issue of genetic testing will become even more complex as genetic susceptibility factors for so-called sporadic diseases (e.g. Alzheimer's disease, Parkinson's disease) are being defined in an increasing number. The benefits, limitations, and risks are described. First results of genetic testing programs are being discussed in the context of ethical, legal, and social issues.

Predictive Testing for Breast and Ovarian Cancer
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Rund 5% der Brust- und Eierstockkrebserkrankungen sind erblich bedingt und folgen einem autosomal-dominanten Erbgang. Der erbliche Brust- und Eierstockkrebs tritt familiär gehäuft auf und ist durch ein frühes Erkrankungsalter gekennzeichnet. Mutationen in den Genen BRCA1 und BRCA2 sind für rund 50% der Erkrankungsfälle verantwortlich und können mit molekulargenetischen Methoden identifiziert werden. Das lebenslange Risiko einer Mutationsträgerin beträgt 80-90% für Brustkrebs und 30-60% für Eierstockkrebs. Das Risiko für ein Zweitkarzinom der Brust oder der Eierstöcke liegt bei 50%. Auf Grund des hohen Erkrankungsrisikos der Mutationsträgerinnen ist ein standardisiertes Früherkennungsprogramm (sekundäre Prävention) zu empfehlen, welches die speziellen Charakteristika des erblichen Brust- und Eierstockkrebses berücksichtigt. Für die Verhütung (primäre Prävention) des erblichen Brust- und Eierstockkrebses gibt es operative und medikamentöse Optionen. Die Inanspruchnahme einer Genanalyse sowie primär präventiver Maßnahmen hängen von der individuellen Situation ab und verlangen eine interdisziplinäre Beratung und Betreuung.
Eine erste Evaluation in unserem Zentrum an über 800 Frauen mit einer familiären Belastung ergab eine hohe Akzeptanz für das Früherkennungsprogramm (>85%), während sich nur wenige Frauen für eine prophylaktische Operation entschieden (5%). Die Effizienz der Früherkennung wurde an 429 Frauen, die seit mindestens einem Jahr an dem Früherkennungsprogramm teilnehmen, untersucht. Die prospektive erhobenen Inzidenzraten für das familiäre Mammakarzinom lagen 40fach höher als in der Allgemeinbevölkerung. Die bisherigen Daten belegen, dass die Detektion früher Karzinome durch eine multimodale und engmaschige Früherkennung möglich ist.

S7

Molecular basis of common forms of human idiopathic epilepsies
Armin Heils
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Epilepsy affects about 2-3 % of the world´s population. Idiopathic epilepsies account for 40% of all cases. During the last decade several genes causing monogenic forms of epilepsy have been identified, however, the molecular basis of common subtypes of idiopathic generalized epilepsy (IGE) remains elusive. Common forms of IGE including idiopathic absence epilepsy (IAE), juvenile myoclonic epilepsy (JME), and epilepsy with grand-mal seizures on awakening (EGMA) are characterized by unprovoked seizures in the absence of any detectable brain lesion. Family studies and segregation analyses have clearly shown, that common forms of IGE are inherited as genetically complex traits. Results of a recent genome search for IGE susceptibility loci provided significant evidence for the existence of a novel IGE locus on chromosome 3q26 (Sander et al., 2000). This chromosomal region harbors several potential candidate genes including CLCN2, encoding the voltage-gated chloride channel CIC-2. Since CIC-2 is essential for an inhibitory response towards GABA-ergic transmission in the brain, we followed a positional candidate gene approach searching for mutations and common sequence variants in 46 IGE index cases. We identified three disease-causing mutations as shown by the results of segregation analyses and heterologuos expression of mutant alleles followed by classical patch clamp techniques. Furthermore we identified a common polymorphism which is associated with IGE as shown by the results of a population-based as well as family-based association study comprising two independent samples. Thus, our results highlight CLCN2 as the first gene of which rare mutations can cause the whole spectrum of common IGE subtypes as well as a common sequence variant conferring susceptibility to IGE probably in a significant number of patients.

Genome scan and families with myocardial infarction
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Myocardial infarction is a trait with a strong familial component. Especially, manifestation at an age of 60 years or less points to a genetic background. However, generally speaking, the pathophysiology of myocardial infarction is rather complex and involves several environmentally and genetically determined risk factors. Moreover, haemodynamic, metabolic, inflammatory, coagulatory, and apoptotic mechanisms are activated to a variable extent. With such complexity the prediction of candidate genes that may explain the familial background of myocardial infarction is extremely difficult. In fact, prioritizing of candidates into more or less important genes should be considered to be rather speculative.
We thus preformed random genome screens in pairs of siblings with premature myocardial infarction (n = 1406 individuals) and large families with up to 20 affected members with coronary artery disease. We identified a chromosomal locus (14 q 32) with a significant LOD score for myocardial infarction (Nat. Genet. 2002; 30:210-4). In addition, the family studies suggest that further loci may contribute to the heritability of myocardial infarction. Of note, we also identified chromosomal loci for genetically determined cardiovascular risk factors. However, there was no overlap with the MI locus on chromosome 14. This suggests that the genetic risk of myocardial infarction is partially independent from traditional risk factors. This finding is not surprising given that epidemilogical surveys already demonstrated the independent nature of the familial contribution irrespective of potential covariates. However, without knowledge on potential mechanisms the functional implications of the gene variant related to myocardial infarction in our families will be difficult to predict. We therefore currently engage in further phenotypical characterization of our sample including analysis of coronary angiographies.

S8

Genetics in a Post-Genomics Era: Lessons from Model Systems
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The human genome project has reached its midpoint. Its initial goals were to complete genetic and physical maps of the human genome in anticipation of completing the primary nucleotide sequence. The project was initially aimed at providing the infrastructure necessary for the identification of the genetic basis of common disease. This effort spawned „sister projects“ focused on model systems (mouse, rat, zebrafish, arabidopsis, Drosophila, C. elegans, yeast, multiple bacterial species etc.) as well as stimulating the growth of our technology base. An important consequence of this effort was the application of high throughput process technologies to discovery phase of research, specifically the introduction of automation and informatics into the biology work place. The project has also stimulated a paradigm shift in research as the gathering and presentation of information has become an end itself, resulting in the dissociation of data acquisition from classic hypothesis based research.
The goal of this talk will be to review the utility of available genetic systems for target discovery and target validation. Special emphasis will be placed on invertebrate model systems as they offer the opportunity for systematic genetic screening in the context of well established understanding of organismal biology, the availability of high quality genomic/genetic information and tools as well as advanced technology for germline modification. I will also discuss the translation of fundamental approaches, first pioneered in simpler genetic systems, into tools for genetic dissection of vertebrate models.

SLC12A7 encoding KCl co-transporter KCC4: another gene associated with deafness and renal tubular acidosis
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Hearing depends on a high K+ concentration of the endolymph bathing the apical membranes of sensory hair cells. According to the K+ recycling model K+ that has entered hair cells through apical mechanosensitive channels is transported to the endolymph producing epithelium (stria vascularis) for re-secretion into the scala media. K+ probably exits outer hair cells by basolateral KCNQ4 K+ channels, and is then transported via a gap junction system connecting Deiters' cells, which support outer hair cells, and fibrocytes back to the stria vascularis. We show here that mice lacking the K-Cl co-transporter KCC4 (encoded by SLC12A7) are deaf because their hair cells degenerate rapidly after the beginning of hearing. In the mature organ of Corti, KCC4 is restricted to supporting cells of outer and inner hair cells. Our data suggest that KCC4 is important for K+ recycling by siphoning K+ ions after their exit from outer hair cells into Deiters' cells, where K+ enters the gap junction pathway. Like in some human genetic syndromes, deafness in Kcc4-/- mice is associated with renal tubular acidosis. It probably results from an impairment of Cl- recycling across the basolateral membrane of acid-secreting a-intercalated cells of the distal nephron. Screening of patients suffering of deafness and renal tubular acidosis so far did not reveal any mutations in SLC12A7.

Mouse model for X-linked mental retardation by targeting the Arhgef6 gene
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Mutations in ARHGEF6, the gene encoding a guanine nucleotide exchange factor for Rho GTPases, have been reported to cause X-linked non specific mental retardation. We have characterized the orthologous mouse gene. Arhgef6 is expressed in various mouse tissues suggesting an ubiquitous expression pattern, as found for ARHGEF6. The mouse gene product shows high homology to the human protein with all structural motifs highly conserved. A genomic cosmid clone containing Arhgef6 exons 1 and 2 has been isolated and used to map the gene to the mouse X chromosome. Subsequently, to establish a Arhgef6 knock-out mouse, we replaced exons 1 and 2 by the tau-lacZ marker gene and a neo cassette. The start codon of the lacZ marker was placed exactly over the start codon of the endogeneous Arhgef6 gene. Detection of homologous recombination events in ES cell clones was done by PCR and Southern blotting. Two independent recombinant ES cell clones were used to generate chimeric mice and germ line transmission was achieved. By PCR analysis on tail DNA, F1 females were found to be heterozygous for the Arhgef6 mutation whereas all F1 males carried only the wild-type allele. Heterozygous F1 females were mated to C57bl/6 males. Among the offspring, males and females were almost equally represented. Males of the F2 generation carrying the targeted Arhgef6 gene showed no gross morphological abnormalities. By RT-PCR, we confirmed the absence of exon 1 and 2 in the Arhgef6 transcript indicating that the full length Arhgef6 cDNA is not present in these mice. The Arhgef6 deficient male mice will be tested for the presence of behavioural and cognitive deficits as well as possible structural abnormalities in the brain.

Inducible mice model of Spinocerebellar Ataxia Type 3

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Spinocerebellar Ataxia Type 3 (SCA3) or Machado-Joseph-Disease (MJD) is an autosomal dominantly inherited neurodegenerative disorder caused by the expansion of a CAG stretch in the MJD1 gene encoding a polyglutamine repeat in the respective ataxin-3-protein. In order to study the course of the disease we generated an inducible transgenic mouse model using the „Tet-Off-System“ developed by Dr. Bujard (Heidelberg). This system is based on two constructs: The promoter construct controls the expression of the so called tTA (Tetracycline transactivator) gene product. The binding of this protein to a Tetracycline responsive element (TRE) in the responder construct induces the transcription of the gene of interest. The expression can be blocked by the addition of Tetracycline which allosterically inhibits the tTA protein.
For the ataxin-3-responder mouse lines two different full length constructs containing 15 repeats (control lines) and an expanded repeat with 77 glutamines (disease model) were used. So far three founders for the control line and six founders for the disease model were generated and stable mouse lines established. In order to verify the function of these mouse lines we crossbred the ataxin-3 responder lines with a well-characterized prion protein promoter mouse line (kindly provided by Dr. S. Prusiner). Double transgenic mice of two disease model mouse lines express the ataxin-3 protein with an expanded polyglutamine repeat. Studies are ongoing to characterize these mouse lines and to define their suitability as a model of SCA 3. These models will allow us to turn off or on ataxin-3 expression at different developmental stages and will demonstrate whether or not the disease phenotype will be reversible.

Transgenic rat model of Huntington's disease

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Huntington's disease is a neurodegenerative CAG trinucleotide repeat disorder in humans. We report a rat model transgenic for Huntington's disease, which carries a truncated Huntingtongene fragment including 51 CAG repeats under control of the native rat Huntington promoter. These rats exhibit an adult onset neurological phenotype with reduced anxiety, cognitive impairments, and slowly progressive motor dysfunction as well as typical histopathological alterations in the brain. As in Huntington's disease patients, in vivo imaging demonstrates striatal shrinkage in magnetic resonance images and a reduced brain glucose metabolism in high-resolution fluor-deoxy-glucose positron emission tomography studies. This is the first transgenic rat model of a neurodegenerative disorder that expresses an intermediate number of CAG repeats. This model allows longitudinal in vivo imaging studies and is therefore ideally suited for the evaluation of novel therapeutic approaches in Huntington's disease.

S9

Gene Therapy of Haemophilia

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Hemophilia A and B are X-linked bleeding disorders caused by mutations within the factor VIII and factor IX-gene, respectively. Although both disorders can be excellent treated by substitution of factor VIII and factor IX-concentrate, considerable efforts are undertaken to develop a gene therapy for haemophilia in order to improve patients' life quality and also to reduce high costs of therapy. The principle of gene therapy is the introduction of an intact copy of the factor VIII factor/factor IX gene in somatic cells compensating the defect cell gene. For this purpose vectors systems based on e.g. retroviral, adenoviral and adeno-associated virus are used. Encouraged by the results of many animal experiments comprising preliminarily mice and canine models several clinical phase I studies on haemophilia A and B patients have been initiated of which one preliminarily was reported to be successful.

Molecular therapeutic approaches for muscular dystrophies

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We will give a review on preclinical experimentation that will hopefully result in safe and effective therapy of muscular dystrophies. At present,different approaches such as gene replacement therapy, utrophin upregulation and muscle progenitor cells are investigated. Adenoviral transfer of therapeutic genes such as dystrophin is hampered by low transduction efficiency of adult skeletal muscle. This is largely due to the lack of appropriate virus attachment receptors on the myofiber surface.
Recent studies in transgenic mice revealed that upregulation of CAR (Coxsackie- and Adenovirus Receptor) improves gene transfer efficiency by approximately 10-fold. Conversely, the vector load that needed to be administered to achieve sufficient gene transfer could be lowered significantly. Reduced viral vector loads may help to control virally mediated toxicity and immunogenicity. To date, there are no drugs or methods known to increase CAR expression in skeletal muscle that would be easily applicable in humans. However, alternative strategies such as vector retargeting are currently being investigated that may allow for an increase in binding of adenoviral vectors to skeletal muscle. Therefore, vector retargeting may be achieved by directed genetic alteration of adenoviral capsid proteins.

Genetic modifications of hematopoietic stem cells as treatment strategy for disorders of the blood and immune system

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Ex vivo genetic modification of autologous hematopoietic stem cells (HSCs) and their subsequent transplantation is a promising therapeutic strategy for a variety of inherited hematopoietic and metabolic disorders. Retroviral vectors have been the most widely used vectors for gene transfer because the vector genome integrates into the target cell chromosomes resulting in stable expression of the transgene(s). In the past years, several improvements for the transduction of HSC with type C oncovirus-based vectors were implemented in clinical protocols leading to successful treatment of two types of inherited immunodeficiencies by stem cell gene therapy. In addition, there are other hematopoietic disorders such as Fanconi anemia where in vivo and in vitro survival advantages of corrected cells suggest that these diseases will be amendable to stem cell gene therapy. However, the major problem with currently used recombinant replication-incompetent oncoretroviruses are that efficient transduction with these vectors requires cytokine prestimulation of the target cells for breakdown of the nuclear membrane during cell cycle and extended in vitro manipulation periods with multiple infection cycles. Since these protocols interferes with the

engraftment capacity of HSCs, the initial two successes in long-term genetic correction of the defective lymphoid lineage within the hematopoietic system were only possible due to a strong selective advantage for the corrected progency of few transduced HSCs. An alternative solution to this obstacle is the development of gene transfer systems that efficiently transduce quiescent cells. Lentivirus-based vectors allow efficient transduction of non-dividing cells including hematopoietic stem cells. However, the toxicity associated with the lentiviral protease requires the use of complex inducible systems for production of replication defective vectors in stable packaging cell lines. These difficulties so far prevented the clinical exploration of lentiviral vectors in humans, e severe nature of diseases associated with lentiviruses. Finally, the use of recombinant vectors based on wildtype viruses that are absent in humans and are not accociated with any disease in their natural animal hosts or in accidentially infected humans would add an additional safety level for human somatic gene therapy approaches. These criteria are fulfilled by foamyviruses (FV), a family of complex retroviruses whose members are widely spread among mammalians and are apathogeneic in all hosts. Findings on foamyvirus-based gene transfer into hematopoietic stem cells in surrogate assays for human hematopoietic stem cells will be discussed.

S10

Visualizing Genome-wide Gene Expression: From Highthroughput Tools (GenePaint) to the Internet (GenePaint.org)

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The human and mouse genomes are now sequenced to an extent that had not been expected until the middle of this decade. As a result of this remarkable progress, the need to decipher gene function has become even more pressing. To advance understanding of gene function it is necessary to study gene expression at tissue and cellular levels. We have deveoped a unique and powerful tool (GenePaint) to study and document gene expression on tissue sections. Specifically, we have

- constructed instrumentation for high-throughput, automated in situ hybridization on tissue sections that yield data with cellular resolution,
- assembled an automated high-throughput slide scanning microscope that digitalizes tissue sections and their gene expression patterns,
- developed programs for automated annotation and quantitative analysis of digitized gene expression patterns,
- established a database (GenePaint.org) that makes accessible annotated gene expression patterns.

We will discuss the individual components of GenePaint hardware and chemistry, compare its performance with conventional low-throughput procedures, discuss our techniques for annotating gene expression patterns and discuss the salient aspects of the Genepaint.org database. We shall illustrate the power of the integrated system by describing the results of expression analysis of regulatory genes and of all known mouse orthologues of human chromosome 21 genes. These studies demonstrate that it is now possible, contingent on appropriate funding, to determine the expression pattern of all genes of the mouse genome.

Sox9-deficient mouse models for mild and severe forms of campomelic dysplasia

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Campomelic dysplasia (CD) a semilethal human skeletal malformation syndrome with XY sex reversal, is caused by heterozygous mutations in the SRY-related transcription factor gene SOX9. The SOX9 gene is expressed during chondrogenesis and male gonadogenesis and acts as a key regulator of cartilage differentiation and testis development. SOX9 is also expressed in a variety of other embryonic tissues, suggesting further functions during organogenesis. Indeed, CD patients show malformations in non-skeletal organs like brain, heart and kidneys.

To provide tools for the analysis of Sox9 functions in skeletal development and the formation of other organs, we have generated a conditional Sox9 mutant allele in mice using the Cre/loxP system. Crossing of Sox9 +/flox mice with Cre deleter mice results in Sox9 +/- mice that die shortly after birth and that recapitulate defects observed in CD patients such as respiratory failure, malformed scapulae and bending of long bones.

We have also established two hypomorphic Sox9-neo lines that, in the heterozygous state, show a 30% reduction of wildtype Sox9 transcript resulting from aberrant splicing into the neo cassette in intron 1 of the gene. These mice have a milder phenotype with little or no bowing of the long bones, reminiscent of the acampomlic form of CD; most mice develop kypholordosis as seen in some surviving CD tranlocation patients. In the homozygous state, normal Sox9 transcript is reduced by 70% and the severity of the phenotype is correspondingly increased, with marked dwarfism, drastic shortening of the long bones, and perinatal lethality, resembling the most severe forms of CD.

The Mouse Mutant „Short Digits" (Dsh) as a Model for the Role of Sonic Hedgehog during Limb Development

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Sonic hedgehog (Shh) plays a central role in vertebrate patterning and is a key signal during limb development. The mouse mutant „short digits" (Dsh) is a semi-dominant, lethal in the homozygote mutant with a limb reduction phenotype in the heterozygote. The homozygote is characterized by multiple internal and skeletal malformations and strongly resembles the Shh k.o. mouse. We have mapped Dsh to a 16 centimorgan (cM) region comprising the Shh gene. Northern Blot analysis revealed that Shh expression is absent in the homozygote and reduced in the heterozygote. However, no mutation was detected within the Shh coding or surrounding genomic region. Using a complementation assay we show that Dsh and Shh-/- are allelic. The heterozygous mutant exhibits foreshortened digits caused by missing/fused second phalanges of all digits and missing interphalangeal joints. Using in situ hybridization for Dsh/+ embryos of stages 10.5 to 14.5 we show that Shh expression is reduced in limbs of Dsh/+ mice at stage E11.5 compared to wt. However Ptc, Gli1, 2,and 3 Bmp2, 4, 7 and Fgf8 expression were not altered at this stage. At E12.5 expression of Ihh, Ptc, Gli1, 2, 3 and Noggin and Gdf5 were subsequently altered or reduced, reflecting the alterations in chondrogenesis and joint development. Our data indicate that the underlying defect is expected to be a regulatory mutation leading to disruption of a cis- or trans-enhancing element affecting Shh expression. Dsh/+ provides a model to analyze human Brachydactyly A1.

S11

Genetic testing and insurance - no interest in see through patients

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Contrary to the spirited debate that is currently being conducted about genetic testing in insurance, these tests to date play virtually no role in risk assessment in life and private health insurance. Nevertheless, insurers are taking the population's concerns seriously and have therefore decided to ease the pressure that is being exerted by the general public on legislators in connection with this issue by drawing up a declaration of commitment, which regulates the use of predictive genetic tests.

This extensive declaration of commitment presents an opportunity to discuss the issue at length in society and to include in this discussion the trend and results of future medical developments in the field of genetic engineering.

The declaration of commitment issued by the German Insurance Association and the Association of Private Insurers essentially comprises the following points:

1. Predictive genetic testing shall not be a prerequisite to the conclusion of an insurance policy.
2. Prospective clients shall not be required to submit to the insurer the results of voluntary predictive genetic tests, except when very high sums insured are concerned (in excess of Euro 250,000 in life insurance and annual payments above Euro 30,000 in occupational disability, general disability and long-term care annuity insurance).
3. In addition, special data protection regulations shall apply. Premium rebates based on genetic tests shall not be allowed. Where policies involve high sums insured, only physicians may take the findings of genetic tests into consideration.

The declaration of commitment will be valid for an initial period of five years. All life and private health insurers in Germany have signed the declaration in the meantime.

The medium- and long-term future of molecular medicine cannot be predicted at present, as the extensive findings of the Human Genome Project have yet to be implemented. One thing already appears to be certain, however: it is unlikely that a general genetic test providing clues as to a person's life expectancy will exist.

Frequently, the uneasiness about providing insurers with access to the results of genetic tests that have already been conducted springs from the fear that insurers might also be able to draw conclusions as to a person's identity. Every human being has a distinctive genotype that makes him or her unique. Today, the judicial systems of several countries are already taking advantage of this knowledge (e.g. DNA fingerprinting in the USA), which raises the question whether this information might also be of interest to insurance companies. However, insurers are sure not to use or evaluate this data because it is completely irrelevant to a policy. Insurance companies will not look into the genotype of a new client, let alone that of an existing one, just because they are curious or want to be on the safe side. Not only would this overstrain their capacity but it would also not make any sense. After all, the actual purpose of insurance is to cover unforeseeable and uncertain risks. Nevertheless, when a person applies for insurance, the insurer has to request information on all of the factors relevant to the risk to be insured, including diseases, provided of course that these factors are known to the applicant. This so-called disclosure requirement that is imposed on the applicant is regulated in Art. 16 of the German Insurance Contract Act. If the applicant fails to disclose any information, even in part, for instance by holding back relevant data or because of a legal restriction, this is likely to lead to antiselection, with adverse consequences for the insured community as a whole.
A genetic test that produces conspicuous findings is practically always equated in public discussions with a potential declinature of life and health insurance. There are even fears that a social group will be formed that is no longer deemed insurable by life and health insurers because ist genetic test results are positive. What is often overlooked, however, is that, even now, without the use of genetic testing in risk assessment, not all applicants are deemed insurable. The concern that the currently low declinature rates (less than 2% of new clients) might rise in future as a result of genetic testing is understandable but given the rapid progress made in medicine and in view of developments in the 20th century, the insurance industry is in fact expecting the opposite to occur.

Actual and future discrimination
Christiane Lohkamp
The increasing knowledge in genetics and further discoveries of genetic dispositions for diseases and handicaps open new perspectives of possible discrimination in the fields of reproduction, social context, employers interests, jurisdiction, creation of legal precedence, legislation and insurance matters, not only in refusing life and disability insurance but also in refusing medical treatment. The necessity of awareness by all groups involved like self-support groups, counsellors, and public awareness in general can help to define actual and future menacing discrimination. Self-support organisations play the key-role in detecting and monitoring discrimination and in defending and protecting individuals against it.

GENETIC DIAGNOSIS IN OCCUPATIONAL MEDICINE - DO WE WANT IT?
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Genetic diagnosis is quite usual in modern occupational medicine and it has been many decades before. Phaenotypical realisation of genetic disposition has to be recognized during every examination as well as all that is told about family anamnesis. Biochemical analysis of gen-products is helpful to identify hidden risks that will find their manifestation far in the future, for example deficiency of Alpha-1-Antitrypsine. But: The employee is not yet ill and he will not get ill immediately if he would work for example as a fire fighter. But doing his job with heavy breath-protection will double his risk to get a lung emphysema. We have to give him a knowledge of that and a warning, of course - or not? And what about recent methods of genome analysis? They will give us far more answers to our questions and we will be able to protect people against much more health risks in worklife than today. Do we want that? Do they want that? This will be subject to many and long discussions. Staff manager will ask us if they can employ a certain man without risk of severe illness in 20 years. They know that we could know about that, if we would. Should we, would we answer?

The Association of German Occupational Health Physicians has developed a distinct position to these questions, that is presented here.

S12

Molecular mechanisms of blood vessel growth in health and disease
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Endothelial cells maintain oxygen and nutrient supply, manage waste removal, play a critical role in hemostasis, and control cell trafficking across the vessel wall. Blood vessel growth is essential for normal development, wound healing and plays a crucial role in many diseases. We have developed an in vitro system of endothelial progenitor cells to address the molecular basis of endothelial cell differentiation and activation. We have found a large number of genes expressed in endothelial cells that take part in angiogenesis, thrombosis or inflammation. We have identified Raf/Mek/Erk as a critical signaling knot that connects these genetic pathways and thus regulates the dynamic balance of endothelial functions. We currently focus on a genome wide functional analysis of the identified genes associated with this critical signal transduction pathway, in order to validate in vivo individual components in animal models of myocardial infarction, stroke and tumorigenesis. The aim of this work is to identify a „new generation" of drug targets that can be used to design therapeutic approaches to safeguard and specifically adjust the diverse functions of endothelial cells.

Characterization of possible signal transduction pathways leading to cardiac structural changes in renal failure
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Death from cardiac causes is the most common fatality in uremic patients. In particular, left ventricular hypertrophy (LVH) is frequent. The cardiac alterations develop very early in renal insufficiency. However, the pathomechanisms involved are currently not fully understood. Thus, additional information about the expression level of the genes in the affected tissue could be extremely helpful in identifying the pathogenesis of these lesions. Here, Sprague-Dawley rats were subjected to subtotal nephrectomy (SNX) or sham operation (SHAM), serving as a model system for a gene expression profiling analysis. The animals were followed for 2 and 12 weeks, respectively. Blood pressure was monitored at regular intervals and the experiment was terminated by retrograde perfusion fixation with ice-cold NaCl. Poly(A)+ RNA was isolated from the hearts of SNX and SHAM rats, 33P labeled, and hybridized with RZPD Rat Unigene-1 cDNA nylon arrays containing about 27.000 gene and EST sequences (Bento Soares clone collection, Univ. of Iowa). Phosphorimaging of the radioactive signals and extensive interpretation of the data with array analysis software and database tools revealed substantial changes in gene expression in SNX compared to SHAM animals. Interestingly, some extracellular matrix genes, motor protein genes genes as well as growth and differentiation markers were upregulated in SNX rats. Activation of some of these genes could be involved in expansion of the non-vascular interstitial tissue, reduced capillary supply and increased wall thickness of intramyocardial arteries in uraemic animals. Initiated by the activation of the renin angiotensin system (RAS), at least two pathways seem to be involved in ECM activation, one going directly via G-proteins, second messengers, MAP kinase, proteoglycans, cadherins and catenins (short term signaling), the other via G-proteins, second messengers, motor proteins, actins, focal adhesions, and integrin (long term signaling). Some of the gene expression profiling results, s and integrin, could be confirmed by in situ hybridization and immunohistochemistry. These results are an important pre-requisite for the ongoing work with a newly designed sub-array for the investigation of special cell types, time points, and treatments, respectively, but also for more specialized experiments on candidate genes involved in cardiac structural changes in chronic renal failure.

Improved DNA-Microarrays for SNP Detection and Transcriptional Profiling based on Nanoparticle Monolayers
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We have developed a new type of microarray that can be used for SNP detection based on

APEX or LDR methodology, as well as for transcriptional profiling. The array is based on nanobeads that are fixed to a solid support, e.g. microscopic slides, using the Layer-by-Layer (LbL) method. With this technique, polyelectrolytes are deposited as nanoscopic layers, forming the binding support for the stable immobilization of nanoparticles. The surface of the particles is designed for improved DNA-immobilization. This approach allows for tailor-made surface chemistry and enhances the active surface area by its specific morphology.
Initial experiments showed that signal intensities for detection of SNP´s using the APEX method is significantly improved if compared to conventional polylysin slides. The probe can be fixed on the nanobead-modified surface by spotting the respective oligonucleotides using contact or non-contact spotters or by fixing the DNA separately to the nanobeads and spotting of the functionalized nanobeads, thus enabling a more flexible design of the arrays. These nanobeads are currently also tested to design protein arrays.

Autosomal dominant inherited obesity by mutations in the Melanocortin-4 Receptor Gene
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Introduction: Mutations in the melanocortin-4 rezeptor gene (MC4R) are associated with early onset obesity. 41 muations are known so far, however the effects on receptor function of several mutations remain undetermined. Excluding two polymorphisms, almost all mutations were found in severely obese indexpatients. Family studies revealed rare cases of mutations in lean subjects.Methods: We screended 887 severely obese children for mutations in MC4R by single strand conformation polymorphism analysis. The following phenotypical data were collected: Weight history, eating behavior, body mass index (BMI), waist hip ratio, body-composition by bioelectrical impedance analysis, serum leptine levels.Results: We identified 17 different mutations in 27 heterocygous mutation carriers. Two subjects werde compound heterocygous, no homocygous mutation carriers were detected. In 9 patients we found a nonsense mutation at codon 35 (Y-35-stop), in two cases we discovered a frameshift mutation (4-bp deletion at codon 211).We recontacted the families and investigated 22 pedigrees, including 189 subjects (82 mutation carriers, 107 wildtype subjects). 71 of the 82 mutation carriers had a current BMI exceeding the 90th percentile (39 exceeded the 99th percentile), whereas only 57 of the 107 wildtype subjects exceeded the 90th percentile. 61% of the female haploinsufficiency mutation carriers had a BMI above the 99th percentile. We could not find additional common abnormalities in phenotype in the mutation carrier s apart from obesity.Conclusion: Our family studies demonstrate a major gene effect of MC4-R in early onset obesity.

2. Workshops

W1

Computer-Assisted Recognition of Syndromic Faces
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Syndromes are often defined by a specific facial appearance. Experienced geneticists usually make a diagnosis through immediate pattern recognition. We have investigated how well a computer can do this. In view of the fact that patients with a syndrome look more similar than unrelated individuals do, we have chosen a pattern recognition program that was developed to identify a person by matching his face to faces stored in a database. It is not based on anthropometric measurements, but uses digital photographs of 256x256 pixels which are subjected to a Gabor Wavelet Transformation to create a vector with 40 complex coefficients (jet) for every pixel. For the purpose of this study, each face was automatically labeled with 48 nodes. The jets attached to each node of a face were then compared to the jets of all nodes at the same fiducial points of every face in the data base (bunch graph). Classification was based on a majority decision of all analysed nodes of a face (jet voting). Analysis of 32 innerfacial nodes from 55 frontal view photographs of patients with mucopolysaccharidosis type III (n=6), Cornelia de Lange (n=12), fragile X (n=12), Prader-Willi (n=12), and Williams-Beuren syndrome (n=13) revealed correct syndrome recognition in 42/55 (76%) of the patients. In another four patients (7%), a correct and an incorrect diagnosis scored equally well. Our results indicate that it may be feasible to develop a program which may aid the clinical diagnosis of genetic syndromes and the study of genetic variation of facial patterns.

Genotype-phenotype correlation in Alagille syndrome resulting from JAG1 mutations
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The Alagille syndrome (AGS, MIM 118450) is an autosomal dominant disorder with reduced penetrance and significant variable expression. It is characterized by 5 major symptoms: chronic cholestasis, typical face and variable heart, sceletal and ocular anomalies. Minor symptoms and various other features have been reported as well. So far, no explanation can be given for the variable expression and the occurence of symptoms not considered to belong to the spectrum of AGS. In approx. 70% of classical AGS patients disease-causing mutations within the JAG1 gene are being found. For analysing a genotype-phenotype correlation we identified 51 different JAG1 mutations in our sample of nonrelated patients with a mutation and correlate the mutation spectrum with clinical features. Based on a standardized questionnaire we evaluated the occurence of the 5 major symptoms as well as additional features like kidney disease, vascular anomalies, pancreatic insufficiency, deafness, short stature etc. For the 5 major symptoms we did not observe any correlation between phenotype and genotype, i.e. type (e.g. truncating mutations versus others) or localization of the mutation within the JAG1 gene at 20p12. A novel significant correlation was found, however, for kidney anomalies and cerebrovascular disease. Kidney anomalies have been reported only for patients with truncating mutations (n=24) with 25% of them being affected, e.g. by hypoplastic kidney or even Wilms tumor. On the other hand, about 22% of all patients with missense or splice site mutations (n=18) developed cerebrovascular diseases (e.g. Moyamoya disease), features having not been reported, however, for patients with truncating mutations. Our data provide also evidence that atypical symptoms like deafness or mental retardation tend to be associated with respective „private mutation“ within a given pedigree.

Subtelomeric screening in clinical practice
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Mental retardation is one of the main reasons for parents to present their child in a Department of Clinical Genetics. In up to 10 % submicroscopic subtelomeric chromosome defects have been found as a cause of mental retardation. Recently, fluorescence in situ hybridisation (FISH) has become available to investigate these subtelomeric rearrangements. Because of the technical complexities and cost of screening, a clinical preselection including an indication checklist with a minimal cut-off score of three points was suggested (de Vries et al., 2001).
We present data of 120 FISH assays we performed during the last six months. We found seven positive cases (6%); whose average checklist score was 3.7 (range 2-7). Two cases only had two points. The family history was positive only in one case. Besides mental retardation facial dysmorphies, especially apparent hypertelorism, were always present. Mental retardation ranged from severe (2/7) to mild (5/7). Postnatal growth retardation was more frequent than prenatal growth retardation. Chromosomal abnormalities comprised single deletions (4/7) and complex rearrangements (3/7).
Conclusion: Since the suggested clinical preselection may lead to underdetection of subtelomeric rearrangements, the FISH assay should be performed in patients with mental retardation in combination with facial dysmorphies. A positive result in subtelomeric screening in the early stage of the diagnostic procedure could avoid cost intensive further diagnostics.

Molecular analysis of TP53: Identification of familial and sporadic cases of german origin
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Li-Fraumeni syndrome (LFS) is an autosomal dominant disorder with high occurrence of malignant neoplasms caused by mutations in the TP53 tumor-suppressor gene. Inherited germline TP53 mutations predispose to an increased risk for cancer at an early age and for developing multiple primary cancers such as sarcomas, brain tumors and breast cancer. The 12 exons of TP53 encode a phosphoprotein which is implicated in cell cycle arrest, DNA repair and apoptosis. The majority of mutations are clustered between exon 5 and 8 preferentially affecting

codons 248, 273, and 245. More than 80% of detected alterations are missense mutations, whereas deletions (~ 9%) and nonsense mutations (~ 6%) are less common. De novo germline TP53 mutations are rarely reported in the literature.
We have analyzed three index cases with conspicuous anamnesis of cancer for germline mutations in TP53. In family A, a 12 month old boy was affected with rhabdomyosarcoma, while no other relative developed cancer. In this patient a nonsense mutation in codon 196 was identified, while neither his parents nor his sister carry this change. Paternity was confirmed with several highly polymorphic DNA markers suggesting a de novo mutation in the patient. Alternatively, a germ cell mosaic could be present in one of the parents. In family B, two relatives are affected with brain tumors at the ages of 12 and 34 years and three siblings under 30 years with breast cancer. Testing of BRCA1 and BRCA2 did not reveal disease-associated alterations. Subsequently, a novel germline mutation in TP53 was detected in codon 105 leading to an amino acid change from glycine to cysteine. In family C, although fullfilling the classic criteria of LFS, most family members developed cancer at a relatively old age (ranging from 12 to 70). We found a deletion of eleven base pairs encompassing the splice acceptor sequences in IVS 5. The pathologic consequences of this intronic deletion on the splicing of the gene is currently being examined in vitro. Our findings in the three cases represent the first report of germline TP53 mutations in patients of German origin.

Three patients with minimal expression of the holoprosencephaly spectrum and cytogenetic rearrangements involving the loss of the Sonic Hedgehog gene at 7q36

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Among the heterogeneous causes of holoprosencephaly (HPE), mutations and deletions affecting the Sonic Hedgehog (SHH) gene at 7q36 have been identified. The phenotypic anomalies of HPE are extremely variable, with symptoms ranging from the most severe form, alobar HPE to microsigns such as single maxillary incisor.
We report on clinical, cytogenetic, and molecular cytogenetic studies of three patients with subtle or submicroscopic 7q36 deletions showing only microsigns of the HPE spectrum.
The absence of a single copy of the SHH gene in the first patient with a de novo 7q36.1-qter deletion did not have gross consequences for structural brain development except for microcephaly. Further anomalies of this 37 month old patient included bilateral ptosis and sacral agenesis. The loss of the homeobox gene HLXB9 which is located telomeric to SHH may cause this bony sacral anomaly as a minimal form of Currarino syndrome.
The second patient, a 13 year old female with a 7q36 microdeletion, showed microcephaly, bilateral iris coloboma, absence of maxillary and mandibular incisors, short stature, mental retardation, and normal brain findings. The 7q36 microdeletion in this case was found by FISH analysis and is the unbalanced product of a t(7q;10q)mat.
In the third patient, a 13 year old male, the de novo 7q36 microdeletion was detected by FISH. He had with a single maxillary central incisor, ptosis, microcephaly, partial agenesis of corpus callosum, short stature, and mental retardation. The clinical presentation of our patients expands the spectrum of symptoms seen in the mildest expression of HPE. Our results stress the importance of 7q36 microdeletion studies in patients with even minimal symptoms of HPE. All patients with terminal 7q deletions should be screened for symptoms of Currarino syndrome.

Zimmermann-Laband syndrome associated with a balanced reciprocal translocation t(3;8) (p21.2;q24.3): delineation of the breakpoint regions by FISH

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Zimmermann-Laband syndrome (ZLS) is a rare disorder characterized by coarse facial appearence including bulbous soft nose, thickened lips, thick and floppy ears, gingival hypertrophy, aplasia or dysplasia of hand- and toenails, various skeletal anomalies including hypoplastic changes in the terminal phalanges, hyperextensibility of joints, and, in some cases, hepatosplenomegaly, hypertrichosis, and mental retardation. The genetic basis of ZLS is unknown whereas autosomal dominant inheritance has been suggested. Here we report on an apparently balanced chromosomal aberration, 46,XX, t(3;8) (p13-p21.1;q24.1-q24.3), in an affected mother and daughter. Neither the healthy son nor one of the healthy parents carries the translocation. In order to define the cytogenetic breakpoints more precisely, we performed FISH experiments with BAC clones widely spaced across the entire chromosome bands. By using 10 BACs for chromosome 3p13-p21.1 and 6 BAC clones for the region 8q24.1-q24.3, we successfully refined the breakpoints to 3p21.2 and 8q24.3 and, thereby, narrowed down both breakpoint regions to approximately 1.5 Mb. In conclusion, our data suggest that the ZLS gene maps to one of the above given breakpoints and that the trait follows autosomal dominant inheritance. Further FISH experiments will identify BAC clones overlapping both breakpoint regions. Subsequent database searches should reveal whether a known or a putative gene is disrupted by one or both breakpoints. Finally, mutation analysis of any candidate gene(s) identified in the breakpoint regions in five sporadic ZLS patients with normal karyotype will be performed.

W2

Analysis of mitotic non-disjunction in binucleated cells: evidence for a genetic disposition

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The rate of mitotic non-disjuncton was studied on interphase nuclei of cytochalasin B-blocked binucleated lymphocytes and lymphoblast cells (LCLs). The study included 1. heterozygote NBS1 women who show a significantly reduced rate of spontaneous abortions which might be due to a particularly low rate of meiotic non-disjunction and 2. a woman (A.B.) from a consanguineous Arab family with three trisomy 21 children suggestive for an increased rate of meiotic non-disjunction. No evidence was found for gonadal mosaicism or premature centromere division. FISH analysis was performed with pericentric probes for chromosomes 18, 21, 22 and X, and 1000 binucleated cells per individual were scored. The frequency of non-disjunction for chromosomes 21 and 22 was in the normal range in lymphoblast cells of NBS1 heterozygotes and homozygotes as compared to normal control subjects. However, in the lymphocytes and the lymphoblast cell lines of the woman with the three trisomy 21 children, the frequency of non-disjunction of chromosome 21, but not of 18, 22 or X, was significantly increased compared to all other individuals studied so far. In contrast, it was in the normal range in her mother, grandmother and, surprisingly, also in her three trisomic children who carry the same chromosomes 21. Thus, the high rate of maternal meiotic non-disjunction is paralled by an increase of mitotic non-disjunction, however, confined to chromosome 21. Experiments are in progress to study the basis of this new phenomenon by treating these cells with various agents that affect cell cycle progression and chromosomal non-disjunction and measuring the rate of segregation of chromosomes 21 and 22.

Partial deletion of the common 1,5 Mb critical region in an infant with classical Williams-Beuren syndrome

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Williams-Beuren syndrome (WBS; OMIM 194050) is a contiguous gene deletion disorder with a variable clinical phenotype that is caused in most cases by a heterozygous microdeletion in 7q11.23. Due to two highly homologous flanking ~300 kb duplicons, the microdeletion is usually of similar size in almost all cases and encompasses a common ~1,5 Mb interval that contains at least 17 genes mostly of uncertain pathogenetic relevance. Phenotype-genotype correlation studies for WBS are hampered by the uniform size of the microdeletion. Here we report the case of a 1-year-old boy with a full WBS-phenotype that is caused by a partial deletion of the common ~1.5 Mb interval. Initial analysis with two sets of commercially available FISH-probes (Appligene/Oncor and Vysis) yielded conflicting results. We therefore carried out deletion mapping with microsatellite markers and an array of targeted FISH probes. We mapped the proximal and distal breakpoint of the deletion on two BAC clones. The deletion spans a region between the elastin gene (ELN) and the distal duplicon and therefore excludes STX1A and FZD9 from the WBS minimal critical region of deletion. Fine mapping and sequencing of the breakpoints is in progress. Evidence from two similar cases in the literature also suggests that the centromeric portion of the 1,5 Mb interval is not always deleted in patients with full WBS-phenotype, defining a WBS critical region estimated to be less than 1 Mb. The conclusions from our work concern diagnosis and molecular

etiology of WBS: 1. Quantitative differences in signal intensity after FISH analysis with commercial probes for a WBS microdeletion should be followed up carefully if partial deletions are not to be missed. 2. Even in patients with full WBS-phenotype the underlying molecular defect may be more variable than previously thought.

Characterization of human small marker-chromosomes by centromere-specific multicolor-FISH (cenM-FISH) and high resolution multicolor banding (MCB)

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The origin of marker-chromosomes is nearly impossible to establish by banding cytogenetics alone, while FISH methods are highly suited for that purpose. 24-color-FISH approaches using whole-chromosome-painting-probes can only be used successfully for the determination of the markers chromosomal origin if it is larger than 17p. Thus, smaller supernumerary marker-chromosomes (SMC) found in clinical cytogenetics in 0.01-0.05% often cannot be characterized by those FISH analyses. For rapid characterization of small SMC recently a technique was established which allows a one-step identification of all human centromeric regions, excluding #13/21, by their individual coloring (centromere-specific multicolor-FISH = cenM-FISH [Nietzel et al., 2001, Hum Genet 109:199-204]). To clarify if euchromatic material is present on the SMC the recently developed multicolor banding (MCB) technique has been shown to be suited. The reliability of cenM-FISH for the characterization of small SMC has been proven in 50 prenatal, postnatal or tumorytogenetic cases. Thus, cenM-FISH fills a gap in multicolor karyotyping. As clinical findings associated with SMC may also be related to uniparental disomy (UPD), UPD analyzes have been done in about 10 cases with SMC. UPD has been detected in 2 of the tested cases (partial UPD4 and UPD12). The characterization of small SMC can help to explain clinical symptoms of a patient, or, if there are none, provide important information about genetically inactive regions in the human genome. Moreover, in case of identification and characterization a small SMC by GTG banding and cenM-FISH and the exclusion of partial trisomy by MCB, a test for UPD of the two normal sister chromosomes by molecular genetic methods is reasonable [e.g. Liehr et al., 2001, Clin Genet 60:83-85]. Supported by the Wilhelm Sander-Stiftung (99.105.1) and the EU (ICA2-CT-2000-10012).

An isochore transition in the NF1 gene region coincides precisely with a switch in replication timing.

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The mammalian genome is a mosaic of long stretches of alternate DNA sequence composition. This so-called isochore structure is correlated, on one hand, with the visible banding pattern of chromosomes - G-bands being composed of GC-poor and R-bands of GC-rich isochores - and, on the other hand, with a number of functional features like gene density, recombination frequency and replication timing. Within the human NF1 gene region a 350 kb stretch with an average of 39% GC is immediately followed by a stretch of several 100 kb with 51% GC. Recently, we have shown that this isochore boundary marks exactly the transition from low to high recombination frequency. To study the correlation between sequence composition and replication timing in detail a 300 kb DNA-stretch containing the isochore transition was analysed by performing interphase FISH for a number of cosmid clones from the respective region. The technique applied results in single fluorescent signals, if the target sequence is still unreplicated and in doublets in the case of replicated sequences. The results clearly demonstrate that GC-rich sequences are replicated early during the S-Phase, whereas neighbouring GC-poor sequences are replicated late. The boundary between early and late replicating sequences is sharp and precisely coincides with the boundary between the GC-rich and the GC-poor isochores. Moreover, fiber-FISH experiments with a cosmid contig spanning the isochore boundary revealed, apart from linear structures, a number of Y-shaped structures, directly demonstrating that the replication fork is arrested for a longer time period within the isochore transition region.

Experience with subtelomeric screening in over 150 prospective patients with developmental delay

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Since the group of Jonathan Flint and others have shown that subtle subtelomeric rearrangements may account for more than 7 % of patients with unexplained moderate to severe mental retardation, we started a prospective investigation to evaluate the incidence and significance of subtelomeric aberrations in children with unexplained developmental delay. The study included all consecutive patients referred for developmental delay, in whom after clinical evaluation and karyotyping by GTG banding at app. 500 bands resolution the reason for developmental delay was still unknown. So far we have analysed 139 patients seen personally by the first author in our genetic clinic and 13 patients referred from other centres. Subtelomeric screening was performed with two colour FISH for each chromosome separately. Initially we used a set of BAC/PAC probes described by Knight et al. (AJHG 2000). With this set we found a 2q deletion in 5 of the first 65 patients (7.7%). Further studies showed that all of these were simple polymorphisms. After delineation of the size of the deletion polymorphism against the deletion size in true „2q-" patients we subsequently used BAC RP11-11B2 for the 2q subtelomeric region. In the highly selected 13 cases from other centers we found 3 significant aberrations (23 %), while in our consecutive patients only in 2 % kryptic aberrations were detected. All in all we found 6 pathological aberrations (3,9 %): two deletions, three unbalanced translocations, and one de novo balanced translocation. Further investigation of genotype-phenotype correlations revealed a 3 Mbp 5q deletion adjacent to the Sotos syndrome deletion region and allowed the delineation of a novel, clinically recognizable subtelomeric 5q microdeletion syndrome. Breakpoint mapping of the balanced translocation is in progress.

Assignment of normal diploid metaphases to the leukemic cell population by chromosome banding analysis of immunomagnetically selected cells in acute lymphoblastic leukemia

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In about 40% of the patients with acute lymphoblastic leukemia (ALL) no clonal chromosome aberrations are detectable using chromosome banding analysis (CBA). With molecular cytogenetic methods masqued and submicroscopic aberrations may be identified. However, after chromosome preparation an assignment of normaldiploid cells to the leukemic cell population is not possible anymore. For a well directed use of molecular cytogenetic methods for the detection of cryptic chromosome rearrangements, the information would be usefull, if the normal diploid metaphases do represent the leukemic cell population. Therefore, immunomagnetic cell selection using anti-CD19 and anti-CD7 coated immunomagnetic particels prior to CBA was chosen to try to assign the respective cytogenetic finding to the leukemic cell population. To adjust the separation procedure the purity of the target cells in the positively selected cell fraction was determined in titration experiments with CD7+ and CD19+ cell lines using different beads concentrations and beads: target cell ratios. The proportion of the target cells was evaluated by interphase FISH and chromosome banding analysis. A purity of the separation procedure of up to 100% was achieved. The procedure was tested on 20 bone marrow/and or blood samples including 11 B-cell precursor, 5 pre-T/T-, and 1 mature B-ALL as well as 3 AML. Chromosomes of the positive selected fraction, of the supernatant, and of control cultures were prepared. Metaphase cells were present in 6 CD7+ and in 7 CD19+ selected fractions. In no case metaphase cells were found in CD19+ as well as in CD7+ fractions. Clonal chromosome aberrations were present in all CD19+ fractions. Most excitingly, only normal diploid metaphases were found in 3/6 CD7+ fractions. Thus, the normal diploid cells in these CD7+ fractions most likely represented leukemic cells. In all cases with metaphases detectable in positively selected cell fractions, flow cytometric analysis revealed expression of the respective antigen on the lehromosome analysis after immunomagnetic cell selection (MCIC) may be used for combined immunologic and cytogenetic analyses in ALL. This method allows the aim directed use of additional cytogenetic techniques in the analyses of normal diploid cells with respect to the detection of cryptic rearrangements. In addition, it facilitates the interpretation of normal diploid chromosome findings with respect to their representativeness of the leukemic cell.

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W3

Large Scale Genotyping in Haemophilia A - An update on 1350 patients

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The phenotype of haemophilia A is due to the deficiency or absence of coagulation factor VIII (FVIII) caused by a great number of heterogenous mutations within the large FVIII gene. The gene consists of 26 exons comprising a cDNA of 7.1 kb that encodes a mature FVIII protein of 2332 amino. Our consortium is supported by the German Human Genome Project and aims to determine the genotype in a substantial proportion of the about 6000 German haemophiliacs as a basis for further studies on phenotype/clinical course - genotype correlations.
So far more than 1350 patients from 900 families with severe and non-severe haemophilia A have been analysed. 35.5% of the patients showed an intron 22 inversion, 0.9% an intron 1 inversion, 47.2% a point mutation (38.0% missense, 9.1% nonsense), 10.2% a small insertion/deletion, 3% a large deletion and 2.4% a splice site mutation. Patients in whom no mutation could be initially identified by the screening methods were diagnosed by the GSF (Munich; Uen, Graw et al., for details see separate abstract). The causative mutations could not be identified in about 2% of the patients, even by sequencing the complete cDNA, which may be due to mutations that are located outside the F8 cDNA (either allelic or non-allelic). Remarkably, about one third of all point mutations have not been described before, thus underlining the great variety of the mutations in the F8 gene. Three mutation hot spots could be verified: i) the prevalent intron 22 inversion, ii) CpG sites and iii) two series of adenines in exon 14 that accounted for 25% of all small deletions /insertions. A total of 56% of point mutations resulted from either C>T or G>A nucleotide exchanges. Consequently, arginine that is encoded by CpG containing codons was affected in 37.4% of all point mutations. Missense mutations were highly underrepresented in exon 14 that forms the middle third of the FVIII gene.
In conclusion our high throughput mutation screening approach allowed successful characterisation of the underlying genotype in 900 haemophilic A families representing 1350 patients. This database will represents an important tool for future large scale genotype - phenotype correlation studies in haemophilia A.

Specific binding of HuD to the NF1 3´UTR; analysis of RNA stabilizing effects

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The 8.5 kb coding sequence of the neurofibromatosis type1 (NF1) gene is followed by an unusually long 3´-untranslated region (3.5 kb) which is highly conserved between mouse and human. 3´UTRs have been found 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. Performing electrophoretic mobility shift assays we identified five protein regions (NF1-PBR1-5), two of them (PBR1 and 2) carry AU-rich elements (AREs). On the one hand AREs were shown to be cis-acting elements which target specific mRNAs for rapid degradation. A family of four proteins, the neuronspecific proteins HuD, HuC and Hel-N1 and the ubiquitously expressed HuR were shown to be involved in mRNA destabilisation via binding to AREs. On the other hand the binding of HuD to specific AU-rich sequences in 3´UTRs can also prolong the mRNA lifetime. Using electrophoretic mobility shift assays we were able to demonstrate the specific interaction between HuD (a kind gift from Dr. Furneaux) and NF1-PBR1 but not with PBR5, one of the other RNA fragments with protein-binding capacity, not containing an ARE. To determine the physiological role of the observed PBR1/HuD interaction we performed northern-blot experiments after co-transfection of HeLa cells with HuD cDNA and reportergene-constructs carrying various parts of the NF1 3´UTR.

Mutated human neural cell adhesion molecule L1 affects neurite outgrowth in a cell culture-based model of L1 disease

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Mutations in L1CAM, the gene encoding the multifunctional neuronal adhesion molecule L1, are associated with neurodevelopmental disorders including X-linked hydrocephalus and mental retardation. It is largely unknown how these mutations result in neurodevelopmental disturbances and whether the effects of mutations on neurodevelopment can be modeled in vitro. We stably expressed full-length human wild type L1 and known pathogenic missense mutations I179S, R184W, Y194C, and C264Y in NIH-3T3 cells and used them as substrate for primary murine cerebellar neurons. Two of the four mutations affected posttranslational processing and surface localization of L1, whereas all four mutations showed reduced stimulation of neurite outgrowth. Measurement of neurite outgrowth of neurons grown on transfected substrate cells may be a suitable model for studying neurodevelopmental disturbances associated with various mutations affecting extracellular domains of L1. Supported by DFG, SFB444 C3.

Expression of Proteases in Cell Culture Influenced by Incubation with Fibrillin-1 Fragments

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The Marfan syndrome (MFS) is an autosomal dominant heritable disorder of connective tissue, caused by mutations in the gene for fibrillin-1. Fibrillin-1 is a large (320kDa) multidomain glycoprotein that is a main component of a class of 10 to 12-nm extracellular microfibrils. The microfibrils are thought to be important for elastogenesis, elasticity, and homeostasis of elastic fibres. The pathogenesis of MFS is still unclear, but some evidence has suggested that a progressive loss of microfibrils may be an initiating factor in the development of aortic dilatation and dissection. Identification of the proteases likely to be involved in the pathogenesis of the Marfan syndrome and the factors that initiate their expression in tissues such as the aorta will represent an important step toward the development of new therapeutic strategies for this disorder.
We and others have shown that fibrillin-1 mutations can increase the susceptibility of fibrillin fragments to proteolysis. We speculate that these proteolytic degradation products may possess signaling properties, and are able to alter the expression of genes -by cells such as medial smooth muscle cells of the aorta- involved in turnover of extracellular matrix components, such as matrix metalloproteinases. To test this hypothesis, we have generated a series of recombinant fibrillin fragments. Fibroblast and other cell cultures have been incubated with the fragments, following which protease activity was monitored by zymography and the mRNA expression of selected proteases by quantitative RT-PCR (TaqMan). Initial results have suggested several alterations in activity following incubation. Current results will be presented.

The abnormal spermatozoon head shape (azh) mutation in the mouse is caused by a deletion in the Hook1 gene

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In mice carrying the autosomal recessive mutation „abnormal spermatozoon head shape“ (azh) all spermatozoa display a highly abnormal head morphology that differs drastically from the compact and hook-shaped head of the normal murine sperm. Moreover, the azh mutation causes tail abnormalities often resulting in coiled sperm tails or in the decapitation of the sperm head from the flagellum. We have isolated and characterized the murine Hook1 cDNA and analyzed the corresponding genomic structure. Furthermore, the Hook1 gene was mapped to the same region on chromosome 4, where the azh locus was previously linked. The Hook1 gene is predominantly expressed in haploid male germ cells and immunohistochemical analysis revealed that Hook1 is responsible for the linkage of the microtulular manchette and the flagellum to cellular structures. Here, we report that the azh mutation is due to a deletion of exons 10 and 11 in the murine Hook1 gene leading to a non-functional protein. Our results indicate that loss of Hook1 function results in ectopic positioning of microtubular structures within the spermatid and causes the azh phenotype. Therefore, the human HOOK1 gene could serve as a candidate gene for male infertility due to teratozoospermia or decapitation defects.

The tumor suppressor gene Patched and rhabdomyosarcomagenesis

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Inherited mutations of Patched (PTCH) in the nevoid basal cell carcinoma syndrome (NBCCS) lead to several developmental defects and contribute to tumor formation in a variety of tissues. PTCH mutations have been also identified in sporadic tumors associated with NBCCS including basal cell carcinoma and edulloblastoma. Mice heterozygous for Ptch recapitulate the typical developmental symptoms of NBCCS and develop rhabdomyosarcoma (RMS) and medulloblastoma. These mice are therefore a useful model to study Ptch function in disease and development.

PTCH is widely assumed to be a tumor suppressor gene. Tumor suppressor genes normally exert a negative control on cell growth and the paradigm is that inactivation of both alleles is required for tumor formation.
To determine if deletion of both copies of Ptch is a prerequisite in RMS development in heterozygous Ptch+/- mice, we examined whether the normal Ptch allele was deleted or inactivated by a mutation in these tumors. Furthermore, we tried to elucidate if both alleles contributed to the high Ptch mRNA expression previously found in RMS of these mice. Our data show that the wild-type Ptch allele is retained in tumor tissue. Interestingly, the high levels of Ptch mRNA in these tumors result from overexpression of the mutant Ptch transcript. Our results suggest that the wild-type Ptch allele might be selectively silenced in RMS tissue or, alternatively, that haploinsufficiency of Ptch is sufficient to promote RMS formation in mice.

W4

GENE EXPRESSION FINGERPINTS IN HUMAN TUBULO-INTERSTITIAL INFLAMMATION AND FIBROSIS

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Gene expression profiles of human kidneys will provide insight into the molecular basis of disease, and may also detect novel diagnostic parameters. To identify molecular markers of renal inflammation and scarring, gene expression screening was performed on human kidney samples.
Total RNA was isolated from 9 hydronephrotic and 4 control kidneys (tumor free, unaffected regions of tumor nephrectomies). Random primed radioactively labeled cDNA probes were hybridized to Panorama Human Cytokine Gene Arrays with more than 350 genes representing cytokines, chemokines, their receptors, cell-cell contact proteins and matrix turnover molecules. In parallel, a pathologist unaware of the gene expression data determined the degree of tubulointerstitial inflammation, fibrosis and tubular atrophy assigning a score value to each kidney. Differential gene expression profiles were processed by self organizing maps and clustering analysis. The dendrogram reflecting the relationship of molecular similarity among the samples generated three distinct groups: (I) 5 kidneys with low inflammation and high fibrosis scores, (II) 4 kidneys with high inflammation and low fibrosis score and (III) the control group. Differential regulation of 7 out of 10 cDNAs could be confirmed by real-time RT-PCR.
28 genes yielding stringent separation between inflammation and fibrosis were selected as potential predictive diagnostic markers, incl. i.e. CXCR2, CXCR5, IL-11, HGF, TIMP-2 and CD40. Results indicate the feasibility of gene expression based disease categorization in a defined set of kidney samples. Analysis in a comprehensive collection of tubulo-interstitial samples from the European renal cDNA bank will help to determine the diagnostic utility of this marker set to separate active inflammation from chronic fibrosis.

FUNCTIONAL ANALYSIS OF MAMMALIAN GENES BY A LARGE SCALE GENE TRAP APPROACH IN MOUSE EMBRYONIC STEM CELLS

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We have established a research centre in the framework of the German HUGO project to perform a large scale functional analysis of mammalian genes taking advantage of the gene trap technology. The gene trap technology is based on insertional mutagenesis in ES cells and provides an important tool for the identification and characterisation of the function of mammalian genes in vitro and subsequently in vivo. The mutated genes are identified using RACE-PCR allowing to establish an archive of mutated genes generated in ES cells. At present, we have established 16 000 mutant ES cell clones. For 7332 clones, the site of gene trap vector integration has been determined using RACE-PCR and direct sequencing of these RACE products resulting in 4530 high quality sequences. From these 4530 sequences, 2463 (54 %) show homology to non-redundant genes, 794 (18 %) to expressed sequence tag's (EST's) and 1273 (28 %) show no homology to sequences present in the NCB1-GenBank database. For about 75 of those lines we have established germline transmission and the we are presently studying the mutant phenotypes. Some represent animal models for human disease e.g. nephrodic syndrom. The individual data for each clone, i.e. sequence, expression pattern and eventually mutant phenotype, are stored in a public database which is accessible to the scientific community (http://genetrap.gsf.de). These data will contribute to unravel gene function genome wide.

Identification and characterization of genes from the retinal pigment epithelium (RPE) as candidates for age related macular degeneration (AMD)

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AMD is the leading cause of visual impairment in the elderly and a major cause of blindness in the developed countries. To date, the molecular mechanisms of the disease are not well understood although in recent years a primary involvement of the retinal pigment epithelium (RPE) has become evident.
The aim of our study is to systematically identify and characterize novel genes active in the RPE and to clarify their role in the pathogenesis of AMD. Towards this goal we have generated 1002 ESTs from an in-house constructed RPE suppression subtracted cDNA library. This has identified 188 known human genes, one partial transcript with exon-intron boundary, 52 predicted proteins in the human genome draft sequence, 14 transcripts without exon-intron boundary and 40 EST clusters without significant homology to known sequences. For all 295 EST clusters, reverse Northern bloting was performed to identify abundantly expressed genes in the RPE and to prioritize subsequent analyses. Representative clones were spotted onto a nylon membrane and hybridized with probes from cDNA of driver (heart and liver) and tester (RPE) used in the cDNA library construction. Subsequently, 107 normalized EST clusters were subjected to Northern blot hybridizations. These analyses resulted in the identification of 6 RPE-specific, 3 retina-specific, 8 RPE/retina-specific, and 8 tissue restricted transcripts, while 33 EST clusters were ubiquitously expressed, and evaluation was not possible for another 49 EST clusters. The genes showing specificity or tissue restriction are currently being analysed further. This include the characterization of their full length and the generation of gene-derived single nucleotide polymorphism (SNP) maps to enable gene association studies in AMD patients and controls.

Protein detection and purification using motif-specfic monoclonal antibodies

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Here we present a novel approach to characterize and purify subsets of functionally or structurally relevant proteins, that greatly reduces complexity of cell extracts or other protein samples for further analysis of signal-dependent changes.
First, we have optimized the existing methods for cell fractionation by both physical and biochemical criteria enabling us to primarily focus on nuclear and chromatin-associated proteins. In order to identify proteins of interest we have developed a number of antibodies that bind proteins according to their catalytic domains or structural features. Functional domains of proteins frequently contain a set of well-conserved amino acids accompanied by residues, which are more variable among proteins of the same type. Monoclonal antibodies raised against a specimen of a functional domain may or may not discriminate between these amino-acid substitutions. By selecting for antibody clones of lower specificity a considerable number of proteins containing the domain can be detected in immunoblots of cellular extracts. So far we are able to demonstrate this principle for the ATP-binding domains of Kinases and Helicases and other motifs of lower complexity. The respective monoclonal antibodies we have developed bind several proteins in a T-cell nuclear extract. Some of these proteins are apparently modified or differentially expressed upon T-cell activation, which we use as a model system for signal induction. Our approach could be very helpful to purify novel enzymatic activities and also to identify disease-related proteins by their differential regulation.

W5

Molecular characterisation of two novel genes disrupted in a mentally retarded patient with a 46,X,t(X;8)(p11.2;p22.3) balanced translocation

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Mild mental retardation (MR) is likely to be a multi-factorial condition, whereas severe cognitive disorders are believed to predominantly involve single gene defects. It has been realised for a long time that mental retardation has a higher occurrence in males than in females. The observation of a skewed sex ratio implies an involvement of genes located on the X-chromosome in at least part of the cases with MR.

By combining the arguments of 'single gene' involvement and X-linked MR (XLMR), investigation of mentally retarded patients with balanced X;autosome rearrangements is a promising starting point in the search for genes important in brain function and/or development.

Here we report the study of a balanced 46,X,t(X;8)(p11.2;p22.3) translocation in a mentally retarded girl suffering from epileptic seizures.

Using FISH we recovered a breakpoint spanning BAC clone on the X-chromosome. Southern blotting with a X-specific probe narrowed down the breakpoint region sufficiently to enable chromosome walking from X to 8. On the X-chromosome the translocation disrupts a novel gene with a predicted PDZ and Leu-zipper domain and on chromosome 8 a novel gene with a predicted Fbx domain is disrupted. Genomic organisation of these two genes was established and expression analysis was performed. For both genes splice variants were recovered.

Mutation screening of the X-chromosomal gene in the >300 unrelated patients of the European XLMR consortium is in progress. This screen is of utmost importance as it is a powerful approach to show the involvement of this gene in XLMR.

The novel Rho-GTPase MEGAP has a putative role in severe mental retardation

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In the last few years, several genes involved in X-specific mental retardation have been identified using genetic analysis. Although it is likely, that additional genes responsible for idiopathic mental retardation are also localized on the autosomes, cloning and characterisation of such genes have been elusive so far. Here we report the isolation of a novel gene, MEGAP, which is disrupted and functionally inactivated by a translocation breakpoint in a patient, who shares some characteristic clinical features, such as hypotonia and severe mental retardation, with the 3p- syndrome. By FISH and LOH analysis, we demonstrated that this gene resides on chromosome 3p25 and is deleted in 3p- patients. MEGAP mRNA is predominantly and highly expressed in fetal and adult brain and specifically in the neurons of the hippocampus and cortex, structures known to play a pivotal role in higher cognitive function, learning and memory. We describe several MEGAP transcript isoforms and show that MEGAPa and b represent functional GAP proteins by an in vitro GAP-assay. We propose that haploinsufficiency of MEGAP leads to abnormal development of neuronal structures that are important for normal cognitive function.

Identification of a novel polymorphism that affects an exonic splicing enhancer in exon 3 of the SMN1 gene and causes mild SMA in heterozygous individuals

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Proximal spinal muscular atrophy (SMA) is a neuromuscular disorder caused by homozygous deletions/mutations within the survival motor neuron gene 1 (SMN1). All SMA patients retain at least one of the SMN2 copy gene which produces mainly alternatively spliced transcripts lacking exon 7. This is the result of a silent nucleotide exchange in exon 7 that disrupts an exonic splicing enhancer.

Here we describe an additional silent nucleotide exchange (G to A transition) in exon 3 responsible for alternative splicing of exon 3. Minigenes containing genomic DNA from exon 2 to exon 4 with either the G or the A variant were constructed and the splicing pattern analysed by RT-PCR after transient transfections in HEK293 cells. Although both minigenes show alternative splicing of exon 3, the amount of variant A is ~10x higher. The G-to-A transition lies within a GA-rich exonic splicing enhancer that is Htra2-ß1 dependent as shown by RNA-protein interaction studies.

We identified 5 type III SMA patients who are heterozygous for SMN1 and carry the A variant as the only mutation within the SMN1 coding region. All show abundant alternatively spliced SMN transcripts lacking exon 3 as compared with control individuals with the G variant. Also the SMN protein level is significantly reduced.

Exon 3 encodes the essential Tudor domain of the SMN protein that interacts with the Sm proteins, important components of the splicing machinery. The amount of full-length SMN1 protein is significantly diminished through the A variant in exon 3 and leads even in the presence of one SMN1 copy to a mild SMA phenotype.

The exon 3 polymorphism will be analysed and discussed as an additional SMA-modifier, as significant genetic implications are expected.

Towards a cell culture model of Spinal Muscular Atrophy

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Spinal Muscular Atrophy (SMA) is an autosomal recessive disease caused by loss of functional survival of motor neuron gene (SMN) product. SMA ultimately leads to progressive loss of motor neuron function and muscular atrophy. Although the SMN gene is ubiquitously expressed, the cause for selective motor neuron loss is unknown. Study of the disease has been hampered by the fact that the condition is embryonal lethal for mice, and other currently available transgenic mice models are not viable for long periods of time. Goal of this study is to develop a cell culture system in which the SMN gene expression can be varied using catalytic nucleic acids and double stranded RNA interference. Catalytic nucleic acids are short sequences of RNA (ribozymes) or DNA (DNAzymes) capable of sequence specific cleavage of a target mRNA, thus downregulating gene expression. RNA interference is the phenomenon by which double stranded RNA complementary to a specific mRNA sequence can strongly inhibit gene expression. We designed three ribozymes and three DNAzymes targeted against the murine Smn RNA sequence. All ribozymes and DNAzymes effectively cleaved the full length Smn RNA in a sequence specific manner, while inactive versions of the molecules had no effect. Cleavage of target RNA was observed at magnesium concentrations as low as 2 mM, which corresponds to the intracellular Magnesium concentration of mammalian cells. Cleavage increased in a time and concentration dependent manner. Preliminary RNA interference results show a reduction in SMN expression in NIH 3T3 cells when treated with double stranded RNA constructs. These results indicate that catalytic nucleic acids and double stranded RNA have the potential to effectively cleave Smn target RNA in the cell and could be valuable tools to study SMN function in different cell types.

Establishing reliable diagnosis of PROMM/MD2 - experience with the first 44 cases

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Myotonic dystrophy is a multisystem disorder and the most common form of muscular dystrophy in adults. In contrast to myotonic dystrophy type 1, proximal myotonic myopathy/myotonic dystrophy type 2 (PROMM/DM2) is characterised by a proximal rather than a distal muscle weakness with sparing of the facial muscles, muscle pain, and absence of congenital cases and mental deterioration. The underlying gene defect could recently be identified as an expansion of a (TG)n(TCTG)n(CCTG)n repeat tract in intron 1 of the zinc finger protein 9 (ZNF9) gene localised on chromosome 3q21. The pathologic expansions show a great variation from 75 to more than 11,000 CCTG repeats. Intraindividual variation can be explained by extensive somatic mosaicism and instability. Therefore, expanded alleles can often only be visualised as faint smears that could easily escape detection in Southern blot hybridisation experiments. In our laboratory, we achieve the most unequivocal results by a combination of pulsed field gel electrophoresis and quantitative Southern blot analysis. Additional information can be obtained in the majority of cases by using the linkage disequilibrium reported. Four flanking markers were tested in DNA samples of all our PROMM/DM2 patients (n = 44) and in samples from 100 unaffected controls. For the most informative marker CL3N59, we observed an allele that was not seen on any of the control chromosomes in about 85 % of our patients, confirming a strong linkage disequilibrium. Interestingly, in one of our patients with a severe manifestation no normal fragment could be seen in Southern blot hybridi-

sation experiments. Furthermore, amplification using a repeat specific marker failed repeatedly in this patient. Since he is heterozygous for all four flanking markers a large deletion could be ruled out. Further Investigations are in progress.

Identification and functional characterization of an R621C mutation in the synphilin-1 gene in Parkinson's disease

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The search for interacting proteins of alpha-synuclein, a protein involved in the pathogenesis of Parkinson's disease (PD), yielded in the identification of synphilin-1, which is also a component of Lewy bodies.

In addition synphilin-1 was identified as a substrate for parkin, an ubiquitin ligase, mutated in an autosomal recessive form of PD. Since synphilin-1 forms a direct link between these proteins involved in PD we analyzed the synphilin-1 gene in 416 familial and sporadic PD patients of German origin for mutations. In two patients we identified an amino acid substitution from arginine to cysteine in position 621 (R621C) of the peptide sequence, that was not found in 350 healthy German controls. Because synphilin-1 produces cytoplasmic inclusions in transfected cells, we tested the inclusion-forming capacity of wild type (R621) and mutant (C621) synphilin-1 in dopaminergic SH-SY5Y cells. Cells expressing C621 synphilin-1 displayed a significantly reduced number of aggregates compared with cells expressing wt synphilin-1, when subjected to proteasomal inhibition. In viability assays C621 synphilin-1-transfected cells were more susceptible to staurosporin-induced cell death than cells expressing wt synphilin-1. Thus the C621 mutation in the synphilin-1 gene may be causative for PD due to a deterioration of cell viability possibly mediated by the accumulation - not aggregation- of toxic intermediates.

W6

The tumor suppressor mechanism localized in chromosome band 13q14.3 involves downregulation of genes in B-cell chronic lymphocytic leukemia

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A critical region distal to the retinoblastoma locus is frequently deleted in a variety of tumor entities. In B-cell chronic lymphocytic leukemia, more than 50% of patients have lost genomic material in this critical region. Three candidate tumor suppressor genes are localized in the 400kbp large region: B-cell neoplasia associated gene with multiple splicing (BCMS), BCMS upstream neighbor (BCMSUN) and Ret finger protein 2 (RFP2). Whereas the RFP2 gene has an open reading frame (ORF), the BCMS and BCMSUN genes probably exert their function as non-coding RNAs (ncRNAs). We isolated the very large BCMS gene, which consists of at least 50 exons and spans more than 560kbp. In addition, it is excessively spliced, giving rise to more than 20 different splice variants. While tissue-specific expression of RNA variants was observed, there was no evidence for the expression of a variant specific for B-CLL.

DNA sequence analyses have failed to detect small mutations in one of these genes, suggesting a different pathomechanism, most likely haploinsufficiency. We therefore tested B-CLL samples for epigenetic aberrations by measuring expression of the majority of genes localized between the Retinoblastoma gene (RB1) and the chromosomal marker D13S25 located 3MBp distal to the RB1 gene, including the critical region. With two exceptions, all genes were significantly downregulated in B-CLL-patients, with RFP2 showing the most pronounced loss of expression. To test whether this loss of gene expression is associated with methylation of CpG-islands in the respective promotor-regions, we performed methylation-sensitive quantitative PCR-analyses and bisulfite sequencing on DNA from B-CLL samples. No difference in the methylation patterns could be detected in any CpG-island of the minimally deleted region. Downregulation of genes within chromosomal band 13q14.3 in B-CLL is in line with the concept of haploinsufficiency, but this tumor specific phenomenon is not associated with DNA methylation.

Genomic and Expression Profiling of Pleomorphic Xanthoastrocytomas

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Pleomorphic xanthoastrocytomas (PXAs) are astrocytic neoplasms that mainly affect children and young adults. PXAs usually show a circumscribed growth and favorable prognosis despite exhibiting a high degree of cellular pleomorphism. We report on the first comprehensive analysis of PXAs for aberrations at the chromosome, gene and mRNA levels. Comparative genomic hybridization of 50 PXAs revealed a distinct pattern of chromosomal imbalances. The hallmark alteration detected in 48% of PXAs was loss on chromosome 9. Less common recurrent losses were on chromosome 17 (10%); 8, 18 and 22 (4% each). Recurrent gains were identified on chromosome X (14%); 7, 9q, 20 (8% each); and 19 (4%). Amplifications were found in 2 tumors and mapped to 2p23-p25, 4p15, 12q13, 12q21, 21q21 and 21q22. Molecular genetic analysis of selected candidate genes revealed TP53 mutations in only 3 of 62 (5%) PXAs analyzed. The CDKN2A, CDKN2B and p14ARF genes on 9p21 did not show homozygous deletion, mutation, promoter hypermethylation or complete loss of mRNA expression. None of the tumors showed amplification of the EGFR, CDK4 or MDM2 genes. Expression profiling of 1188 cancer related genes using cDNA array analysis revealed a distinct set of genes that were differentially expressed in PXAs versus diffuse astrocytomas and/or non-neoplastic brain tissue. Taken together, our study indicates that PXA are associated with genetic and transcriptional aberrations that differ from those in diffuse astrocytomas which may account for their more circumscribed growth and better prognosis.

Microarray Technology; Applications in Cancer Research

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The Division of Functional Genome Analysis at the DKFZ is involved in the development of technologies for the analysis of large genomic areas or the entire genome with respect to encoded functions and their regulation. Functional aspects are being studied by means of DNA-, protein- and peptide-microarrays. Beside other applications, analyses on disease-relevant polymorphisms, comparative studies on transcription levels, the actual protein expression and epigenetic variation are performed. Also, systems are being developed toward early diagnosis, prognosis and evaluation of the success of disease treatment.

(http://www.dkfz-heidelberg.de/funct_genome)

Caveolin-1 gene silencing by methylation at four CpG sites in the promoter region

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The CAV-1 promoter contains seven CpG dinucleotides four of which are methylated in two human breast cancer cell lines. Both cell lines do not express CAV-1 mRNA, thereby suggesting CAV-1 gene regulation by CpG methylation. Here we report on CAV-1 expression in both, human prostate cancer and normal prostate cell lines. The methylation status of the promoter was found to vary at the first four CpG sites and may correlate with differing CAV-1 activity in these cell lines. CAV-1 transcription was completely absent from the human prostate cancer cell line LNCaP. In these cells, the distal part of the CAV-1 promoter was methylated at the first CpG site suggesting this CpG to be part of a main regulatory element. Methylation-dependent transcription compatible with the repression of CAV-1 in LNCaP cells was also revealed by reporter gene assays. Demethylation by 5-aza-2'deoxycytidine restores CAV-1 transcription demonstrating the presence of an intact gene copy in this cell line and confirming a possible role of methylation in gene silencing. EMSAs detected a nucleoprotein interacting with the methylated CAV-1 promoter and this protein is different from MeCP2. In conclusion, silencing of the Caveolin-1 gene in LNCaP cells is associated with CpG methylation in the promoter region, suggesting this epigenetic event playing a potential role in prostate cancer progression.

Epigenetic inactivation of the RASSF1A gene in human cancers

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Loss of genetic material from chromosome 3p21.3 is one of the most common and earliest events in the pathogenesis of lung cancer and

many other solid tumors. The chromosomal area 3p21.3 is thought to harbor at least one important tumor suppressor gene, which despite many years of investigation, has remained elusive. In our previous studies, we have identified and cloned a gene from the common homozygous deletion area at 3p21.3. The gene, named RASSF1A (Ras Association domain Family 1A), has homology to a mammalian Ras effector. The RASSF1A gene is epigenetically inactivated in a large percentage of human lung cancers, in particular small cell carcinomas. A high frequency of methylation of RASSF1A is found also in breast cancers, renal cell carcinomas and ovarian cancer. Recently, we have analyzed the epigenetic inactivation of RASSF1A in thyroid carcinomas. In 9 thyroid cancer cell lines and in 71% (27/38) of primary thyroid carcinomas the RASSF1A CpG island was hypermethylated. Methylation frequency was higher in the aggressive forms of thyroid carcinoma. Furthermore, we have investigated the methylation status of RASSF1A in pancreas carcinoma. In 64% (29/45) of pancreas carcinoma RASSF1A was silenced. Interestingly, RASSF1A methylation was detected in 41% (7/18) of pancreatitis samples. Thus, RASSF1A inactivation may play a crucial role in the malignancy of human carcinoma. This work was supported by the BMBF (NBL3 - FKZ 01ZZ0104) and in part by Land Sachsen-Anhalt.

A possibly new hereditary tumor predisposition syndrome characterized by pancreatic cancer and basaloma

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Pancreas cancer (PC) is the fifth leading cause of cancer-related mortality with a very poor prognosis. Its etiology is still largely elusive. The only consistent environmental risk factor is cigarette smoking. A family history of PC provides a 18- and 53-fold risk among first degree relatives of PC patients with two and with three or more affected family members, respectively. It is estimated that 3-5% of all PC cases are caused by a genetic predisposition. In July 1999 the German National Case Collection for Familial PC (FaPaCa) was set-up to collect families with PC (FPC) and with pancreas cancer-melanoma syndrome (PCMS) for the evaluation of the clinical and genetic characteristics of familial PC. The prevalence of PC as well as of other tumors and diseases was studied in families with at least 2 first degree relatives with histologically confirmed PC or with at least two first degree relatives with PC or MM. At total of 32 families has been collected so far. A combination of PC and breast cancer, which may indicate a predisposing BRCA2 germline mutation, was found in 4 (12.5%) families. PC and melanoma, which may point to a CDKN2A germline mutation, was present in 6 (18.8%) families. Most surprisingly, in three families an accumulation of PC and basaloma was identified. The median age at diagnosis of PC in these families was 67 years (range 43-78 years). The median age at diagnosis of basaloma was 63 years (range 56- 74 years). All basaloma patients were first degree relatives of PC patients. Most interestingly, one patient developed basaloma at first and PC 5 years later. No other tumors had occurred in all three families. A familial accumulation of PC and basaloma has not been described as yet. Thus, our three families may represent a new hereditary tumor predisposition syndrome.

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W7

The Leri-Weill and Turner-Syndrome homeobox gene SHOX encodes a cell-type specific transcriptional activator

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Functional impairment of the human homeobox gene SHOX causes short stature and Madelung deformity in Leri-Weill syndrome and has recently been implicated in additional skeletal malformations frequently observed in Turner syndrome. To enhance our understanding of the underlying mechanism of action, we have established a cell culture model consisting of four stably transfected cell lines and analysed the functional properties of the SHOX protein on a molecular level. Results show that the SHOX encoded protein is located exclusively within the nucleus of a variety of cell lines, including U2Os, HEK293, COS7 and NIH3T3 cells. In contrast to this cell-type independent nuclear translocation, the transactivating potential of the SHOX protein on different luciferase reporter constructs was observed only in the osteogenic cell line U2Os. Since C-terminally truncated forms of SHOX lead to Leri-Weill syndrome and idiopathic short stature, we have compared the activity of wild-type and truncated SHOX proteins. Interestingly, C-terminally truncated SHOX proteins are inactive with regards to target gene activation. These results for the first time provide an explanation of SHOX related phenotypes on a molecular level and suggest the existence of qualitative trait loci modulating SHOX activity in a cell-type specific manner.

Report of a novel TRPS1 missense mutation and functional analysis of the two predicted nuclear localization signals of the TRPS1 transcription factor

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The TRPS1 gene on human chromosome 8q24.1 encodes a nuclear zinc-finger transcription factor. It is the only known zinc-finger protein with an unusual combination of nine zinc-finger motifs of four different types (Momeni et al., 2000). One of them is a GATA-type DNA-binding zinc-finger. It is flanked by basic amino acids stretches predicted as nuclear localization signals (NLS1: LRRRRG and NLS2: RRRTRKR).
In our ongoing attempts to augment the spectrum of TRPS1 mutations, we identified two mutations which affect the predicted NLSs in patients with TRPS type I. One is a mutation of the splice-donor of intron 6, IVS6+1G>T, causing an in-frame skipping of exon 6. The resulting truncated TRPS1 protein lacks the entire GATA-type zinc-finger as well as the last four amino acids (RRRG) of the predicted NLS1. Fusion of exons 5 and 7 does not create a novel NLS. The second is a transition G>A at position 2885 in exon 7, found in two unrelated patients. It leads to the single amino acid change R952H. Arginine 952 is the last of the seven amino acids which represent the predicted NLS2.
To analyze the functional consequences of these mutations, we constructed expression plasmids of wild-type and mutant TRPS1-GFP fusion proteins. As expected, the wild-type TRPS1-GFP fusion protein was only detectable in the nucleus. Interestingly, the deltaexon6-TRPS1-GFP construct was still able to enter the nucleus, whereas the R952H-TRPS1-GFP construct was exclusively located in the cytoplasm.
Our data indicate that the predicted NLS1 is not a nuclear localization signal, at all, and that the predicted NLS2 is the only true NLS in TRPS1. Furthermore, it shows that the deltaexon6-TRPS1 behaves like a functional null-allele like alleles with premature truncating mutations. This is in contrast to the TRPS1 proteins with missense mutations in the GATA-type zinc-finger which lead to the more severe TRPS type III phenotype.

Imprinting defects in Prader-Willi syndrome and Angelman syndrome: a molecular study of 133 patients

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The Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are neurogenetic disorders which are caused by the loss of function of imprinted genes in 15q11-q13. The most frequent lesions are a deletion of the entire region or uniparental disomy. In a small group of patients, the disease is due to aberrant imprinting and gene silencing. Incorrect imprints can occur de novo without any mutation in the DNA sequence or as the result of a microdeletion affecting the imprinting centre (IC), which controls the whole imprinted domain. The IC maps to the SNURF-SNRPN locus and appears to consist of two elements. One element is required for the maintenance of the paternal imprint during early embryogenesis. The second element is required for maternal imprinting in the female germline.
Here we describe the molecular analysis in a series of 133 patients with an imprinting defect. Fifteen (12 %) of these patients were found to have an IC deletion. Sequence analysis in 62 AS non-IC deletion patients and 27 PWS non-IC deletion patients did not reveal any point mutation in the critical IC elements.
The presence of a faint methylated band in 25% of AS non-IC deletion patients suggests that these patients are mosaic for an imprinting defect that occured postzygotically. This is in agreement with the finding that in AS patients the imprinting defect occured on the chromosome that was inherited from either the maternal grandfather or grandmother. In contrast, in all informative PWS non-IC deletion patients the imprinting defect always occured on the chromosome inherited from the paternal grandmother. These data suggest that the parental identity of the two homologues is or can be maintained through spermatogenesis.

The IC-SNURF-SNRPN transcript serves as a host for multiple small nucleolar RNA species and as an antisense RNA for UBE3A

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The imprinted domain on human chromosome 15 consists of two oppositely imprinted gene clusters, which are under the coordinated control of an imprinting centre at the 5' end of the SNURF-SNRPN gene. One gene cluster spans the centromeric part of this domain and contains several genes that are transcribed from the paternal chromosome only (MKRN3, MAGEL2, NDN, SNURF-SNRPN, HBII-13, HBII-85, HBII-52, HBII-436, HBII-437, HBII-438A and HBII-438B). Apart from the HBII small nucleolar RNA (snoRNA) genes, each of these genes have a 5' differentially methylated region (DMR), and paternal-only expression appears to be regulated by methylation of the maternal allele. The second gene cluster maps to the telomeric part of the imprinted domain and contains two genes, UBE3A and ATP10C. For ATP10C preferential maternal expression has been demonstrated in fibroblasts, cultured lymphoblasts and brain, whereas maternal-only expression of UBE3A is restricted to brain. As there is no evidence for a DMR at the UBE3A locus, maternal-only expression of UBE3A may be regulated indirectly through a paternally expressed antisense transcript. We report here that a processed antisense transcript of UBE3A starts at the imprinting centre. The paternally expressed SNURF-SNRPN sense/UBE3A antisense transcription unit spans more than 460 kb, contains at least 154 exons and serves as the host for all snoRNAs, which are encoded within introns of this transcript. To find out if and how the antisense transcript might regulate imprinted expression of UBE3A we have compared expression levels of both transcripts in human blood and brain. In blood, where UBE3A is biallelically expressed, we found very low expression of the antisense transcript compared to UBE3A. Interestingly, in brain, where UBE3A expression is imprinted, an equal expression level of both transcripts could be detected. This suggests that upregulation of the antisense expression may lead to silencing of paternal UBE3A expression.

Gene expression changes in brains of MECP2 knockout mice

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Rett syndrome is caused by mutations in MECP2, a gene that encodes methyl-CpG-binding protein 2. MeCP2 has been shown to act as a transcriptional repressor, and the Rett syndrome phenotype is assumed to result from changes in the expression pattern of MECP2 target genes. We have used cDNA microarrays to search for gene expression differences in brains from Mecp2 -/y knockout mice and normal age-matched controls. Several differentially expressed mRNA species were identified in this way, and the expression changes were found to coincide with the onset of symptoms. Possible implications of these findings for the pathogenesis of Rett syndrome will be discussed.

A 1.4 Mb deletion within the 5´ control region of SOX9 causes acampomelic campomelic dysplasia and XY sex reversal

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Campomelic dysplasia (CD; MIM 114290), an autosomal dominant skeletal malformation syndrome with XY sex reversal, is caused by heterozygous mutations in and around the SOX9 gene on 17q. SOX9 has an extended control region as indicated by translocation breakpoints that scatter over 1 Mb 5´ to the gene. We have screened a sample of 13 classical and unclassified campomelic cases with no SOX9 coding region mutation or translocation for deletions in the SOX9 5´-flanking region by comparative genomic hybridization (CGH) on a DNA microarray. Using large-insert clones covering a 2.5 Mb region around SOX9, a deletion was thus detected in an XY female patient with features of CD such as Robin sequence, tracheobronchiomalacia and cervical kyphosis but who lacks bending of the long bones (acampomelic CD). Size and location of the deletion were refined by STS content mapping on DNA from a somatic cell hybrid clone that carries the deleted chromosome 17 in the absence of the normal chromosome 17, placing the deletion breakpoints at 380 +/- 5 kb and at 1830 +/- 10 kb 5´ to SOX9. The deletion does not remove the five evolutionary conserved sequence elements previously identified by us that are up to 290 kb 5´ to SOX9. This indicates that one or more additional cis-regulatory elements must exist within the deletion interval that are essential for correct SOX9 expression during chondrogenesis and gonad development. The milder phenotype and the acampomelia seen in the patient, who is now over 4 years of age, may be attributable to residual SOX9 expression from the deletion chromosome.

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EnsEMBL, a genome information system

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EnsEMBL (www.ensembl.org) is a joint project between the Sanger Institute and EBI funded by the Wellcome Trust to provide a bioinformatic framework for handling genomic information. It currently contains annotation for human, mouse, mosquito and zebrafish. All data is publicly available through a website interface and flat files via an ftp site. EnsEMBL offers a variety of gene prediction methods which utilize different degrees of similarity to existing peptide and dna sequences. One method places known genes using a fast, exact dna/protein matcher (pmatch) to match known human proteins. This is followed by a refining of the genestructure with genewise. Another method uses aligned non human proteins to get novel gene predictions. Alignment supported ab initio predictions (genscan) and est based genes complete the set of the current prediction methods. Sequencing projects like human and mouse go through frequent iterative improvements of the sequence data. Identifiers for genes, transcripts and peptides are maintained throughout these updates where possible. The EnsEMBL website displays genomic data for multiple species. All genomes are presented with the same user interface. Human and mouse displays are thoroughly interlinked, providing easy species comparison information by mouseclick. Third party data can easily be added using the DAS protocol. EnsEMBL is an open source software engineering project and all code and data is made available for download with a non-restrictive license. The development process is open to everyone and involves a number of international collaborations. The whole system is fully portable. The web interface contains advanced visualisation and search tools.

The GenomeMatrix Information Retrieval System

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A number of databases hold information on genomes, genes, gene products and their function. Most of these are either restricted to one organism or to one type of information. Interfaces allow access to information on one gene/gene product at a time. Few databases allow an overview of many genes at a time. The functional analysis of a specific gene requires information on the function of orthologs of the gene in other species. To simplify multi-gene, cross species analyses, GenomeMatrix (www.genome-matrix.org), a new database/interface system, able to integrate a wide range of information resources, was established. Information on genes and the different types of information is displayed as a matrix of colored boxes, where columns represent the different genes, and rows the different information types linked to the genes. Depending on the type of information the color of a box encodes either its presence or by a spectrum of colors within a type gives insight into the underlying information. Diverse types of information are linked to genes, e.g. related diseases, protein interactions, mouse gene traps and biological material available at the RZPD (www.rzpd.de) to facilitate the work of the scientific community. To improve the list of biological materials a gene-specific product portfolio is developed. Whereas some parts of the product portfolio are already available, others are under development. Genome matrices are currently available for man, mouse, worm and fly. Orthology relationships between genes of different organisms are used to combine information on gene function across species.

The human endogenous retrovirus family HERV-K(HML-3) and its genomic impact during primate evolution

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A substantial amount of the human genome comprises human endogenous retroviruses (HERVs). Manifold HERV families have been identified and are represented in Repbase, among them several HERV-K families. While the HERV-K(HML-2) family has been studied in detail by us and others in recent years, other HERV-K families are much less characterized. The availability of the human genome draft se-

quence now provides an excellent source of information to study HERV families at the nucleotide level regarding structure and specific evolution. We here describe the HERV-K(HML-3) family in more detail. We analysed proviral loci regarding structure and variations. We estimate about 140 proviral loci or remains of such per haploid genome. Most loci are severely mutated. Proviruses displaying larger deletions in gag and pol are common. A multiple alignment of 73 HERV-K(HML-3) sequences displays several potentially important differences compared to HERVK9I, the HERV-K(HML-3) corresponding consensus sequence found in Repbase. We generated a consensus sequence from the multiple alignment displaying open reading frames for all retroviral genes. As reported for HERV-K(HML-2) and (HML-6), for instance, dUTPase motifs indicating a formerly active dUTPase enzyme were observed within the protease ORF. Variants of the envelope gene displaying different coding capacities were observed. Phylogenetic analysis shows near-monophyly with distinction of two closely related subgroups. From LTR-LTR comparisons it is concluded that HERV-K(HML-3) homologous proviruses formed about 36 million years ago, immdiately after the split of Old World from New World primates. However, and in contrast to the HERV-K(HML-2) family, for instance, no continuous activity throughout primate evolution is indicated in terms of generation of new proviruses. Our analysis contributes to the understanding of repetitive elements, specifically human endogenous retroviruses, as important factors for the evolution of the host genome.

Approaching virtual organism by bridging of cellular actions

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With the steadily emerging number of interaction data gained by genome-wide methods such as the chip technology, mass spectrometric analysis and two-hybrid technique, interaction databases become more and more important for the classification of the individual components into the network of regulatory systems. Several computational approaches have been presented addressing the problem of signal interaction and signal transduction. The main focus of all these interaction databases is to provide knowledge about interaction profiles on molecular levels. Seizing the idea of system-level thinking we have to take into account that interactions take place within distinct biological compartments and that organism function is managed by the concerted action of a multitude of different cell types. Therefore, to attack the problem of virtual network analysis on a systemic level it is crucial to link the individual interactions to their respective cell systems and to express signal transduction events as cell-specific phenomena. In order to approach automated network analysis on organism level the relational platform PheGe was generated. PheGe is a pilot scheme for virtual biology on a systemic (multi-cellular organism) level. It represents a prototype of knowledge base platforms that organize data-driven interactions in a way that attack the problem of digital recording and simulation of cell-overlapping signal cascades and cellular differentiation programs. By means of directed graph technology one can follow up signaling stepwise by an automated data-driven organization of interaction profiles. As an example, someone can pursue the fate of a pluripotent pancreatic progenitor cell during development, and switching from the insulin-producing ß-cell to insulin signaling will then provide the necessary information to understand the meaning of this cell type for the regulation of energy metabolism on a cellular and systemic level. Moreover, PheGe will guide through the peripheral regulation of energy homeostasis, the central control of food intake and the supply of nutrition to the organism.

A Simplified Computer readable Cytogenetic Notation (SCCN) for the automated analysis of tumor cytogenetic findings

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The aim of the project „Zentrale Zytogenetik“ of the Competence Net „Akute und chronische Leukämien“ is to identify new rare chromosome aberrations, which are relevant for leukemogenesis, and to evaluate them with respect to their clinical significance. Additional aberrations and hot spots of chromosomal rearrangements, gains and losses should be found. Therefore, flexible definitions of aberration keys, which describe distinct chromosomal aberrations and combinations of it, are neccessary to make cytogenetic information available for the correlation with results of therapy studies. The analysis of distinct leukemia entities presenting with identical chromosome aberrations should contribute to the clarification of the relation between genotype and phenotype in leukemias. Type and frequency of therapy-related chromosome abnormalities should be evaluated in different leukemias in order to clarify the mutagenic effects of the drugs in different leukemias and to give clues to the genetic mechanisms of acquired resistance against different cytostatic drugs. A large amount of cytogenetic findings is needed for these studies which will be generated by the compilation of the data from cytogenetic centers involved in therapy trials of leukemias. This includes data (>10000 patients) from chromosome banding analysis and molecular cytogenetic methods. The manual analysis of such a large amounts of cytogenetic data is time consuming. Automated procedures are not available as yet. The ISCN-karyotype of unbalanced aberrations includes more information than written in signs. Therefore, we developed a Simplified Computer readable Cytogenetic Notation (SCCN) to allow an automated analysis of cytogenetic findings. The information of the ISCN karyotype is splitted into the qualitative and quantitative contents. Using predefined criteria the ISCN karyotyp is manually converted into this two types of data strings which are now ready for computer analysis with two special software modules programmed in VBA (Visual Basic for Applications). After successful analysis, results and error prone findings are reported in tables or graphs. Every step of the analysis can be retraced by the operator. In future, the manual conversion of ISCN into SCCN will be replaced by automated procedures.

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Screening for Down’s syndrome - a bioethical point of view -

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Prenatal screening strategies for Down’s syndrome have become an established part of obstetric practice and a frequent subject of genetic counselling services. They are offered with the aim of minimising the number of „affected“ pregnancies that are missed, reducing the number of miscarriages due to amniocentesis or chorionic villus sampling and reducing costs. Today, antenatal care providers have several strategies in addition to screening based on maternal age (e.g. foetal nuchal translucency measurement combined with maternal serum tests) at their disposal.

A comparative analysis of screening strategies regarding their consistency with principles of medical ethics is performed. Implications and consequences relating to the availability and utilisation of screening for Down’s syndrome are elucidated. Implications concern physician-patient interactions as well as interactions between parents and children or between health care professionals and people with Down’s syndrome. It is stressed that routine screening for Down’s syndrome - apart from screening based on maternal age - is particularly conflicting with regard to the principle of respect for autonomy. Besides, it might lead to a remarkable shift in physician-patient interaction in suggesting that selection of children with Down’s syndrome and termination of affected pregnancies is medically justified or authorized.

In conclusion, it is pointed out that several screening strategies for Down’s syndrome are potentially inconsistent with the principles and standards of medical ethics if offered as part of routine antenatal care. They should therefore be embedded in a process of shared decision-making between the health care professional and the patient. Standards of disclosure and informed consent prior to antenatal screening procedures need to be developed to encourage autonomous, competent choices and prevent paternalistic physician-patient interactions.

Genetic counseling following prenatal diagnosis of chromosomal abnormality - a support in deciding a conflict situation?

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The empiric study focuses on the special counseling situation that follows after prenatal diagnosis of chromosomal abnormality and the conflict for those who seek for advice. About 200 case histories were retrospectively surveyed since 1993. The common basis of all cases was chromosomal abnormality and the fact that there was no genetic counseling prior to prenatal diagnosis. It was shown that factors like age of parents, number of common children, stage of pregnancy at time of diagnosis, indication for and method of prenatal diagnosis and especially the results of the chromosome analysis have an enormous influence on the parents´ decision wether or not to continue the pregnancy. Effects of the decision are mostly mourning and feelings of guilt. In consequence of this study we recommand that all parents should be offered a qualified genetic counseling before prenatal diagnosis. During the coping process they should also

be accompanied by an interdisciplinary team (geneticists, obstetricians, psychologists) to come up to medical, psychological, ethical and social aspects of the individual decision.

An unbalanced translocation t(X;15)(q22;q12) in one fetus of a dizygotic twin pregnancy diagnosed after pathologic ultrasound findings: a case report

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An ultrasound scan performed on a 39 year old woman pregnant with twins at 11+6 weeks revealed a pathological nuchal translucency in one of the twins. Therefore, diagnostic amniocentesis was performed at 16+3 weeks of gestation. GTG-banding showed a cytogenetically normal male karyotype in one twin, whereas the twin with the suspicious ultrasound findings showed a female karyotype with additional material on the long arm of one X-chromosome [46,X, add(X)].

Characterization of the origin of th is chromosomal material using M-FISH and centromeric probes revealed a translocation der(X)t(X;15). Multicolor banding (MCB) performed according to Liehr et al. (Int J Mol Med 2002, 4:335-339) allowed the specification of breakpoints: der(X) t(X;15)(q22;q12). Thus, the fetus had beside a partial monosomy Xq (deletion of the region Xq22—>Xqter) a nearly complete trisomy 15q (15q12—>15qter). The parents' karyotypes showed that the aberration occurred de novo. FMR 1 repeat analysis revealed the paternal origin of the X-chromosome involved in the translocation.

Although there were nearly no information about the expected clinical outcome of this specific aberration, the pregnancy was continued. After premature birth at 31+4 weeks because of severe maternal heart insufficiency, the affected twin showed apart from mild growth retardation and developmental delay no facial dysmorphic features and no further external or internal abnormalities. To the best of our knowledge this is the first case of an almost complete non-mosaic translocation trisomy 15. According to t he literature, patients with unbalanced X-autosomal translocations can have attenuated phenotypes because of the complex mechanism of inactivation of autosomal material attached to the X chromosome. This leads to varying effects on the patients' phenotype (Abuelo et al. Am J Med Genet 2000, 94: 392-399).

W10

Pharmacogenetics of Antiemetic Drug Treatment

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Nausea and vomiting still belong to the feared side effects of cancer chemotherapy. 5-Hydroxytryptamine (5-HT3) receptor antagonists like ondansetron or tropisetron play the major role in the current antiemetic treatment of acute emesis. However, about 20-30% of the patients do not respond satisfactorily. These individual differences in drug response may be explained by variation in drug biotransformation by genetically polymorphic enzymes, such as the cytochrome P450 2D6 enzyme (CYP2D6) and by genetic variations in the 5-HT3 receptor gene. Therefore we analyzed the antiemetic outcome in 270 cancer patients. Nausea and vomiting was documented by using standardized interviews before and in two observation periods following the chemotherapy. We genotyped all individuals for CYP2D6 and measured the tropisetron serum concentrations in 42 patients. We sequenced the whole 5-HT3B and 5-HT3A receptor gene of all patients. About 30% of all patients suffered from emesis. Genetically defined poor metabolizers of CYP2D6 had higher serum concentrations of tropisetron than all other patients ($p<0.03$). Ultrarapid metabolizers of CYP2D6 had higher scores of vomiting in both observation periods than all other patients ($p<0.001$, $p<0.03$). Sequencing of the 5-HT3B receptor gene revealed 13 polymorphisms, two amino acid exchanges (Tyr129Ser, Ala223Thr) and two deletion variants. Patients homozygous for the -100AAG deletion variant showed significantly more vomiting in both observation periods than all other patients ($p<0.001$, $p<0.04$). In conclusion, ultrarapid metabolizers of CYP2D6 and individuals homozygous for the -100AAG deletion variant of the 5-HT3B receptor gene had the highest severity of nausea and vomiting. These patients need a different antiemetic approach.

Molecular genetics as an alternative diagnostic tool for maligant hyperthermia

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Malignant hyperthermia (MH) is a pharmacogenetic and potential life-threatening disorder triggered by inhalation anaesthetics and depolarizing muscle relaxants. The disposition to MH is inherited in an autosomal-dominant manner. The objective of the presentation is to give a survey of experiences and results obtained within a period of 15 years of diagnosis of malignant hyperthermia in the MH-centre in Leipzig. The new branch of MH-diagnosis - the molecular genetics - and its general diagnostic potential shall be presented more in detail.

Methods: The halothane/caffeine in vitro contracture test of a skeletal muscle specimen represents the standard method for determining the MH disposition. In 1999, the MH diagnosis in our centre was supplemented by molecular genetic examinations of the skeletal ryanodine receptor gene RYR1 (direct sequencing of the three hot spot regions). This fast calcium releasing receptor (RYR1) located in the membrane of the sarcoplasmic reticulum appears to play the most important role in the pathogenesis of MH. More than 20 RYR1-mutations were found to be associated with the disorder.

Results: A total of 1456 muscle tests (carried out from 1986-2001) in patients having a potential MH disposition provided 376 MHS, 121 MHE and 921 MHN results. In 99 independent MH families the RYR1 was genetically screened identifying a mutation in 46. This indicates a high success rate of the mutation screening (46,6%) as compared to other studies. 18 different RYR1-point mutations were found, out of these four have not yet been published. 38 RYR1 mutation carriers were classified as MH positive without the invasive muscle test.

Conclusions: Under exact and critical evaluation each of its potential, one of the three pillars of MH diagnosis - the clinical phenotype, the IVCT or the DNA analysis - can preferentially be used to obtain certain information. However, these methods should not be regarded as competing with each other but should be complementary used to increase sensitivity and specificity of MH diagnosis. In MH families in which a MH related mutation is detectable the molecular genetics may represent a less invasive and specific diagnostic tool for determining the MH disposition.

Prospects and Limits of Pharmacogenetics: The TPMT Experience

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An increasing number of pharmacogenetic studies is rapidly elucidating the genetic influence on drug safety and efficacy and may help to improve future drug therapy. Thiopurine drug metabolism is a quintessential case of pharmacogenetics and has repeatedly been brought forward as a perfect example for the promises of pharmacogenetic diagnostics. A wealth of experimental and clinical data on polymorphisms in the thiopurine metabolizing enzyme thiopurine methyl transferase (TPMT) has been generated in the past decade. Pharmacogenetic testing prior to thiopurine treatment is already being practiced to some extent in the clinical context, and it is likely that it will be amongst the first pharmacogenetic tests applied on a regular basis.

Here, we analysed the published TPMT data to identify some lessons to be learned for the future implementation of pharmacogenetics for thiopurines as well as in other fields. While there are undoubtedly clear benefits of pharmacogenetic TPMT-testing, we identified some problems and pitfalls which should be taken into account in future studies. These include

- the appreciation of the limits of pharmacogenetics, which may be able to avoid some, but by far not all adverse side effects of drug therapy; clinical studies with thiopurine drugs showed that an average of 78% of side effects in heterozygous patients were not associated with TPMT polymorphisms;
- the need for comprehensive and unbiased data on allele frequencies relevant to all populations worldwide; the vast majority of TPMT studies published so far are restricted to the most frequent alleles from one or two populations, which entails the risk of generating ethnically biased data;
- a careful approach towards dose increases for patients with high enzyme activity, which may increase adverse drug reactions due to an increase of toxic byproducts.
- the necessity to address the dual information problem and thoroughly study possible disease susceptibiliy information provided by a pharmacogenetic test, which may entail serious ethical, social and legal issues;

The TPMT-experience provides some valuable insights into the power and the limits of pharmacogenetics. A careful analysis of this experience

may help to avoid some of the shortcomings and pitfalls in the future.
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Dynamics of DNA Topoisomerases I, IIalpha and beta in Living Human Cells
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Constitutive expression of active human DNA topoisomerases as fusion proteins with green fluorescent protein enabled us to monitor these enzymes in living cells going through mitotic cell cycles. Using photobleachimg techniques we determined enzyme mobilities and resident times at various nuclear locations. Our key finding is that each topoisomerase species constitutes a single diffusible population in interphase and mitosis. The heterogeneous subnuclear and chromosomal distribution is determined by differences in residence times, resulting for instance in upconcentration in nucleolar substructures.
During mitosis, almost the whole complement of topoisomerases I and IIa localizes to chromosomes throughout the cycle, whereas topoisomerase IIb can not be discriminated at chromosomes before anaphase. Such an appearance implies that only topoisomerases I and IIa function in chromosome condensation, segregation and decondensation. Moreover, the entire population of topoisomerase IIa was found to be mobile between individual chromosomes and nuclear substructures. Thus the concept of topoisomerase II participating in the building of a fixed chromosome scaffold or nuclear matrix must be reconsidered.

Subtelomere COBRA FISH: A novel multicolour FISH method for the efficient detection of subtelomeric rearrangements
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Cryptic subtelomeric chromosomal rearrangements represent a significant cause of unexplained mental retardation and are also being described increasingly in neoplasia. FISH using subtelomeric probes is the most widely used technique to detect such rearrangements. Since for a complete investigation of all euchromatic human subtelomeres 41 different FISH probes must be analysed, multicolour techniques have been developed for subtelomeric probes to facilitate a more efficient analysis. COBRA (COmbined Binary RAtio) labelling combines ratio and combinatorial labelling to attain especially high multiplicities [Ref. 1]. With „Subtelomere COBRA FISH“, the specific detection of all FISH probes is possible in only two hybridisations using four spectrally separated fluorochromes. By strict probe selection mostly from the „second generation probe panel“ [Ref. 2] and by using the ULS / Universal Linkage System ® labelling technique, high labelling specificities and hybridisation efficiencies could be reached. This allows the unequivocal analysis of 21 respectively 20 probes per hybridisation with two to four metaphases each. In contrast to other subtelomeric multi-colour techniques, the high multiplicities of Subtelomere COBRA FISH make it possible to differentiate e. g. long arm and short arm subtelomeric regions of a given chromosome thus permitting the diagnosis of cryptic pericentric inversions in addition to translocations and deletions. The technique and its validation on known aberrations as well as on normal controls will be presented. References: [1] Genome Res. (2000) 10:861-865, [2] Am J Hum Genet (2000) 67:320-332

Screening for subtle subtelomeric rearrangements in 75 mentally retarded and dysmorphic children
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Subtle chromosomal rearrangements have been found to be an important cause of human genetic diseases. Especially among moderate to severe mentally retarded children without any explanation for their handicap a frequency of 7.4% chromosomal rearrangements involving the ends of chromosomes has been described (1). In this study we analysed 75 children with mental retardation and dysmorphic features with the Chromoprobe Multiprobe-T system (Cytocell). It is a FISH based approach, which has a predefined hybridisation area for the p and q telomeric probe of every single chromosome from 1 to 22, and X and Y. We found 4 subtelomeric rearrangements according 5.3%. Case 1 was the unbalanced offspring of a mother carrying a cryptic balanced translocation 4p;20q. Case 2 was a subtle de novo deletion 8p. Case 3 was a partial duplication 7p and a partial deletion 7q resulting from a very large pericentric inversion 7 of the father. Case 4 was a de novo unbalanced translocation 1;22 leading to partial trisomy 22q and partial monosomy 1p. None of the chromosomal aberrations could be seen by high resolution banding. Only two of the cases (cases 1 and 4) could have been detected also by other molecular cytogenetic techniques like SKY or MFISH. In case 1 only the derivative chromosome 4 was recognisable with painting probes while the mother's derivative chromosome 20 appeared inconspicuously. While handling and hybridisation efficiency of the FISH-chip proved to be sufficient for routine diagnostics there is one major pitfall: The probe for the subtelomeric region of 2q (marker D2S2986) does not hybridise with a polymorphism known to occur in about 5% of the population (2). In 3 patients a „deletion“ was indicated that had to be ruled out by a second hybridisation with another probe for 2q (VYSIS TelVysion Probe; D2S447). In all three cases the polymorphism could be shown to be familiar and without clinical relevance. By PCR analysis of D2S2986 it was furthermore excluded that the polymorphism is based on a microdelet Ref.
1. Subtle chromosomal rearrangements in children with unexplained mental retardation. Knight et al. Lancet. 1999; 354: 1676-81.
2. he promise and pitfalls of telomere region-specific probes. Ballif et al. Am. J. Hum. Genet. 2000; 67:1356-1359.

A new subcentromeric probe set for the characterization of centromere-near rearrangements
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A variety of FISH approaches have been developed in the last decade, covering the entire human genome in multiple ways. Nonetheless, there is still a chromosomal region which is not well investigated with the presently available probe sets: the pericentric region. This region is of special interest when small supernumerary marker chromosomes (sSMC) shall be characterized. Neither whole nor partial chromosome painting (wcp and pcp) probes are informative if centromere-near euchromatic material is present on a sSMC. This question can be answered best when hybridizing centromere-near probes like BACs. At present, we are working on 24 probe sets, each for one human chromosome, which consists of a centromere specific satellite probe, one centromere-near BAC in q and p (excluding the acrocentric chromosomes) and arm-specific pcp probes. Finished probe sets are used after characterizing the sSMC by cenM-FISH (Nietzel et al., 2001, Hum Genet, 108, 199-204). Up to now two cases with cat eye syndrome (CES) chromosomes [inv dup(22)(q11.2)] and four sSMC derived from #2, #16, #19 and #22, respectively, have been characterized by centromere-near BAC probes. As expected, centromere-near euchromatic material was present in the CES-chromosomes. For the derivative #2 q-arm specific centromere-near material could be detected, while the derivatives #16 and #22 had no detectable euchromatin. Additionally, a clinical case with a normal karyotype apart from the very small pericentric inversion could be detected using the chromosome 2 specific pericentromere probe set. Recently, a case with a centromeric duplication including the centromere-near region of 5p could be identified, as well. In summary, we present a probe set useful to characterize centromere-near rearrangements. Supported by Herbert Quandt Stiftung der VARTA AG and the EU (QLRT-1999-31590).

The establishment of high-resolution phenotype-genotype maps
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In the „post-genomic“ era one of the most important challenges is to link genomic to functional data. Patients with chromosomal aberrations can facilitate this task if the chromosomal defect is corroborated with the corresponding clinical data. However, in order to establish high-resolution phenotype-genotype maps G-banding may be too imprecise. The use of publicly accessible BAC-clone resources should allow the fine-mapping of chromosomal aberrations to the kb-resolution range. As a consequence, strategies are needed to utilize both appropriate technologies and probe sets to work up cases in an efficient way. We propose a hierarchical approach. In a first step a screening method (e.g. M-FISH) is used to identify the involved chromosomes. Subsequent steps are aimed to fine map breakpoints. (e.g. application of BAC-clones, mi-

crodissection of chromosomes, etc.). To exemplify our point we demonstrate two examples. The first is a familial terminal deletion on the long arm on one chromosome 6 which segregated through at least two generations in several family members. Contrary to the common believe that subtelomeric imbalances result usually in severe malformations affected family members had no dysmorphic features and were only mildly mentally retarded. The BAC tools allowed to map the deletion to a 2.8Mb region which harbors 15 genes. The second example exemplifies that chromosomal aberrations may be much more complex than initially thought. The initial diagnosis of an inv ins(5;7)(p13;qterq31) turned out after detailed characterization with a YAC/BAC-clone cocktail to involve not 3 but at least 6 breakpoints including a deletion in the Mb-range. The deletion would had been completely missed by standard cytogenetic methods. These fine mapping efforts will provide if done on a large scale a wealth of information and should improve our understanding of genotype/phenotype relationships.

Chromosomes are chromosomes throughout the whole cell cycle

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Interphase chromosomes analysed with currently available techniques do not present any recognizable structures such as bands, centromeres, telomeres, or specific shapes. Microirradiation experiments and molecular cytogenetic investigations with whole chromosome paints and region specific microdissection probes have confirmed a territorial organization of chromosomes in interphase nuclei. Until now, however, their structure is not well understood. Using laser scanning microscopic examination and the high-resolution DNA-based multicolour banding (MCB) technique, we have generated a banding pattern and have determined the length of human chromosome 5 in lymphocyte interphase nuclei, and in nuclei of HeLa cells arrested at different phases of the cell cycle. The shape and MCB pattern of chromosome 5 in interphase nuclei is similar to that of metaphase chromosome 5 at all stages of the cell cycle. The length of the chromosome axis is comparable to that of a metaphase chromosome at a 600-band resolution. Therefore, the concept of chromosome condensation during mitosis has to be reassessed. Interphase chromosome banding can be used to identify chromosome aberrations and opens new fields in cytogenetic analysis.

W12

Analyzing Human Cell Division by Chemical Genomics

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The field of chemical genomics proposes to use small molecule inhibitors to study gene and protein function. Chemical compounds that interfere with a biological process can be obtained in high-throughput screens. These compounds can then be used to identify the enzymes required for the biological process of interest, much like a mutant in classic forward genetics. We have used chemical genomics to study chromosome segregation during mitosis in human cells. I will use this project as an example to illustrate the technical challenges and the potential of the chemical genomics approach.

W13

Molecular genetic findings in obesity

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Obesity in adulthood is defined via a body mass index (BMI; kg/m²) ³ 30 kg/m². Approximately 50% and 20% of the German population is overweight (BMI ³ 25 kg/m²) and obese, respectively. Obesity is associated with an increased risk for development of type 2 diabetes mellitus, cardiovascular disorders, specific types of cancer and other disorders. Both environmental and genetic factors predispose to overweight. Heritability estimates for the quantitative phenotype BMI typically range from 50-80%; non-shared environment is considerably more important than shared environment. Genetic research into human obesity has greatly profitted from the similarity of regulatory pathways in mammals. Both spontaneous mutations in mice and transgenic models have led to the identification of single monogenic forms of human obesity, which are all inherited autosomal recessively and associated with diverse endocrinological abnormalities. Mutations in the melanocortin-4 receptor gene have been detected in 2-4% of obese probands in different European populations. Current evidence suggests that these mutations underly an autosomal dominant form of obesity, which is not associated with other symptoms; these same mutations have only rarely been detected in non-obese individuals. However, because non-obese mutation carriers have been identified among family members of index patients the associated relative risk for development of obesity are is unclear. Several genome scans have been performed in different populations.
Chromosomal regions of interest include 1p, 3q, 4q, 10p, 11q and 20q. Fine-mapping of these regions and identification of candidate genes is ongoing.
Our molecular genetic studies are being supported by the Bundesministerium für Bildung und Forschung (DHGP and NGFN).

A GENOME SCAN IN FAMILIES WITH BIPOLAR AFFECTIVE DISORDER

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Bipolar affective disorder (BPAD) is a severe psychiatric disorder that has a lifetime prevalence of about 1% in all human populations. In order to identify chromosomal regions containing BPAD-related genes we have conducted a complete genome screen at an average marker spacing of 9.3 cM in a sample of 75 BPAD families. Pedigrees were of German, Israeli, and Italian origin, respectively. The highest two-point LOD score was obtained on 8q24 (D8S514; LOD score = 3.62), in a region that has not attracted much attention in previous linkage studies of BPAD. The second best finding was seen on 10q25-q26 (D10S217; LOD score = 2.86) and has been reported in independent studies of BPAD. Other regions showing „suggestive" evidence for linkage localized to 1p33-p36, 2q21-q33, 3p14, 3q26-q27, 6q21-q22, 8p21, 13q11, and 14q12-q13. In addition, we aimed at detecting possible susceptibility loci underlying genomic imprinting by analyzing the autosomal genotype data with the recently developed extension of the GENEHUNTER program, GENEHUNTER-IMPRINTING. Putative paternally imprinted loci were identified in chromosomal regions 2p24-p21 and 2q31-q32. Maternally imprinted susceptibility genes may be located on 14q32 and 16q21-q23.

The QTc interval duration is correlated to KCNQ1 polymorphisms in a population based MONICA survey

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Prolongation of the QTc interval is known to be a risk factor for ventricular arrhythmias. The influence of polymorphisms in genes encoding for ion channels on QTc duration in the regular population without structural heart disease or inherited long QT syndrome is not known.
Digitized ECG's of 1516 probands from the MONICA-Augsburg survey were used to calculate QTc interval duration. Furthermore, the results were correlated with echocardiographic and electrocardiographic parameters as well as with age, gender, and medication. The coding region of the KCNQ1 gene was analyzed by single strand conformation polymorphism (SSCP) in all probands with a QTc above (group 2, n=44) and below (group 1, n=38) the second standard deviation as well as in a sample of probands with a median QTc interval (group 3, n=55).
The table summarizes the significant differences between the three groups, while ejection fraction, left ventricular diameters and blood pressure were not different.

	1	2	3	p
n	38	44	55	
Age	42+14	60+13	54+12	0.01
Heart rate	57+8	76+18	67+9	0.01
QT interval	367+25	397+43	377+26	0.01
QTc interval	345+9	444+17	397+1	0.001

Next to 9 known polymorphisms we identified four new intronic polymorphisms in KCNQ1 (IVS 10 +46A->T; IVS 11 +14T->C; IVS 12 +36G->A; IVS 14 +32G->T). Three different haplotypes, differing in polymorphisms in exon 10 (+46A->T)

and exon 13 (+43T->C), were identified. The resulting haplotype frequency results in a significant correlation with QTc and heart rate (0.58 and 0.57, p<0.01).
The QTc interval duration as well as resting heart rate are modified by polymorphisms in the KCNQ1 gene in the general population. These results provide evidence for a genetic influenced QTc duration independent of gender and age.

Common haplotypes in five genes influence genetic variance of LDL and HDL cholesterol in the general population

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We studied the association between common haplotypes in six relevant lipid metabolism genes with plasma lipid levels. We selected SNPs in the cholesteryl ester transfer protein (CETP), lipoprotein lipase(LPL), hepatic triglyceride lipase (HL), low density lipoprotein cholesterol receptor (LDLR), apolipoprotein E (ApoE), and lecithin:cholesterol acyltransferase (LCAT) genes and studied 732 individuals from 184 German families. Total cholesterol (TC), low-density lipoprotein cholesterol (LDL), and high-density lipoprotein cholesterol (HDL) were similar to those reported in other European and American populations. Haplotypes derived from SNP combinations resulted in more significance and of a higher degree than did single SNPs in the genotype-phenotype association analysis. Reduction of the polygenic variance attributable to haplotypes was estimated using variance components analysis. Under the biometrical genetic model, allelic association of haplotypes was highly significant for HDL, LDL, and the LDL/HDL ratio. The residual kinship correlation was reduced accordingly. The ApoE gene had a strong effect on trait variation; however, the other genes also contributed substantially. An epistatic interaction could not be demonstrated in this sample. The data are consistent with the notion that common genetic variants influence common traits.

W14

ICSI and congenital malformations: infertility- or technique-related risks?

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The course of pregnancies and health of children born after assisted reproductive technologies are two of the most important outcome parameters of the quality of the techniques. There is an ongoing discussion, whether these parameters may show worse results as compared to spontaneous conception. Recent studies have shown increased risks for the pregnancy course following conventional IVF (e.g. premature birth, low birthweight), and a higher rate of major malformations after conventional IVF as well as after ICSI. Of course, especially in the situation of ICSI there have to be taken several genetic risks of the father into account. However, even if there are studies - especially in animal models - which might rise the theory, that ICSI itself is responsible for these problems, other studies may support the idea, that these risks are not related to the techniques used, but to parental background factors. Data from surrogate motherhood also demonstrate, that here the risks are lower as compared to pregnancies from IVF mothers, who carry there own child to birth. Therefore, there are more infertility related problems than technique related ones. Finally, however, a risk related to the techniques itself cannot be excluded totally by the actual available data.

Morphological anomalies in spermatozoa reflect chromosomal aneuploidies

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Recent studies have shown that reproductive difficulties can be associated with cytogenetic abnormalities like chromosomal aneuploidies. Therefore intracytoplasmic sperm injection (ICSI) as an treatment for severe male infertility may lead to an increased incidence of chromosomal abnormalities in the resulting offspring. So far, Fluorescence in situ hybridisation (FISH) studies with probes specific for chromosomes 1, 7, 18, X and Y were performed to analyse the aneuploidy rate in morphologic al aberrant spermatozoa of infertile men. Other studies focused on the correlation between sperm motility and nondisjunction. The aim of our study was the comparison of aneuploidy rates in spermatozoa of infertile men prior and after selection according to morphology and motility, used in practise for ICSI. Specific probes for chromosomes 13, 21 and 16 served for FISH as disomy 13 and 21 could lead to trisomic severe effected live-births and trisomy 16 ist one of the frequently found cytogenetic aneuploidie in abortions. Four infertile patients were included in this preliminary study. The inclusion criteria were: oligoasthenoteratozoospermia with sperm concentration lower than 10x106/ml, sperm motility <30% and normal sperm morphology <10%. Sperm selection was performed under 400xmagnification, using Hoffman modulation contrast. FISH procedure was made by standard protocol, using probes for chromosome 13 and 21 (locus specific probes) and the probe for chromsome 16 (centromeric satellite probe). For each patient, we analysed 100-150 spermatozoa prior and after selection, respectively. The significanty lower aneuploidy frequencies were observed in the selected group in comparison to native sperm population (3-4% vs. 4-31%). Incidence of disomy in native sperm ranged from 2% to 7%, 2% to 10% and 1% to 4% for chromsome 13,16 and 21, respectively. In the selected group, the rates of disomy ranged from 0,7% to2%, 0% to 0,7% and 0,7% to 1% for chromosome 13, 16 and 21, respectively. The frequency of diploidy was 1-5% in ected in the selected group of sperm. Our results support the hypotesis of positive correlation between abnormal sperm morphology and motility and their cytogenetic anomalies. Spermselection prior ICSI using 400 x magnification significantly reduces the incidence of aneuploidy in gametes of infertile men. However, this method cannot exclude aneuploid offspring of paternal origin.

Establishing an aneuploidy screening for single cells using CGH

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Several methods are currently used for the characterization of aneuploidy in single cells. FISH and multiplex PCR with polymorphic markers enable the analysis of small cell or DNA amounts, however only a limited number of chromosomes can be simultaneously analyzed. In contrast, comparative genome hybridization (CGH) is a powerful method to detect gain or loss of chromosomal regions or entire chromosomes ideally covering the whole set of chromosomes. Limitations of the current CGH protocols include the necessary amount of probe DNA as well as the time consuming hybridization steps. Here we report an optimized CGH protocol based on whole genome amplification enabling us to analyze small cell amounts of up to one cell for aneuploidy. Initially, single mononuclear blood cells were isolated by FACS, lysed and subsequently whole genome amplification was performed by ligation mediated PCR. After biotin- and digoxigenin-labeling of the resulting PCR products of probes and controls respectively, CGH was carried out on metaphase spreads of male control individuals. Following hybridization for up to 3 days, washing and immunodetection slides have been evaluated using the Metasystems ISIS/CGH module. Results of the analysis of cells with different gonosomal constitutions will be presented, demonstrating the potential of this assay to detect aneuploidies in single cells. In addition to mononuclear blood cells this optimized single cell CGH has been successfully applied to first polar bodies from unfertilized oocytes. Potential applications might include the evaluation of the frequency of aneuploidies in polar bodies of women with an increased risk for unbalanced chromosomal aberrations in their offspring as well as - after further optimization of the protocol - as an alternative procedure to detect aneuploidies prior to fertilization of the oocyte during assisted reproduction.

Intracytoplasmic sperm injection (ICSI) may increase the risk for imprinting defects

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In germ cells and the early embryo, the mammalian genome undergoes widespread epigenetic reprogramming. Animal studies suggest that this process is vulnerable to external factors. Abnormal DNA methylation and gene expression were found in cloned animals. In addition, cloned sheep preembryos showed hypomethylation of the imprinting control element of the maternal Igf2r allele, thus demonstrating that maternal imprinting in the preembryo is vulnerable to external factors.

We report two children who were conceived by intracytoplasmic sperm injection (ICSI) and found to have a chromosome 15 imprinting defect (ID) causing Angelman syndrome (AS). Mutations of the imprinting centre were excluded.
Observing 2 IDs in ICSI children by chance appears unlikely. One would expect 2 ICSI AS ID individuals in a sample of 40 ICSI AS patients. However, we do not know of any AS ICSI patient with a 15q11-q13 deletion, a uniparental disomy or a UBE3A mutation.
The ID occurred on the maternal allele, thus providing two explanations: (i) The failure occurred in the oocyte (imprint switch failure), i.e. the maternal chromosome carried a paternal imprint that is maintained in the zygote. (ii) The failure occurred in the zygote, i.e. the oocyte carried a normal maternal imprint, that was not maintained in the zygote.
In summary there are some indications that ICSI might interfere with the establishment of the maternal imprint in the oocyte or preembryo and increase the risk for imprinting defects. We suggest to address this question in a long-term follow-up study of a large cohort of children. One may also consider to perform a prenatal methylation test in all foetuses conceived by ICSI.

W15

Privacy Protection and Human Genome Testing
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Personal data, gained by genome testings, are different: They reveal possible or probable future incidents more than actual facts. Nevertheless they can determine life – not only the life of the tested person, but also that of untested relatives. This has consequences for the right to ignorance as well as to the requirement of technical data protection.
Predictive genome testings for medical purposes need a medical indication, profound informations about how and how long the institution will deal with the sample and with the data, and an explicit consent.
Genome testings for research purposes raise different questions: Is it allowed to use samples obtained for medical reasons for basic genetic research without the consent of the person affected? What does anonymity really mean in regard to genome data? Which procedure is safe enough to prevent the deciphering of a pseudonym?
In October 2001 the conference of the data protection commissioners in Germany again demanded clear binding rules in order to protect privacy and self-determination in human genome testing. It published a detailed proposal of legal regulations, which now is discussed within the political parties and the responsible administration.

Genetic discrimination - a challenge for medical education of general public and lawgivers action
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Genetic tests are becoming a routine tool for medical diagnosis. Information revealed allows individuals and their families to understand and sometimes to control their inherited health risks. Genetic testing to be judged a worthwhile enterprise has to provide results, which from the recipients' point of view have to increase their ability to enrich their lives.
Parallel to medical issues with questionable benefit for the individual patient a whole class of public policy questions emerge: genetic information has the potential to be used out of medical context in ways that are contrary to the interest of patients. In general DNA profiles can provide insights into many intimate aspects of a person and their family as possession of and susceptibility to certain medical conditions. It becomes increasingly necessary to decide whether and to what extend third parties as employers, healthcare and insurance companies etc. may get access to an individual's genetic information thus providing them with an opportunity for genetic discrimination.
Steps to be taken to prohibit and to eliminate genetic discrimination include action of lawgivers in developing legislation to protect individuals from it an action of medical professionals to teach general public about it.
We contacted ministries of health and law in several relevant countries to collect information about awareness of the problem, legislation projects and laws prohibiting genetic discrimination. Over the past years the Internet has become an effective medium for reaching large numbers of health consumer and the general public. Health related Web sites are among those pages of the Internet with the highest rate of interest. An analysis of Web sites with genetic discrimination as topic was performed using predefined criteria for the evaluation of the content.
According to our preliminary analysis neither action of lawgivers nor information of general public are satisfying and appropriate for the magnitude of the problem.

For-Client Letters in Genetic Counseling for Hereditary Breast/Ovarian (HBOC) and Colorectal Cancer (CRC): How can their Communicative Value be Enhanced?
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The knowledge about hereditary cancers is growing rapidly. However, the social, ethical and legal consequences of this knowledge are hard to assess. The concept of informed consent and the intention to help clients by genetic counseling to arrive at valid decisions obviously come under pressure. Hence ensuring the quality of counseling is of great importance. Our project is concerned with for-client letters, serving as a medium for recording the actual state of knowledge as well as its individualized interpretation, facilitating clients' deliberation beyond the temporal limitations and other constraints of the actual counseling session. It studies the effectiveness, the scope and the ethical significance of enhancing the flow of communication by for-client letters. An established standard format for such letters is varied and enriched systematically. One half of the clients receive a standard letter, the other an enriched letter (randomized, double blinded). For this purpose information gained by tape-recording the whole counseling session is used. Comprehensibility and use-value of the standard format and the enriched format are empirically compared, both by quantitative (interview and questionaire with a 20 point score respectively) and qualitative methods. The cases come from three diagnostic conditions in which increases in diagnostic complexity and predictive power translate into individual persons' practical problems of valid decision-making: Counseling concerning i) family-related cancers, ii) anomalous findings in prenatal diagnosis and iii) infertility disorders. To date we counseled 61 families with suspected HBOC and 27 families with suspected CRC. Analysis in the first 30 families (2x20 clients) counseled because of suspected HBOC revealed that clients receiving the enriched format had a significantly better knowledge and comprehension of relevant facts than clients receiving the standard format (Kruskal-Wallis One-Way Analysis of Variance: $p < 0{,}001$). The preliminary results in the subgroup of clients counseled because of suspected HBOC indicate that i) for-client letters can improve client´s knowledge and comprehension of relevant facts and ii) form and content of the letter influence this effect.
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W16

Patent on method of diagnostic, the BRCA1 gene - the real problem
Jacques Warcoin, Paris
In vitro diagnostic tests are patentable subject matter. Example of such type of patent is BRCA1 diagnostic tests. But what is the situation vis-à-vis of the patent dealing with mutated genes per se. Is it an infringement to use the knowledge of the mutated gene itself or of the normal gene itself ? Is there any remedy if no solution is found with the owner of the patent ? French government has proposed a solution for patented inventions dealing with human health, are these solutions
satisfactory or not ? and what happen if these tests are used for research purpose only ?"

3. Quality Workshops

QW3

QUALITY CONTROL IN TUMOR CYTOGENETICS – CAN IT BE PERFORMED AT ALL?
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External quality control assessment in the cytogenetic analysis of constitutional chromosome aberrations was implemented in Germany more than ten years ago. A comparable enterprise in tumor cytogenetics was started last year, when participants of the tumor cytogenetics workshop have decided that quality control in tumor cytogenetics is an essential prerequisite of this discipline.In the meantime, Harald Rieder and Reiner Siebert, who have been elected as the leaders of the quality control group, have dispatched a questionnaire to all laboratories in Germany and Austria that are concerned with tumor cytogenetics. This is the first step in our quality assessment strategy and corresponds to the phase I of the quality assessment in cytogenetics of constitutional chromosome abnormalities.

The problems and limitations of the standardization of quality in cytogenetics in general are due to the used different protocols for cytogenetic analysis, and limited tissue availability. In tumor cytogenetics,however, the spectrum of methods for cell cultivation and harvesting, as well as of metaphase staining, is much broader than in „constitutional „ cytogenetics, and the amount of material available for cytogenetic analysis is, in most cases of leukaemias, lymphomas and solid tumors, very restricted. Thus a standardization of techniques in tumor cytogenetics is very difficult to perform.
One of the intentions of this session is to demonstrate that for different tumor types different procedures have proved most successful.To prepare and stain chromosomes, but also to evaluate the karyotype, a number of different methods have to be used even within a group of related diseases, such as myelodysplastic syndromes and acute myeloid leukaemias.
Another important aspect is related to the institutions where tumor cytogenetics is performed. Therefore representatives of institutes of human genetics, of hematology and oncology clinics and of private laboratories will present their specific points of view of tumor cytogenetics. Our primary aim is to provide clinicians with correct cytogenetic diagnoses that may be of great impact on prognosis and therapy planning. Secondly, the cytogenetic findings constitute the base for the isolation and characterization of affected genes, and thirdly, specific chromosome anomalies may give hints to substances and mechanisms associated with chromosome aberrations. In order to achieve these goals, quality assessment in tumor cytogenetics is of pivotal importance.

A survey about tumor cytogenetic diagnostics concerning hematological malignancies in Germany and Austria
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The assessment of the genetic changes using metaphase chromosome and interphase nuclei analyses has become a mandatory part of the diagnostic requirements in hematological malignancies. Because the techniques as well as the procedures may vary depending on the disease in question, the type and the spectrum of the analyses and also the operating expenses may differ between the cytogenetic institutions. Therefore, we started a survey among the tumor cytogenetic institutions in Germany and Austria to collect information about the diagnostics concerning hematological neoplasias. Investigations of metaphase and interphase chromosomes were addressed and the persons responsable for the respective methods were asked to fill in a mailed questionnaire. Data about the spectrum of the ages of the patients, of the diseases and of the samples used for the analyses were collected. Detailed information was requested concerning cultivation procedures for metaphase chromosome preparation including precultivation treatment of the samples, cultivation media, cultivation time, and the use of cytokines. The spectrum of the chromosome banding techniques, the avarage time spent on the analysis, and the type of the documentation of the findings were asked. The extend of the use of molecular cytogenetic methods in metaphase chromosome analysis was addressed, e.g. the types of probes, the analytical procedure, and the documentation and interpretation of the findings. Questions about interphase molecular cytogenetic analyses included detailed information about the types of samples, types of probes, and of the analytical procedures. Over 75% of the institutions answered the questionnaires. Personal and institutional identifying information was removed from the forms prior to evaluation. The results of the survey will be presented and discussed in detail.
This work was supported in part by the Bundesministerium für Bildung und Forschung, competence net ?Akute und chronische Leukämien?, project ?Zentrale Zytogenetik?, grant 01GI9974

THE PROBLEM OF SMALL CLONES – HOW MANY METAPHASES NEED TO BE ANALYZED FOR AN ACCURATE CYTOGENETIC DIAGNOSIS IN MDS?
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Hematological diseases like MDS, typically show mosaic karyotypes at the timepoint of first diagnosis correlating with a minor infiltration of the bone marrow by the malignant clone.
The karyotype in MDS is one of the most significant prognostic markers and has a strong impact on the differential diagnosis, especially if non-clonal reactive bone marrow dysfunctions have to be discriminated from clonal myelodysplastic syndromes. The therapeutic spectrum is extremely broad, ranging from supportive care to intensive „AML-like“ regimens or myeloablative radioch-chemotherapies with retransfusion of autologous or allogeneic stem cells. Therefore, it is of major interest, how much metaphases have to be analyzed to detect even smaller cell clones with an acceptable expenditure.
In 1986 Heim and Mitelman calculated that - if 25 metaphases are completely analysed - the risk to miss an existing cell clone is 10% related to a clone size of 9% and 1% related to a clone size of 17%.
In a pilot study we examined 77 pts. with suspected or verified MDS who displayed mosaic karyotypes in their bone marrows. We retrospectively determined the analytical effort for the verification of an abnormal cell clone. In the mean, 2.8 metaphases were needed to detect the first - (range: 1 to 25) and 5.8 (range 2 to 26) metaphases were needed to detect the second abnormal cell. Our analyses showed an increase of sensitivity depending on the amount of metaphases analyzed. 5 metaphases: 81% of abnormal clones were detected, 10 metaphases: 84% detection, 15 metaphases: 94% detection, 15 metaphases: 94% detection, 20 metaphases: 97% detection, 25 metaphases: 100% detection.
Summarizing, at least concerning MDS, it has to be discussed critically, to which extent the analytical effort can be reduced without endangering the accuracy of cytogenetic analysis and possibly missing a clonal disease which might need intensive treatment.

Necessity for high quality in cytogenetic results in acute myeloid leukemia
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The karyotype of the leukemic blasts is the most important, independent prognostic parameter with respect to response to therapy and survival in acute myeloid leukemia (AML). During the last years knowledge about karyotype abnormalities and treatment outcome has increased and led to treatment decisions based on the karyotype of AML blasts. Therefore, a high quality of culture techniques, metaphase preparation, chromosome banding and karyotype interpretation is necessary in order to provide a correct cytogenetic result as a reliable basis to guide treatment decisions. Since October 1998 bone marrow and/or blood samples of 1753 AML at diagnosis were sent to our laboratory for chromosome banding analysis. More than 85% of samples were sent overnight. Cells were cultured in RPMI 1640 medium with 20% fetal calf serum and the addition of antibiotics and antimycotics. Five cultures were set up in parallel for each patient: one culture without further supplements, one with the addition of thymidine and three cultures with the addition of a cytokine cocktail (CC) containing erythropoietin, G-CSF, GM-CSF, SCF and IL-3. One plain culture (R24) and 3 stimulated cultures were cultivated for 24h, colcemid was added for 2h followed by standard slide preparation. One culture was cultivated for 24h without colcemid and then another 24h after the addition of colcemid followed by standard slide preparation. Metaphases were analyzed for G-bands using a modified GAG-banding technique. In cases showing an aberrant karyotype 20 metaphases were karyotyped, in cases with no chromosome abnormalities 25 metaphases were karyotyped. 947 cases (54%) showed clonal chromosome abnormalities. In 21 of 1753 cases (1.2%) no analyzable metaphases could be obtained. In another 26 cases (1.5%) less than 10 normal metaphases could be analyzed and were scored as no result. A series of 273 cases with AML M0, M1 or M2 with normal karyotype were investigated using FISH with a variety of different probes. In 9 cases (3%) karyotype abnormalities were detected. Furthermore 25 AML with normal karyotype were analyzed using 24-color-FISH, in one patient a t(17;21) was identified. In conclusion, in 97.3% of AML at diagnosis a valid result from chromosome analysis can be obtained. With high quality chromosome banding analysis a screening with FISH is not necessary in every case. In cases with less than 10 analyzable metaphases or poor chromosome quality a screening with FISH probes covering the most frequent abnormalities in AML is necessary in order to provide the clinician with the best possible information.
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Quality control in solid tumor cytogenetics
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Solid tumors display a variety of recurrent cytogenetic markers, some of which are of prognostic and therapeutic significance. Unlike for hematological malignancies, however, clinically sound cytogenetic classification schemes are scarce for solid tumors. Major reasons for this are difficulties in cell culturing that make some tumor types virtually inaccessible to chromosome analysis, as well as the sometimes extremely complex patterns of aberrations in solid tumors that hamper the establishment of clinically valuable diagnostic schemes. This is why cytogenetic laboratories working on solid tumors are mostly specialized on few and sometimes rare tumor types. Consequently, quality manage-

ment strategies for solid tumor cytogenetics face some specific challenges:
- Multicenter evaluation studies for a given tumor entity are not feasible,
- The prognostic relevance of findings often remains elusive due to limited published data, and
- Results may be biased by specific biological and technical problems of solid tissue cytogenetics, like intratumoral heterogeneity and clonal selection in vitro, that sometimes lead to conflicting results between different techniques. Nonetheless, these shortcomings must not give rise to nihilism in quality management, because there are in fact rules of good practice that must be obeyed and can, in part, be externally assessed even in this field:
- Laboratory procedures using up-to-date techniques aiming at the best possible number and resolution of analyzed cells,
- Correct documentation and interpretation of data with particular caution on the background of clinically valid experience on the given tumor entity,
- Internal evaluation of results by clinical follow-up within multidisciplinary quality circles including clinicians and pathologists, and
- Continuous education in tumor cytogenetic methodology.

Standardisierte tumorzytogenetische Analysen im Routinelabor
Elisabeth Gödde, Datteln

Tumorzytogenetische Analysen sind inzwischen Teil der Differentialdiagnostik bei der Abklärung unklarer hämatologischer Befunde in onkologisch-hämatologischen Schwerpunktpraxen sowie der Kontrolle ambulanter Therapien chronischer Leukämien. Dementsprechend sollten tumorzytogenetische Befunde qualifiziert, zügig und kostengünstig in die kassenärztliche Versorgung integriert sein.
Bei den Proben zur tumorzytogenetischen Analyse handelt es sich in der Regel um Blut- oder Knochenmarkproben, die auf der Basis der „Leitlinien zur tumorzytogenetischen Labordiagnostik“ (Berufsverband Medizinische Genetik e.V.) bearbeitet werden. Ausgehend von der (Verdachts)-diagnose werden Laborprotokolle gewählt, die so in die Routine integriert sind, dass systematischen Fehlern vorgebeugt und gleichzeitig die Qualität der Arbeitsprozesse nicht beeinträchtigt wird. Hierzu gibt es zum einen des Basishandbuch zu den Laborprozessen und zum anderen Entscheidungsdiagramme zur Festlegung der Kultur-, Färbe- und Analyseschritte für den Einzelfall, die auf der Basis aktueller wissenschaftlicher Erkenntnis jeweils nach den anamnestischen Angaben abgearbeitet werden.
Die Standards eines genetischen Labors im Rahmen einer humangenetischen Kassenpraxis, das auch tumorzytogenetische Analysen durchführt, werden vorgestellt.

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P1-03 01

Frameshift mutations of RIZ, but no point mutations in RIZ1 exons in malignant melanomas with deletions in 1p36
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Recently, the retinoblastoma protein interacting zinc finger gene RIZ has been proposed as a candidate for the tumor suppressor locus on 1p36, because of the common loss of RIZ1 RNA in human tumors. In addition, frameshift mutations of this gene have been demonstrated in a variety of tumors with microsatellite instability. Since alterations in this region have been described in malignant melanomas, we investigated DNA of paraffin-embedded sections from 16 typical naevi, 19 atypical naevi, 33 primary melanoma lesions and 25 metastases by PCR and direct sequencing analysis of RIZ. Frameshift mutations in the RIZ gene were found in 17% of melanoma samples and 8.6% of naevi, but we could not demonstrate any missense mutations in the exons of RIZ1. No LOH of the RIZ gene nor any microsatellite instability in six dinucleotide markers or in the mononucleotide repeats IGFIIR, hMSH3, and hMSH6 could be demonstrated in the samples with RIZ frameshift mutations. Although our results do not explain the high rate of deletions in 1p36 found in this tumor, they assign RIZ a potential role in the multi-step tumor forming process of malignant melanoma of the skin.

P1-03 02

Expression profiling of pediatric rhabdomyosarcoma versus ewing sarcoma using oligonukleotid microarrays
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The aim of this study was to identify patterns of differentially-expressed genes in pediatric rhabdomyosarcoma (RMS) in comparison with pediatric ewing sarcoma (EWS). We used Affymetrix Human Genome U95A oligonucleotide microarrays carrying approximately 12,000 genes in order to investigate the expression profile of 23 pediatric primary tumors [12 RMS (3eRMS, 9 aRMS), 11 EWS]. Subsequent hierarchical clustering identified 22 of 23 tumor entities and revealed a group of 33 gene probe sets exhibiting tumor-type specific up-regulation and clearly differentiating the two tumor entities. Verification of the mRNA levels of genes in the microarray experiments was carried out by duplicate hybri zations and quantitative real-time polymerase-chain-reaction (PCR).
The 12 RMS exhibited up-regulation of genes encoding the C-terminal binding protein (CtBP2), a co-repressor associated with mammalian transcriptional regulators, the tumor suppressor gene and trigger of terminal muscle differentiation cadherin-15, as well as numerous other muscle-specific genes. We observed down-regulation of the glutathione-S-transferase M1 (GSTM1) associated with drug metabolism.
The 11 EWS showed up-regulation of genes encoding the inhibitor of DNA binding 2 (Id-2H), probably an inhibitor of tissue-specific gene expression, as well as cyclin D, a cell cycle regulator and oncogene. Beyond it, we observed down-regulation of genes encoding the bridging integrator binding protein 1 (BIN1), a tumor suppressor gene and inducer of apoptosis.
In summary, two clusters totaling 33 differentially-expressed genes are able to differentiate RMS from EWS. These genes are likely to be instrumental in maintaining tumor-specific characteristics and, therefore, participate in key signaling processes. RMS associated genes may inhibit terminal muscle tissue differentiation, whereas EWS associated genes may have profound implications on cell-cycle regulation.

P1-03 03

Interphase-FISH using YAC-derivated probes reveals losses on 10p14-15 in cervical cancer
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In a previous study we had demonstrated that putative senescence genes are located on the short arm of chromosome 10 (10p14-15). The high frequency (38,7%) of LOH in this region in cervical cancers (including early stage tumors) suggests that a loss of gene function at this particular locus may be significant for the development of cancer.
In this study we used 10 different YAC-derivated probes for interphase FISH of paraffin sections in order to detect or exclude allelic losses within 10p14-15 in a large number of cervical tumors and cervical high grade lesions (CIN3).
So far we could confirm frequent loss of 10p in 20 cervical cancers. The analysis of 20 CIN3 lesions is under way. Those YAC clones which detect the most frequently deleted region are suitable for further functional analysis (complementation of gene defects by spheroblasts/cell fusion). None of the genes so far mapped to chromosome 10p14-15 represent obvious candidates for tumor suppressor genes.

P1-03 04

Evaluation of the potential significance of a senescence gene locus on chromosome 4 (q31->4qtel) involved in cervical carcinogenesis
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Putative senescence genes can be mapped to specific chromosomes by the introduction of chromosomes obtained from normal cells into immortal or tumour cells via microcell fusion. For HPV-induced cervical cancer cells we had demonstrated via microcell fusion that putative senescence genes may be located on the long arm of chromosome 4 (4q31->4tel). In the present study we are evaluating the potential significance of loss of gene function in this region by microsatellite-PCR and interphase FISH of paraffin sections using YAC-derivated probes.
Paraffin-embedded tissue sections from cervical cancer (25 cases) were selected. Specific FISH-probes from 4q were generated from YAC-clones (MPI Berlin). After slide preparation fluorescence - in situ - hybridization (FISH) was performed. For microsatellite PCR only histologically verified microdissected areas consisting of 103 - 104 dysplastic or tumour cells were used. Cases with smaller amounts of material were amplified by DOP-PCR first.
Thus far interphase FISH analyses revealed allelic losses in the 4q terminal region in about 12% of cervical cancer cases. Data from LOH analysis is still being generated.

P1-03 05

Breast cancer in young women: Loss of chromosome 8p23 reveals strong association with metastatic phenotype and disease free survival

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Breast tumors occuring in young women are generally more aggressive and have a higher metastatic potential than tumors of older women. So far, the genetic alterations that may be responsible for these differencies are largely unknown. Therefore, we applied comparative genomic hybridization (CGH) to analyze DNA copy number changes in breast tumors of 88 young women (21-35 years at time of diagnosis). The most frequent gains of chromosomal material were found on 1q (64.8%), 8q (61.4%), 17q (50%), 20q (33%) and 3q (20.5%). Losses of genetic material affected chromosomes 8p (19.3%), 11q (11.4%), 16q (11.4%) and 17p (11.4%). Gaines occured significantly more often than losses compared to CGH findings in the cohort of older patients. Loss of 8p23 and gain of 14q were strongly associated with positive lymph node metastasis, suggesting that these genetic alterations are involved in the metastatic process of breast cancer in young women. Univariate analysis of our patients revealed a higher risk of relapse for tumors with loss of 8p23, gains of 18q and positive lymph node metastasis. Using a multivariate analysis loss of 8p23 emerged as significant predictive variable ($p<0.05$). These findings indicate that 8p23 plays an important role in breast cancer of young women and may harbor a novel tumorsuppressor gene.

P1-03 06

Various LOH events on chromosome 11 lead to oncogenesis of paragangliomas

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Paragangliomas of the head and neck region are usually benign tumors developing from chemoreceptors of paraganglionic origin in the majority of patients. These receptors play an important role in sensing and regulation of the blood CO2-level. Genetic alterations in the mitochondrial enzyme complex II (SDH), which is involved in respiratory chain and citric acid cycle reactions, have been shown to lead to sporadic as well as familiar cases of these tumors. Therefore we analyzed our collective containing sporadic cases of patients with paragangliomas for genetic changes in the SDH-genes SDHD, SDHC and SDHB. We detected several new DNA mutations in samples derived from tumor patients. Furthermore we demonstrated loss of heterozygosity (LOH) usually connected with oncogenesis of various tumors. Elucidation of the genetic regions involved in tumor development is a basis for understanding their contribution to normal and pathogenic cell physiology.

P1-03 07

Chronic myeloid leukemia with a variant Philadelphia translocation: t(9;22;11)(q34;q11.2;q13)

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We report a 57-year-old female chronic myeloid leukemia patient, first diagnosed in March 2000, who was admitted to the Department of Hematology in June 2001 for further evaluation and therapy adaptation. Cytogenetic analysis revealed the following karyotype: 46,XX t(9;22;11)(q34;q11.2;q13). Detailed molecular cytogenetic studies showed that the BCR/ABL rearrangement is present and BCL 1 (CCND1) is completely translocated to the derivative chromosome 9. The prognostic implications as well as the relevance of the involvement of the additional breakpoint region 11q13 and the BCL 1 gene are discussed.

P1-03 08

Frequency and Parental Origin of de novo APC Mutations in Familial Adenomatous Polyposis (FAP)

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A predominance of de novo mutations in the paternal germline has been reported for several disorders, but parental origin of APC mutations in familial adenomatous polyposis (FAP) has been systematically examined scarcely so far. FAP is an autosomal-dominant inherited precancerous condition characterized by the occurence of hundreds to thousends of colorectal adenomas and extracolonic manifestations. Germline mutations in the tumor suppressor gene APC are responsible for FAP. We examined 916 unrelated FAP families for germline mutations. Family history was known sufficiently in 552 families; in 8,5% out of them both parents of the index patient had not developed colorectal adenomas suggesting a de novo mutation. A germline mutation was detected in 45 patients with suspected de novo mutation, in 38 of them the mutation was excluded in both parents. The 5 bp deletion at codon 1309 was overrepresented in the group of patients with proven de novo mutation (17/38 = 45%), when compared with the group as a whole (58/916 = 6%), thus 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 13 families, including 3 families with large, cytogenetically detectable deletions. 6 mutations were of maternal and 7 mutations of paternal origin. Interestingly, the large deletions were of paternal origin only. Mutations in the paternal germline included two base exchanges (R564X; S1201X), a 5 bp deletion at codon 1061 and a 2 bp deletion at codon 1465. The 5 bp deletion at codon 1309 was found three times, but only in the maternal germline. In addition in the maternal germline a 2 bp deletion at codon 1186, a single bp deletion at codon 1548 and a splice site mutation at codon 653 were detected. In conclusion, in our sample de novo APC germline mutations show almost the same frequency in the paternal and maternal germline, but we observed a difference in parental origin regarding the 1309 mutation and large del

P1-03 09

Juvenile Polyposis: Massive Gastric Polyposis is More Common in MADH4 Mutation Carriers than in BMPR1A Mutation Carriers

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Juvenile polyposis syndrome (JPS) is an autosomal-dominant predisposition to multiple juvenile polyps in the gastrointestinal tract. Germline mutations in the MADH4 or BMPR1A genes have been found to be causative of the disease in a subset of JPS patients. So far, no genotype-phenotype correlation has been reported. We examined 29 patients with clinical diagnosis of JPS for germline mutations in the MADH4 or BMPR1A genes and identified MADH4 mutations in seven (24%) and BMPR1A mutations in five patients (17%). A remarkable prevalence of massive gastric polyposis was observed in patients with MADH4 mutations when compared to patients with BMPR1A mutations or without iden-

tified mutations. This is the first genotype-phenotype correlation observed in JPS.
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P1-03 10

Laser capture microdissection in 2-D coculture models as a novel tool to study tumor-stroma-interactions

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Cellular adhesion, migration and invasion are essential processes in tumor progression including actions by malignant cells as well as by the stromal microenvironment. In vitro, tumor cell - stromal fibroblast interaction can be studied by 2-D and 3-D co-culture models. In conventional coculture systems, the different cell types are grown together in glass-slide based chambers with medium. Immunohistochemical detection at the protein level can be easily performed, however, for downstream protein and gene expression analysis the cell compartments have to be dissociated and sorted, for instance by fluorescence activated cell sorting or magnetic cell separation. These procedures are labor intensive and could possibly result in stress-induced alterations in RNA expression.
Here we present a novel technique for co-culturing and separating fibroblasts and carcinoma cells. It is based on cell co-cultivation on a PALM LCM membrane followed by rapid immunostaining and laser capture microdissection of the cell compartments. The 1.35 µm thin membrane has been originally used for the mounting of histological tissue sections on regular glass slides for the PALM LCM System (P.A.L.M. GmbH, Bernried, Germany). For identifying the tumor cell compartment, immunolabeling for the laminin gamma 2 chain was performed, a marker that is expressed only in epithelial tumor cells.
Laser capture microdissected tumor and stroma cells from the presented membrane based coculture model can be used for gene expression profiling and DNA based analysis. Isolation of amplificable DNA from the microdissected cells can be performed with standard procedures. Analysis of RNA molecules has been more troublesome and can be a major limiting step for downstream applications because of loss of RNA quality and quantity during RNA sample preparation from a limited number of immunostained cells.The RNA quality from our microdissected co-cultured cells was successfully proved by RT-PCR for a housekeeping gene transcript and for the laminin gamma 2 chain gene transcript used in immunostaining the tumor cell compartment The laminin cDNA was amplificable only in tumor cells and not in the co-cultivated fibroblasts indicating no cell-cross-contamination during microdissection.

P1-03 11

TP53 mutation and LOH analysis as a non-invasive diagnostic tool in serum and urine samples of bladder cancer patients

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Molecular markers are needed for better distinguishing non-invasive papillary (pTa) and minimally invasive (pT1) bladder carcinomas and for identifying individual tumors with a high risk of recurrence or disease progression.
First aim of our study was to evaluate TP53 microsatellite and mutation analysis as an effective concept for the characterization of superficial bladder tumors with different biological aggressiveness. Mutation screening in the TP53 hot spot region was performed in 55 microdissected superficial bladder tumor samples by direct genomic sequencing. PCR based LOH analysis was done with two markers at 17p13.
Second, there is considerable interest in the development of non-invasive techniques that would detect recurrent bladder neoplasia. In order to evaluate TP53 alterations as a potential marker for a non-invasive diagnosis of recurrences or residuals and to determine whether tumor-specific DNA exhibiting LOH or sequences harbouring a mutation can be detected in body fluids, mutation screening was performed in urine, plasma and serum of patients with a mutated primary tumor. LOH analysis with two markers at 17p was done in the corresponding urine and blood samples of 31 primary tumors.
As seen from our results, TP 53 inactivation by mutation seems to characterize higher malignant superficial bladder tumors which tend to recur and in which the probability is higher that the rezidives progress to muscle invasive growth pattern.
Only in 2/8 cases, the TP 53 mutation from the primary tumor could be redetected in patients urine and blood. 17 p microsatellite changes with at least one marker were found in 30/31 body fluids of the tumor patients (97%). Correlating the 17 p status found in body fluids to the status of the primary tumor, the concordance was only about 52%.
We conclude that TP 53 genotyping as a non-invasive diagnostic tool in outpatient samples is of limited value for clinical practice.

P1-03 12

Chromosomal aberrations in patients with B-CLL - the role of DICE1

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B cell chronic lymphocytic leukemia (B-CLL) is the most common type of leukemia in adults in Western countries. Clonal chromosomal aberrations are found in about 50% of cases using conventional chromosome analysis (CCA) and in more than 75% using molecular cytogenetic methods, such as fluorescence in situ hybridisation (FISH). The most frequent changes are deletions in 13q14, 11q22~23, trisomy 12q, deletions 17p13 and 6q21. Deletions in chromosomal band 13q14.3 may indicate the involvement of a tumour suppressor gene. The DICE1 (deleted in cancer-1) gene is a tumour suppressor gene deleted in various sporadic cancers, including carcinomas of the head and neck, breast, ovary, prostate, and others and is localised in 13q14.3. DICE1 maps app. 4 Mb distal from the retinoblastoma gene (RB1). CCA and FISH analyses were performed on bone marrow cells and unstimulated lymphocytes from 14 B-CLL patients. Locus-specific FISH probes were used for ATM (Ataxia teleangiectasia mutated gene) in 11q23, RB1 and DICE1 in 13q14.3 and p53 in 17p13.1. Three patients showed a deletion of ATM, two a deletion of p53 and five patients a deletion of RB1 and DICE1. The same percentage of RB1 and DICE1 deletion was detected in 3 of them. Two of them showed a higher percentage of RB1 deletion than DICE1 deletion. This study indicates, that the DICE1 gene also may be involved in B-CLL patients. As the rule, patients with 13q deletions have a favourable outcome. For delineation of the prognostic factor of DICE1 deletions in B-CLL patients further patients have to be examined.

P1-03 13

Constitutional Hemimethylation of the STK11/LKB1 Gene Promoter Predisposing to Peutz-Jeghers Syndrome - a Single Case?

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We report the first case of Peutz-Jeghers syndrome (PJS)due to constitutional hemimethylation of the serine/threonine protein kinase 11 (STK11/LKB1) gene promoter. The severely affected young female exhibited characteristic symptoms of PJS including pigmentation abnormalities and multiple intestinal lesions. Typical mutations inactivating the STK11/LKB1 gene were excluded at the RNA and DNA level (Abed et al., 2001). However, hemimethylation of the STK11 gene was identified in mononucleated peripheral blood cells, and also detected in exfoliated oral squamous cells, and in normal gastric and duodenal mucosa. Interestingly, mucosa of two polyps resected from the antrum and the duodenum demonstrated complete biallelic methylation of the STK11/LKB1 promoter. However, examination of microdissected stromal and epithelial cells of one harmartomatous polyp revealed complete methylation restricted to the epithelial fraction, while stroma cells showed hemimethylation in accordance with the constitutional situation. Hence, functional loss of STK11/LKB1 due to two consecutive epigenetic mutations was demonstrated as a very early event in the initiation of the hamartoma-adenoma-carcinoma sequence in PJS tumors. Since constitutional hemimethylation was not found in any of 10 PJS patients, who did not harbour a typical germline alteration in STK11/LKB1, it could be of significance that our sporadic PJS patient simultaneously exhibited a de novo Xq+ chromosomal aberration resulting from a X/Y translocation.
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P1-03 14

Hereditary Nonpolyposis Colorectal Cancer: Frequent Occurrence of Large deletions in MSH2 and MLH1 genes

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Hereditary nonpolyposis colorectal cancer (HNPCC) is due to a deficiency in DNA mismatch repair. In a considerable proportion of HNPCC patients no germline mutation in DNA mismatch repair genes can be uncovered. However, large genomic deletions cannot be detected by the methods currently used. In this study we used a semiquantitative multiplex PCR to detect the proportion of large genomic deletions in patients suspected of HNPCC. Of 368 unrelated colorectal cancer patients fulfilling the Bethesda criteria for HNPCC, 180 exhibited microsatellite instability in their tumor tissue. Among these patients 68 disease-causing point mutations (38%) had previously been detected in the MSH2 and MLH1 genes by SSCP, heteroduplex analysis or DHPLC followed by direct sequencing. The remaining 112 patients were examined for large genomic deletions. We identified deletions in 19 patients (10.6%). 11/19 (58%) deletions were located in MSH2, and 8/19 (42%) in MLH1, respectively. The size of deletions ranged from one exon to a deletion of a whole gene. Five breakpoints of deletions were sequenced. Alu-repetitive elements were involved in all of them. In patients meeting the Amsterdam criteria the proportion of genomic deletions was 12.6%. A similar proportion of deletions was found in the group of patients with a positive family history for colorectal cancer and MSI-H tumors, not meeting the Amsterdam criteria. The results of this study suggest that genomic deletions in both MSH2 and MLH1 genes play a considerable role in the pathogenesis of HNPCC and should be part of the routine HNPCC mutation detection protocols.

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P1-03 15

Attitudes towards molecular genetic testing and coping strategies of persons at risk for hereditary cancer diseases and those of their partners

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Introduction: Gene analysis has been possible for several hereditary cancer diseases: HNPCC, HBOC, FAP and MEN. As there is a dominant cancer predisposition, for persons at risk predictive diagnosis can help to decide to take part in early diagnosis. However, the social and psychological problems may grow with this new knowledge.

The aim of our study was to investigate the attitudes towards molecular genetic testing and coping strategies of persons at risk (RP) for hereditary cancer diseases and those of their partners (P).

Methods: The investigation was carried out by questionnaires. We received 67 questionnaires completed by RP and 30 questionnaires completed by P.

Results: Nearly 90% of persons in both groups accept predictive diagnosis. Only less than a fifth see problems in taking it. Those were psychological problems and problems with insurance companies. Coping strategies are mainly active and related to the disease: 2.4 for RP and 2.1 for P on a five degree rating scale. Other coping strategies are distraction and self esteem upgrading: 2.8 for RP, 2.9 for P. The profile of mood states (POMS) show less dejected (1.7 for RP, 1.5 for P) and disgruntled mood (1.9 for RP, 1.8 for P), but more thirst for action (3.5 for RP, 3.2 for P).

Conclusions: Compared with coping strategies of persons at risk for late onset neurodegenerative disorders, persons at risk for hereditary cancer diseases show a more positive thinking, diversion and active coping. For them genetic counselling plays a more important role than offered psychological care. They know that in case of positive predictive diagnosis specific prophylactic and treatment programmes are available.

P1-03 16

INTERPHASE CYTOGENETICS IN CHRONIC LYMPHOCYTIC LEUKEMIA USING SEVEN DIFFERENT DNA PROBES

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B-cell chronic lymphocytic leukemia (CLL), the most frequently diagnosed leukemia in adults, is characterized by a low mitotic activity of the leukemic cells. Therefore by means of classical cytogenetics clonal chromosome aberrations can be revealed in only 40-50% of cases. It has been demonstrated that fluorescence in situ hybridization (FISH) on interphase nuclei allows the detection of genomic anomalies in up to 80% of patients. Chromosome aberrations in CLL have proven to be important predictors of disease progression and survival time (Döhner et al., 2000). Thus for risk-adapted clinical management it is of great importance to characterize the leukemic B-cells by FISH analysis at diagnosis. In our study peripheral blood mononuclear cells of 50 patients with CLL were analyzed by FISH for deletions in chromosome bands 11q22~23 (ATM locus), 13q14 (Rb1) and 17p13 (p53), for trisomy of band 12q13 and for translocations involving band 14q32 (IgH gene). With this DNA probe panel we could detect at least one chromosome aberration in more than 70% of the blood samples, two changes were observed in about 25% of the aberrant cases. Consistent with the data from a larger study, the most frequent anomaly was a deletion in 13q14 followed by the loss of one 11q22~23 signal and trisomy of 12q13.

P1-03 17

DICE1 gene expression is regulated by CpG methylation in the promoter region

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The candidate tumor suppressor gene DICE1 (DDX26; OMIM *604331) is located within a previously reported LOH critical region telomeric to the RB1 gene on human chromosome 13q14.3. Expression of DICE1 is downregulated in lung and prostate tumors. To elucidate the reduced DICE1 expression in tumor cells the putative promoter sequence upstream of the DICE1 transcription start site was analyzed. This sequence shows a high GC content and is rich in CpG sites. Several fragments of the putative promoter sequence were analyzed for promoter activity by transfecting chimeric ß-galactosidase reporter constructs into COS-7 cells. Promoter activity was identified within three overlapping fragments. When reporter constructs exhibiting promoter activity were methylated by SssI methylase decreased promoter activity was observed in COS-7 cells. Band shift assays demonstrated that the predicated DICE1 promoter binds proteins from HeLa nuclear extract enriched for transcriptional factors. By analysis of lung and prostate tumor cells with methylation sensitive enzymes, a methylation pattern of the CpG sites in the upstream sequence of the DICE1 gene corresponding to the expression level was found. Prostate tumor cell lines LNCaP and Du145 as well as lung tumor cell line Calu-3 and SK-Mes1, which show low DICE1 expression, are hypermethylated at all investigated restriction sites. In contrast, the higher DICE1 expressing lung tumor cell line SW900 is hypomethylated. From these results we conclude that 1) the DICE1 promoter is rich in CpG sites and shows promoter activity; 2) DICE1 is downregulated in lung and prostate tumor cell lines by hypermethylation of the DICE1 promoter and may associate with tumor progression.

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P1-03 18

Detection of altered CpG methylation by denaturing high-performance liquid chromatography (DHPLC)

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Background: Promoter methylation at CpG dinucleotides is an established mechanism of tumor supressor genes (TSGs) down-regulation in cancer development and progression. DHPLC is a reliable, highly sensitive technique primarily developed for mutation detection. In this study, the use of DHPLC as a prescreening method for detection of CpG methylation was investigated.

Methods: The method was established by analyzing the methylation pattern of the CDKN2A p16 promoter using DNA samples with different, well characterized methylation patterns, either from tumor specimens or generated artificially by treatment of unmethylated blood DNA with DNA methylases. Bisulfite treatment of genomic DNA was used to deaminate unmethylated cytosines converting them to uraciles. A 392 bp fragment of the p16 promoter region containing 35 CpGs, was PCR-amplified with primers suitable for unmethylated as well as methylated alleles. PCR products were denatured and renatured permitting the formation of heteroduplex DNA detectable by DHPLC. To verify DHPLC positive results, PCR products were subcloned and sequenced.

Results: After establishing the method, DNA samples from sporadic breast and ovarian cancers were analyzed, revealing hypermethylation of the p16 promoter in 10% of cases. While complete methylation results in a peak shifted in retention time compared to the unmethylated blood DNA, partial methylation can be distinguished by appearance of additional signals representing diverse heteroduplex structures.
Conclusions: DHPLC is suitable as a prescreening technique to detect promoter methylation of presumable tumor suppressor genes. The method is now used for a methylation screen analysis of several putative TSGs associated with sporadic breast cancer.
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P1-03 19

Variations of DNA double strand break repair genes in 120 patients with bilateral breast cancer

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Defective DNA double strand break repair has been implicated in the etiogenesis of breast cancer. We are investigating a hospital-based series of 120 consecutive patients with bilateral breast cancer for the prevalence of inherited mutations and variants of genes involved in chromosome break repair. Truncating mutations of the BRCA1 and BRCA2 genes were identified in eight unrelated patients (4 BRCA1, 4 BRCA2). After a median follow-up of 72 months (first cancer) and 30 months (second cancer), local recurrence was observed in 3 out of 8 identified BRCA1 and BRCA2 mutation carriers (38%) whereas this was the case in 8 out of 103 non-BRCA1 and BRCA2 carriers (8 %). Two further patients carried truncating ATM gene mutations. We also identified several patients with common missense substitutions of the p53, XRCC2 and XRCC3 proteins, or with the promoter variant 135g>c in the RAD51 gene. Some patients harboured alterations in more than one gene. One particularly young patient who had her first breast cancer by the age of 29 and her second by the age of 30, was found to be heterozygous for BRCA1 mutation 4184del4 as well as for the RAD51 gene variant 135g>c and for a XRCC3 missense substitution. Homozygosity for a XRCC2 missense substitution was found in the only affected patient from a family segregating the BRCA1 mutation 5382insC. Our data suggest a genetic defect in DNA double strand break repair in at least 10% of cases with bilateral breast cancer and a potential interaction of different DNA repair gene variants in these patients.

P1-03 20

ALTERATION OF THE FRAGILE HISTIDINE TRIAD (FHIT) GENE IN INTRAHEPATIC CHOLANGIOCARCINOMAS

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Intrahepatic cholangiocarcinoma (ICC) is the second most common intrahepatic neoplasm accounting for 10-30% of primary liver cancers. Since the carcinogenic mechanisms leading to the development of ICC are poorly understood we examined whether alterations of the fragile histidine triad (FHIT) gene, a putative tumor suppressor gene on chromosome 3p14.2, contribute to the carcinogenesis of ICCs. Additionally, oncogenic mutations within exon 3 of b-catenin which essentially lead to a constitut ively activated Wnt signaling pathway and microsatellite instability (MSI) in the tumors as a consequence of mismatch repair deficiency were examined. LOH at the FHIT/FRA3B locus was detected in two of 10 (20%) informative cases using the marker D3S1300 and in one of seven (14%) informative cases by marker D3S1234. Furthermore, an altered expression pattern characterized by an unusual intracellular distribution of FHIT protein was detected by immunohistochemistry analysis in these cases with LOH. In contrast, oncogenic mutations were excluded in exon 3 of the b-catenin gene and MSI could not be detected in the tumor specimens tested by appropriate markers. In two of nine (22%) informative cases loss of heterozygosity (LOH) was displayed by marker D5S346, which is localized nearby the adenomatous polyposis (APC) gene. Thus, the Wnt pathway may be affected in ICCs rather by the APC gene than by mutations within exon 3 of the b-catenin gene. MSI seems not to contribute to the development of ICCs in our cohor t of tumors analyzed. Our results of parallel investigations of several genetic alterations in ICCs provide further insights in the carcinogenesis of ICCs. The detection of allelic losses of genomic DNA in intronic intervals of the FHIT gene is the first demonstration of FHIT alterations occuring in the carcinogenesis of ICCs. Therefore, the FHIT gene could be identified as a new player in the multistep carcinogenesis of ICCs.

P1-03 21

Molecular analysis of differentially expressed candidate genes in breast and ovarian tumors

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Putative tumor suppressor genes (TSGs) and oncogenes were identified as differentially expressed by „in-silico“ database searches. In order to characterize the TSGs a strategy including expression analysis, LOH (loss of heterozygosity) analysis and mutation screening were applied. To confirm the electronic Northern data TSG candidates were investigated by hybridization of cancer profiling arrays (CPA; Clontech) with gene-specific probes. Two TSGs (bn39, on78) were found to be differentially expressed in up to 80% of breast (BC) or ovarian cancer (OC) samples, respectively. In a set of 200 BC and 70 OC samples gene specific LOH analysis was performed to find at least 20 tumors with allelic loss of the corresponding TSG. LOH was found in 20% to 47% of informative cases. For two TSGs (bn39, on98) LOH positive tumor samples were screened for mutations by DHPLC (denaturing high performance liquide chromatography). Several missense mutations could be determined and characterized as so far unknown polymorphisms. No functional mutations were found. To investigate altered promoter methylation as an alternative mechanism for observed TSG silencing a methylation screening analysis by DHPLC has been established. To characterize activation of putative oncogenes, gene expression was analysed by hybridization of CPAs or RT-PCR. One presumable oncogene (bt11) was shown to be frequently overexpressed especially in OC but gene amplification determined by quantitative differential PCR could not be shown in BC and OC samples. The pathohistological evaluation of TSG bn39 and oncogene bt11 in breast and ovarian tumors and the functional characterization of both genes in BC and OC cell lines will further elucidate the role of these genes in development and progression of breast and ovarian tumors. (This project is part of the DHGP „Gynecological Cancer Consortium“, coordinated by Edgar Dahl, metaGen Pharmaceuticals GmbH)

P1-03 22

A physical map for the rat genome

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One of the key goals of the German Human Genome Project is the identification of genes that cause or contribute to multifactorial diseases and the characterization of disease-associated allele variants. The rat provides the most thoroughly studied experimental models for common human complex genetic diseases. A large number of quantitative trait loci (QTL) have been identified in the rat, which lead to the subsequent generation of numerous congenic and sub-congenic rat strains in QTL regions Tantamount to the identification of the underlying disease genes is the establishment of a powerful set of genomic tools and reagents for this species.
We have built a physical map covering the rat genome by integrating marker-content information with the constructed radiation hybrid map. The integrated map shows the location of ~5500 BAC and PAC loci, providing BAC and PAC landmarks with an average spacing of approximately 600 kb across the rat genome. YAC coverage extends well beyond 90% of the rat genome represented by some 30,000 clones. The cloned proportion of the rat genome in BAC and PAC clones affords to 27%.
We will discuss the integration of the physical map with large scale genomic sequencing efforts in this species. We propose to utilize the established physical map for the generation of region specific BAC/PAC contigs providing a minimal tiling path for the fine mapping, sequencing, and cloning of disease genes in rat models.

P1-03 23

Detection of loss of heterozygosity in primary bladder carcinoma

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In bladder carcinoma, loss of heterozygosity (LOH) is a common phenomenon in chromosomal loci harboring tumor supressor or related genes. In a pilot study we analyzed 20 microsatellite markers from various chromosomal regions in primary bladder carcinoma specimens of 11 patients. Microsatellites were analyzed using PCR and fluorescence-based detection system ABI 310. All aberrations detected in a primary screen were checked twice by repeating the complete assay. This eliminated several suspected positive results, particularly additional or aberrant peaks previously reported to be associated with bladder carcinoma. LOH or allelic imbalance (AI) was found with one or more of 10 markers in a total of 7 out of the 11 samples. The 10 markers included one, four, and five on chromosomes 2, 8, and 9, repectively. In a second series of tumor specimens from 10 additional patients we used the 10 markers with positive findings in the pilot study. Seven out of the 10 samples showed LOH or AI in one or more of the markers also verified by replication. None of the 10 markers showed signs of LOH or AI in surgical bladder tissue specimens of 21 control patients without bladder carcinoma. In summary, using the 10 markers selected allowed the detection of LOH or AI in 14 of 21 tumor specimens (67%) but in none of 21 control specimens (two-tailed Fisher exact test $p < 0.0005$). Detection of LOH/AI in tumor tissue may be helpful for confirmation of histopathologically suspected carcinoma.

P1-03 24

Alterations in 5 chromosomes define high risk groups in neuroblastoma

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Introduction: Amplification of the MYCN-oncogene and deletions in 1p36 in a subgroup of neuroblastoma (NB) are well known factors of poor prognosis. The prognostic impact of other non-random aberrations (17q-gain, deletions in 3p and 11q) as determined by LOH-analyses or CGH-data is not yet clear.
Methods: Using interphase-FISH on large series of primary NB the frequency and prognostic potential of aberrations in the following chromosomal regions were investigated: 1p36 (n=191), 2p23-p24(n-myc) (n=202), 3p26 (n=188), 11q23 (n=202) and 17q21.3-q23 (n=141). Deletion events were defined as: deletion (=monosomy of a specific region) and imbalance (at least two intact and additional truncated copies of the investigated chromosome).
Results: Of al1 analyzed NB, aberrations were found in: 1p36: 19% deletions, 9% imbalances; MYCN-amplification: 19%; 3p26: 15% deletions, 4% imbalances; 11q23: 19% deletions, 8% imbalances; 17q-gain: 62%. Aberrations were highly associated with unfavorable clinical stage 4 and occurred rarely in favorable localized stages 1-3 and 4s. The age of patients showing chromosomal alterations was significantly increased. MYCN amplification, 1p36-alteration and 17q-gain on the one hand as well as 3p- and 11q-aberrations on the other were highly associated with each other ($p<0.001$). Contrary, MYCN-amplification and 11q-alterations tend to show an inverse correlation ($p=0.07$). The outcome of patients with alterations in 1p, MYCN-amplification and 17q-gain was inferior compared to those without aberrations ($p=0.01$ and $p<0.001$). Chromosomal changes in 3p and 11q were determined as markers of poor prognosis in localized and 4s NB ($p<0.01$).
Conclusion: Investigation of 5 chromosomal regions allows to define high and low risk subgroups in NB to forecast disease progression and to optimize therapy.

P1-03 25

Interphase FISH in myelodysplastic syndrome with normal conventional cytogenetic result

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Acquired loss of a great portion from the long arms of chromosome 5 or 7, loss of whole chromosomes 5 or 7 and trisomy 8 are frequent cytogenetic aberrations associated with myelodysplastic syndromes (MDS) or myeloid leukemias (AML). In this study we performed fluorescence in situ hybridisation (FISH) analysis on interphase nuclei of 32 patients with AML secondary to MDS and a normal karyotype in conventional analysis prior to and after progression of disease. BAC probes mapping to two critical intervals on 5q and 7q as well as centromeric FISH probes for chromosomes 7 and 8 were analysed in order to search for hidden chromosomal aberrations of these chromosomes. In one patient a hidden terminal deletion of the long arm of chromosome 5 was present. Monosomy of chromosome 7 was found in 4/32 cases. All these clonal aberrations occurred after progression to AML. Deletions of 7q31 and trisomy 8 were not detectable. The presented ?7 and 5q- cases could only be detected by interphase FISH and were not assessed in conventional metaphase analysis. Such hidden anomalies may contribute to the poor outcome in cases of myeloid disorders with progression of disease and a normal cytogenetic result in dividing cells.

P1-03 26

Validation and functional analysis of candidate tumour suppressor and oncogenes in sporadic breast cancer

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Background: Over 600 putative tumour suppressor and oncogenes were identified as being differentially expressed in gynecological tumours by an „in silico" approach. This was based on the analysis of more than 4 million ESTs derived from either normal or tumour tissue data bases (metaGen Pharmaceuticals).
Aim: To validate differential gene expression of a subset of 15 tumour suppressor gene candidates and 5 oncogene candidates by real-time-RT-PCR and reverse Northern analysis.
Results: For this purpose total RNA was extracted from histologically verified ductal breast cancer tissue and corresponding normal tissue. RNA from the two tissue types was then subjected to real-time RT-PCR. RNA from the same tissues was also used as hybridisation probe for reverse Northern analysis (cDNA arrays derived from IMAGE clones). Twenty one different tumour samples were analysed. Differences in gene expression in relation to a pool of normal ductal tissue (n=5) were scored as
1) no difference,
2) up- or down-regulated by a factor of 2-4, and
3) up- or down-regulated by a factor of > 4.
Six of 20 genes showed to be of particular interest because a high percentage of cancers were consistently either up- or down-regulated by a factor > 2. These are bn22, bn39, pct56, ot59 and ot89 (all down-regulated) and bt11 (up-regulated). According to the in silico analysis of ot59 and ot89 we would have expected to observe an up-regulation of RNA in tumour tissues.
Ongoing research: For further analysis, the full length cDNAs from the six candidate genes were amplified by RT-PCR and were cloned into pET 29a (Novagen) and pBK (Stratagene) for expression of their protein in bacteria (generation of antisera for immunohistochemical analysis) and eukaryotic cells (functional tests) respectively. Future functional tests will focus on the putative senescence or immortalization properties of the genes under investigation.

P1-03 27

Tissue-specific microdissection coupled with protein chip array technology to analyse proteomic differences between normal and highly proliferative non-invasive squamous epithelium of the hypopharynx and invasive hypharyngeal carcinomas

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For the molecular pathological analysis of epithelial hyperproliferation and invasive carcinoma of the hypopharynx 20 deep-frozen (-80 C) tissue samples from normal epithelium and hypopharyngeal cancer as well as one sample of a non-invasive but highly proliferative epithelium (HPE) were microdissected from unstained histological sections with Laser Capture Microscope (Palm). The crude protein extracts were analyzed on a strong anionic exchanger (SAX2) ProteinChip Array in a ProteinChip Reader (SELDI PBS II; Ciphergen Biosystems). In HPE prominent new protein peaks -not detectable in the normal epithelium- appear at 3533 Da, 5207 Da, 9955 Da, 10462 Da and 13606 Da. A peak at 10171 Da is in comparison to the normal epithelium considerable increased. In the invasive tumor tissue the protein of 3533 Da is in comparison to HPE massively overexpressed. The expression of the 5207 Da, 10464 Da and 13606 Da proteins however is downregulated. The proteins of 9955 Da and 10171 Da stayed quantitatively unchanged. The results indicate that protein pattern correlate with epithelial hyperproliferation and malignant growth in the hypophar-

ynx, allowing the identification of proteins associated with hyperproliferation on the one hand and invasion on the other. Further analyses to identify these proteins are in progress. Moreover the results demonstrates the suitability of the combination of tissue microdissection and protein chip array technology as a new powerful tool in cancer research.

P1-03 28

MEN1 gene mutations in 11 MEN1 families and their associated tumors

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MEN1 is an autosomal dominant disorder characterized by the combined occurrence of tumors of the parathyroid glands, the endocrine pancreas, the duodenum, and the anterior pituitary gland. With the cloning of the MEN1 gene, we are able to identify the carrier status of individuals at risk. The MEN1 gene contains 10 exons and encodes a ubiquitously expressed 2,8-kilobase transcript. The predicted 610-amino acid protein product, termed menin, exhibits no apparent similarities to any previous known proteins.

Eleven unrelated German MEN1 families and their associated tumors were characterized using PCR-Single-strand conformation polymorphism (SSCP), DNA-sequencing and LOH studies on chromosome 11q13. All probands were clinically evaluated at university hospitals. Familial MEN1 was defined as endocrine tumor in two of the three principal MEN1-related tissues plus at least one first degree relative with an MEN1 related endocrinopathy. All individuals were also screened biochemically, with measurements of intact parathormone, gastrin, insulin, c-peptide, and prolactin. LOH studies revealed deletions at 11q13 in tumor samples in 8 cases.

We identified 7 novel mutation (2 deletions, 3 nonsense, 1 spliceside, 1 missence) in exons 2, 8, 10 and intron 3. MEN1 mutations are distributed throughout the open reading frame with no apparent hotspots for mutation. Mutation screening can be offered to affected families. As there were no clinical signs of MEN1 in members at low risk in many of the families studied, it should be possible to identify persons at risk with a mutant allele for regular clinical and biochemical examinations.

P1-03 29

Incidence and significance of a deletion in der(9)t(9;22) in children with CML

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Data obtained with FISH probes targeting ABL1 and distal to BCR located sequences on chromosome der(9)t(9;22) subdivide CML patients in two distinct clinical subgroups: Patients with a deletion have median survival of about 38 months, while the median survival of patients without a deletion is about 88 months (Sinclair et al, 1999). Virtually all adult patients with a deletion in der(9) lack the ABL1-BCR-transcript and the respective gene. Inversely, only 50% of the ABL1-BCR-transcriptional negative patients exhibit a deletion in der(9) by FISH (Loncarevic et al, 2002). We examined the incidence of ABL1-BCR gene deletion and gene transcription in 54 children with CML. Lack of an ABL1-BCR-transcript was found in 18/54 (33%) individuals. Among the 18, 10 (55%) exhibited a deletion in der(9) by FISH. This data show for the first-time a deletion in der(9)t(9;22) in childhood CML and demonstrate that this deletion occurs at similar frequency (~33% (18/54)) as in adults. None of the children showed variants of standard t(9;22). This stays in contrast to the data we obtained with adult ABL-BCR RNA negative patients. Therefore, variants of standard t(9;22) do not contribute significantly to ABL1-BCR-RNA negativity in childhood CML. Moreover, we found only one variant t(9;22) among 137 children with CML after GTG-based karyotype analysis. The clinical significance of the deletion in der(9) could not be evaluated in a long time follow up, as 75 % of the children where subjected to stem cell transplantation within a period of 1.5 years after diagnosis. Our data indicate a common mechanism that acts in both children and adults generating the deletion in der(9). The deletion affecting all of the ABL1-BCR gene in our cohort of patients occurs most likely simultaneously with the t(9;22) in the double strand break repair process. (Sinclair PB et al (2000) Blood 95:738-743 / Loncarevic IF et al (2002) Genes Chromosome and Cancer 34, 2, 193-200)

P1-03 30

Characteristic genetic changes in childhood adrenocortical carcinoma and pheochromocytoma detected by comparative genomic hybridization

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Pheochromocytoma and adrenocortical carcinoma in children are very rare. Pheochromocytoma occur sporadically or within the scope of familiar disease like VHL, MEN 2a/b or NF1. Differentiation of benign and malignant lesions is often difficult by standard criteria. In order to detect characteristic chromosome imbalances that may serve as a clinical parameter 12 pheochromocytoma and 11 adrenocortical carcinoma were analyzed by comparative genomic hybridization (CGH). In Pheochromocytoma loss of chromosome 3/3p and 11/11p was found always together and in 10 of the 12 patients analyzed. Gain of chromatin was found less frequently and affected chromosome 6,7,12,15,17. The data differ from those obtained with adult patients, who show losses of 1p and 3q as the most frequent anomalies (Edström et al 2000). One tumor showed an near tetraploid karyotype and a relatively underrepresentation of chromosome 3 and 11 as determined by interphase FISH. In addition we examined 11 childhood adrenocortical carcinoma and one adenoma. This group of tumors showed gains of chromosomes 1, 3, 5, 6, 7, 9, 12, and 15. Chromosome losses were found less frequently and affected chromosome 2, 3, 4, 11q, 16 and 17. All patients in this study are recorded in the interdisciplinary and multicenter trial study GPOH-MET 97 (German Society of Pediatric Oncology and Hematology - Malignant Endocrine Tumors). Evaluation of the clinical data and correlation to specific genotypes are part of our ongoing work.

P1-03 31

Frequency of the Heterozygous Germline NBS1 Mutation 657del5 in Cancer Patients from Poland

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Blood relatives of patients with Nijmegen breakage syndrome were reported to have an increased risk of malignancy, implying that heterozygous carriers of the 657del5 mutation of the NBS1 gene should be more frequent among cancer patients than in the general population. Following molecular screening of blood samples from 1683 non-selected patients with malignant tumors, mostly from Central Poland, we found 16 carriers of the 657del5 mutation vs. approximately 9 expected. Germline 657del5 mutation was found in 5/145 patients with melanoma, 4/232 patients with breast cancer, 3/246 patients with colorectal cancer, 2/49 patients with non-Hodgkin's lymphoma and in 1/183 patients with soft tissue sarcoma. No carriers were found among 164 patients with malignant tumors of the testis and only one carrier was identified among 388 patients with various gynecologic malignancies. The average age of carriers did not differ from the mean age of non-carriers with the same tumors. Of 11 carriers interviewed, 9 reported 1-4 cancers in 1-st and/or 2-nd degree relatives. One of the probands with melanoma had a first degree relative with melanoma, two probands reported lymphoma in a relative and one proband with breast cancer reported two cases of prostate cancer in the family. In addition 6 patients (3 colorectal tumors, 1 melanoma, 1 non-Hodgkin's lymphoma and 1 with sarcoma) were carriers of the R215W aminoacid exchange, which is believed to be a polymorphism. These results imply that heterozygous carriers of the 657del5 mutation may have indeed an enhanced risk to develop tumors, in particular melanoma and breast cancer, and perhaps also colorectal cancers and lymphomas. To asses the potential tumor supressor function of the NBS1 gene and hence the risk factor of the 657del5 mutation for the Slav population, tumor material of the heterozygous patients found here was further analysed for loss of heterozygosity.

P1-03 32

Genomic heterogeneity in advanced head and neck cancer detected by comparative genomic hybridization

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Although the knowledge of genetic changes during carcinogenesis of head and neck squamous cell carcinomas (HNSSC) has enlarged during the last decade, very little is known about the extent of intratumoral genomic heterogeneity. Comparative genomic hybridization (CGH) provides an excellent tool to gain more detailed insight in genomic differences between multiple biopsies from advanced HNSSC. Therefore, 79 HNSSC samples stage III and IV from 35 patients were investigated for DNA copy number changes. In 32 cases, the biopsies were taken from the primary tumor and a corresponding ipsilateral lymph node metastasis. In 6 cases, additional biopsies came from the primary tumor and in 6 cases, contralateral lymph nodes were analysed. In both primaries and metastases the most frequent gains of chromosome material were 3q, 11q, X, 19, 5p, 8q, 1q, 7q, and 17. The most frequent losses affected chromosomes 3p, 9p, Y, 18q, and 8p. There was a slightly higher mean total number of aberrations per tumor in primaries (11.6) compared to metastases (10.2). Losses were 1.5 times more frequently detected in primary tumors than in metastases. Between primaries and metastases a more extensive heterogeneity (average discordance 32.8%) was found compared to multiple synchronous metastases (26.5%) or within primaries (24.3%)

P1-03 33

Keimbahnveränderungen in beiden BRCA-Genen bei Ratsuchenden aus Hochrisikofamilien mit Mamma- und Ovarialkarzinomen

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Weltweit wurden nur wenige Familien publiziert, in denen in beiden BRCA-Genen Mutationen auftreten und darunter nur wenige Mitglieder, die Träger dieser beiden Mutationen sind. Die meisten Familien gehören der Gruppe der Ashkenasi Juden an, in denen die drei Gründermutationen 185delAG und 5382insC in BRCA1 und 6174 delT in BRCA2 relativ häufig vorkommen. Eine Möglichkeit für die geringe Detektionsrate in nicht Ashkenasi-Familien kann darin bestehen, dass die Stammbäume nicht gründlich genug erhoben und nach Entdeckung einer BRCA1-Mutation die Analyse nicht fortgeführt wurde. Im Rahmen unserer Analyse bei Mitgliedern aus Hochrisikofamilien für hereditäres Mamma- und Ovarialkarzinom fanden sich nun zwei Familien, in denen Mutationen in beiden Genen auftreten. In einer Familie wurde die 185delAG in BRCA1 und die 5950delCT Mutation in BRCA2 und in der anderen Familie die 1081G>A Trp321Stop in BRCA1 und die 2459C>G Ser744Stop in BRCA2 nachgewiesen. Bei der ersten Familie trat die BRCA2-Mutation in der väterlichen (nur Mammakarzinome) und die BRCA1-Mutation in der mütterlichen (Mamma- und Ovarialkarzinome) Linie auf. In der zweiten Familie traten alle berichteten Mammakarzinomfälle nur in der mütterlichen Linie auf. In beiden Familien gibt es eine Patientin, die Mutationen in beiden BRCA-Genen trägt und in einer Familie noch einen männlichen nicht erkrankten verwandten mit Mutationen in beiden Genen. Die anderen Mutationsträger in den beiden Familien haben entweder in dem einen oder anderen Gen die Veränderung. Im Gegensatz zu den bisher publizierten Fällen zeigen in diesen Familien die Doppelmutationsträgerinnen einen früheren Erkrankungszeitpunkt und einen schwereren Krankheitsverlauf als die alleinigen BRCA2-Mutationsträgerinnen. Insbesondere kann in der ersten Familie gezeigt werden, dass die Mutationen in den einzelnen Anlageträgern zu unterschiedlicher Expression führen und die BRCA2 Mutationen mit einem späteren Erkrankungsalter einher gehen. Die Daten zeigen, dass BRCA1- und BRCA2-Mutationen in nicht Ashkenasi-Familien häufiger als bisher angenommen auftreten können und deshalb eine gründliche Stammbaumerhebung unabdingbar ist. Sollte der Stammbaum Hinweise ergeben, dass in beiden Linien mit BRCA-Veränderungen assoziierte Tumore auftraten, sollte nach Detektion einer BRCA1-Mutation die Analyse des BRCA2-Gens ebenfalls durchgeführt werden. Die unterschiedliche Expressivität der einzelnen BRCA-Mutationen in diesen und weiteren Familien aus unserem Beratungskollektiv stützt die Beobachtung, dass sich, im Gegensatz zu einer BRCA1-Mutation, die Mammakarzinome, die auf eine BRCA2-Mutation zurückzuführen sind kaum von den sporadischen Karzinomen unterscheiden.

P1-03 34

DNA bank of familial adenomatous polyposis and mutation spectrum of APC gene in Polish population

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Familial adenomatous polyposis (FAP) is inherited as autosomal dominant predisposition to initiate numerous polyps in colon that lead to development of colorectal carcinoma. FAP is caused by heredited or germ line mutations in APC gene. Early recognition of mutation carriers is very important for medical treatment of persons from high-risk group. DNA bank for Polish FAP patients at the Institute of Human Genetics in Poznan was established in 1996. Central registry of genetic data prevents from double analysis of mutations for the same families living in different regions of country. Performing genetic tests in single laboratory covering large part of Poland may lead to significant reduction of the analysis costs due to lower investment in laboratory equipment and analytical tools. DNA samples from 233 persons affected with FAP and 141 their family members belonging to 145 FAP families were collected. In these group 212 cases with typical FAP, 11 cases of attenuated FAP and 10 cases of Gardner syndrome were collected and evaluated. All available DNA samples from FAP patients and their family members were isolated from peripheral blood. FAP patients and families come from North-West, Central, South-West and South-East regions of Poland. Institute of Human Genetics cooperates with clinics of medical schools in Gdansk, Cracow, Poznan, Szczecin and Wroclaw. Molecular diagnostics of collected DNA samples was focused on APC gene and involved PCR-HD, PCR-SSCP, PTT and DDF methods. Genetic testing involved APC gene fragments encompassing exons 5, 8, 11, and 3'-end of exon 15. The study allowed us to establish distribution of mutation of APC gene in Polish population and identify mutations in 38% of Polish FAP families.

P1-03 35

Complex karyotypes in two cases of gastrointestinal stromal tumors

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Gastrointestinal stromal tumors (GIST) constitute the most common mesenchymal group of tumors of the gastrointestinal tract. Cytogenetic studies of this entity are rare, and only two molecular cytogenetic studies using different chromosome-specific probes have been conducted. We performed detailed karyotypic analysis of two malignant GISTs (case 1: f/80; 30mitoses/50HPF; 22x16x8cm, liver metastases; case 2: m/60; 170 mitoses/50HPF; 30x45 x15cm). Both tumors were CD117-positive immunohistochemically. Mutational analysis of c-kit gene detected a 6-bp insertion in exon 9 in the first case, whereas the second GIST had a duplication of codons 574-586 in exon 11.
Both GISTs revealed losses of chromosomes 14, 15, and 22, alterations, which are commonly reported in GISTs. Formation of a dicentric chromosome involving chromosome 1 was another feature in common to case 1 and 2. In case 1 a stable dicentric chromosome (1;15) was characteristic. In case 2 the rearrangement of chromosome 1 involved a different acrocentric, presumably chromosome 14. In addition, a second dicentric chromosome - dic(6;11) - was remarkable due to its high variability within the fusion region. Supernumary small variable rings and an obligatory marker chromosome indicate a more complex and heterogeneous karyotype than in case 1. By use of whole chromosome painting and centromer probes the composition of the marker chromosome was shown to contain parts of chromosomes 15 and 22 and a piece of the rearranged chromosome 6. Exclusive partial monosomy of chromosomes 15 and 22 represents a novel observation in GISTs.
These results indicate that chromosomal rearrangements in malignant GISTs are much more complex than assumed to date. Increased complexity of the karyotype in case 1 may reflect an extraordinarily high tumor growth.

P1-03 36

The breakpoints of the benign thyroid tumors with 2p21 aberrations cluster to a region of less than 450 kb

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Cytogenetic studies of about 450 hyperplasias and adenomas have been reported in the literature. Cytogenetic aberrations occurred in about 20% of these lesions (Belge et al., 1998) and

were divided in different subgroups. One of these subgroups is characterized by structural rearrangements involving the chromosomal band 2p21. To narrow down the breakpoints of these aberrations, we established two cell lines from benign thyroid tumors showing translocations involving 2p21. These two cell lines and one additional primary tumor were used for FISH-studies with 18 BAC-clones derived from band 2p21. The results show that the breakpoints of these tumors map within a segment of about 450 kb flanked by the two BAC-clones 339H12 and 1069E24. As to known genes in the breakpoint region, a database search allowed us to identify the zinc finger gene ZFP36L2, a member of the TIS11 early response family, within BAC clone 339H12. This makes it a well-likely candidate gene for the 2p21 aberrations seen in benign thyroid tumors.

P1-03 37

An infrequent haplotype of the PCTA-1 gene, located in the susceptibility region 1q42.2-43 (PCaP), indicates linkage and association to prostate cancer

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Prostate cancer (PCa) is a complex disease with various genetic factors involved in its etiology. While familial aggregation of PCa, observed in about 10% of all affecteds, could be best explained by the cosegregation of rare high risk mutations, common gene variants may effect sporadic disease risk with a low penetrance contribution. Within the susceptibility region PCaP (1q42.2-43), expected to harbour a high risk gene predominant in the population of South-West Europe, we characterized the candidate gene locus of the prostate carcinoma tumor antigen-1 (PCTA-1) by SNP genotyping and identified five gene haplotypes coding for four different PCTA-1 proteins. The rarest of these variants, which we termed haplotype C2, was associated with both sporadic and familial PCa. In a population based association study involving 265 controls and 263 cases, heterozygous carriers of the C2 haplotype carried a relative risk of 2,9 (CI: 1,39-6,13) in sporadic cases and 3,6 (CI: 1,30-9,83) in familial index patients. In order to elicit transmission disequilibrium of haplotype C2 in high risk prostate cancer, familial based association tests were in performed 57 prostate cancer pedigrees, revealing an extent of cosegregation exerted by haplotype C2. Parametric linkage analyses indicated no linkage to the PCaP region in the total set of families, but a LOD score of 1,6 was obtained from the seven pedigrees with a C2-haplotype carrier as index patient. These positive association and linkage results concerning haplotype C2 may be due to the conspicuous allele itself, because it codes for a unique PCTA-1 protein, or could reflect linkage disequilibrium to a neighbouring gene predisposing for prostate cancer.

P1-03 38

Evaluation of automated MNT by computer based image analysis

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The micronucleus assay (MNT) in human lymphocytes is a frequently used tool to assess chromosomal damage or instability. It has been suggested to discriminate between cancer patients and controls. In order to eliminate interobserver differences from MN scoring we started to establish an automated scoring procedure for the cytokinesis blocked MNT based on computerized image analysis (Metafer 4 vers 2.12 by Metasystems).
The evaluation was based on slides of blood samples after irradiation with gamma-rays with 2 Gy. The slides were stained with DAPI and counted automatically. Our sample consisted of 26 (13 patients with sporadic breast cancer and 13 age matched controls) whom we compared to 328 counts by human observers in order to obtain information on the origin of interobserver variability.For automated analysis two classifiers with different symmetry criteria were used to identify binucleated cells.The mean values of the human observers (range:284-320) and the two classifiers (102-106) had a large variation (coefficient variation: 0.3)and they varied concerning their „discriminative“ power. Classifier 1 showed a clear-cut differentiation between carcinomas and controls (logistic regression: OR:13.16,p-value:0.0029), whereas classifier 2 did not. We therefore assume that automated scoring has the advantage of invariant standards which allow reproducible results whereas human observers may vary in their scoring criteria and as a result end up with different numbers in MN scoring. The variation between human observers which impairs any use of the MNT seems rather due to the identification of binucleated cells than on the definition of micronuclei.

P1-03 39

First results on the use of the G0 Micronucleus Test to detect cancer patients.

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There is a considerable number of studies demonstrating a difference in micronucleus induction and other mutagenicity tests between controls and a variety of cancers. However, the results of these studies are frequently conflicting (Berwick and Vineis). Since the G0 Micronucleus Test (MNT) has been shown to detect BRCA 1 and -2 mutation carriers with high efficiency we decided to look whether this test can be used for sporadic breast cancer and prostate cancer as well.
The blood samples where either used directly or irradiated with 2 Gray at the beginning of the culture and the MNT was performed using cythocalasin B. The slides were stained with Giemsa and counted in the microscope by different observers.
Our female sample consisted of 11 sporadic breast cancer patients and 10 controls (mean age 45,6, range 24-81) and the sample of male patients included 9 sporadic prostate cancers and 10 controls (mean age 54, range 27-74).
The breast cancer patients could be identified by higher micronucleus counts compared to controls in non-irradiated and irradiated cells (logistic regresion OR:10.6, p<0.02). The prostate cancer patients did not differ from the controls in the MN counts of irradiated cells but were almost completely separated when looking for the spontaneous MN-frecuency (contingency table chi2-Test:13.38, p>0.0003; due to the separation of the groups no OR could be calculated).
Our results are too preliminar to draw a conclusion out of the conunted slides, but demonstrate that it may be promising to evaluate further the G0 Micronucleus Test and to see whether or not it is suitable for predictive testing.

P1-03 40

Characterization of chromosomal aberrations in 23 diffuse astrocytoma using cytogenetic and molecularcytogenetic methods

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Informations on combined cyto- and molecular-cytogenetic analyses of astrocytomas are rare.
We analyzed 337 metaphases of 23 primary cell cultures (5 astrocytomas WHO grade II, 2 astrocytomas WHO grade III, 16 glioblastomas) using classical karyotyping (CK), additionally spectral karyotyping (SKY) was performed in two, multicolor- FISH (m-FISH) in three and Comparative Genomic Hybridization (CGH) in eight of the cell cultures.
We found 155 structural aberrations with highest frequency on regions 2p21->p22 and 7q32 (CK).
The following translocations were identified using CK and SKY: t(1;10)(p32;q32); t(2;13)(p21;q22), t(2;17)(?;?), t(2;17)(p23;p11), t(3;5)(q29;q33), t(7;8)(q10;p10), t(8;9)(q11;q12), t(8;12)(q11;p12), t(12;20)(p13;q13), t(15;21)(p10;q10). The majority of these translocations are reciprocal and the t(3;5)(q29;q33) occured in some metaphases as a balanced change. Some small intrachromosomal aberrations could only be specified by CK.
Conclusions: The broad spectrum of aberrations encountered in astrocytomas requires a combined approach. We were able to identify a sufficient number of high quality metaphases in spite of in several studies described difficulties. To our knowledge most of the identified translocations and the aberrations on 2p21->p22 are described for the first time in astrocytomas.

P1-03 41

Protein Profiling of Head and Neck Cancer using protein chip arrays and SELDI technology

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The analysis of the proteome is a possibility to monitor specific changes in physiological different tissues. The surface-enhanced laser desorption/ionisation – mass spectroscopy technology (SELDI-MS) is a new tool to investigate particularly these differences by using protein chip technology. In this respect we compared protein profiles of head and neck cancer (HNC) to normal tissue. In the present study we examined fifteen normal and seventeen HNC tissues, respectively. The protein samples of both tissue species were generated and separated using laser capture microscopy (LCM) and studied accordingly concerning signal constitution and intensity. Rough data were subjected to a evaluation by the Ciphergen Biomarker Wizard software. Differences in signal intensity could be detected. Namely, sample deduced from HNC tissue showed peaks that were overexpressed, compared to the same signal in normal tissue. These peaks could be mainly found in the range of 3 to 10 kDa. A proper identification of these absorbing peaks as well as a further analysis of the data with bioinformatic tools is in progress. The here described technology represents a powerful feasibility for the discovery of specific signals in unregulated proliferating cells as well as in cancer.

P1-03 42

FISH-analysis detects amplification and deletion of the NBS1-gene in tumor samples from NBS-heterozygotes

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There is still the open question, whether the NBS1-gene has a tumor-supressor gene (TSG) function. Recently, we could demonstrate that NBS1 gene deletions are not a major cause or primary event in tumorigenesis of human B- and T-cell non-Hodgkin lymphomas (NHL) (Stumm et al., 2001). These results were in line with the findings of Stanulla et al. (2000), who did not found NBS1 mutations in NHL of childhood and aldolescence. In contrast, Varon et al. (Cancer Res 2001) detected missense-mutations in the NBS1-gene in childhood acute lymphoblastic leukemia (ALL) patients. These latest results led us to perform further studies wether NBS acts as TSG. For this purpose we have investigated by FISH analysis 9 tumor samples (5 colorectal tumors, 2 melanomas, 1 Hodgkin`s lymphoma, 1 neuroblastoma metastases) of NBS-heterozygotes from Poland and Czech Republic (see also abstract from R. Varon). Interphase FISH was performed on isolated nuclei from parafin embedded tumor samples, by means of a Cy3-labeled BAC-probe (BAC159I23), containing the whole NBS1 gene region. A FITC-labeled BAC-probe flanking the NBS1-region and a chromosome 8 centromeric probe were hybridised as controls. Three samples failed, because they did not contain tumor cells or there were not enough nuclei for FISH analysis. Three tumor samples (1 colorectal tumor, 1 melanoma, 1 Hodgkin`s lymphoma) showed a deletion of the NBS1 gene in about 20% of the cells due to a monosomy 8. The Hodgkin`s lymphoma showed aditionally a homozygous deletion of the NBS1 gene in about 45% of the cells. Three tumors (1 colorectal tumor, 1 melanoma, 1 neuroblastoma metastases) demonstrated an amplification of the NBS1 gene due to a trisomy to pentasomy of chromosome 8. These results suggest a „second hit" causing a dominant negative effect of the mutant NBS1 allele or loss of the NBS1 wild-type allele, as possible mechanisms for tumorigenesis in NBS-heterozygotes. CGH analyses and moleular analyses are in progress to get additional information about the mechanisms gtes.

P1-03 43

Expression analysis of mt101, a new tumor suppressor candidate gene, in sporadic breast cancer

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A candidate for a novel tumor suppressor gene, called mt101, has been identified as differentially expressed in breast carcinomas by analyzing four million ESTs with software tools developed at metaGen Pharmaceuticals. The candidate gene, mt101, is located on chromosome 5q34. This region is often deleted in patients with invasive breast carcinomas. (Richard et al., 2000). LoH (loss of heterozygosity) analysis of 32 malignant ovarian germ cell tumors revealed a deletion in 5q34 region in 46% of the tumors (Faulkner, 2000). Here we present expression analysis of mt101 on RNA level.
Mt101 encodes a transmembrane protein that is expressed in the hippocampus and in several non-neuronal tissues including the lung, stomach, uterus, ovaries and in the epithelial cells of mammary gland. The mRNA is 3,3kb in size and is specifically down-regulated in breast tumors. Northern blot hybridization on cancer profiling array (Clontech) showed in 75% of mammary carcinomas a more than two fold down-regulation of mt101. Real Time PCR analysis on 6 matching patient pairs (normal and tumor tissue) and 10 tumor samples with invasive mammary carcinoma confirmed the significant down-regulation in breast tumors.
We further examined the expression and localization of mt101 transcript by RNA in situ hybridization. A strong expression was observed in normal epithelial breast cells and in benign papilloma epithelial cells but no signal could be detected in invasive ductal carcinoma.
Future experiments will include antibody design and analysis of the protein expression pattern in breast tumors. The anti-proliferative potential of mt101 will be evaluated after forced expression in established human tumor cell lines. Additional functional studies of mt101 using cell culture assays will be performed.
The presented study is a research project within the gynecological cancer consortium (GCC).

P1-03 44

A regulator of the Wnt signaling pathway is strongly down-regulated on RNA and protein level in breast cancer

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Candidates for novel tumor suppressor genes have been identified as differentially expressed in gynecological carcinomas by analyzing four million ESTs with software tools developed at metaGen Pharmaceuticals. The selected genes are validated on the RNA and protein level to confirm the in silico expression data.
One of the candidate genes, secreted Frizzled related protein 1 (SFRP1), encodes a regulator of the Wnt pathway. The dysregulation of the Wnt signaling pathway is known to play a role in cancer development. Among the target genes of this pathway are the known oncogenes c-myc and cyclinD1. SFRP1 is abundantly expressed on the RNA level in many human tissues, the strongest expression is observed in heart, kidney and mammary gland. The chromosomal localization of SFRP1 on chromosome 8p12 is a site of frequent alterations in various tumors including sporadic breast carcinoma. The SFRP1 transcript is specifically down-regulated in breast tumors. Hybridization of RNA from microdissected normal and tumor tissues to a custom designed Affymetrix chip revealed a two-fold down-regulation of SFRP1 in 72% of the examined breast carcinomas. To investigate the protein expression pattern of SFRP1 in breast cancer, we characterized an SFRP1-specific antibody. A down-regulation on the protein level was observed in breast tumor cell lines. Protein expression of SFRP1 was analyzed by immunohistochemistry on paraffin sections of 83 breast tumors. Strong immunostaining is detected in epithelial, stromal and endothelial cells of normal mammary tissue. We observed a loss or reduction of SFRP1 expression in 73% of the examined breast carcinomas. Future experiments will include functional studies to evaluate the anti-proliferative potential of SFRP1 after forced expression in established human tumor cell lines.
This study is a research project within the gynecological cancer consortium.

P1-03 45

Rare MSH2 and MLH1 missense variants are a frequent finding in HNPCC patients, but should be treated with reserve in predictive testing

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Identification of germline mutations in HNPCC patients is of major importance for predictive testing in family members at risk. From a sample of 569 unrelated index patients 169 meet the Amsterdam criteria for HNPCC (AC+), 400 meet

other Bethesda criteria for HNPCC. Tumour tissue was available from 115 AC+ patients and from 277 AC negative patients. High microsatellite instability (MSI-H) was found in 92 (80%) of the tumours from AC+ patients and in 125 (45%) of tumours from AC negative patients, 10 tumour had low microsatellite instability (MSI-L). Screening for germline mutations in the MSH2 and MLH1 gene was performed in 227 patients with MSI-H or MSI-L tumours and 21 AC+ patients, of whom no tumour tissue was available. We found 101 definitely pathogenic germline mutations (61 mutations in MSH2, 40 in MLH1). The mutation MSH2,c.942+3A>T was found in 9 unrelated index patients and represents a hot spot. 38 rare missense variants (18 in MSH2 and 20 in MLH1) and 8 intronic variants of unknown relevance were detected. In some families segregation of a variant with HNPCC tumours could be demonstrated, and in most of these cases no other mutation could be identified; immunohistochemistry data point towards a mutation in the gene harbouring the variant. On the other hand, the MLH1 variant T682I was identified in a patient, who was later found to carry a MLH1 nonsense mutation as well. The MLH1 variant Q701H apparently segregates with the HNPCC phenotype in two generations, but not in the youngest generation. These observations demonstrate, that before offering genetic testing for persons at risk to families with unclear variants, very careful evaluation of these variants has to be undertaken.

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P1-03 46

Comparative analysis of serum proteins from patients with Renal Cell Carcinoma (RCC) and normal controls using protein chip arrays and SELDI technology

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The common aim in molecular analysis of malignant tumors is to find specific markers which allow an early diagnosis, the monitoring of patients after primary therapy and the optimization of individual therapy regimes. In renal cell carcinoma (RCC), 30 % of patients had already developed metastases at the time of primary diagnosis. Since 40 to 50 % of patients develop metastases in course of disease, a precise prediction of the metastatic potential of primary tumors by reliable markers may help selecting patients for adjuvant therapies. In this context we tried to establish a serum cancer assay to detect protein markers in serum specific to RCC. As an alternative to 2-DE in this preliminary study a new technique was used to generate protein expression patterns from serum of patients with RCC and controls. Surface-enhanced laser desorption and ionization (SELDI) allows the retention of proteins on a solid-phase chromatographic surface (ProteinChip Array) with direct detection of retained proteins by time-of-flight mass spectrometry (TOF-MS). Up to now we analyzed blood sera from ten patients with RCC and ten controls. The serum mixed with a special binding buffer was directly applied to different chip surfaces (anion/cation exchanger, hydrophobic) and analyzed by the SELDI system. Differences between controls and RCC patients were explored by cluster analysis. Differentially expressed proteins were found by comparing the serum proteins from controls and patients with RCC. Further examinations will comprise screening of a valid number of cases including the comparison of metastatic and non-metastatic RCC. It can be concluded that applying this fast and powerful ProteinChip array technology it becomes possible to find tumor specific markers in serum.

P1-03 47

BCR/ABL D-FISH should be a mandatory examination for primary diagnosis of CML

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Imatinib and Interferon-alpha alone or in combination with cytostatic drugs can induce major and durable cytogenetic responses in chronic myelogenous leukaemia (CML) patients. Since these patients have a significant survival benefit, frequent follow up investigations have become clinically important. It has been shown by Duba et al. [Int J Oncol 17; 1245-1249; 2000] that fluorescence in situ hybridisation (FISH) strategies reveal results comparable with conventional cytogenetics. In a recent report Huntly et al. [Blood, 98, 1732-1738, 2001] have demonstrated that a highly sensitive BCR/ABL D-FISH probe detects deletions of ABL and BCR sequences on the derivative chromosome 9. These deletions provide a powerful and independent prognostic indicator in CML. We have therefore performed FISH with the highly sensitive LSI® BCR/ABL Dual Color, Dual Fusion Translocation Probe probe from Vysis in CML patients treated with Interferon-alpha and YNK1 (n=147), Imatinib (n=22) and on archived cell suspensions from CML patients at first diagnosis (n=22). We have detected deletions in 12% (22/191) of patients. A BCR/ABL sensitive D-FISH for detection of the Philadelphia chromosome and deletions of the derivative chromosome 9 should therefore be incorporated into future diagnostic strategies as well as management decisions of CML.

P1-03 48

Linkage of aggressive prostate cancer to chromosome 7q31-33 in German prostate cancer families

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Chromosome 7q32 has been suggested to contain genes that influence the progression of prostate cancer from latent to invasive disease. In an attempt to confirm linkage to prostate cancer aggressiveness 108 German prostate cancer families were genotyped using a panel of 8 polymorphic markers on chromosome 7q. We used a multipoint allele sharing method based upon a likelihood ratio test implemented in GENEHUNTERPLUS v1.2 in order to calculate the nonparametric Zlr and the associated LOD scores.

We applied the aggressiveness of prostate cancer given by the pathological tumor grade of each individual and the mean age of onset of a family as covariates and constructed two weighted models. The first (weight0-1 model) puts weights on families with at least two cases of GIII prostate cancer. The second (weight 0-2 model) also adds weights to families with early and late onset cancer respectively. The unweighted analysis gave no evidence of linkage to chromosome 7q. The Zlr scores increased when including the covariates, to 2.60 (p=0.005) using the weight0-1 and to 3.02 (p=0.001) using the weight 0-2 model for late onset prostate cancer. The associated LOD scores were respectively 1.47 (p=0.009) and 1.98 (p=0.002). The markers that gave most evidence for linkage were exactly in the range of the published prostate cancer aggressiveness region. Our results support a widespread relevance of this locus and suggest that aggressive and late onset prostate cancer is linked to chromosome 7q31-33 in the German population.

P1-03 49

Mutation and expression analysis of TRAIL-receptors in human breast cancer

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The chromosome region 8p12-p22 shows frequent allelic loss in a variety of human malignancies, including breast cancer (BC). The tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) receptors TRAIL-R1, -R2, -R3 and -R4 are located on 8p21-p22 and might be candidate tumor suppressor genes in this region.

We studied the mRNA expression of the four receptors in a group of BC cell lines and tumors. In most of the cell lines the expression of the TRAIL receptors was reduced. In cancer tissue a 1.7-, 1.2-, 3.6-, and 3.5-fold reduction of TRAIL-R1, -R2, -R3, -R4 mRNA levels was observed when all cancer tissues were compared with the corresponding normal samples.

To understand the tumor-specific down-regulation of TRAIL receptors we have analyzed the entire coding region of TRAIL-R2 and the death domain regions of TRAIL-R1 and -R4 for the detection of mutations in a series of breast tumors and BC cell lines, lymph node metastases and patients with a family history of BC. Although functional studies have not yet performed we assume that most of the alterations found do not alter the function of TRAIL-receptors and, consequently, do not play a significant role in BC. Taken together, our mutation studies indicate that death domain receptor mutations occur at a low frequency and are not the primary cause for the altered mRNA expression of the TRAIL-receptors in BC cell lines and breast tumors.

P1-03 50

Quantitative expression of p16INK4a and p19ARF in blood leukocytes of patients with chronic myelogenous leukaemia (CML)

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Recent studies revealed the CDK-I p16INK4a and p19ARF, potent blockers of p53-inactivation, as important inhibitors of cell cycle progression. The p16 gene encoding both proteins is located on chromosome 9p21. In many solid tumours both cell cycle inhibitors are inactivated. The primary aim of this work is to find a correlation between p16INK4a/p19ARF expression pattern and disease progression in CML as it could be shown in many other tumours. Valid quantification results are obtainable using real time PCR for amplification of p16-mRNA. For our experiments we used the LightCycler instrument with the SYBRgreen detection system and examined blood cell derived cDNA of about 60 patients in different leukaemic stages. 13 samples derived from chronic phases with tendency of progression, 26 showed no progression, 9 were cytogenetically in remission and 10 were staged as blast crisis. Because specimen spanned a wide range of mRNA concentration we decided to use crossing points (CroP; calculated cycle number, at which a critical fluorescence level is reached) as an indirect value of concentration. p16 expression level was determined as a ratio of crossing point of sample to crossing point of house keeping gen. We found different levels of p16INK4a and p19 ARF -expression in various phases of CML. To correlate distinct expression values further data analyses with different CML stages or clinical outcome are in progress. The median survival of CML patients varies significantly between individuals. Expression analysis of p16 and p19 may provide a new clinically relevant parameter for individual prognosis.

P1-03 51

Amplification of the Serine/Threonine kinase STK15 gene in low grade and high grade gliomas

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Searching for amplifications in low grade and high grade gliomas we observed an interesting correlation between the recurrence and progression of astrocytic low grade gliomas and the amplification of the STK15 gene located in the chromosomal region 20q13. Chromosome copy gains in this region have been reported in astrocytic gliomas and glioma cell lines before as well as in many cancer types including breast, colorectal and ovarian cancers. The serine/threonine kinase STK15 has been reported to be amplified and overexpressed in breast cancer cell lines and colorectal cancer. Another candidate gene located in this region is PTPN1, a protein tyrosine phosphatase non-receptor type 1 that might play a role in cell cyclus control. We used comparative PCR for quantitative DNA analysis to search for STK15 and PTPN1 amplification in gliomas previously characterized by CGH. Five out of 16 tumours (31%) of different grade (1x II, 1x III, 3x IV) showed low-level DNA amplification of STK15 whereas we could not detect amplification of PTPN1. We hypothesize that amplification of the STK15 gene may be a nonrandom genetic alteration in human gliomas playing a role in the genetic pathways of tumourigenesis.

P1-03 52

Interaction of chromosome 20p11.2-12 and Xq27-28 in German prostate cancer families

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Chromosome 20q13 has been suggested to harbour a prostate cancer (PC) susceptibility locus in families that were characterized by a low number of affected familiy members, late onset disease and no male-to-male transmission. As this epidemiologic profile is characteristic for many German PC families we performed linkage analysis using 9 markers on chromosme 20. There was no evidence for linkage in the whole sample of 108 PC families and in the subsets with mean age of onset < or > 66 or the number of affecteds/family < or > 3 as criteria for stratification. In a previous study we identified a subset of families that were linked to chromosome Xq27-28 (NPL > 0) but at the same time had an affected individual in the paternal line of their pedigree. In this subset with the conflicting characteristics of male-to-male transmission and X chromosomal allele sharing, two point parametric linkage analysis (Genhunter 1.3) showed a maximum LOD score of 3.47 at D20S112. We correlated the nonparametric NPL Z scores of the two loci using the Kong and Cox allele sharing model (ASM) implemented in Genehunterplus v1.2. We observed an interaction of chromosomes 20p11.2-12 and Xq27-28 in pedigrees whith male-to-male-transmission and early onset disease ($p<0.001$).

P1-03 53

Comparative analysis of proteins in urine from patients with bladder carcinoma and normal controls using protein chip arrays and SELDI technology

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The development of non-invasive methods for the diagnosis of transitional cell carcinoma (TCC) of the bladder is required, because 70 % of the cases show relapse in the first two years. Therefore, the aim is to find specific markers, which allow an early diagnosis, the monitoring of patients and the optimization of individual therapy regimes. Consequently, we tried to establish an assay to detect protein markers in urine specific to TCC. As an alternative to 2-DE a new technique was used in this preliminary study to generate protein expression patterns from urine of patients with TCC and controls. Surface-enhanced laser desorption and ionization (SELDI) allows the retention of proteins on a solid-phase chromatographic surface (ProteinChip Array) with direct detection of retained proteins by time-of-flight mass spectrometry (TOF-MS). Up to now we analyzed free protein in urine from eight patients with TCC and eight controls. For that purpose the urine was centrifugated to remove cells, supernatant was mixed with binding buffer, then directly applied to strong anion exchanger chip surfaces and analyzed by the SELDI system. Differences between controls and TCC patients were explored by cluster analysis. Four differentially expressed proteins were found by comparing the urine proteins from controls and patients with RCC. Especially one cluster with about 17 kDa displayed a high significance. Further examinations will comprise screening of a valid number of cases and the identification of differentially expressed peaks. It can be concluded that due to the application of this fast and powerful ProteinChip array technology tumor specific markers could be also detected in urine.

P1-03 54

Different amplification pattern of the STK15 gene in cancer cells

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Gene amplification is found in many tumor cell types and is thought to be associated with tumor progression. Amplification of STK15, a centrosomal serine/threonine kinase located at 20q13, is particularly interesting because this aberration is commonly detected in breast cancer and correlates with poor prognosis.
In previous investigations we detected an amplicon at 20q in gliomas by comparative genomic hybridization. The aim of our study was to detect STK15 amplified cells, especially their frequency and the delineation of the amplification.
We examined the breast tumor cell line MCF7 and three primary cell cultures of gliomas (T5135, T3868, TX3868) by using simultaneous fluorescence in situ hybridization (FISH) with the centromeric probe for chromosome 20 and a STK15 gene specific probe.
We found in 50% of the MCF7 cells increased copy numbers of the STK15 gene in comparison to the signal numbers of the centromeric probe. Surprisingly in 29% of the T5135 glioma cells we observed a cytogenetic visible high level amplification of the STK15 gene. T3868 and TX3868 cells did not show an increase of STK15 signals. Interestingly we found two complete different pattern of the copy number gain of the STK15 gene. In the MCF7 cells we could detect additional distinct single signals, in contrast to the glioma cells, where we saw an expanded amplification signal. These different pattern of STK15 gene copy gain may implicate different pathways of tumor cell development, which have to be proved in further investigations.

P1-03 55

Germline mutations in both BRCA genes found in members of two high risk families with hereditary breast and ovarian cancer

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To date, only a few families have been reported with two mutations in the BRCA genes and also individuals with two mutations. Most of the reported families belonged to the Ashkenazi Jewish population were three founder mutations, 185delAG and 5382insC in BRCA1 and 6174delT in BRCA2 occur relative frequently. One reason of the low detection rate in non-Ashkenasi families could be a not extensive evaluated pedigree and a termination of analysis after the detection of a deleterious mutation in the BRCA1 gene. During the mutation analysis on members of hereditary breast and ovarian cancer families we found two families with the presence of two mutations, one in BRCA1 and the second in BRCA2. The alterations in the first family were 185delAG in BRCA1 and 5950delCT in BRCA2 and in the second family 1081G>A W321X in BRCA1 and 12459C>G S744X in BRCA2. The BRCA2 mutation in the first family was detectable only in the paternal branch (only breast cancer cases) whereas the BRCA1 mutation segregated from the maternal branch (breast and ovarian cancer cases). In both families double heterozygote affected females and one unaffected double heterozygote male were observed. Other affected and unaffected family members harbored the mutation either of BRCA1 or BRCA2. In contrast to the up to now reported cases the affected double heterozygote patients displayed an earlier onset and severe progression of disease than the members with the solely BRCA2 mutation. In particular in the first family further analysis revealed differential expression of the BRCA2 mutation especially a later onset of breast cancer. Thus previous reports and the present study emphasize the fact that the presence of two independent mutations sepsiegating in the same family may not be infrequent outside the Ashkenazi Jewish community. It is important to obtain an accurate family history in the context of genetic testing. 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. Without the knowledge of the second mutation in these families, many assymptomatic individuals would have been given a negative test result and be falsely reassured. The variable expression of the different BRCA mutations in the reported and other families of our cohort support the evidence that in contrast to breast cancers due to BRCA1 alterations tumors attributable to BRCA2 mutations are barely distinguishable from sporadic cases.

P1-03 56

Denaturing High Performance Liquid Chromatography (DHPLC) in Screening for Mutations in the APC (Adenomatous Polyposis Coli) Gene

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FAP (OMIM: *175100, McKusick 1986) is a rare form of hereditary colorectal cancer. Germline mutations of the APC gene were reported in patients with Familial Adenomatous Polyposis (FAP). Inactivation of the APC tumor suppressor gene plays a significant role in the development of early onset colon cancer based on a polyposis of the colorectum. The location of germline mutations in the APC gene appears to correlate with the clinical phenotype (number of colorectal adenomas, concomitants like occurence of further adenomas in other digestive organs, desmoid tumors, osteomas, thyroid cancer and Congenital Hypertrophy of the Retinal Pigmental Epithel [CHRPE]). The APC gene encodes for 2843 amino acids and more than 800 mutations are described. To provide a fast mutation screening we analyzed the APC gene by using DHPLC (Denaturing High Performance Liquid Chromatography) mutation analysis followed by automated sequencing of suspicious fragments. We investigated the genomic DNA of 11 patients with a clinical diagnosis of FAP. 16 different sequence variations could be identified: 8 mutations and 8 polymorphisms. All mutations lead to a truncation of the predicted protein and have disease causing character. According to our results DHPLC is an efficient and fast screening method to identify mutations in the APC gene which can be applied to optimize further diagnostic and therapeutic strategies in families with hereditary colon cancer.

P1-03 57

Molecular-Cytogenetic Analysis of 38 Gastrointestinal MALT Lymphoma

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We are studying archival paraffin embedded tissues from 38 well documented cases of surgically resected primary gastrointestinal lymphomas of the mucosa associated lymphoid tissue (MALT) for the occurrence of different reported cytogenetic abnormalities. The aim is to correlate cytogenetic aberrations with the tumour grading, -staging and the clinical outcome, and to establish the prognostic value of the aberrations detected. The tumour samples included 15 classical marginal zone B-cell lymphomas (low grade MALT), 6 lymphomas with low and high grade component (mixed type), and 17 diffuse large B-cell lymphomas (high grade MALT). The lymphomas were staged according to the modified Ann Arbor system by Musshoff: 17 patients presented with stage EI, 19 with EII, and 2 with EIV. So far we tested for some of the most frequently reported genetic abnormalities, i.e. trisomies 3, 7, 12, 18, and translocations or aberrations including the MLT, IgH, BCL6, BCL10, p53, MYC locus by FISH on isolated nuclei preparations. Using centromere probes only trisomies 3 and 18 (but not 7 or 12) were found in 29% of the cases. The trisomies were most often associated with tumour stage EII, however, without prognostic value. Using a new 18q21 breakpoint flanking probe set for the MLT locus we were able to reliably detect the translocation t(11,18) on paraffin embedded material. The t(11;18) was found as the sole aberration in one low-grade MALT only. Interestingly a higher rate of trisomic patterns was found with this probe set as compared to the centromere 18 probe. Similar results were also obtained with a BCL6 probe set for 3q27. Additionally we observed an unexpected high rate of a pathological probe patterns at the IgH locus on 14q23 that does not seem to result from a MALT specific t(1;14). The results from the ongoing study will allow to evaluate the prognostic value of the detected aberrations.

P1-03 58

VHL2C Phenotype in a German von Hippel-Lindau Family with Concurrent VHL Germline Mutations P81S and L188V

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VHL germline mutations predispose to von Hippel-Lindau disease (VHL), a tumor syndrome involving multiple sites including eyes, cerebellum, spinal cord, kidney, adrenal gland, pancreas and epididymis. VHL is distinguished into type 1 disease without pheochromocytoma, and type 2 disease with pheochromocytoma. The VHL protein (pVHL) is a key player of a regulatory pathway of controlled protein degradation. Some VHL germline mutations show a preponderance for adrenal gland tumors. They are located at nucleotides affecting amino acid changes in the pVHL beta- or alpha - domain critical for protein - protein interaction. Phenotypically these mutations differ with respect to a low or high risk for additional renal cell carcinoma. Here we describe a familiy with two concurrent VHL germline mutations affecting both the pVHL beta and alpha - domain resulting in a pheochromocytoma only phenotype. Among 16 members of a VHL family 9 members were previously diagnosed for pheochromocytoma and identified to carry a nucleotide 775 C>G (L188V) germline mutation (Glavac et al. Hum Genet 98:271-280, 1996). Refined VHL mutation analysis including new exon 1 primers 1-3F and 1-3R, SSCP, DHPLC and sequencing analysis detected a 454 (C>T; P81S) mutation that co-segregated with the 775 (C>G; L188V) mutation in all patients. The concurrent P81S may influence the phenotypic consequences of VHL disease resulting in the rare pheochromocytoma only phenotype (VHL type 2C) of this family.

P1-03 59

Evidence of hidden monosomy 13 in a cohort of 32 multiple myeloma patients

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We analysed 32 multiple myeloma (MM) patients in part with complex cytogenetically visible aberrations not involving chromosome 13, for the presence of deletions in the chromosomal region 13q14 or monosomy 13. For the FISH analyses we used BACs from the critical region shown to be deleted in CLL covering the markers D13S663E, D13S1168, D13S1220, D13S319 and D13S272; parts of this region have also shown to be deleted in multiple myeloma patients. Close to this region, overlapping with D13S272, the BCMS gene was identified, which is discussed as a candidate tumor suppressor gene for B-CLL. The following 13q14 BACs were used: RPCI-11 236M15, RPCI-11 346I19, RPCI-11 34F20 and RPCI-11 480P3. We performed FISH on interphase nuclei of methanol/acetic acid fixed bone marrow samples using one 13q14 BAC indirectly labelled with digoxigenin together with a 13q telomeric probe indirectly labelled with biotin to prove the presence of monosomy 13. Hybridization of the probes was detected with anti-DIG-FITC and avidin-Cy3 respectively. Only bone marrow samples of the first aspiration were analysed, containing >20% plasma cells. We scored 300 nuclei for each probe set. Cut off values for all probes were determined on 4 control samples. We found monosomy 13 in 46,8% (15) of the patients. 7 of the patients had a poor prognosis and 6 of them showed monosomy 13. A deletion was found only in one patient, where the 4 BACs used, were absent. Further analysis of the extension of the deletion is in work. The results indicate a correlation of monosomy 13 with poor prognosis in MM. Statistical computation has to be done.

P1-03 60

Hereditary colorectal cancer: two different entities in regard of genetics and clinic

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The families described here are taken from a large cohort (254) of suspected HNPCC families. Here we report about the microsatellite analysis, the immunohistochemistry (IH), age of onset, tumorspectrum and tumorlocalisation of two different entities: First, 25 families with truncating mutations in hMLH1(H1) or hMSH2 (H2) and microsatellite instability in the corresponding tumors and second, 20 Amsterdam positive families without mutations in H1 or H2 and without MSI in the tumors. The 25 mutations described, include 14 different new mutations for H1 and H2 respectively. Regarding the Amsterdam I and II families only, the percentage of mutation positive families is 13/33. Additional 12 truncating mutations were found in families or patients fulfilling the Bethesda criterialncluding these families, the percentage of mutation positive families in the cohort of families with hereditary colorecatal cancer is 25/45. All tumors with truncating mutations were MSI-H whereas none of the tumors without mutation in H1 or H2 was MSI-H. IH however gave a protein expression for H1 in more than 10% of the tumor cells for two tumors caused by mutations in H1, one tumor without mutation showed a loss of expression for H1. The tumor with mutations in H1 or H2 were found in the whole colon, whereas tumors of the mutation negative group clustered in the rectum and sigma. H1 and H2 negative families show the highest relative percentage of colorectal tumors (79%), compared to families with mutations in H1(68%) and families with mutations in H2 (58%). Families with mutations in H2 show the most associated tumor diseases. The mean age of onset for colorectal cancer in the first group was 39 years versus 48 years in the second.
Of the families with a hereditary predisposition for colorectal cancer, only one third carry mutations in the DNA mismatch repair genes. The residual families most likely carry mutations in genes not involving the DNA repair system as the tumors are MSS. These results show that there are at least two entities of hereditary colorectal cancer and that these entities show different clinical features.

P1-03 61

A comparison of methods to determine the risk to develop breast cancer and to predict the BRCA1/2 mutation status

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The Deutsche Krebshilfe has granted a multicenter project to 12 German centres. The centres offer persons from breast and/or ovarian cancer families qualified genetic and medical counselling, participation in an intensified early cancer detection program, and provision of psychological support. Families who satisfy the inclusion criteria are offered genetic testing. At present more than 1000 families are under study; in about 1 out of 3 families a mutation in BRCA1 or BRCA2 has been detected (German Consortium for Hereditary Breast and Ovarian Cancer, Int. J. Cancer 97: 472-480, 2002).
One aim of this project is to develop criteria that better identify BRCA1 or BRCA2 positive families. A better identification of those families could allow a more selective referral for genetic counselling, intensified surveillance, and genetic testing.
As yet data of 1001 persons with BRCA1 and 731 persons with BRCA2 testing completed have been reported to the central register. The risk of breast and ovarian cancer was evaluated in the reported families using various instruments (Claus tables, MLINK as incorporated in Cyrillic 2.1, BRCAPro, Gail model). We also tested the accuracy to predict the mutation status using standard methods (MLINK, Couch, Shattuck-Eidens, Frank, BRCAPro).
The preliminary results demonstrate that the various methods produce quite different probabilities of becoming affected by breast or ovarian cancer. Also neither method could accurately predict whether or not a BRCA1/2-mutation is present.
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P1-03 62

Familial occurrence of low-grade astrocytomas

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Brain tumours like astrocytomas are most often sporadic diseases and show two age peaks in frequency, namely in childhood and in the sixth to seventh decade, respectively. Mortality due to brain tumours accounts for about three per cent of all tumours in men. In most cases, a multifactorial genesis is thought to be causative. Less often, brain tumours occur because of an inborn, genetic susceptibility to tumours of different tissues (e. g. Li-Fraumeni syndrome, Turcot syndrome) or as part of a syndrome with features in addition to tumour predisposition (e. g. the neurofibromatoses, tuberous sclerosis, and Cowden syndrome). We report on a family with four affected males diagnosed with low-grade astrocytomas but without any other symptoms suggesting a syndromic disorder. An astrocytoma was first observed in a 12-year-old boy, later in his brother at age 19 and his son at the age of 8 years. The second, today 56-year-old brother of the index patient has not suffered from any tumour, whereas his son recently developed an astrocytoma at age 22. The tumour of the latter patient can be analysed for chromosomal imbalances by comparative genomic hybridisation (CGH). All patients were operated on successfully and are alive. Male-to-male transmission and the healthy 56-year-old male suggest autosomal dominant inheritance with incomplete penetrance. To our knowledge, familial occurrence of dominantly inherited low-grade astrocytomas is exceedingly rare. We are currently investigating the TP53 tumour suppressor gene and would continue to perform CGH analysis mentioned above to reveal the genetic aberration underlying the disease in this family.

P1-03 63

Detection of Complex Germline Rearrangements of the BRCA1 Gene Using Semiquantitative Multiplex PCR Method

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Most described germline BRCA1 mutations are single base pair substitutions or small sequence deletions and insertions causing frameshift and truncation of the BRCA1 protein. However, at present only two-thirds of expected mutations are detected in high risk breast and ovarian cancer families. One reason for this is the existence of complex germline rearrangements in the BRCA1 gene that are not detectable by standard

diagnostic techniques like direct sequencing. To detect such large deletions or duplications encompassing one or more exons of the BRCA1 gene we developed a semiquantitative Multiplex PCR method. Short exon fragments corresponding to the 22 protein-coding exons and one fragment containing sequence of the 5?untranslated region of the BRCA1 gene were PCR-amplified from genomic DNA using fluorescent labeled primers and a limited number of cycles. The method was tested with the study of eight complex germline BRCA1 rearrangements previously reported. In order to assess the possibility that German families with a strong history of breast and/or ovarian cancer negatively tested for coding-region mutations in BRCA1/2 by direct sequencing could carry complex germline BRCA1 rearrangements, we screened them using Multiplex PCR method. Data as to the frequency of germline BRCA1 rearrangements in German breast and/or ovarian cancer families will be presented and discussed with respect to their relevance in BRCA1 diagnostic.

P1-03 64

Mutation analysis of BRCA1 and BRCA2 genes in Iranian breast and ovarian cancer families

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Germline mutations in either BRCA1 or BRCA2 genes are responsible for the majority of hereditary breast and ovarian cancers. Most disease causing mutations spread throughout the genes and generate stop codons. However, specific mutations are found to be common within particular populations, resulting from genetic founder effects. So far, germline mutations in the BRCA1 and BRCA2 genes in families with a strong history of breast and/or ovarian have not been identified within the Iranian population. To estimate the frequency and spectrum of BRCA1/2 mutations in Iranian families we collected a large number of breast and ovarian cancer families in co-operation with Tehran University of Medical Sciences, Department of Human Genetics. Ten families with more than five breast cancer cases have been chosen for BRCA1/2 mutation screening by direct sequencing of the protein coding regions including all exons and exon flanking regions of the introns as well as the 5' and 3' untranslated regions of both genes. Results as to novel sequence alterations and common BRCA1/2 polymorphisms defining haplotypes will be presented and discussed.

P1-03 65

Relevance of BRCA UVs for familial breast cancer and CHK2 analysis in BRCA1/2 negative families

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The German Consortium for Hereditary Breast and Ovarian Cancer (granted by the Deutsche Krebshilfe) has recently published BRCA1 and BRCA2 mutation profiles and frequencies for breast and ovarian cancer families (Int. J. Cancer 97: 472-480). This comprehensive study (to date more than 1700 distinct families have been investigated) revealed that further predisposing genes have to be identified in the German population and that rare sequence variants (unclassified variants = UVs) with unknown relevance for tumorgenesis have to be validated further (60 from about 100 families).
Recently, a novel predisposing gene for breast cancer has been published (The CHEK2-Breast Cancer Consortium, Nat. Genet. 31, 55-59, 2002). The CHEK2 gene encodes for a cell-cycle checkpoint kinase that participates in DNA repair processes and a single frameshift mutation (1100delC) was found in about 5% of BRCA1/2 negative families tested. Our group and members of the GCHBOC have started to screen over 600 families where BRCA1/2 mutations were excluded. Preliminary analysis of 80 families, mainly from Southern Germany, has not revealed a 1100delC mutations so far.
To further characterize UVs found in the BRCA1/BRCA2 proteins from families with breast/ovarian cancer, we have collected the corresonding microdissected tumor samples. The UVs identified either in the BRCA1 (T1685A; I1385A) or the BRCA2 (R2502H; K1286del; S1221Y) protein, respectively, are rare variants which to date have been twice, once or not listed in the International BIC database. Currently, the tumors are being tested for LOH in the BRCA1 region in 17q21 or BRCA2 region in 13q13. After demonstration of LOH in the according tumor, the remaining allele will be sequenced for the presence or absence of the missense mutation.
Conclusions: A low prevalence (<5%) of the 1100delC variant in the CHEK2 gene in the German population is very likely and the relevance of UVs for tumor initiation can be addressed by the characterization of microdissected tumor samples.

P1-03 66

Differentially expressed genes from gynecological tumors: approaches and perspectives

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Differentially expressed genes in tumors can be identified by several approaches that include microarray, SAGE or „in silico" techniques. Much of the information available in literature and public databases is varying based on the source material and bioinformatic programs. Indeed, so far only a few of the candidate genes defined by these approaches are overlapping. In order to increase the reliability of the data, laser capture microdissected material should be used. In addition, to increase the likelihood of identifying interesting candidates from the complex data, genes can be restricted to known regions of LOH or amplification. By implementing these strategies, major genes involved in tumorgenesis and metastasis may be identified.
Using virtual northern, the metaGen company has identified over 600 genes differentially expressed in gynecological tumors and 50 of them, either putative tumor suppressor genes or oncogenes, could be localized to regions of LOH or amplification. Initial experiments with 25 of these genes indicate that one third of them could be confirmed after characterization by hybridization on array filters or by real time PCR. In a similar approach, we plan to construct SAGE libraries from microdissected material to identify differentially expressed genes in gynecological tumors. The resultant list of candidate genes will be limited to genes localized to regions of interest.
This work will be done in conjunction with the group in Bonn who is currently investigating differentially expressed genes located in an LOH interval on chromosome 15. Eight prognosis genes for metastasis, defined by array techniques in recent publications, and 20 candidate genes, selected due to their involvement in tumor associated pathways, are being tested by real time PCR on microdissected material. The results of these ongoing experiments will be presented.

P1-03 67

The Role of Trisomy 7 in Thyroid Neoplasms

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AIM: Trisomy 7 is often found in short term cultures of different epithelial neoplasms, including malignant tumors of the thyroid, and also in benign lesions such are thyroid hyperplasias and adenomas. This trisomy has also been reported in normal cells from different tissues. As the role of trisomy 7 in the malignant process from hyperplasia to adenoma and finally to folicular carcinoma of thyroid or from hyperplasia to papillary carcinoma of thyroid is still discussed, we present this study.
METHOD: We performed fluorescence in situ hybridization (FISH) using probes for chromosome 7 in paraffin-embedded samples of 2 hyperplastic thyroids, 6 papillary carcinomas and 4 folicular adenomas. Four samples of the normal thyroid were used as controls. Evaluation of results was done by counting at least 200 nuclei per slide according to the description in recent literature.
RESULTS: We found no trisomy 7 in the normal and hyperplastic samples, but tumors with and without this trisomy were found in the both remaining groups. It suggests that trisomy 7 does not specify the pathway of malignant development, it may be included either in the process leading to the formation of papillary carcinoma or to the folicular carcinoma. The findings of cases without trisomy 7 demonstrate that it does not imply the development of tumor cells in the thyroid.

P1-03 68

Deletions of the long arm of chromosome 10 have no prognostic impact in t(14;18)-positive lymphomas

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The t(14;18)(q32;q21) translocation initiates lymphomagenesis via deregulation of the BCL2 oncogene. Nevertheless, the sole presence of a t(14;18) is not sufficient to render cells oncogenic and secondary chromosomal alterations are necessary for malignant transformation. Deletion of 10q is observed in around 10-15 % of the t(14;18)-positive lymphomas. The commonly deleted region has been narrowed down to 10q24 by cytogenetics and FISH. To study the clinical impact of 10q24 deletions in t(14;18)-positive lymphomas, we correlated the 10q24 deletion status -as detected by cytogenetics and FISH- with the most relevant clinical parameters by uni- and multivariate statistical analyses in a retrospective multi-center survey of t(14;18)-positive follicular and diffuse large B-cell lymphomas.

The survival analyses showed well known prognostic factors like histology or clinical stage to clearly differentiate groups with different prognosis in t(14;18)-positive lymphomas ($p<0.01$). Different treatment regimens did not significantly influence overall survival of the population under study. Regarding 10q24 deletions, the median survival in patients with and without 10q24 deletion was comparable ($p>0.5$). After adjusting the 10q24 deletion status for important clinical parameters by multivariate analysis, its impact on survival was not significant either. Restricting the analysis to the follicular lymphoma patients, the major prognostic parameters were retained and 10q24 deletions remained not associated with prognosis. Our analyses show that deletions in 10q24 are not related with the outcome or other clinical parameters in t(14;18)-positive lymphomas. Therefore we speculate that 10q24 deletions are involved in the early onset of t(14;18)-positive lymphomas or represent a very late event common to different prognostic groups.

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P1-04 01

A new case of OFCD-Syndrome

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We report on a 2 5/12 year-old girl, first child of healthy non-consanguinous parents, with bilateral microphthalmia, bilateral congenital cataract, coloboma of the left iris, submucous cleft palate, small median cleft palate, bifid uvula, gothic palate, broad nasal tip, long philtrum, delayed dentition, syndactyly of the toes 2-3 and retarded psychomotor development. The clinical examinations during the first weeks of life showed neither abnormalities of the cardiovascular nor cerebrovascular system. Chromosomal analysis of lymphocytes at the age of 10 weeks showed a normal female karyotype. The panoramic radiograph at the age of 2 3/12 years depicted an oligodontia. OFCD-syndrome was suggested because of the girl's eye anomalies, dental abnormalities, her syndactyly of toes 2-3 and her facial appearance. Oculo-facio-cardiodental (OFCD)-syndrome is a very rare probably X-linked dominant inherited disorder. Because of the small number of all patients with OFCD-syndrome described in the literature and the report of a female patient with the typical findings of OFCD-syndrome and consanguinous parents autosomal recessive inheritance could not be excluded. The term OFCD-syndrome summarises the combination of congenital cataract, dental abnormalities like root gigantism and delayed dentition, characteristic facial findings and cardiac abnormalities. Despite the absence of cardiac abnormalities our patient fullfills the other criteria for OFCD-syndrome.

P1-04 02

UPD and pregnancy complications: The resulat of a parent-offspring conflict or simply of a chromosomal disturbance?

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Uniparental Disomy (UPD) has been observed for almost all chromosomes. In some instances, it gives rise to distinct phenotypes (UPDs 15, 11, 7), UPD of others is not associated with a clinical phenotype (UPDs 13, 21, 22). In most syndromes associated with UPD, pregnancy complications include intrauterine growth retardation (IUGR). This indicates that genomic imprinting plays a fundamental part in regulating antenatal growth. However, the influence of imprinting is not obligate considering that trisomic rescue is the most frequent UPD formation mechanism: trisomic rescue might lead to either placental or fetal tissue mosaicism. This mosaicism may remain undetected and renders the delineation of a phenotype more difficult.

We tested a large cohort of IUGR patients with and without further clinical features for UPD of chromosomes (namely 2, 6, 9, 14, 16, and 20) the imprinting situation of which is ambiguous. The heterogeneous clinical pictures of UPDs 2, 9 and 16 and the frequently detected additional chromosomal mosaicisms suggest that placental mosaicism is the underlying mechanism causing the phenotype. Paternal UPD6 is restricted to IUGR in association with transient neonatal Diabetes mellitus. In case of UPD14, a maternal UPD14 syndrome could be delineated which includes IUGR. An imprinting effect should also cause the clinical features of UPD20 since prenatally detected mosaic trisomy 20 does usually not affect the pregnancy outcome. In conclusion, most of the known UPDs with clinical abnormalities can be divided in two groups: the ones belonging to imprinting syndromes and the ones caused by placental dysfunction as the result of a chromosomal aberration.

Both groups include growth abnormalities and, in case of putative imprinting syndromes, specific clinical features. However, there remain UPDs such as maternal UPD7 in Silver-Russell syndrome in which both imprinting and chromosomal aberrations appear to influence the phenotype.

P1-04 03

Clinical and molecular cytogenetic analyses of three patients with interstitial deletion of chromosome 6q

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Interstitial deletions involving chromosome 6q are rare. Only 11 patients with deletions of the segment 6q13-6q21 have been reported. Characteristic features correlating with the specific region are: low birth weight, mental retardation, hypotonia, microcephaly, mild facial dysmorphisms with prominent forehead, hypertelorism, low set and malformed ears, depressed nasal bridge. Respiratory distress is described in nearly half of these patients. Prader-Willi-like phenotype (feeding problems in infancy, polyphagia, adipositas) is reported in 5 patients with interstitial deletion of 6q16 or q21. We present the molecularcytogenetic investigation of three patients with interstitial deletion of the q15-q21 region of the long arm of chromosome 6. The karyotypes were analysed at the 500 band level. The breakpoints have been determined accurately by using Micro-FISH. Patient 1 is a 3-year-old girl with an interstitial deletion, del (6)(q15q16.3), developmental delay, low birth weight, short stature, mental retardation and minor craniofacial anomalies. Patient 2 is a 2 5/12-year-old boy with facial dysmorphisms, high arched palate and hypotonia. Reverse chromosome painting identified an interstitial deletion del(6)(q15q21). In the third case of a 5-year-old girl with developmental delay, VSD, respiratory problems, and discreet facial stigmas an obviously balanced complex structural aberration with insertion of chromosome 22 material into a chromosome 6 and simultaneous inversion of chromosome 6 was prediagnosed. Reverse painting with a microdissection library probe, which was generated out of the derivative chromosome 6, however, showed that in the area of the distal breakpoint in 6q21 genetic material is deleted, so that partial monosomy exists. Interstitial deletion of the precise segment (q21) deleted in our patient has not been reported previously. The investigations showed that through chromosome microdissection in combination with the usual cytogenetic examination, as well as FISH analyses with locus spefication of chromosome structural aberrations can be achieved. Such investigations will allow a direct comparison of reported cases and enable a more accurate diagnosis as well as prognosis in patients with 6q deletions.

P1-04 04

Multicolor chromosomal bar coding characterizes a de novo interstitial deletion (5)(q33.3q35) in a child with multiple congenital malformations

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Phenotype correlations of partial chromosome 5q deletions are yet poorly defined.
We describe a boy with multiple congenital anomalies including a severe complex heart defect, club feet, adducted thumbs and facial dysmorphic features. He died at the age of two months following cardiac surgery. G-banding revealed an abnormal chromosome 5q interpreted as an interstitial deletion (5)(q23.2q31.3). Breakpoints of the deleted segment were adjusted to del (5)(q33.3q35) by multicolor fluorescence in situ hybridization (FISH) using two sets of combinatorially labeled band specific YAC clones.
Our findings underscore the importance of exact breakpoint analysis for genotype-phenotype correlations in patients with uncertain chromosomal aberrations such as interstitial deletions and show the importance for genetic counseling.

P1-04 05

Partial monosomy 20q13.1-qter and 2p24.1-pter is associated with multiple abortions: molecular characterization and segregation analysis in a family bearing a t(2;20) translocation

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Fetal chromosome aberrations account for about 50% of first-trimester pregnancy losses. Numerical abnormalities represent the bigger part (86%) while a low percentage of abortions is caused by structural chromosomal rearrangements (6%). About half of the structural abnormalities are inherited from a parent carrying a balanced chromosomal translocation or an inversion. We analysed a novel reciprocal translocation t (2;20)(p24.1;q13.1) and its segregation in a three generation family. The rate of miscarriages in pregnancies from male translocation carriers of 5 out of 10 could clearly be explained by adjacent 1 misbalanced translocation bearing spermatozoa found with a frequency of 48.2% in the entire sperm population of one of the t (2;20)(p24.1;q13.1) carriers. While the translocation is clearly associated with an increased risk of early abortions (7/12) in male and female carriers, no malformed livebirths were observed. This suggests complete embryonic lethality of 2, der(20) and der(2), 20 imbalanced offspring. With respect to known cases of partial trisomy 2p and 20q we consider that their corresponding monosomies provoce fetal wastage. This is the first study describing multiple abortions associated with a partial monosomy 20q13.1-qter and 2p24.1-pter and the first report on the meiotic segregation pattern of a t(2;20) (p24.1;q13.1) in a male carrier.

P1-04 06

Deletion of 16q23: A recognizable facial phenotype

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We report on a 9 6/12-year-old boy with short stature, normal head circumference and developmental delay. He shows specific facial features such as a long, narrow face, high forehead, small palpebral fissures and low set, posteriorly rotated ears. In addition, the examination of the limbs revealed short and broad thumbs and first toes as well as a decreased length of the distal phalanges. Retardation of speech and delayed motor milestones were present. Due to his behavioural problems, especially aggressiveness, he was accommodated in an institution for handicapped children.
Cytogenetic banding analysis and molecular cytogenetics using the multicolor banding technique (MCB) and region specific BAC probes revealed a distal interstitial deletion of segment 23 on the long arm of chromosome 16 [karyotype: 46,XY,del(16q23)]. Werner et al. (1997) previously described a case of a 5-year-old boy with deletion 16q23.1 ->q24.2. The propositus showed striking facial similarities to our patient. In contrast to previous reports we speculate that a deletion of 16q23 leads to a clinically recognizable facial phenotype.

P1-04 07

13 novel point mutations in the Factor VIII encoding gene lead to severe or moderate Hemophilia A

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Hemophilia A is caused by heterogeneous mutations in the gene coding for factor VIII (gene symbol: FVIII); it maps to Xq28 and consists of 26 exons. In Germany, approximately 6000 patients are suffering from Hemophilia A. In a systematic large-scale analysis we will identify the genotype of all severe cases (approximately 3000 patients). A first screening for mutations causing Hemophilia A analyzes the exons from genomic DNA by methods like DGGE, SSCP or dHPLC. Since this approach covers only approximately 97% of the mutations, the FVIII-gene of the remaining patients have to be sequenced in total. Therefore, all exons including their flanking regions were amplified and subsequently sequenced. Here, we describe the results for a set of 54 patients. We tested our patients for the intron-1 inversion, 5 (#3, 14, 33, 76, 79) patients are characterized as carriers of this inversion. In 11 patients mutations have been identified, which have been described previously. Additionally, 13 novel mutations have been characterized during this study in 14 patients. The mutations are mainly base pair substitutions. The second group are deletions or insertions of 1 bp; in two cases, 4 nucleotides have been inserted. The mutations are predicted to cause amino acid exchanges or frameshifts leading to premature stop codons close to the mutation points.
During the systematic sequencing of the 54 patients, three known and 1 novel polymorphic sites were identified: the first one at codon 1241 (C to G; Arg to Glu) occurs five times in this set of patients. The silent polymorphic site in exon 14 at codon 1269 (A to C, Ser to Ser) was observed in four patients and does not have any consequence for the amino acid composition. Moreover, the known polymorphic site in the 3'-UTR of exon 26 (position 8728 A to G) was confirmed in 8 patients. The novel polymorphic site T to A is located in intron 19 at position 86.nt and occurs two times (patients 68, 69).
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P1-04 08

Clinical, cytogenetic and molecular investigation in a fetus with Wolf-Hirschhorn syndrome and loss of paternal 4p16 alleles

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Wolf-Hirschorn (4p-) syndrome (WHS) has been extensively described in children and young adults. Knowledge on fetuses with WHS is still limited due to the small number of published cases. We report on a fetus with club feet deformity and intrauterine growth retardation on prenatal ultrasound. Chromosome analysis after amnioncentesis at 23. gestational weeks displayed a de novo deletion of chromosome 4p; 46,XX,del(4)(p16.1->pter). The terminal deletion was confirmed by FISH with a WHSCR probe (4p16.1) (Vysis), a subtelomere probe (4p16.3) (Vysis) and a D4S96 chromosome 4p16.3 specific probe (Oncor). By use of highly polymorphic DNA microsatellite markers we determined the extent of the deletion. The markers D4S2366 (4p15.3-16.2) and D4S3023 (4p16.2) showed lack of paternal alleles. Correct paternity was demonstrated by use of D4S2623 markers lying in 4q25-26. Parents decided for termination of pregnancy. Post mortem examination confirmed a WHS phaenotype, the female fetus showing characteristic features like „greek warrior helmet-facies", carp-shaped mouth, micrognathia and low set simple ears. Our findings in the fetus are in agreement with the observation of loss of mainly paternal 4p16 alleles in female WHS patients, while male WHS patients show loss of maternal 4p16 alleles.

P1-04 09

PARTIAL TRISOMY OF CHROMOSOME 22 DUE TO INTERSTITIAL DUPLICATION OF 22q11.2 IN A CHILD WITH TYPICAL CAT EYE SYNDROME

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Background: Cat Eye syndrome (CES) presents as a genomic disorder with a variable combination of congenital features including ocular coloboma, anal atresia, defects of heart and kidney, craniofacial anomalies, and mild to moderate mental retardation. CES is usually characterized by the cytogenetic finding of a bisatellited, dicentric marker chromosome containing material of the CES critical region on proximal 22q (inv dup(22)), resulting in a partial tetrasomy. We report here a patient with a rare interstitial duplication of 22q11.2 and typical features of Cat Eye syndrome. Clinical findings: The patient was born after uneventful pregnancy, parents are healthy and unrelated. The boy presented at the age of 3 weeks with a combined heart defect, kidney anomalies, and anal atresia. Characteristic craniofacial features included preauricular pits, colobomata of iris and uvea, downslanting palpebral fissures and ptosis, short nose with flattened nasal bridge, hypertelorism, long philtrum, high arched palate, and retrognathia. Re-examination at the age of 8.5 months showed moderate delay of psychomotor development. Cytogenetics: Standard GTL banding techniques revealed a structural anomaly of one chromosome 22 (46,XY,add(22)(q11.2)). FISH on metaphase spreads with probes for the DiGeorge critical region (D22S553, D22S942) showed only one signal on each chromosome 22. However, FISH with YAC probes for the paracentromeric region of 22q11.2 showed hybridization patterns suggestive for an inverted interstitial duplication of proximal band 22q11.2. Conclusion: To our knowledge this is the first case of CES with the complete pattern of anomalies including coloboma, preauricular anomalies, heart defect and anal atresia caused by interstitial duplication of the CES critical region on 22q11.2, resulting in partial trisomy 22q11.2.

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Split hand/split food malformation with Mondini dysplasia

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Split hand/split foot malformation (SHFM) occurs either as isolated birth defect or as feature of different malformation syndromes. Most cases are inherited as an autosomal-dominant trait, but X-linked and autosomal-recessive inheritance also have been described. SHFM loci have been mapped in 7q21 (SHFM1), Xq26 (SHFM2), 10q (SHFM3); as well as 3q27 (p63 gene), 6q and 2q for EEC syndrome. We report on a 3 years-old boy with split hand/split foot malformation as well as syndactyly of the 3rd and 4th digits of the right hand and deafness because of Mondini dysplasia of the cochleas. Cochlea implantation was performed on the left side. Recently, in two families with SHFM and hearing loss the underlying gene has been mapped in 7q21(Tackels-Horne et al., 2001) and a deletion in 7q21 has been detected in a child with SHFM and Mondini dysplasia (Haberlandt et al., 2001). In our case, no deletion could be detected by conventional chromosome analysis. Therefore, molecular cytogenetic studies are in progress in order to detect a possible microdeletion.

P1-04 11

Autistic disorder and chromosomal mosaicism 46, XY [120] / 46, XY, del(20)(pter>p12.2) [10]

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Autism is characterized by impairments in social interaction and communication, stereotyped patterns of interests and activities as well as developmental abnormalities. It is estimated to affect approximately 5 per 10000 individuals with a male to female ratio of 4:1. Mental retardation occurs in approximately 75 % and seizures in about 15-30 % of autistic patients. In most cases of infantile autism the behaviour of affected children is abnormal from early infancy. We report on a 3-year-old boy with a moderate to severe mental retardation, autistic behaviour patterns and myoclonic epilepsy of early childhood. Facial dysmorphic signs are a flat nasal bridge and prominent ears. Magnetic resonance imaging of the brain, electrophysiological examinations as well as metabolic sreening and molecular testing for Angelman syndrome and FraX syndrome showed normal results. The cytogenetic analysis of blood lymphocytes revealed a deletion of chromosome 20p12.2 occuring as mosaicism in 8 % of the analysed metaphases: 46, XY [120] / 46, XY, del(20)(pter>p12.2) [10] by conventional banding analysis and fluorescence in situ hybridization. Several studies lead to the conclusion that many cases of autism are caused by chromosomal disorders involving chromosome 15 and the sex chromosomes. To date it is not clear whether genes on other chromosomes like chromosome 5, 8, 13, 17 and 18 or on chromosome 20 as in our case play a role in the development of this disease.

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Deletion 2p12-2p11.2 without abnormal clinical findings detected over three generations

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We report on a family with a deletion in chromosome 2 - del(2)(p12p11.2) - without apparent phenotype. The deletion of the short arm of chromosome 2 was initially detected in the fetus of a 38 year old woman referred for amniocentesis. Karyotypic analysis of the mother and the grandfather showed a morphologically altered chromosome 2, as well. According to GTG-banding the breakpoint was thought to be in 2p12. Using high resolution multi color banding (MCB) applying the probe set for chromosome 2 in combination with region specific YAC and BAC probes, the breakpoint could be corrected and refined to 2p12-p11.2. The missing chromosomal material could not be detected in the karyotype of any of the three studied probands using these FISH techniques. The reported deletion is located more proximal than a known case of a familial deletion without phenotypic penalties [Lambert et al., 1991, J Med Gent Vol. 28, p62: del(2)(p13p12.2)]. As the region 2p12p11.2 is known to be gene rich it is surprising that no phenotypic consequences can be observed. Thus, a new region within the human genome is identified, which seams to have no clinical consequences when present in one copy, only. Supported by the DFG (PO284/6-1) and the EU (ICA2-CT-2000-10012 and QLRT-1999-31590).

P1-04 13

The role of the BamHI-polymorphism of heparan sulphate proteoglycan 2 (HSPG2) in the development of CAD

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HSPG2 is one of the three major classes of heparin sulphate proteoglycans acting within the cardiovascular system and controlling various aspects of vascular development. It's involved in the lipid metabolism by binding lipoprotein lipase and apolipoprotein B and may therefore be related to vascular disease. Methods: In a case control-study we examined the interactions of the BamHI-polymorphism (PM) of HSPG2, lipid metabolism (LDL, HDL, triglycerides, total cholesterol) and coronary atherosclerosis (CAD). We investigated 353 patients with angiographically confirmed coronary diagnosis depending on their angiographically confirmed coronary and lipid state, 126 patients without any coronary symptoms having an abnormal lipid metabolism (mean age: 51.4y) and 227 CAD patients having a distinct pathological lipid profile (mean age: 50.6y). As controls we examined 300 long-standing healthy blood donors with normal lipid metabolism (mean age: 43y). Results: In order to evaluate the interrelation of the HSPG2 BamHI-PM, lipid metabolism and CAD the genotype frequencies were examined according to a dominant, codominant and recessive genetic model within in three proband groups. The analysis of the codominant genetic model showed significantly decreased frequencies of the homozygous mutation-carriers TT among CAD patients (0.02) and patients without coronary afflictions (0.04) compared to healthy blood donors with normal lipid profile (0.07, $p<0.032$). Based on a T-recessive genetic model a comparison of CAD patients showing the most pathological lipid profile and healthy blood donors revealed a 4.2 times higher risk for TT-carriers of developing an unbalanced lipid metabolism connected with severe coronary atherosclerosis ($p>0.006$; 95% CI: 1.42-12.4). Conclusions: Depending on the pathological lipid profile the TT-genotype frequency of the BahmHI-PM of HSPG2 decreased significantly. Beside the pathological lipid profile the angiographically confirmed coronary state seems to be only a more minor fact in this asso

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Genetic analysis of patients with hereditary hemorrhagic telangiectasia (HTT)

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Hereditary hemorrhagic telangiectasia (also known as Osler-Weber-Rendu Syndrome or Morbus Osler) is a rare autosomal dominant disorder that affects 1 in 10000 individuals. It is heterogeneous both between and within families in age of onset and severity of clinical manifestations. The first symptom is generally epistaxis, in most cases followed by arteriovenous malformations (AVM) of different inner organs (lung, liver, brain, gastrointestinal tract).
The heterogeneity can be explained in part by mutations in at least three different genes, two of them are known. Mutations in the Endoglin gene on chromosome 9 are associated with HHT type 1, which is a more severe form with a higher incidence of pulmonary AVMs. The milder form of HHT (type 2) is associated with mutations in the ALK-1 gene, which is located on chromosome 12. Both gene products are transmembrane proteins, they are expressed in endothelial cells and are connected to the TGF? signalling pathway.
We screened six single patients and two families with six members for mutations in both genes. We amplified the 10 (ALK-1) or 14 (Endoglin) exons by PCR followed by direct sequencing. Beside several polymorphisms we observed missense and nonsense mutations as well as deletions. All mutations are located in the extracellular domain, but no hot spot region could be identified.

P1-04 15

Fetal alcohol syndrome in association with Rett syndrome

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Fetal alcohol syndrome (FAS) is a common syndrome which affects about 1/1000 livebirths. It is recognizable by characteristic craniofacial abnormalities, malformations, growth retardation and deficits in intellectual functioning. Severe FAS is mostly diagnosed in the first days of life. On the contrary, patients with Rett syndrome develop normally until the age of 6 to 18 months. Thereafter, a stagnation of development occurs followed by regression of mental and motor abilities and autistic features. Rett syndrome is less frequent than FAS but with an incidence of 1/10000 in girls it belongs to the more common genetic diseases.
We report on a girl who was born in the 36th week of gestation with hypotrophy, microcephaly, ASD and VSD and typical features of alcohol embryopathy. The mother conceded a daily consumption of about 150 to 200 g pure alcohol. During the first months of life the child progressed in her motor and psychomotoric development but was at the very beginning of her life severly retarded.
At the age of twenty months regression and loss of already acquired skills was obvious. At the age of 30 months the girl has lost purposeful hand function, had intermittent episodes of hyperventilation and was gritting with her teeth. Chromosome analysis, molecular analysis for Fragile X as well as for CATCH 22 showed normal results. By sequence analysis of the MECP2 gene the 808 C?T mutation, a mutation which leads to a stop codon, was detected. The diagnosis Rett syndrome was therefore stated.
Conclusion: Even if the symptoms of a patient have already been adjoined to a known syndrome it has to be kept in mind that the syndrome may be superimposed by symptomes of a further disease.

P1-04 16

Pure trisomy 12pter-12p11.21 in a girl with X-autosomal translocation: minor congenital anomalies and moderate developmental delay

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Trisomy 12p has a distinct chromosomal phenotype including heavy birth weight, macrocephaly, turri-brachycephaly with high and prominent forehead, midface hypoplasia, broad nasal bridge, upturned tip of the nose, long philtrum, everted prominent lower lip, short broad hands and fingers, early hypotonia, severe mental retardation and seizures. Over 35 cases have been reported, including 10 observations of complete pure trisomy 12p and 4 cases of trisomy 12p involving mosaicism. We now report the first patient with trisomy 12p in conjunction with an X-chromosomal translocation.
Our patient was born at 42 weeks of gestation (birth weight 4.800g, length 53cm, OFC 37cm). She had unilateral postaxial hexadactyly as sole malformation. Dysmorphisms included hypertelorism with inner epicanthus, thin upper lip, drooping lower lip, sparse eyebrows, hypopigmented skin areas, bilateral inverse nipples and prominent great toes. Mental retardation was obvious at the age of 3 years. Motor development was in the normal range. Seizures have not been observed. At the age of 4 3/12 years (height 105cm, weight 18,7kg, OFC 49,5cm) she had a vocabulary of 200?300 words.
Cytogenetic analyses revealed an X-autosomal translocation which was characterised as der(X)(Xpter->Xq28::12p11.21->12pter) by various molecular-cytogenetic techniques. A subtelomeric probe Xq (cosmid CY29) hybridised to the derivative X-chromosome. X-inactivation studies showed that the normal X-chromosome was inactive in 41/57 analysed lymphocytes.
The patient gives us the opportunity to show the role of X-inactivation in mitigating the effect of chromosomal imbalance. Different X-inactivation ratios in various tissues could explain non-concordance of the phenotypic expression with the inactivation status in peripheral blood cells. The clinical picture of the patient will be demonstrated in comparision to cases with pure trisomy 12p and cell line mosaicism.

P1-04 17

DNA-Diagnostik for Noonan-Syndrome: Mutation-Screening in the PTPN11-Gene

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The Noonan Syndrom is a complex syndrome with a highly variable phenotype, typically comprising hypertelorism, low set ears, pulmonary stenosis, sternum anomalies. Though phenotypically similar to the 45,X Turner-Syndrom it affects males and females and chromosomes are normal. Recently, mutations in the SHP-2 Gene have been reported to account for approximately 50% of all Noonan-Cases.
We have performed mutation screening in the SHP-2 gene in patients referred to our department with the clinical diagnosis of Noonan-Syndrom. Here, we report on the mutation spectrum and genotype-phenotype correlation in our patient population. All mutations were missense mutations. Few recurrent and mostly private mutations were identified. Our data indicate a higher rate of cardiac hypertrophy in those patients were mutations could be identified.

P1-04 18

A disease associated balanced chromosome rearrangement (DBCR) in a girl with multiple congenital anomalies (anal atresia, colon aplasia) and normal mental development

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Disease associated balanced chromosome rearrangements (DBCRs) are a powerful tool for the identification and isolation of disease genes and for our understanding of their physiological and pathogenetic role as well.
We report a 12-year-old girl with multiple congenital anomalies and a balanced translocation 46,XX,t(12;14)(q24.1;q22.1) de novo. The proposita was born at 42 weeks of gestation as the second child to healthy unrelated parents. She had mildly dysmorphic facial features with slight asymmetry, a prominent left zygomatic bone, strabismus divergens, high nasal root, short philtrum, and a thin vermilion border. She also had anomaly of the iris, hearing loss of the right ear, horseshoe kidney with nephrocalcinosis, colon aplasia, anal atresia, right hammer toe and streak-like hypopigmented areas on both arms and legs. Her mental development was normal.
Detailed FISH analysis was performed. For chromosome 12, we used YAC probes 855e2, 979a5, 753g1, 654b2 (order, cen—>qter) and found the breakpoint between probes 979a5 (locus data not available) and 753g1 (D12S354). For chromosome 14, we used YAC and BAC probes 959 b11, RP11-2G1, RP11-58E21, RP11-286 O18, and 772d1 (order, cen—>qter). BAC RP11-286 O18 (AL133485) showed signals on the normal chromosome 14 and both derivative chromosomes and is therefore breakpoint-spanning.
We suggest that her phenotype resulted from the inactivation of a gene(s) at one or both breakpoints, either by direct disruption or possibly by

an undetected minimal unbalanced rearrangement at the molecular level.

P1-04 19

Dup(13)(q14.2q21.1) - yet another differential diagnostic aspect for short stature like phenotype

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We report on a case of a healthy pregnant woman with hyposomia. She was 23 years old and it was her first pregnancy. The short stature was conspicuous (length only 146 cm). No other clinical symptoms and no menstrual irregularities were registered. 15 years ago she has been suspected having a Turner syndrome and chromosomal analysis revealed a very mild mosaic 46,XX/45,X (72/2). Now the woman requested counseling for a potential recurrence risk for the unborn child having a Turner syndrome.
In gestational week 16+1 she was referred to amniocentesis. Prenatal cytogenetic diagnostics showed a fetal karyotype of 46,XX,dup(13)(q14.2q21.1) ish.13q14(RBx3) In this context familial chromosome analysis have been carried out. The mother had the same karyotype as the fetus - caused by a de novo aberration. Additionally a very mild Turner-mosaic was found - conforming with the earlier analysis. The father had a normal karyotype. The parents decided to continue the pregnancy. Growth arrest and polyhydramnion were detected by ultrasound. At 35 weeks of gestation the female child was born by cesarean section with apgar scores 6/8/9, 2750g weight and only 44cm length. Postnatal examinations revealed a healthy girl with normal development apart from the short stature and very mild dysmorphic signs. Mainly phenotypic correlation of the presented unbalanced partial trisomy 13 is a short stature. The reported chromosomal aberration was not described previously. The case report presents detailed phenotype descriptions and advice for a differential diagnosis and genetic counseling in rare chromosomal aberrations associated with short stature.
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Mapping of Brachydactyly A2 to Chromosome 4q22-26

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Brachydactyly is due to abnormal development of the phalanges and/or metacarpals. As an isolated feature, the different types of brachydactyly were classified according to the anatomical location by Bell in 1951. Type A2 brachydactyly is a rare hand malformation characterized by brachymesophalangy and lateral deviation of the index finger and the second toe. Brachymesophalangy of the 5th finger with clinodactyly can occur and the big toe is often short and broad. Other fingers or toes are usually not affected. Brachydactyly A2 follows an autosomal dominant mode of inheritance; the causal gene is unknown. Only a few families with brachydactyly A2 have been published in the literature (Mohr and Wried, 1919; Freire-Maia et al.,1980). We present a large German kindred with brachydactyly A2 (Mohr-Wriedt) and typical clinical findings. The manifestations show a high variability among the affected individuals. The most severely affected person is characterised by a missing middle phalanx of the index finger. We have performed a genome scan using a set of 395 polymorphic microsatellite markers to map this type of brachydactyly. Two point linkage analysis (maximum LOD score [Zmax] 5.2 at recombination fraction [theta] 0.00) located brachydactyly A2 in this family to chromosome 4q. Haplotype analysis of the pedigree confined the locus within an interval of 21.6 cM to chromosome 4q22-q26 on the cytogenetic map. Candidate genes are currently being investigated in this region.

P1-04 21

Three particular cases with p63 mutations

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Mutations in the p63 gene have been established as the molecular basis in a number of human syndromes (EEC syndrome, AEC syndrome, Limb-mammary syndrome, ADULT syndrome) and non syndromic split hand and foot malformation. Genotype-phenotype correlations have been described for EEC (Ectrodactyly, Ectodermal dysplasia, Clefting) syndrome and AEC (Ankyloblepharon, Ectodermal dysplasia, Cleft lip and palate) syndrome. Heterozygous missense mutations in the SAM (Sterile Alpha Motif) domain are the cause for AEC-syndrome; missense mutations in the DNA-binding domain of p63 have been found in the vast majority of individuals with EEC-syndrome.
We present one familiar and two sporadic cases with unusual mutations in the p63 gene and discuss the genotype-phenotype relations.
(1) Father and son presenting ectrodactyly, oligodontia and lacrimal duct stenosis which are features of EEC syndrome. A deletion of one nucleotide was detected in exon 14 (1913delA) resulting in a frameshift. This kind of mutation is not typical for EEC syndrome.
(2) A 38-year-old man with features of AEC syndrome such as chronic scalp erosions in childhood, partial hair loss, dystrophic nails, teeth abnormalities, partial anhidrosis, lacrimal duct atresia, hypoplastic uvula and a mild form of brachydactyly. Interestingly, we detected a frameshift mutation in exon 13 (1615-1616delCAinsG) which stands in contrast to previously described missense mutations (SAM domain) in patients with Hay Wells syndrome.
(3) A 13-year-old girl with typical AEC syndrome. We have identified a novel missense mutation in exon 14 (I558N) that appears to be characteristic for the class of mutations found in persons with AEC syndrome.

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A comprehensive molecular cytogenetic analysis of a familial complex chromosome rearrangement involving four chromosomes and at least 8 breakpoints

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We report a complex chromosome rearrangement (CCR) involving 4 chromosomes and at least 8 breakpoints which was observed in both a mother and her daughter. This is to the best of our knowledge the first case in which such a complex rearrangement was transmitted through two generations.
An extensive molecular cytogenetic work-up of this CCR was appropriate for two reasons: Firstly, while the mother is phenotypically normal the daughter suffers from mild mental retardation. This suggests that a difference may exist between the chromosome complements of mother and daughter. Secondly, there is no single method which on its own can elucidate such complex rearrangements completely.
Standard chromosome CGH showed a balanced profile for all chromosomes. Investigation with M-FISH confirmed the rearrangement of the aberrant chromosomes. YAC clones were then used to determine the orientation of the chromosome fragments. This higher resolution analysis also identified small insertions which had not been detected previously. A study using ordered BAC clones was then initiated to identify breakpoint spanning clones. As this is a time consuming process, we employed a rapid method utilising DNA microarrays which has been developed to facilitate breakpoint mapping. The translocation chromosomes were isolated by flow sorting and hybridised to a 1 Mb BAC array (Fiegler et. al. in press). Only two hybridisations of the flow sorted derivative chromosomes onto the BAC arrays were required to provide localization of all breakpoints to within a resolution of 1 Mb. This clearly demonstrates the power of this approach to map breakpoints rapidly. Currently we would describe the four translocation chromosomes as der(6)(6pter->6q21::11p13->11p13::20p13->20pter), der(9)(11pter->11p14.1::11p11.2->11p13::6q23.2->6q22.1::11p14->11p14::9p21.3->9qter), der(11)(9pter->9p21.3::6q22.1->6q22.1::11p11.2->11qter), der(20)(6qter->6q23.2::11p14->11p14::20p13->20qter).
All breakpoints will be further refined to within breakpoint-spanning clones as our mapping efforts continue.
This extraordinary case sets a striking example for the effectiveness of different molecular cytogenetic tools and how, if applied in concert, an extremely complex rearrangement can be resolved efficiently.

P1-04 23

DiGeorge/velocardiofacial syndrome: FISH studies of chromosomes 22q11 and 10p14, and clinical reports of the proximal 22q11 deletion

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The DiGeorge/velocardiofacial syndrome (DGS/VCFS) may occur with different deletion intervals on 22q11 or 10p14. The clinical outcome with the common 22q11 deletion (CDEL; size 3 Mb, some 30 genes) is well known, but the outcome with the other deletion types is less well documented. We hypothesized that there could be differences with respect to intelligence and/or behaviour disorder/psychosis (polygenic traits). From 1997 to 2001, we studied a series of 295 patients with suspected DGS/VCFS.

No 10p deletion was observed, adding evidence to that the 10p deletion is extremely rare. We found the CDEL in 52 patients and the proximal deletion (PDEL; size 1.5 Mb, some 15 genes) in 5 patients. Clinical data of 4 patients with PDEL were available. Their malformations represented the DGS/VCFS spectrum, as could be expected from previous studies, but two patients (age, 6 and 25 years) had a normal mental development. Normal mental outcome may also occur with the CDEL, but not frequently. This study may provide a first evidence for a better intellectual and/or behavioural outcome with the proximal vs. the common 22q11 deletion.

P1-04 24

Female child with Duchenne muscular dystrophy and double recombination in X-chromosome

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Over the past decade geneticists have searched for rapid and efficient means of identification of genetic diseases. Our laboratory is involved in mutational analysis, carrier detection, and prenatal diagnosis in DMD/BMD families for the last 11 years. Till now, we have analyzed over 200 DMD patients and 15 BMD patients. Studied group involved 15 familial cases. Out of these, 51% were found to have intragenic deletions and 49% were nondeletional cases. 70% of deletions were located in central deletion prone region, with 35% deletion originating in intron 44. In approximately 10% of nondeletional cases point mutations were detected. Molecular diagnostic studies of carrier status were performed for 39 DMD/BMD families. In all studied families at least one marker was informative. Additionally, a few cases of intragenic recombination event were detected. Analysis of (CA)n repeats located in the deletion prone region of the DMD gene detects de novo mutations and provides direct information about carrier status. Occurrence of symptomatic disease in girls can be explained by the Lyons hypothesis. The full clinical picture of DMD has been described in children with Turner syndrome. A 12-year-old girl was born to parents with no history of disease. There was history of repeated falls, difficulty in walking and getting up and normal performance in the school. On clinical examination she was mentally normal, had muscle pseudo-hyper-trophy and was Gower's sign positive. Her clinical symptoms were less severe than typical for boys of the same age. Her creatinine kinase level was elevated, X-ray chest and ECG were normal, and myogenic EMG. Her brother had normal creatinine kinase level and no signs of disease. Molecular analysis revealed double recombination event in patient's X-chromosome.

P1-04 25

IDENTIFICATION OF RARE FORMS OF AUTOSOMAL DOMINANT HERITABILITY OF MYOCARDIAL INFARCTION

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Myocardial infarction (MI) is considered to be a multifactorial disease that results from an integrated vascular response to common cardiovascular risk factors. Interestingly, some families show a high incidence of MI that cannot be explained by these traditional risk factors. Thus, the aim of this study was to investigate whether MI can also be inherited in a Mendelian pattern. Methods: In cooperation with 17 in-hospital cardiac rehabilitation centers, the Regensburg MI family study group screened over 200.000 medical reports of patients with coronary artery disease in order to find extended MI families.
Results: We identified 124 MI families with at least 3 or more siblings who survived a MI. In 13 of these families without bilinearity, a parent, an aunt or an uncle, as well as a minimum of 2 cousins had the history of MI. Within these families, 85 living MI patients and 365 unaffected family members were recruited. Moreover, 49 patients had died from MI in these families. The largest family had 22 affected individuals in five generations. In the age group from 50 to 70 years, 47.7% of the family members were affected. Traditional cardiovascular risk factors, such as smoking, hypercholesterolemia, hypertension and diabetes mellitus, had the same frequencies as a population-based sample of sporadic MI (MONICA MI-Registry-Augsburg, Germany, n=609, $p>0.1$ for all risk factors). However, the predominance of male gender in this age group as usually seen in MI registries, was less pronounced in MI families (69.4% vs. 87.4 % in the population based KORA MI registry, $p<0.001$). Furthermore, the patterns of inheritance argued against X-chromosomal disorder. In the MI families, power simulation using the SLINK software package and applying several potential models of inheritance consistently suggested that an autosomal dominant trait explained the heritability in these families with the highest probability.
Conclusion: A minority of MI patients shows an autosomal dominant pattern of heritability which cannot be explained by traditional cardiovascular risk factors. The data suggest the exsistence of yet unknown monogenic forms of MI.

P1-04 26

Severe, neonatal onset OTC deficiency in twin sisters with a X;5 translocation

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OTC deficiency is the most common inborn urea cycle defect inherited as a X-linked trait. Female carriers may manifest mild to moderate metabolic insufficiency but the severe neonatal form of the disease is normally restricted to hemizygous males having less than 5% residual enzyme activity. Case report: We report on monozygotic female twins born to healthy unrelated parents with an unremarkable family history. Both infants developed hyperammonemic coma on the 5th day of life. The metabolic profile suggested OTC deficiency confirmed in one of the patients by liver biopsy (residual OTC activity less than 5% of normal). Because of the unusual manifestation of a X-linked disorder in females, genetic anaylsis was started with karyotyping. Chromosome analysis revealed a de novo, balanced X;5 translocation (karyotype: 46,X,t(X;5) (p21.1; q11.2)) in both patients. No mutation could be detected by subsequent direct sequencing of the entire OTC gene. No abnormality was either found by Southern blot using the OTC cDNA. Discussion: Manifestation of X-linked recessive disorders in females is very rare. When it occurs it is mostly associated with cytogenetical abnormalities of the X-chromosome or abnormal X-inactivation pattern. In the present case the almost complete absence of OTC activity might be explained by two effects due to the chromosomal rearrangement: The first is presumably a disruption of the OTC-gene by the chromosomal break occurring exactly in the subband of the X-chromosome where the OTC gene has been mapped (Xp21.1) - although Southern blot did not suggest a breakpoint within the OTC gene. Secondly, the translocation of a large autosomal fragment to the X-chromosome results in skewed X-inactivation in favor of those cells in which the derivative X is the active one and the normal X is inactivated.

P1-04 27

Homozygoter LDL-Rezeptormangel bei eineiigen Zwillingen

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Wir berichten von zwei identischen Zwillingen türkischer Herkunft, bei denen im Alter von zwei Jahren ausgeprägte Xanthome erkannt wurden. Lipidanalysen zeigten eine massive Hypercholesterolämie (Gesamt-/LDL-Cholesterol >1100/1000 mg/dL, Lp(a) 130 mg/dL). Beide Eltern zeigten mäßig erhöhte Cholesterinspiegel, die auf medikamentöse Behandlung gut ansprachen. Die Familienanamnese war

bezüglich kardiovaskulärer Erkrankungen unauffällig. Molekulargenetische Analysen im LDLR-Gen ergaben bei beiden Zwillingen Homozygotie für die bislang nicht beschriebenen Mutation W556R (c.1729T>C). Beide Eltern sind heterozygot für diese Mutation. W556R betrifft eine von fünf hochkonservierten YWTD-Motiven des LDLR-Proteins und beeinträchtigt vermutlich den Transport vom ER zur Zelloberfläche (Mutationsklasse 2A). Funktionelle Studien in Fibroblasten bestätigten eine fehlende Bindung und Internalisierung von LDL. Eine konservative Behandlung der Zwillinge brachte keine wesentliche Senkung der Cholesterinspiegel. Im Alter von drei Jahren waren die Xanthome deutlich vergrößert, und eine Behandlung mit Lipidapharese wurde begonnen. Dadurch wurde eine akzeptable Senkung der Cholesterinspiegel errreicht; die Kinder sind jetzt 4 1/2 Jahre alt und altersentsprechend gesund ohne Zeichen einer kardiovaskulären Schädigung. Der molekulare Befund erklärt sowohl die massive Hypercholesterolämie bei den Kindern als auch den milden Phänotyp und die gute Therapieantwort bei den Eltern. Das Auftreten symmetrischer Hautläsionen bei beiden Kindern war ein wichtiger Faktor für die frühe Diagnosestellung - manchmal kann es unerwartete Vorteile haben, ein Zwilling zu sein.

P1-04 28

A Novel Mutation in AIRE Gene in a Patient with Atypical Presentation of Autoimmune Polyglandular Syndrome Type 1

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Autoimmune polyglandular syndrome type 1 (APS-1) is a rare autosomal recessive disorder, characterised by presence of two of the three major clinical symptoms: hypoparathyroidism, Addison's disease and mucoutaneous candidiasis. Several other endocrine or nonendocrine disorders may be present. The disease usually occurs in childhood, but new tissue-specific symptoms may appear throughout life. APS-1 cases are reported worldwide, with higher incidence in some genetically isolated populations (Finland 1/25000, Iranian Jews 1/9000, Sardinia 1/14400). The aetiology of APS-1 is associated with mutations in AIRE gene encoding a protein predicted to be a transcriptional regulator.
The patient was born in 1976. He presented with alopecia at the age of 9 years, folowed by Addison's disease at the age of 11.5 years and IDDM and vitiligo at the age of 21 years.
Genomic DNA was extracted from peripheral blood lymphocytes by salting out procedure. All exons and the exon/intron boundaries of the AIRE gene were individually PCR amplified and directly sequenced. Nucleotide sequences were compered to the sequence of the normal gene (GeneBank Access No. AB006684).
A novel deletion of 1 G in exon 5 and common Finnish mutation R257X were identified. The novel 540delG (counting from AUG codon, Acc. No. AB006682) is a frameshift mutation, allowing the synthesis of 180 amino acids, followed by 196 unrelated amino acids and then premature stop codon in exon 10. R257X is the most common mutation in Slovenian population.
Although the patient is a compound heterozygote for two serious mutations resulting in shorter and probably unfunctional protein AIRE, he only developed one out of three major clinical characteristics. On the contrary Iranian Jewish patients are known to have milder phenotype with only one major symptom (often hypoparathyroidism) associated with Y85C mutation causing just mildly abnormal protein AIRE. Other genetic and/or environmental factors must influence the phenotype and disease progression of individual APS-1 patient.

P1-04 29

Mutation 314del14 in GJB2 is the second mutation in patients with non-syndromic hearing loss in Poland

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Hearing loss is the most frequent disease of human senses. At least 60% of all cases are attributed to genetic factors in developed countries. During last few years several genes causing hearing loss have been discovered and beyond any doubt the most important one is GJB2 gene coding connexin 26. Connexin 26 is a component of gap junctions in the organ of Corti.
At first in our study we have tested 262 patients with different levels of non-syndromic hearing loss. In order to detect 35delG mutation, which is the most frequent in Caucasian populations we have used a method based on PCR mediated site mutagenesis. Among tested patients 137 (52,3%) probands did not display 35delG, 92 (35,1%) were homozygotes for 35delG and 33 (12,6%) were hetorozygotes for this mutation. The next step consisted in sequencing of the coding region of GJB2 gene in heterozygous patients. In 13 cases mutation was found in the second allele. In 8 cases it was 314del14 and in two cases 333-334delAA. Having regard to the frequency of mutation obtained, a new method was elaborated permitting to display the three most common mutations at the same time. This method consists in simultaneous amplification of the regions 12-72 and 306-464 of the coding region of GJB2 with the use of labelled primers. Products obtained are next subject to size analysis with the application of ABI 377. 857 patients were examined with this new method. Both methods were used on 1119 patients. 314del14mutation was found in 21 compound heterozygotes for 314del14 and 35delG, and in one case homozygote for 314del14. 256 patients were homozygotes for the 35delG mutation and 132 heteorozygotes for this mutation. Therefore 314del14 mutation is second most frequent mutation in the coding region of GJB2 in patients with hearing loss in Poland and this fact should be taken into account when setting diagnostic methods in patients with hearing loss also in neighbouring countries.

P1-04 30

Congenital disorder of glycosylation Type Ie (CDG-Ie) - 4 patients have been reported so far. The 5th case in the literature?

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Congenital Disorders of Glycosylation (CDG), a recently described group of multisystem disorders, characteristically are associated with major nervous system impairment. The underlying pathomechanism consists of an abnormal glycosylation of glycoproteins and glycolipids. CDG comprises two major subentities: Type I (CDG Ia-e) concerns the synthesis and transfer of the carbohydrate chain. In type II CDG the processing of the carbohydrate chains is altered. CDG-Ie is characterized by mutations the dolichol-phosphate-mannose synthase-1 gene leading to a deficiency of DMP1.
Here we report on the first child born to healthy parents of Sicilian origin. After an uneventful pregnancy the boy was delivered by cesarean section because of shoulder dystocia. He presented with the clinical picture of macrosomia (4700g) and arthrogryposis multiplex with clasped thumbs. In his first weeks of life he developed seizures. Several EEGs demonstrated the pathologic pattern of burst suppression. An MRI of the brain showed a bilaterally reduced volume of the cerebral hemispheres. Screening for common metabolic diseases showed normal results. Cytogenetic studies carried out on peripheral blood lymphocytes proved a normal male karyotype. His motor and mental development was profoundly retarded. At examination with 4 years he only turned from his back onto his stomach lacking any speech development. His body measurements were below the third centile. Hypertelorism, beaked nose, high arched palate and small teeth were observed. Additional investigations included a serum transferrin isoelectric focussing (IEF), which serves as a convenient marker of glycosylation abnormalities. Results showed a significant amount of tetrasialo- and disialotransferrin but hardly any asialotransferrin. Clinical features and serum IEF pattern strongly suggest CDG Type Ie as the underlying cause. Currently, further biochemical investigations on cultivated skin fibroblasts and molecular genetic analysis of the dolichol-phosphate-mannose-synthase1 gene are under way.

P1-04 31

Artificial neural networks in malformation syndrome diagnosis - don't believe and take for granted, but weigh and consider

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In this study, we developed an artificial intelligent diagnostic system based on an artificial neural network (ANN) to support the diagnosis of multiple congenital malformation syndromes caused by chromosomal structural mutations. As a software tool a commercially available shell was used, which was suitable for development of feedforeward ANNs trained using backpropagation of errors. Several ANNs were built up using standard training procedures with different sets of training data. For the analysis of the re-

sulting diagnostic tools predefined criteria were used for the evaluation. Decisions for the correct syndrome with a high probability were favoured, while undecisiveness of the network and a differential diagnosis without the correct diagnosis led to a devaluation. Using different orders of cases within a training set we succeeded in the optimisation process of different diagnostic tools. The numbers of symptoms/combinations for the description of cases were limited to 28, 55 and 78 in different series of tests. the most efficient ANN yielded a diagnosis in more than 95% of all tests - the correct diagnosis was produced in 74% of the analyses. A test of the final ANN with more than 70 different clinical cases yielded no correct answer in two cases and seriously wrong diagnoses (the correct diagnosis not within the differential diagnosis and high probability value for a wrong decision) in additional two cases. The significance of an ANN is to a great extent influenced by the experience of the developer.

P1-04 32

Clinical and molecular-genetic findings in 6 families with cherubism

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Cherubism (MIM 118400) is rare autosomal dominant inherited syndrome characterised by excessive bone degradation of the upper and lower jaws followed by development of fibrous tissue masses, which causes a characteristic facial swelling. The bone lesion may cause premature loss of deciduous teeth and displacement of the permanent dentition.

All affected family members were examined clinically and radiographically. We detected 5 mutations in exon 9 of the gene coding the c-Abl-binding protein SH3BP2 by DNA-sequencing in five of six families investigated.

The destruction and expansion of the jaws is most active in patients between the age of 3 and 7 years. The spectrum of severity may range from extreme bilateral expansion to subclinical unilateral involvement. In one family we observed craniosynostosis and clubbing of the fingers in two patients. There was no difference in frequency of affected males and females among 52 probands, but the onset of the disorder occurred in females at an older age. In contrast to previous reports we observed incomplete penetrance also in males.

P1-04 33

Search for Deletion 22q11.2 in Interphase Nuclei of Buccal Mucosa of Patients with Apparently Isolated Cleft Palate: A New Diagnostic Approach

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A new approach for the detection of chromosome deletion 22q11.2 in interphase nuclei from buccal mucosa cells obtained by a non-invasive procedure is decribed. FISH analysis has been performed on samples from a group of 101 patients that presented consecutively for speech therapy and/or surgical correction of cleft palate. 92 patients had overt cleft palate (hard/soft), in 9 patients, the cleft was submucous. At the time of the investigation, most of the patients had already received surgical correction. Ages ranged from three months to 20 years, mean age was 7.1 years. 37 patients were female, 64 were male. A normal result has been obtained in 98 patients; a deletion 22q11.2 was present in three patients (2.8%), one with no other major clinical finding than cleft palate. In one case, the deletion turned out to be familial. With the investigation of interphase nuclei from buccal mucosa cells, screening for 22q11.2 deletion also in groups with minor prior risk becomes feasible. The present investigation shows that in about 1% of patients with isolated cleft palate a deletion 22q11.2 occurs which may have consequences to the families due to its heritability.

P1-04 34

Hereditary motor and sensory neuropathy (HMSN) IA, developmental delay and autisme related disorder in a boy with duplication (17)(p11.2p12)

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We present a 6-year-old boy with moderate developmental delay, gait disturbance, autisme related disorder and mild dysmorphic features with a triangular face, hypertelorism and a preauricular pit. He was seen for evaluation of his retardation since the age of 2 years. At first sight, a cytogenetic analysis showed a normal 46,XY karyotype. Neurologic examination at the age of 5.5 years revealed a motor and sensory polyneuropathy. FISH with probe PMP22 and VAW409 specific for Charcot-Marie-Tooth type 1 (CMT1) disclosed a duplication which confirmed the diagnosis HMSN IA. Subsequently, GTG banded metaphases were re-evaluated and a small duplication 17p was seen on retrospect. Additional FISH with probe LSISMS (Vysis) specific for the Smith-Magenis region at 17p11.2 again showed a duplication. Cytogenetic investigation including FISH for CMT1 was normal in both parents. The boy had a de novo 46,XY,dup (17)(p11.2p12) karyotype.

Up to now, 4 patients have been reported with the same duplication. The present case confirms previous findings of mild to moderate delay, neurobehavioural features and minor craniofacial anomalies as the major phenotypic features. Furthermore, it illustrates the need of a thorough cyto- and molecular genetic work-up on clinical grounds.

P1-04 35

MeCP2 related disorders in males

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Mutations in the MeCP2 (methyl-CpG-binding protein 2) gene are the cause of Rett syndrome which is a well-known and clinically defined neurodevelopmental disorder. Rett syndrome occurs almost exclusively in females and for a long time was thought to be an X-linked dominant condition with lethality in hemizygous males. Since the discovery of the MeCP2 gene as the cause of Rett syndrome in 1999, a series of publications reported the occurrence of MeCP2 mutations also in males. These males phenotypically have Rett syndrome when the mutation arises as somatic mosaicism or when they have an extra X chromosome. In all other cases, males with MeCP2 mutations show diverse phenotypes which are different from Rett syndrome and cover a spectrum from severe congenital encephalopathy via mental retardation with different neurological symptoms up to mild retardation only. In this group, no de novo mutation has been reported up to now.

We present a 22 year old male with severe mental retardation, spastic tetraplegia, dystonia, apraxia and neurogenic scoliosis. A history of early hypotonia emerging into severe spasticity, slowing of headgrowth, breathing irregularities and a good visual interactive behaviour were reminiscent of Rett syndrome. He had a de novo missense mutation in exon 3 of the MeCP2 gene (P225L).

P1-04 36

Photoanthropometric Investigation of Facial Structures

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The photoanthropometric method suggested first by Stengel-Rutkowski et al. (1984) was used to study the facial features in 136 Iranian children with Down syndrome, aged 4 to 14 years. Nineteen parameters were investigated and compared to an age related control group of 100 normal Iranian children. The obtained measurements were related to reference values in the same faces. The normal range was defined by age related index values between the 20th and 80th percentile in the collective of normal Iranian children. Five parameters were considered as characteristic facial traits of Iranian children with Down syndrome by index values outside these percentiles in ¡Ý50% of the studied collec-

tive: low midface; narrow, upslanted palpebral fissures and short, anteriorly rotated ears. Twelve parameters were considered as additional facial traits by index values outside these percentiles in ¡Ý30% <50% of the studied collective: broad inner canthal distance; prominent nose root; short nose back; everted nasal base; long nasolabial distance; forwards inclined integumental upper lip; narrow mouth fissure; high, prominent chin; high-set, narrow ears and narrow conchae. These results contribute to an objective definition of facial traits in children with Down syndrome in a homogeneous ethnic population.

P1-04 37

CAMURATI-ENGELMANN DIAPHYSEAL DYSPLASIA - familial occurrence with anticipation

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We report on 3 sibs affected by muscular hypoplasia and weakness, broad-based waddling gait, hyperlordosis, leg pain, and reduction of subcutaneous fat, who had been diagnosed with autosomal recessive myopathy. Family History – The parents are young and non-consanguineous. The mother and oldest brother are healthy. The father has unilateral deafness and received surgery for coxa valga and genua vara, cortical and endosteal hyperostosis were demonstrated radiographically. His mother was asymptomatic, but had severe hyperostosis on radiographs. Clinical picture – The onset of the disorder was reported in the sibs at the age of 2-2.5 years during different febrile illnesses, after which muscular hypotrophy and subcutaneous lipoatrophy were noted. All show muscle weakness, joint hyperexcursibility, lumbar hyperlordosis and waddling gait, muscular pain in the legs, and have frequent headaches. 2 of 3 siblings suffer from microcystic anemia. Mental development is quite normal in all 3 children. EMG findings are normal, serum CK, LD, and alkaline phosphatase are in normal ranges. Radiographic findings – There is striking thickening of the cortices of the long bones, particularly involving the diaphyses with irregular bone contours in all affected persons. All show sclerosis of skull base. Analysis of the phenotype, family history and radiological findings leads to the diagnosis AUTOSOMAL DOMINANT CAMURATI-ENGELMANN DYSPLASIA (CED). DNA analysis confirmed CED by detection of the most common R218C mutation in TGFB1 gene. Conclusion – Variable expression is presented in the family with manifestation of CED in 5 members of 3 generations. The age of onset and severity of manifestation in successive generations which show an anticipation had led to the mistaken clinical impression of autosomal recessive muscular dystrophy in the youngest generation.

P1-04 38

Overexpression of Xq28 causing retardation in a boy with der(Y)t(X;Y)

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Deletions of the long arm of the Y-chromosome (Yq-) cause a wide phenotypic spectrum. Loss of the heterochromatic region is usually not associated with abnormalities. Deletions of the euchromatic region are found in individuals ascertained for short stature and azoospermia, but also several patients with dysmorphism, microcephaly, short stature, and retardation have been described.
We report on a boy with microcephaly, failure to thrive, and retardation. MR imaging of the brain showed atrophy, retarded myelinisation, and a hypoplastic corpus callosum. The face was regarded as „funny looking“ with no discernible dysmorphism. At the age of 15 months he was not able to sit and required a gastrostomy tube for sufficient feeding. Chromosomal analysis on peripheral blood lymphocytes of the patient showed a male karyotype with a deleted Y-chromosome described as del(Y)(q11). Multiplex PCR for the AZF-regions showed complete loss. The paternal Y-chromosome was normal.
In 1994 Lahn et al. described three microcephalic and retarded boys with a der(Y)t(X;Y)(q28; q11.1). Molecular studies showed a paternal and a maternal allele for markers localised in Xq. All patients had an elevated glucose 6-phosphate dehydrogenase (G6PD)-activity. Overexpression of genes localised in Xq28 was regarded as causative for the phenotype. The syndrome was named XYXq-syndrome. As our patient showed a phenotype comparable to that syndrome, we performed FISH with several clones mapping to Xq28.
The probes for the genes FVIII, G6PD, TMG3, MTM1 hybridised not only to the X- but also with the terminal portion of the derivative Y-chromosome. The duplicated fragment was determined to have a size of about 4 Mb. Functional studies for the G6PD-activity showed a two- to threefold elevation in the patient.
To exclude the XYXq-syndrome FISH with clones of Xq should be offered to all patients with a Yq--karyotype.
Lahn et al., Nat Genet 8, 243-250 (1994)

P1-07 01

Cytogenetic monitoring database: the assessment of the tendencies and dynamics of mutagenesis in child

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Several of mutagenesis tendencies were discovered during 1986-2002 cytogenetic monitoring studies of Belarus children population. When chronic internal and external exposure of population by low doses of ionizing radiation take place, it is productively to analyze the basic characteristics of cytogenetic status, i.e. frequency of:
chromosome aberrations, aberrant metaphases, chromatide and chromosome aberrations, cytogenetic markers of irradiation, as well as the parameters of a ratio of frequency of chromosome/chromatide aberrations. The dynamics of this ratio changes is informative too.We demonstrated the increased level of chromosomal aberrations due to exceeding of chromatide aberration type in children evacuated 9-10 years ago from contaminated Gomel area to Minsk-city. Taking into account, that irradiation produce the chromosome aberration type mainly, we suppose, that chronic low dose radiation exposure can modify the genetic repair response of cells after another genotoxic influence. As the result, environmental chemical mutagens stimulate the higher frequency of chromatide aberration.Aberrations level in newborns concerned as background pattern reflecting the basic tendencies of mutagenesis in a cohort. Dicentric and ring chromosomes frequency as well as amount of poliploid and aneuploid cells are essentially increased in newborn of contaminated area in comparison with the marched group. That specifies the biologically effective influence of radiation exposure on the hereditary device of blood lymphocytes.Studies of different age/territory groups of Belarus children population showed that children who were born in 1986-88 have undergone most effective influence of ionizing radiation on the somatic cells chromosomes. Thereof the specified cohort should be referred to group of the increased genetic risk.

P1-07 02

Down Syndrome Registry - fields for application in Belarus

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A computer-annotated database - Down Syndrome Registry (DSR) is presented. It includes cytogenetics, phenotypes, genetic data of 953 patients detected by GTG-banding method (1983-2001 years). Unusual karyotypes are collected in CytoVision. Cytogenetics: DSR contains 840 cases full trisomy 21, 79 Down syndrome (DS) cases due to de novo and inherited Robertsonian translocations and other structural abnormalities, 13 complex rearrangements and 21 mosaic cases. Rare aberrations were observed: numerical aberrations: 48,XXY, +21; structural unbalance: 46,XX,psudic(21;21)(21pter ->21q22::21q22->pter); 46,XY,der(9)t(9;21) (p24.3;q11.2); 46,XX,dup(21)(q22q11.2); complex rearrangements: 48,XY,+21,+mar; 47,XX, t(11;21) (q21;q22)mat,+21; 47,XY,t(12;22)(p11.2;q13) pat, +21; 47,XX,t(9;14)(q13;q22)+21; 47,XY, inv(7) (p12q21.1)mat,+21; 47,XX, inv(9)(p11q13), +21. Mosaic forms are presented as numerical abnormalities -47,XY,+21/48,XY+21;+21; 47,XY, +21/ 46,XY, as structural 46,XX, der (21;21) (q10; q10),+21/46,XX and complex rearrangements- 48,XY,+21,+mar/46,XY. Phenotype's data contains clinical, ultrasonography, post mortem findings. DSR includes different proband & parents characteristics like sex, age, origin of mutation (de novo, inherited), consanguinity, ethnic origin, graphic pedigree. Fields for application: DSR is utilized in the scientific researches, genetic counseling and prenatal diagnostics service, congenital malformations monitoring system, in different Teaching Genetics courses for medical geneticists, cytogeneticists, other physicians and students. DSR is an in-progress database. Our next step for update DSR is to built a new „prenatal DS cases“ subgroup. Rare cytogenetical and clinical data of our patients with DS will be discussed. Teaching Genetics courses organization in Belarus will be presented.

P1-07 03

A Phenotypically Normal Liveborn Male after Prenatal Diagnosis of Trisomy 20 Mosaicism

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Trisomy 20 is a common chromosomal mosaicism diagnosed in amniotic cells, constituting 16 % of all such mosaicism, and it is also one of the most common pseudomosaic trisomies. Trisomy 20 mosaicism remains a serious dilemma in prenatal diagnosis and genetic counseling. In prenatal chromosome analysis a true mosaic is present if two different karyotypes are found in at least two different clones or cultures. We report on a case of a prenatally detected trisomy 20 mosaicism and the pregnancy outcome. Cytogenetic analysis was performed on amniocytes (the karyotypes identified in three independent cell cultures were 46, XY[15]/47,XY+20[4]; 46,XY[12]/47,XY+20[6] and 46,XY[20]/47,XY +20[3], respectively)and postnatally on lymphocytes and extra-embryonic tissues.For analysing uroepithelial cells we established a new protocol. We performed cell nuclei preparation by treating with potassium chloride and trypsin solution and after fixation procedure we performed a pepsin digestion, so that we revealed good nuclei preparation of urine sediment for FISH analysis. After birth of a healthy male baby conventional cytogenetic analysis and FISH were performed on umbilical cord blood lymphocytes, on extra-embryonic tissues (three different sites of the placenta) and on an urine sample. The postnatally analysed cells showed disomy 20 by conventional and FISH analysis. The phenotypically normal male baby is developing normal. We compare our data to previously described cases in the literature. The significance of a prenatally analysed trisomy 20 mosaicism is still in doubt. Review of the literature shows that it is not possible to correlate the presence of amniotic fluid trisomy 20 mosaicism with any specific phenotypic abnormalities.

P1-07 04

Follow-up in a Patient with Trisomy 8 Mosaicism over a Period of Eleven Years

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Trisomy 8 estimated to occur in about 0,1% of all clinically recognised pregnancies. In the liveborn population, trisomy 8 is almost always associated with mosaicism, and more than 100 cases have been reported so far. Individuals with trisomy 8 mosaicism may present with a variety of features, including moderate mental retardation, multiple skeletal and joint anomalies, urogenital malformations, congenital heart defects, deep palmar and plantar furrows, distinct and agenesis of the corpus callosum. There is a great phenotypical variability, and several patients displaying normal intelligence and development and normal or near-normal phenotype have also been described.
To date, relatively few long-term studies of probands with trisomy 8 mosaicism have been published. We report on the cytogenetic and molecular-genetic investigations of a child with mosaic trisomy 8, analysed over a period of eleven years. The female patient showed clinical features and facial dysmorphisms characteristic of the syndrome as well as mentally impairment. The mosaic trisomy 8 was diagnosed prenatally in amniotic fluid cells (38%) and fetal lymphocytes (78%) and was confirmed postnatally in the umbilical cord (52%), placental biopsy (40%), lymphocytes (between 55% and 70%) and buccal mucosa cells (between 30% and 42%), demonstrating the overall prevalence of the trisomy 8 cell line in this patient was quiet high. We found no evidence of an appreciable increase or decrease in the frequency of the trisomic cell line over a period of eleven years.
Molecular genetic investigation demonstrated the maternal origin of the additional chromosome 8. It is known from the literature that trisomy 8 mosaicism in liveborns can almost always be traced to a post-zygotic mitotic error.

P1-07 05

Subtelomeric screening: new means to diagnose chromosomal changes in children with malformations of the heart

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Congenital heart defects are heterogeneous. Besides being exclusively exogenous, there exist those caused by genetic factors on the one hand and on the other hand multi- factorial ones adding an exogenous stimulus to an existing genetic disposition.
The latter include the majority of congenital malformations of the heart. The frequency of heart defects among newborns amounts to 0,6% in Central Europe. Already prenatally, a high percentage of severe malformations of the heart can be diagnosed by ultrasound.
Parents of a child with congenital heart defects carry a risk of 2-4%, statistically, to have another child with congenital heart defects. This empirical figure includes couples with a very high risk (50%) and those with a very low one (less than 1%), though. Furthermore, of importance is the indication of additional malformations and/ or dysmorphic features like morphological changes without illnesses. This requires classification of the syndromes.
About 10% of the patients with congenital malformations of the heart show the presence of a chromosomal aberration detectable by conventional cytogenetic analysis. Prenatally performed ultrasound at specialised clinics can even lead to a percentage of 40% of malformations of the heart caused by chromosomal aberrations thus justifying a supplementary chromosomal analysis in all cases of heart defects.
Up to now, there was no way to unequivocally exclude small structural aberrations by means of conventional analyses. The development of fluorescence in situ hybridisation (FISH) which enables the investigation of critical regions with small DNA-probes offered further results concerning submicroscopic microdeletions.
Our study presented deals with the exclusion of subtelomeric deletions leading to congenital malformations of the heart. Preliminary results are being presented.
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P1-07 06

Analphoid de novo marker chromosome invdup(3)(q28qter) with neocentromere in a dysmorphic and dvelopmentally retarded girl

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We describe a marker chromosome from distal 3q which is smaller than those reported earlier. The marker was ascertained during cytogenetic diagnostics of a 5-year-old girl with developmental delay and mild facial dysmorphism. The girl showed slight hirsutism and a bilateral ichthyosiform hyperkeratosis of hands and feet. At the age of 5 years, she showed slight muscular hypotonia and hyporeflexia. Perceptive skills and visiomotoric coordination were retarded corresponding to a developmental age of 3-3.5 years. At the age of 8.5 years the girl attends a special school for mentally handicapped children. She cannot speak properly, seeking for words, can recognize only few letters, and is still unable to write. Chromosome analysis detected a very small supernumerary de novo marker chromosome, which was C-band negative but appeared mitotically stable in cultured lymphocytes. FISH with an alpha satellite probe detecting all human centromeres gave hybridization signals on all chromosomes of the proband, except the marker chromosome. The chromosomal origin of the marker was clarified by microdissection and reverse painting: After microdissection and amplification DNA from the marker hybridized exclusively on the marker and on distal 3q. In order to characterize the extent of the marker chromosome along 3q, FISH with mapped YAC or BAC clones was performed. All clones tested gave either no FISH signal on the marker chromosome, or a double signal. Thus the marker belongs to the expanding category of analphoid marker chromosomes originated by (presumably inverse) duplication of a distal chromosome arm, which must have gained centromere function by neocentromere formation. According to the ENSEMBL database the most proximal clone present on the marker, RP11-634I24, maps to chromosome band 3q28, and the extent of the duplicated segment is approximately 7 Mb.

P1-07 07

Recurrent bone fractures and overgrowth in a retarded boy due to unbalanced t(2;15)(q37;q26)de novo translocation

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Growth retardation is considered a common feature in children affected with developmental delay caused by an unbalanced chromosomal rearrangement. Prenatal onset of tall stature, by contrast, is observed mainly as a result of maternal influence eg gestational diabetes, so called overgrowth syndromes or distinct genetic causes like fragile X syndrome. We report on a now 2 year old boy who showed in the newborn period macrosomia, muscular hypotonia,

long and thin hands and feet with hyperextensible fingers, somewhat unusual facial appearence, and transient hypocalcemia. Later on overgrowth persisted and psychomotor and speech delay was noticed. In the age of 1 year two femoral fractures occurred without an appropriate trauma. Lymphocyte karyotyping revealed a small additional fragment on 2q. Further investigation by means of subtelomeric probes revealed a translocation of the subtelomeric 15q FISH-signal to 2q, in addition to 2 normal signals on both chromosomes 15. Using a panel of 9 FISH probes mapping to the telomeric 2q region, evidence was given for a very small 2qter deletion in addition to the partial trisomy 15qter. The parent's karyotype proved to be normal. To our knowledge, our patient represents the first observation of this unusual clinical picture associated with this particular rearrangement. More detailed analysis of the breakpoints on 15q and the respective genes might elucidate pathogenesis of the phenotype and possible therapeutic approach.

P1-07 08

Confocal laser scanning microscopy on the cytoskeleton and human chromosomes after immunostaining, multicolor FISH and Feulgen reaction

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At present confocal laser scanning microscopy allows an insight into exceedingly complex organization of chromosomes. New results are now possible by analysis of 3-dimensional ultrastructural pictures. Especially the simultaneous presentation of fluorochromed chromosomes in combination with fluorescence-immunohistochemical presentations of the cytoskeleton provides an innovative methodical approach to study 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 developed 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 concen-tration. In the case of simultaneous tested ß-actin such a quantitative increase was not found. The obtained data allow conclusions to be drawn on the intracellular, intranuclear and intrachromosomal localization of elements of the cytoskeleton (e.g. tubuline and tau-protein), and permits analyses of the location-specific interaction between some (e.g., by multicolor FISH). Fluoreszenz analysis of interphase nuclei and metaphase chromosomes after Feulgen staining (DNA-analysis at 63 C; excitation laser wavelength 364nm, 488 nm, 543 nm and 633 nm) allows measurements of the DNA content in the different phases of the cell cycle. The integrated density profiles shows in some of the nuclei chromosomes in a compartment like structure („chromosome territories"), probably in the end of G2 phase. 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.

P1-07 09

A third case of proven mosaicism for del(17)(p11.2p11.2) in a Smith-Magenis-syndrome (SMS)-patient

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Smith-Magenis-syndrome (SMS) is associated with an interstitial deletion involving band 17p11.2. Since its first description in 1982, over 100 patients with SMS have been reported. The incidence is estimated to be 1:25.000 livebirth, but it is believed that this condition is easily overlooked clinically and also be missed cytogenetically.

SMS is characterised by a well-documented but varying clinical phenotype including brachycephaly, prominent forehead, epicanthal fold, broad nasal bridge, ear anomalies, prognathism, short stature, brachydactyly, sleep disturbance, self-injurious behaviour, signs of peripheral neuropathy, delayed speech development, and variable degrees of mental retardation.

We report a third proven mosaic case for the deletion characterising SMS. To our knowledge, proven mosaicism for del(17)(p11.2p11.2) has been reported before only twice. Our patient is the second who is mosaic and displays characteristic phenotypic anomalies of SMS. The first observed mosaicism was a phenotypically normal mother mosaic for del(17)(p11.2) discovered only because she had had a child with SMS.

The detection of mosaic cases not only for SMS but also for other structural rearrangements emphasises the value and usefulness of FISH analyses. Furthermore a generous use of FISH is recommended even though only a part of examined metaphases are suspected to bear a subtle aberration.

It is quite possible that mosaicism for del(17)(p11.2p11.2) and further structural rearrangements may be a significantly underdiagnosed cause of contiguous gene syndromes, particular in patients with mild expression of the phenotype.

The frequency of mosaicism for structural chromosomal abnormalities is unknown but this type of aberration may be much more common than was, is, or ever could be, recognised.

P1-07 10

A Phenotypically Normal Liveborn Male after Prenatal Diagnosis of Trisomy 20 Mosaicism

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Trisomy 20 is a common chromosomal mosaicism diagnosed in amniotic cells, constituting 16% of all such mosaicisms, and it is also one of the most common pseudomosaic trisomies. Trisomy 20 mosaicism remains a serious dilemma in prenatal diagnosis and genetic counselling. In prenatal chromosome analysis a true mosaic is present if two different karyotypes are found in at least two different clones or cultures. We report on a case of a prenatally detected trisomy 20 mosaicism and the pregnancy outcome. Cytogenetic analysis was performed in amniocytes (the karyotypes identified in three independent cell cultures were 46,XY[15]/47,XY +20[4]; 46,XY[12]/47,XY+20[6] and 46,XY[20]/ 47,XY+20[3], respectively), and postnatally in lymphocytes and extra-embryonic tissues. For analysing uroepithelial cells we established a new protocol. We performed cell nuclei preparation by treating with potassium chloride and trypsin solution and after fixation procedure we performed a pepsin digestion, so that we revealed good nuclei preparations of urine sediment for FISH analysis. After the birth of a healthy male baby conventional cytogenetic analysis and FISH were performed on umbilical cord blood lymphocytes, on extra-embryonic tissues (three different sites of the placenta) and on an urine sample. The postnatally analysed cells showed disomy 20 by conventional and FISH analysis. The phenotypically normal male baby is developing normal. We compare our data to previously described cases in the literature. The significance of a prenatally diagnosed trisomy 20 mosaicism is still doubtful. Review of the literature shows that it is not possible to correlate the presence of amniotic fluid trisomy 20 mosaicism with any specific phenotypic abnormalities.

P1-07 11

Partial trisomy 10p and partial monosomy 6p resulting from a paternal translocation - case report and review of the literature

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We report on a 2 year old boy who is the first child of a
healthy not consanguinous couple. At delivery the mother was 34 years old and already had two healthy children from a previous marriage. During pregnancy no adverse events were noted. Delivery was induced because of CTG-abnormalities in the 38th week. Birth weight, length and head circumference were in the normal range. Several craniofacial abnormalities are present: triangular face shape, unilateral flat occiput, frontal bossing, low set ears with preauricular tags, deep hair insertion, long lashes.
Moreover, the boy shows anisocoria with normal reagibility of the pupils. In addition he developed microcephaly (<3rd percentile). The statomotoric development is severly retarded. Development of speech is also severly disturbed although hearing tests were normal. Structural brain abnormalities or organ malformations are not present. On karyotyping we found an elongation of the short arm of chromosome 6 derived from a

previously unknown translocation of the father. The karyotype of the child is 46,XY, der (6) t (6;10) (p25;p13) pat. Compared with cases reported in literature our propositus shares some features with other individuals with trisomy 10p (microcephaly, hypotonia and severe developmental delay). Cleft lip / palate is also reported although in association with the region p11.2 to p12.2. Partial deletion of the distal part of 6p correlates with abnormalities of the anterior eye and hearing loss. The anisocoria of our patient is an interesting finding in this context. In summary, the phenotype of the unbalanced translocation in our case seems to be influenced both by partial trisomy 10p but also by haploinsufficiency for genes located on the distal part of 6p.

P1-07 12

Cytogenetic and molecular cytogenetic study of an infant with MIDAS syndrome: First case of MIDAS syndrome in a 46,XY male

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The MIDAS syndrome (microphthalmia, dermal aplasia, sclerocornea) alias MLS syndrome (microphthalmia, linear skin defects) is a rare X-linked multisystemic disorder occurring exclusively in XX individuals. To date, 32 cases of MIDAS syndrome have been published. In most cases, the MIDAS syndrome was associated with Xpter->Xp22.2 monosomy. These observations suggest that deletions within the critical Xp22.2 area are lethal in males, possibly due to nullisomy for the causative gene.
The patient, a male infant, showed intrauterine growth failure (week 37, birthweight 1640 g)linear skin defects parallel to the nose, and corpus callosum agenesis. Findings strongly suggest MIDAS syndrome. Additionally he suffered from severe congenital lactic acidosis, and cardiomyopathy. High resolution RBG-banding revealed a subtle inversion in Xp22 [inv(X)(p22.33/22.32p22.13)] in 15 % of lymphocytes.
The finding was confirmed by FISH and DNA analyses. Biochemical studies confirmed a deficiency of the pyruvate dehydrogenase complex. To our knowledge, this is the first case of MIDAS syndrome in a 46,XY male.

P1-07 13

Clinical, cytogenetic and molecular analyses of partial 21q monosomy in a girl with mental retardation, marfanoid habitus and minor dysmorphic features

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Phenotypic and molecular analysis of patients with partial monosomy 21 resulting from translocations, ring chromosomes or pure partial monosomy 21 allows to define which regions of chromosome 21 contribute to the generation of specific aspects of the 21q- syndrome. Here we report on the clinical, cytogenetic and molecular characterization of a pure de novo partial monosomy 21 with a deletion of 21q22.2-qter. FISH mapping of cytogenetically and genetically anchored YAC and BAC clones result ed in the identification of a BAC clone clearly spanning the deletion breakpoint. Moreover, we have amplified different single copy DNA probes within exon-intron sequences of the ETS2 (avian erythroblastosis virus E26 oncogene homolog 2) gene for FISH analyses. Interestingly, our results revealed that the deletion breakpoint must lie in a fragment of 5,2 kb, between exon 5 and exon 8 of ETS2. Molecular studies using polymorphic markers supported these findings and showed that the derivative chromosome 21 was of paternal origin. The patient who showed mild mental retardation, marfanoid habitus and minor dysmorphic features, is lacking most of the typical features seen in the 21q- phenotype and thus is quite unique. Our findings support the suggestion that the loss of the region at 21q22.2-qter is critical for only some minor aspects of the 21q-syndrome. Genotype-phenotype correlations of our case and other reported cases will be discussed.

P1-07 14

An Unusual Intrachromosomal Rearrangement in Chromosom 16 in an Azoospermic Man

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Cytogenetic investigation in a couple with fertilization failure revealed a normal karyotype for the woman while the partner showed an aberrant chromosome 16. The banding pattern after GTG-, CBG- and RBG-banding was not compatible with an inversion and more than two breakpoints were suspected in one chromosome 16. After FISH using a WCP 16 translocation involving another chromosome was excluded. Hybridisation using a locus specific probe for 16p13.3 (D16Z71) showed a normal signal localisation on the normal #16 while on the der(16) the signal was observed proximal to the centromere on the p-arm. However, only by multicolor banding (mBAND) it was possible to identify the chromosomal structure and 5 breakpoints were diagnosed. Additional information was obtained by hybridisation using the subtelomeric probeset for chromosome 16 which leads to the diagnosis of altogether 6 breakpoints. The derivative chromosome 16 was described as follows:

der(16)(pter::p12 ->
p11.2::q11.2->p11.2::p12->p13.3::q12.1->q24::q11.2->q12.2::qter)
The couple wished a intracytoplasmatic sperm injection (ICSI) because of desperate desire for children. The family situation and the results of the cytogenetic and molecular cytogenetic investigations will be reported in detail as well as the outcome of the pregnancy.

P1-07 15

Dysmorphic stigmata and psychomotor retardation in a boy with a rare interstitial deletion del(9)(q32q34)

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We report on a 2 year old boy with a very rare deletion 9(q32q34). The 30 year old father and the 29 year old mother are healthy and not consanguinous. The pregnancy and delivery were uneventful, the birth weight was 2080g (< 3 centile), length 48 cm (3 - 10 centile) and head circumference 32 cm (< 2 centile) after 40 weeks gestation. The patient presents at the age of 2 years dysmorphic stigmata including downslanting palpebral fissures, prominent forehead, retrognathia, deep set ears, microcephaly, broad nasal bridge, simian crease, and arachnodactyly. GTG-banding analysis of cultured lymphocytes revealed an aberrant karyotype 46,XY ,del(9)(q32q34). The interstitial break points were confirmed by high-resolution multicolor-banding (MCB) with a chromosome 9 probe mix and FISH-analyses with a ABL gene specific probe [VYSIS] and three different YAC-probes (957k08, 933c05, 750c6). To the best of our knowledge, there are only 3 further patients with del (9)(q32q34) reported in the literature (Farrell et al., 1991). Currently, the small number of cases does not allow the delineation of a characteristic chromosomal syndrome.

P1-07 16

A molecular cytogenetic study of female patients with various Xq deletions

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Efforts of more precise karyotype-phenotype correlations have been made in 10 cases of various Xq deletions. First, the presence of an additional 45,X cell line was evaluated. Second, the breakpoint localization as well as the X inactivation status were studied by high-resolution RBG-banding. Third, the precise breakpoint localization was verified by FISH using a specific set of Xq probes: XIST (q13.2), pcos80II-7 (q13.2-q21/Yp11), and tel Xq/Yq (subtelomeric Xq28). Further, the presence of a minor Xq-/Xp+ or Xq-autosome rearrangement was excluded by FISH using cosmid probes 2.1/2.B (in PAR1) and wcpX probe.
The results were: 1. Nine out of ten cases of our Xq deletions had a breakpoint in the proximal region Xq13 to Xq24; 2. One case was an unbalanced Xq/12q translocation with a distal Xq breakpoint; 3. An additional 45,X cell line was present in four cases; 4. In all cases the deleted Xq chromosome was always late replicating; 5. Interestingly, all Xq deletions tested had the distal breakpoint in the 300 kb subtelomeric region. Finally, we tried to correlate the clinical findings of the female patients with the extent and localization of the Xq deletions. Special attention has been paid to non-mosaic Xq deletions. The results support the notion that the phenotype in Xq patients is inexplicable by the size of Xq deletion and a mosaic constitution alone.

P1-07 17

Two healthy boys with normal karyotype from a mother with isochromosome t(21;21)

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Robertsonian translocations are among the most common balanced structural chromosome rearrangements in the general population with an estimated incidence of 1 in 1000. They can be transmitted through many generations. Carriers of a balanced Robertsonian translocation are phenotypically normal. The majority of balanced Robertsonian translocations involve nonhomologous chromosomes. Homologous translocations are rare and always seen as a de novo event. Most likely homologous Robertsonian translocations are postzygotic and of maternal and paternal origine. Fusions occur in one of the earliest cell divisions. In contrast, most of the t(21;21) chromosomes are isochromosomes i(21q) arising from one homolog, either maternal or paternal. Practically all conceptions of homologous translocation carriers result in either trisomy or monosomy. Monosomy 21 results in abortion, as does trisomy 14, 15, 22 and most of trisomy 13. Trisomy 21 results in Down syndrome. Only one case of a son with ring 13 from a 13;13 translocation mother and one case of a son with ring 15 from a 15;15 translocation mother are known. Here we report a healthy mother with homologous translocation t(21;21). Chromosome analyses in all 60 metaphases from peripheral blood lymphocytes showed a 45,XX,t(21;21)(q10;q10) karyotype. She had five miscarriages, but gave unexpected birth to two phenotypically normal boys with 46,XY karyotypes. This observation confirms the theory of mitotic origine of the t(21;21), formed after conception.
Most likely the mother carries a somatic mosaic 46,XX/45,XX,t(21;21). To our knowledge this is the first report of a homologous Robertsonian translocation carrier with healthy children.

P1-07 18

Mapping the non-recombining region of the human Y chromosome (NRY) by „fiber-FISH“

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The non-recombining region of the human Y chromosome (NRY), which comprises 95% of the chromosome, does not undergo sexual recombination. Thus a genetic linkage map cannot be constructed, and the localization of genes and gene families within this differential region on the Y chromosome depends on physical mapping. Complete physical mapping, however, has been found difficult in regions rich in repetitive sequences. Recently, a first detailed map of the human NRY has been published (Tilford et al. 2001), and nearly one half of the euchromatic portion of the NRY has been sequenced through the publicly funded Human Genome Project. However, there are still four gaps - one in Yp and three in Yq - in this map of overlapping BACs and ordered STSs spanning the human NRY. To overcome the problems of physical gene mapping occurring when building up a Y contig, we made use of the fiber-FISH-technique as a complementary mapping strategy. This technique allows the direct visualization of cloned DNA sequences along chromatin „fibers“ released from interphase nuclei. In the present study we physically mapped the gene families CDY, DAZ, RBMY and TSPY on released chromatin. We used the Y heterochromatin and the Y centromere-specific alphoid sequences, as well as the already mapped XKRY genes (Lahn and Page 1997) as markers along the fibers. Our mapping results received by fiber-FISH allowed the construction of an overlapping fiber-FISH contig spanning the non-recombining region of the human Y. Indeed, the results of our fiber-FISH contig presented here may help to close the gaps in the Tilford-map. Furthermore, it may help to get a more complete picture with respect to position and arrangement of the multi-copy gene families investigated along the human NRY.

P1-07 19

Computer assisted diagnosis of chromosomal aberrations using a Bayesian and a counting approach with the help of the database SYNDROC

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The possibility to diagnose chromosomal aberrations using a computerized database was tested using 101 patients with an established chromosomal aberration using the database SYNDROC. This system provides the user with two different algorithms for the calculation of a diagnosis:
- a descriptive algorithm which proposes a diagnosis counting a set of phenotypic markers all having the same weight.
- a Bayesian-algorithm which, evaluating calculates probabilities for competing diagnoses by analyzing phenotypic anomalies.

Three levels of precision were used assessing the diagnoses: suggestion of the correct (1) chromosome number, (2) chromosome arm, (3) aberration type and rough location.
The combination of both algorithms yielded 51 consensus diagnoses for the level of the correct chromosome, 24 for the chromosome arm, and 15 for the aberration type. Additional diagnoses solely with the descriptive algorithm were yielded for 43, 52 and 47 cases and using the Bayesian-algorithm for 1, 4 and 1 diagnoses respectively. Since with the Bayesian-algorithm, when evaluating an uncertain diagnosis using a combination of symptoms, one does so by calculating the probability of the claim in the light of given information. This seems to be a much more promising for a correct diagnosis than the pure counting of numbers of matches. The Bayesian coefficients were in the range between 0.57 and 0.05; the 0.5 margin as a trustworthy one announced by the authors of SYNDROC was reached by only one of the correctly recognized cases. The prior probabilities for the calculations of the Bayesian-formula do not seem to use serviceable weights.

P1-07 20

From rod to rings: Chromosome 18 replaced by two ring chromosomes of chromosome 18 origin

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In a patient with a single chromosome 18 and two additional ring chromosomes, FISH analysis with a whole chromosome paint specific for chromosome 18 revealed the chromosome 18-origin of the ring chromsomes. FISH analysis with a chromosome 18 specific alpha-satellite probe demonstrated a chromosome 18 specific centromere on both ring chromosomes. The signal on the normal chromosome 18 always presented stronger than the signals on both individual ring chromosomes, respectively. With subtelomeric probes specific for the short and long arm, the normal chromsome showed the respective signals, while the ring chromosomes showed no signal. A chromosome painting probe specific for the short arm of chromosome 18 revealed a positive signal on the small ring chromosome. Molecular studies showed the exclusive presence of paternal alleles distal to short arm locus D18S843 and distal to long arm locus D18S474. This demonstrates 1. the maternal origin of the ring chromosomes, and 2. the loss of the respective short and long arm regions distal to these loci thus indicating the breakpoints in ring formation. The dysmorphisms observed in the patient also indicate deletions of both chromosome arms, as clinical findings partly overlap with observations in 18p- and 18q- syndrome and are similar to some cases of ring chromosome 18. This case is the first example of the replacement of an autosome by two ring chromosomes originating from the missing chromosome. Centromere misdivision is suggested as one mechanism involved in the formation of the ring chromosomes.

P1-07 21

FISH assessment of sperm aneuploidy frequencies in ICSI patients with severe oligoasthenoteratozoospermia (OAT)

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The aim of this study was to examine chromosomes in sperm by fluorescence-in-situ-hybridisation (FISH) with considerable differences in disomy frequency for the chromosomes 13, 16 and 21.
23 patients with oligoasthenoteratozoospermia were involved in our study. FISH procedure was made by standard protocol, using probes for chromosome 13 and 21 (locus-specific probes) and a probe for chromosome 16 (centromeric satellite probe). For each patient 100 - 2000 sperms were analysed. In the OAT-patients, the average incidence of disomy for chromosome 13, 16 and 21 is 2.12%, 1.57% and 1.87%, respectively. In the normospermia patients the average disomy incidence of chromosome 13, 16

and 21 is 0.67%, 0.44% and 1.1%, respectively. The incidence of aneuploidy was significantly higher in OAT-patients (median: 5% vs. 2.8%; Wilcoxon-test: p = 0.028).
Our results support the hypothesis of positive correlation between OAT and higher aneuploidy rates. However, this correlation is not linear and absolute, among patients with severe OAT we can observe also high proportion of men with normal range of diploidy.
According to our results and also results from literature, we recommend a more intensive prenatal control of pregnancies, originating from the ICSI method.

P1-10 01

Expression profiling in murine medulloblastomas by means of cDNA microarrays

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Medulloblastoma is a highly malignant, invasive embryonal tumor of the cerebellum with a preferential manifestation in children. Although the majority of medulloblastomas occur sporadically, this tumor is also associated with familial cancer syndromes including the nevoid basal cell carcinoma (Gorlin) syndrome. Mutations in the tumor suppressor gene PATCHED have been described in both familial and sporadic cases and inactivation of one Patched allele in mice promotes development of medulloblastoma. In order to determine candidate genes involved in tumorigenesis of medulloblastoma we have screened tumors of heterozygous patched mice for differentially expressed genes by means of cDNA microarray technology. Hybridization of fluorescently labeled cDNA probes isolated either from normal cerebellum or medulloblastoma to an array of 1000 genes revealed cell cycle genes, cell adhesion genes and signal transduction genes to be altered in the tumor. Our data suggest that formation of medulloblastoma in Patched mutants is associated with an aberrant activity of mitogenic signaling pathways.

P1-10 02

Identification of Protein-Protein Interactions by Systematic Interaction Mating

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The sequencing of the human genome has resulted in the identification of a large number of novel proteins, whose functions have to be determined. In order to determinate the function of human proteins we will analyse protein-protein interactions on a large scale using the yeast two-hybrid system. For the systematic identification of protein-protein interactions an automated yeast two-hybrid system has been developed in our laboratory. This method is based on interaction mating in 384-well microtiter plates. Currently, we are subcloning ~2000 human cDNA-fragments into DNA-binding and activation domain vectors. These plasmids are then transformed into yeast MATa and MAT alpha strains for interaction mating. Using the automated yeast two-hybrid system we will generate a protein-protein interaction matrix of 2000 human proteins. To verify the protein-protein interactions in vitro binding experiments, co-immunoprecipitations and co-localisation studies are performed. The results of the two-hybrid screens and of the functional assays will be stored in the primary database at the RZPD.

P1-10 03

Decreased decorin transcript levels in fibroblasts of patients with Ehlers-Danlos type I/II syndrome with and without COL V mutations

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Ehlers-Danlos syndrome (EDS) is characterized by loose-jointedness and fragile, bruisable skin. EDS I is the severe form of classic EDS, and EDS II is the milder one. There is evidence that EDS types I and II are allelic, and that mutations in COL5A1/A2 cause the syndrome in about 40% of the reported investigated cases. Another molecule of the extracellular matrix is decorin. Decorin (DCN) is a small leucine-rich proteoglycan. Dcn null mice show an increased fragility of skin and altered shape and interfibrillar distance of collagen fibrils. Therefore, DCN might be a candidate gene for idiopathic fragile skin disorders. We present data about studies in the DCN gene of unrelated patients and familial cases with EDS types I and II, diagnosed according to clinical symptoms and ultrastructural collagen fibril aberrations. We have sequenced all exons with their splice junctions of DCN on genomic DNA of 16 EDS I/II individuals. Not a single mutation nor a polymorphism was found. In addition we measured transcript levels in skin fibroblasts of these patients. There is a significant decrease in transcript levels to an average of about 60 % independent of an intact COL5A1/A2 allele. We suggest that a factor upstream of decorin contributes to the down-regulation of decorin on the mRNA level. The diminished amounts of processed decorin proteoglycan molecules may be a consequence of other disease-causing mutations and co-influence the precise assembly of collagen fibers, leading to fragile skin and other connective tissue disorders.
This work was supported by the Fritz Thyssen Foundation. Grant: 1999 1071

P1-10 04

SELDI protein chip analysis of laser microdissected tissue - optimisation and standardisation

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SELDI-MS (surface enhanced laser desorption /ionisation- mass spectrometry) allows analysing protein extracts from small amounts of samples. Protein profiles of cell lysates, urine and serum, can be generated. To gain small well-defined not contaminated samples, especially for the analyses of tumours, microdissection is inevitable. Therefore our aim was to combine both techniques to an optimised procedure for protein profiling in cancer. The LCM (laser capture microdissection) technology offers the possibility to perform a tissue selection at the microscope level and to cut normal and tumour cell areas accurately by laser. Currently, however, there are no references to what extend the SELDI generated protein spectra gets affected by LCM handling. Consequently we tested potential procedures for handling tissue with LCM to obtain samples that can be analysed by SELDI. The performed experiments involved the creation of tissue slices, different stains, different LCM procedures, transfer of excised tissues by laser or needle into a test tube and the quantifying of the minimum of required tissue. Best results were detected by using non-stained cryostat tissue slices (8 µm thickness) on microscope slides coated with a 1.35 µm membrane. Different tested stains affected the results adversely. The membrane between section and slide did not show influence on the protein profile and thus it is advantageous, because of the better handling of the laser-cut section. To keep the impact of the laser as slight as possible it appeared favourable to transfer the laser-excised areas of tissue slices into test-tube manually by a lancet-needle. By analysing a tissue lysate dilution series we defined the minimal required quantity of tissue that still generates satisfying results by SELDI-analysis as about 700 cells. Using this procedure we are in the position to obtain optimised and standardised results in SELDI analysis.

P1-10 05

The search for proteins interacting with bestrophin

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Best vitelliform macular dystrophy (BMD) is an autosomal dominant disease with a juvenile age of onset that causes loss of visual acuity. BMD is diagnosed specifically by a depressed electrooculogram. The gene responsible for Best disease (VMD2) has been identified by positional cloning and encodes a 585 amino acids transmembrane protein named bestrophin. Bestrophin is preferentialy expressed in the retinal pigment epithelium (RPE) and is localized to its basolateral plasma membranes. Based on amino acid sequence predictions bestrophin contains at least four transmembrane domains (TMDs), with the N- and the long C-terminal portions facing the cytosol. Almost all disease-associated alterations reported to date are missense muta-

tions that cluster in 4 regions near the predicted TMDs.
Our goal is to identify interacting partners of bestrophin and to elucidate the molecular function of the protein. Toward this goal a GAL4-based yeast two hybrid (Y2H) system was used. An oligo(dT)/random primed bovine RPE cDNA library was constructed and cloned as a fusion of the activation domain vector pGADT7 and the bovine RPE cDNAs. In addition, we generated a series of bestrophin-derived baits. Thus far, screenings with N-terminal and loop fragments have been completed by analysing 6 x 105 and 8 x 105 transformants, respectively. This resulted in the isolation of 37 (N-terminus) and 6 (loop fragment) putative positive clones. Further characterization and verification will include reintroduction of plasmids from positive clones into the yeast strain used for screening either (i) alone, (ii) with the bait vector, (iii) with the bait used in the initial search, or (iv) with a plasmid encoding a totally unrelated protein as a DNA-binding domain hybrid. Subsequently, plasmids only positive with the target hybrid will be subjected to GST-fusion assays and coimmuno-precipitation studies. Additionaly we generated a set of human VMD2 N-terminal mutants by site directed mutagenesis in order to asses the effect of the disease-associated mutations on the strength of the protein-protein interactions.

P1-10 06

Monitoring tissue remodelling in human dilated cardiomyopathy (DCM) by gene expression profilling

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In response to various pathophysiological conditions heart undergoes complex remodelling processes, which primarily compensatory, ultimately lead to ventricular dilation and heart failure. Cytoskeletal, apoptotic and calcium-related signalling pathways play a pivotal role in this remodelling gene programme. To monitor gene expression during progression of human dilated cardiomyopathy (DCM), we collected biopsies from 10 patients by routine heart catheterization. An „in silico" normalised human UniGene library containing 33,689 cDNA clones was PCR amplified and robotically arrayed on nylon membranes. The resulting filters were hybridised with 33-P labelled cDNAs derived from RNA isolated from 4 healthy and 10 dilated cardiomyopathy biopsies. We identified ~650 differentially expressed transcripts, from which ~400 were sequence verified, and several (23) confirmed by real time-PCR. Global trends in gene expression patterns were revealed by hierarchical clustering and functional categorisation.
The largest number of differentially regulated genes was found to be involved in energy metabolism, sarcomere architecture and cell signalling. Moreover, several transcripts emerge to be involved in the cytoskeleton-related signalling, myocyte survival/apoptosis and calcium homeostasis. These may be essential for dilative remodelling and heart failure. In conclusion, this study represents the first report on gene expression profiling of >33,000 unique cDNAs using human cardiac biopsies from clinically stable patients. The resulting profiles might enable to describe the pathways involved in the pathophysiology of DCM and heart failure. Finally convenient custom-arrays for routine profiling may be developed on this basis, and provide a useful diagnostic resource.

P1-10 07

Identification of differentially expressed genes in colon carcinoma by pairwise hybridization of normal and tumor tissue on global human cDNA arrays

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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 72.000 non-redundant sequences. 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 in carcinomas e.g. IGF2R (insulin growth factor receptor), several kinases and transcription factors and downregulated e.g. carbonic anhydrases and genes involved in apoptosis. 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 without annotation 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 strategy for testing new targets in colon cancer therapy.

P1-10 08

Characterization of Novel Proteins by high Throughput Analysis of Human Protein Networks (Automated Two-Hybrid Screening)

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As the human genome becomes fully sequenced, our attention turns to proteomics, the large-scale identification and characterization of proteins. Our project is focused on the characterization of unknown human proteins by yeast two-hybrid screening in order to assign them to particular pathways or multimeric structures.
We defined the genes of interest in the human genome that lack annotated function, or that have purely predicted function attached. In total we found 5.200 of these genes, and cloned 3.120 full length ORFs up to now. Most of them have been completely sequence verified and annotated, to characterize differences to the already published sequence data in respect to bp exchanges and alternative splice forms. Only clones showing stop codon mutations and frame shifts were discarded. The information on the cloned material has been passed on RZPD.
As the initial cloning was done in a recombination shuttle vector system, we are moving the ORFs in a second step to bait and prey vectors (1.150 and 96 ORFs respectively). These plasmids will be used to perform an automated two-hybrid interaction screening (see joint abstract by Goehler et al.).
We intend to characterize and verify the protein-protein interactions that result from novel proteins by in vitro methods. Therefore, we have established Baculovirus and E.coli (in vitro and in vivo) expression systems. These systems will allow us to gain access to the respective proteins in question for in vitro binding experiments. The results of these studies will be deposited in the Primary Database at RZPD.

P1-10 09

Heterologous expression of human TIGR/MYOC as well as selected mutations in insect cells results in insoluble protein aggregates

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Several mutations in the TIGR/MYOC gene, also known as myocilin (MYOC), were identified in juvenile open angle glaucoma (JOAG) patients, and more rarely in primary open angle glaucoma (POAG). The biological function of MYOC is unknown. Based on High-Five insect cells, we have investigated the determination of a putative adhesion capability of normal and mutant forms of MYOC as well as the localization of the respective proteins in transfected cells. By means of fusion proteins carrying green fluorescent protein (GFP) as a reporter the intracellular localisation was visualized. First, selected mutations (Pro 370Leu, Gln368X, Lys423Glu) have been introduced into a human MYOC cDNA by PCR directed in-vitro mutagenesis. Subsequently these

mutations have been cloned into pure expression/selection plasmids (pEXIV), as well as into expression/selection plasmids in frame with the GFP-coding region derived from pQI25 (Quantum). To transfect these expression plasmids in High-Five cells we used insect cell specific liposomes, namely insectin (Invitrogen).
Conclusions: The results indicated, that a particle number analysis of the cells, transfected either with wt- or mutant forms of MYOC, gave no significant alteration compared to non-transfected or only GFP transfected controls. Using fluorescence microscopy, no insertion of the MYOC/GFP-fusion proteins into the cell membrane was observed. In all cases, GFP-fluorescence was clearly visible in cytosolic vesicles, but not in the nuclei. GFP-alone transfected cells show fluorescence also in the nuclei as well as in the whole cell. With these experiments we have excluded its putative role as adhesion molecule, and furthermore no secretion of MYOC from this cells was observed. MYOCwt protein as well as mutant expression results in agglutinates and MYOC accumulates insoluble in cytosolic vesicles followed by a preliminary cell death. The MYOC agglutination and insolubilisation after overexpression or mutation represents possibly one of the pathomechanisms.

P1-10 10

The Berlin Protein Structure Factory
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The Protein Structure Factory (PSF, http:// www.proteinstrukturfabrik.de) is a joined initiative of research institutes and companies in the Berlin area covering the fields of protein structure analysis, biophysics, genomics, protein expression and bioinformatics. Is was established to characterise the structures of proteins encoded by the large number of human genes being discovered, using automation technology to accelerate the process from the protein sequence to the structure.
Proteins are selected for structure analysis, which have an unknown structure, do not contain non-globular domains and for which full-length cDNA clones are available. In addition to whole proteins, there is a focus on predicted single domains.
In the E. coli expression project of the Protein Structure Factory, human cDNA sequences are subcloned into expression vectors for over-expression in E. coli. A parallel project for the expression in yeasts has been established for proteins that are difficult to express using bacterial systems. Subcloning is performed by standard techniques, which are adapted to the microtitre plate format to increase the throughput. PCR products are purified and bacterial colonies are picked by robotic systems.
Human cDNA clones as templates for subcloning are obtained either from the Berlin Ressource Center of the German Human Genome Project (RZPD, http://www.rzpd.de) or from expression libraries developed by the E. coli expression project. These libraries are arrayed in microtitre plates and onto high-density colony filters using robot technology. Screening of high-density protein arrays, protein expression and purification in microtitre plates and mass spectrometry identify protein expression clones with the desired properties.

P1-10 11

Expression of the Mtmr1 gene in the Mtm1-knock out mouse and its differential alternative splicing
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X-chromosomal recessive Myotubular Myopathy (MTM1) is a severe congenital myopathy in man. Affected boys present with general hypotonia and muscle weakness after birth (floppy babies). A histological examination of muscle biopsies shows myotube-like fibres with centrally located nuclei. Private mutations distributed all over the myotubularin gene, coding for a phosphatidylinositol-3-phosphate specific phosphatase, are causative. Bujbello et al. (unpublished) established a knock out mouse model for MTM1 with a similar phenotype and histology. We examined by quantitative RT-PCR the expression of the myotubularin gene (Mtm1) and its neighbouring homologue myotubularin related protein 1 (Mtmr1), both originating from a common ancestor gene, in various tissues of Mtm1-knock out mice and wild type mice. Myotubularin mRNA was present in the knock out mice in its full length (without exon 4, which is knocked out), and there was no compensatory upregulation of the Mtmr1 gene. Also, no mutation was found in MTMR1 till now in any patient with Myotubular Myopathy. Consequently, MTMR1 does not play a role in the pathogenesis of the myopathy. We observed three new coding exons in intron 2 of the mouse Mtmr1 gene leading to differential alternative splicing in different tissues. Each organ has its own individual pattern of transcripts. This observation supports the idea of a special function of Mtmr1 isoforms in many tissues.

P1-10 12

Gene Expression Changes in Stem Cell Differentiation studied with DNA Microarrays
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Research on adult stem cells has gained increased attention since these cells have been found to show a high plasticity and differentiate into many different cell types.
Mesenchymal stem cells from bone marrow, so called bone marrow stromal cells (BMSC) differentiate into cells of mesenchymal, but also non-mesenchymal origin, including cells with a neuronal phenotype. Another type of adult stem cell that can differentiate into neurons and glial cells exists in the mammalian brain and proliferates in vitro forming so called neurospheres. BMSC which are easily accessible in humans, but also neuronal stem cells from the brain represent a source for cell replacement / transplantation therapy.
We are analyzing the differentiation pathways of these two types of adult stem cells using the DNA microarray technology. Investigating expression changes in the course of differentiation in mouse models and BMSC from patients with central nervous system disorders will help us to understand underlying pathomechanisms of the diseases.

P1-10 13

Variance stabilization and robust normalization for microarray gene expression data
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Due to variations in the experimental conditions, measurements from different gene expression array experiments are generally on different numerical scales and need to be normalized before further analysis. Furthermore, the analysis of replicate experiments shows that the variance of the measured expression intensities depends on their mean value. Thus the statistical significance of a measured fold change depends on the intensity level at which it was observed. Our approach for dealing with these problems is based on a statistical model of measurement error for gene expression data. From this model, using a mathematical technique, we derive a family of variance-stabilizing transformations of the measured intensities which are of the form y=arsinh(ax+b). Differences on this new intensity scale have nearly uniform statistical significance. For large intensities x, our transformation coincides with the usual logarithmic transformation, such that differences can still be interpreted in terms of fold changes. For small values of x, the transformation diminishes the fluctuation of the intensities that is usually visible in log-transformed data. For the normalization of data from different mRNA samples, we use a robust statistical estimation technique. This is applicable as long as between the samples under consideration, the majority of genes have roughly unchanged expression levels, while possibly different numbers of genes may be up- or down-regulated. Finally, we evaluate the performance of our approach on real data sets, comparing it with standard methods. We find that our method leads to higher power of statistical tests to identify differentially expressed genes.

P1-10 14

OLIGONUCLEOTIDE VERSUS cDNA-BASED MICROARRAY SYSTEMS: A COMPARISON USING NORMAL HUMAN COLONIC MUCOSA
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The human genome project has identified ~32,000 genes, many of which are predicted genes supported by expressed sequence tags. Functional characterisation of unknown genes can be assisted through the use of high-throughput microarray expression screening. In this study, we compare the two main types of expression screening platforms—an oligonucleotide-based and a cDNA-based platform. Ex-

pression profiles of 5 normal controls from sigmoid colon mucosa were investigated using a cDNA filter microarray (Human UniGene Set RZPD1) and an oligonucleotide array (Affymetrix HG-U95Av2). Each platform performed in a similar manner with regard to variability in probe detection (coefficient of variation= 0.26±0.19 and 0.20±0.16 for the Affymetrix and clone-based systems, respectively). Quantitatively, there was no statistically significant rank order correlation between the mean expression data generated by the two platforms (rs=0.140). Replication rates of gene detection on both platforms were related to BLAST scores between cDNA clones and probe sequences on oligonucleotide arrays, indicating that observed differences are influenced by probe design. Overall, probes detected transcripts for genes known to be linked to the function and structure of the colon, such as genes involved in immunity, signal transduction, and transport. This study demonstrates the complementarity and overlap between two different techniques for expression profiling.

P1-10 15

Towards the analysis of the mouse transcriptome - MouseExpress

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RNA from various mouse-sources, derived from the ENU mutagenesis screen (GSF, Munich) are subjected to analyses on DNA-chips. The aim of the collaborative initiative is to get new insights into the interrelations of gene function, expression and networking on the one hand and the (mutagenized) phenotype of an animal on the other. For a global transcriptome analysis more than 60.000 PCR-products from two independent non-redundant clone libraries are used (one being the MouseExpress RZPD library, the other originating from LION Bioscience).
The system was established with a less-complex chip of some hundred PCR-products, which are important in embryonic development of the mouse.
Additionally a set of external controls for normalization has been developed, used for spiking ss-DNA made of phage lambda instead of RNA.
Furthermore, we approached the question of differential gene expression in mutant mice by representational difference analysis (RDA) of cDNA. Using a Delta-(minus) mutant, various modifications of RDA have been tested for improved sensitivity and selectivity.

P1-10 16

DNA-Microarray technology defining the normal variance in mouse gene expression

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High-throughput gene expression has become an important tool to investigate transcriptional activity in a variety of biological samples. Mouse models have become an indispensable and versatile tool to study development, genetics, behavior, and disease. To interpret experimental data, the extend and diversity of gene expression for the system under study should be well characterized. Up to now the vast majority of these experiments have focused on specific biological processes. Unfortunately little is known about the normal variance of mouse gene expression in vivo. To study the normal variance we analyzed the expression profile of normal male C57Bl6, C3H, Balb/c, DBA/2, and FVB mice, respectively. In total, we profiled gene expression of 75 mouse samples of 15 different individuals. We used a U74A mouse Genearray from Affymetrix to quantitate transcript levels in brain, liver, heart, spleen, and kidney in the five genetic backgrounds.
The expression levels of several genes varied significantly in more than one tissue. These variances in gene expression might be one of the reason for observing different phenotypes of transgenic mice based on a different genetic background. These studies will help to define the baseline level variability in mouse gene expression and accentuate the importance of replicated microarray experiments. Furthermore, this dataset will be used as a data base for normal expression profiles, to reveal insights into the mechanisms of transcriptional regulation, and to study disease etiology in future transgenic models. Finally, we are developing a free and publicity database to allow the scientific community to use this resource.

P1-10 17

MouseExpress: In Silico Analysis Of Expression Patterns In Mouse Mutants

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We have implemented a platform for the functional analysis of mouse mutant lines using RNA expression profiling technology on a large scale and high throughput basis. The routine analysis of RNA expression patterns from organs supports the understanding of the underlying molecular biology of such mouse mutants and provides new insights in mammalian gene function. To achieve this goal standardised protocols (SOPs) from mouse husbandry and tissue sampling to the molecular techniques of DNA-chip hybridisations were developed and implemented. We generate comprehensive high quality DNA glass chips with 20.000 and 48.000 3?UTRs and ESTs, respectively. Expression profiles that have been obtained in a first high throughput series were used to establish a comprehensive database system for gene annotation and functional data mining. Genes differentially expressed in mouse mutant lines have been identified and were confirmed by alternative methods. The detection of affected pathways has become an integral part of the molecular phenotypic analysis of mouse mutant resources and, together with mapping data, supports the identification of candidates for mutated genes in critical regions. Data generated in this project are a major tool and have successfully been used to identify new mouse models for human genetic diseases based on the differential expression of known marker genes. In addition, new and functionally not annotated genes have been associated with specific mouse mutant models. One of the largest European resources of mouse models (over 400 mutant lines) from the Munich ENU mutagenesis screen is directly accessible. The MouseExpress Consortium combines the power of mouse genetics, expression profile technology, and bioinformatics. Such comprehensive transcriptome analyses will lead to the identification of new gene functions and co-regulated synexpression groups of genes, which are the basis for the description of regulatory networks.

P1-10 18

Eliminating Cross-Hybridisation: High Quality Expression Profiling Data For The Molecular Phenotypic Analysis Of Mouse Mutants

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The cDNA-chip technology is a powerful tool for the comprehensive analysis of gene expression at the transcript level. The biological significance of such expression profiling analyses critically depends on the quality and specificity of hybridisation data. Based on experimental data, we describe methods to >discriminate between gene specific signals and signals resulting from extensive cross-hybridisation. We identify criteria that can be used for each individual probe on comprehensive DNA-chips to correct expression data to achieve high quality results. For this, we apply in situ fractionation of hybridised targets by means of contiguous washes with increasing stringency. In the course of such washing steps, distinct fractions of hybridised target are washed out at different stringency. The fluorescent intensity data at each step and for each probe of a microarray comprise the fractionation curve. Based on this information, unreliable data can be filtered and gene specific probes relevant for high quality expression data can be identified. In the MouseExpress project we apply this technology for a systematic and comprehensive analysis of expression profiles from a set of organs in a compendium of mouse mutant lines (more than 400 mutant lines) derived from the Munich ENU mutagenesis screen. Such comprehensive transcriptome analyses will lead to the identification of new gene functions and co-regulated synexpression groups of genes, which are the basis for the description of regulatory networks.

P1-10 19

Transcriptional analyses of a Lupusnephritis mouse model

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Systemic lupus erythematosus is a frequent chronic automimmune disease. One of the major and most severe symptoms is an inflammation of the renal glomeruli, called Lupusnephritis (LN). It is one of the most common reasons for renal failure resulting in dialysis and transplantation. LN may be well understood with the help of the MRL-Faslpr-Mouse model, presenting a genetically dependent glomerulonephritis.
In order to get an insight into the progression of the disease and the involved genes, RNA from mouse kidneys at characteristic time points during the manifestation of LN are analysed using DNA microarrays. These arrays are constructed from PCR-products of about 1000 EST-clones representing more than 600 genes that are characteristic for different cell-types in the nephron and for the various cells of the immune system. The aim the project is to profile the inflammation processes on the transcriptional level. A focus is on the characterization of the immunogenic invasion of the renal cortex, which will be adressed based on the analysis of chemokine related and cell type specific housekeeping genes.The respective second kidneys of the mice used in our experiments may serve for parallel histological examination.

P1-10 20

Expression of human growth hormone in milk of transgenic rabbits with transgene mapped to telomeric region of chromosome 7q

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Targeting the expression of sequences encoding non-milk proteins to the mammary gland of transgenic farm animals was proven as effective way for producing pharmacologically active proteins on a large scale. We report here expression pattern of human growth hormone (hGH) in transgenic rabbits carrying hGH genomic sequences driven by the rat whey acidic protein (WAP) promoter. Transgenic founder animal was generated by microinjection of genetic constructs to male pronuclei of fertilized rabbit oocyte and transferred to foster mother. Founder animal transmitted the transgene to the next generations in stable form. Approximately 30% of F1 offspring demonstrated presence of transgene. Ectopic expression of transgene was not observed in brain, heart, kidney, liver and salivary gland indicating that WAP promoter possessed essential sequences for directing expression exclusively to the mammary gland. In fluorescence in situ hybridization (FISH) experiments transgene was mapped to telomeric region on long arm of chromosome 7. Purification of human growth hormone from milk and releasing its active form was performed by affinity chromatography followed by thrombin cleavage. Biological activity of growth hormone was measured by immunoreactivity and adding to culture of hormone dependent NB211 cell line. The results indicate that transgenic rabbits are suitable for the production of recombinant proteins in the milk of lactating females.

P1-10 21

Cloning and expression studies of the chicken homolog for the human ARVCF, a gene from the CATCH22 deletion region

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The human 22q11.2 chromosomal deletion produces haplo-insufficiency for a number of developmentally regulated genes which cause mainly disturbances of the migration and differentiation of neural crest cells. This leads to syndromes of the DiGeorge or CATCH22 spectrum. Still not fully understood are the contributions of each deleted gene towards a pathological phenotype. In our study we use the chicken embryo as a model to study the embryonic expression profiles of the corresponding genes. Our actual work focusses on ARVCF (armadillo repeat gene deleted in velo-cardio-facial syndrome) a member of the catenin family, characterised by armadillo repeats and with a predicted function in signal transduction and cell differentiation. We cloned the chicken homologous gene which is highly conserved to human, mouse and xenopus. By means of whole-mount ISH a ubiquitous, but variable expression can be shown in different organs and stages of the chick embryo. Partially it co-localizes with the expression of N-cadherin, a potential binding partner of ARVCF. However when using RT-PCR we were able to show alternative splicing patterns which are specific for different embryonic tissues. For the 5'-region altogether 5 variants (a-e) were observed mainly due to alternative start sites, while for the 3'- region of the gene only 2 variants (1,2) were found. Heart and liver e.g. present only the d2 isoform, while other organs like eye, kidney, intestine or brain show various combinations. Any functional differences of the different splice variants are still not known, however the armadillo-repeats, and thus the binding site for cadherin molecules, are not affected by alternative splicing.

P1-10 22

Genepaint.org - A Database of Gene Expression Maps

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The genomes of humans and an increasing number of model organisms, including the mouse, have now been sequenced and tens of thousands of mammalian genes have been predicted. However, for many of them little is known beyond their mere sequence. Protein domains can sometimes be identified based on sequence similarities with other known proteins, but there are currently no reliable methods available to predict the spatiotemporal expression pattern of a gene in a living organism, it can only be determined experimentally. We have therefore developed machinery for high throughput gene expression analysis on tissue sections by in situ-hybridization. Automation of various steps of the procedures minimizes human hands-on time, rendering investigations at a genome-wide scale conceivable. The results of our studies are made available for the scientific community via a database at www.genepaint.org. Here we describe the different possibilities for data retrieval using the web-based public interface that can be used by all members of the scientific community to access our data. Searches of the Genepaint database can be conducted based on gene names, descriptions, and accession numbers. In addition, systematic annotation to a tree-like hierarchical list of anatomical structures permits searches for and comparison between expression patterns of genes. The digital images of sections at single-cell resolution are very large, so special attention was paid to providing a convenient way of viewing these images: Along with the annotations of expression patterns, a virtual microscope feature is available that can be used to view and zoom into images down to the original cellular resolution while keeping download times reasonable.

P1-10 23

Characterization of an alternative TSPY transcript (TSPY-L) in LNCaP prostate carcinoma cells

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Recent studies revealed an aberrant expression of TSPY in prostate carcinoma leaving the question of its role in malignancy. We analysed the TSPY expression profile in detail for LNCaP cells and revealed evidence for an alternative spliced transcript, termed TSPY-L, which was cloned and analysed in detail. This variant is transcribed at of least 4 fold lower abundance than TSPY-S, the common form. We were able to show that a presumed alternative splicing takes place in intron 4 using a splice acceptor site just 11 bp upstream from that of TSPY-S. Due to a frame-shift this leads to an altered amino acid sequence and thus to a shortened C-terminus with postulated different cellular functions. There is a predicted difference in molecular weight from almost 2 kD between TSPY-S (35 kD) and TSPY-L (33 kD). To test whether TSPY-L is functional and not a pseudogene we raised an anti-peptide-antiserum in rabbit using a peptide of 16 aa from the TSPY-L C-terminus for immunization. The specificity of this anti-peptide antiserum (a-1026) was tested in immunoblot experiments against GST-fusion proteins of as well TSPY-L (clone pRo-11) as TSPY-S (clone pRo-18). These clones cover the complete coding region of each variant. As a positive control for the detection of both GST-fusionproteins TSPY antibody a-839 (2) was applied which was raised against a N-terminal fusionprotein common to both variants.

Using anti-peptide antiserum a-1026 a specific signal of the predicted size was obtained only for the TSPY-L type, thus confirming the specificity of the antiserum.
As several copies of TSPY with different haplotypes do exist on the human Y chromosome a haplotype analysis was performed on cDNA indicating that the G-G-18 haplotype is absent in TSPY-L transcripts. This suggests that only defined TSPY copies on the Y chromosome can give rise to the alternative splice product TSPY-L.

P1-10 24

Designing customized arrays in the post genome era

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Entering the post-genome era with an increasing amount of sequence data available in public databases, for genome research a complex matrix of possibilities is opened. Up to date more than 500 sequencing projects for the most prominent model organisms have been initiated, from which already hundred have been finished. The outcome of these projects represent the basis of genome research in academia, pharma R&D or the diagnostic industry. To address this vast and complex arena of genomic questions, new flexible analysing tools are desperately needed that translate these into meaningful biological experiments.
Febits Geniom technology represents the first integrated solution to that problem by transforming genomic questions into a digital software file that is used as the design scheme for potentially any genomic assay in a microarray format. Microarray fabrication, hybridisation, detection and analysis takes place within one single benchtop instrument. This setup allows for maximum flexibility in design and realisation of all kind of genomic assays.
Employing proprietary algorithms for calculation of optimal oligonucleotide probes febit has started a broad initiative to make a wide variety organisms available for expression profiling studies on the Geniom platform. So far complete sets for yeast, E. coli and mayor parts of human are available. Further projects include mouse, N. crassa, A. thaliana, drosophila and analysis of splice variants for human genes. Starting from these calculated libraries customized array designs for expression profiling experiments are easily set up following a kind of drag & drop scheme. New array designs - and therefore new genomic assays - maybe developed either from scratch or more simply by combining parts of existing designs stored in the database. This kind of combinatorial approach will simplify accessing the huge amount of data produced by the various genome projects in the nearby future. A further advantage that results from the digital nature of the assay format is that the assays are always backward compatible. To run an assay that was set up years ago it's only necessary to pull up the old array design file from the database and load it onto the system.
Details on the procedures how these probe libraries have been generated will be presented. It will be shown how customized expression profiling arrays are easily selected from these libraries through easy to handle software tools. Furthermore, ways for data analysis of will be described.

P1-10 25

Identifying novel retina-specific genes by characterising a suppression subtracted cDNA library highly enriched for retinal genes

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The human retina is a multi-layered neuronal tissue specialized for the processing of visual information. It is a highly complex system which consequently may be greatly susceptible to genetic defects leading to a wide range of retinal disease phenotypes. To identify novel retinal genes we have generated a human retinal cDNA library by suppression subtractive hybridisation (SSH) techniques. We have sequenced 1113 clones with insert sizes ranging from 150 to 850 bp. On the basis of BLASTN algorithm analysis, the clones were classified into four categories including those with i) significant homology to known human genes (769/1113), ii) significant homology to partial transcripts (43/1113) and hypothetical gene predictions (119/1113), iii) no homology to known mRNAs (149/1113), and iv) vector sequences and clones derived from mitochondrial genes (33/1113). After correcting for redundancy, category 1 represents 236 known human genes and category 2 a total of 94 unknown transcripts. Clones from category 2 were selected for expression analysis by RT-PCR in 20 human tissues. Thus far, RT-PCR has been completed for 46 EST clusters and has revealed 18 transcripts specifically or abundantly transcribed in the human retina. Another 6 EST clusters are expressed in neuronal tissue. Cloning and characterisation of five of the retinal genes is currently in progress. In addition, partial cDNAs of two EST clusters represent novel splicing variants of known genes. Our study demonstrates i) a high enrichment of retinal transcripts in our in-house constructed cDNA library and ii) the presence of many as yet unidentified tissue-specific genes, as well as unknown alternatively spliced variants for known genes. Therefore, isolation and characterisation of these novel genes will further our understanding of retinal physiology and will provide novel candidates for retinal disease genes.

P1-10 26

Construction of a Relational Database Management System (RDBMS) for the analysis of RPE-enriched expressed sequence tags

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Age-related macular degeneration (AMD) is the most common cause of legal blindness in industrialized countries and predominantly affects the elderly population over 75 years of age. Although the primary events in AMD pathogenesis are not well understood, it is thought that the deterioration of the highly metabolic retinal pigment epithelium (RPE) is involved in the degenerative processes.
Our aim is to identify and characterize genes specifically or abundantly expressed in the RPE. Towards this end, a bovine cDNA library enriched for RPE was constructed in-house using a PCR-based suppression subtractive hybridization technique, which normalizes sequence abundance and achieves high enrichment for differentially expressed genes. The individual sequences of 1002 cDNAs were analyzed and querried against sequences deposited in the GenBank and dbEST databases. A RDBMS was designed and developed to organize the storage and retrieval of the sequencing and blast searching information. RDBMS leads to more efficient use of queries, forms, and reports, increases the reliability of the extracted information, and can expand as database information requirements grow and change. Our results demonstrate that 64% (642) of the clones are derived from known genes, while 36% (360) of clones showed no matches in dbEST. In silico expression analysis of the known RPE genes revealed that 7.5% are RPE-specific, 4.2 % are retina-specific, and 4.2% are expressed in both retina and brain. Extrapolation of these data to the EST clusters with no significant homolgy to known genes suggests that the number of novel RPE/Retina-specific genes to be around 10-15. Characterization of the full length human orthologs of the bovine RPE/Retina-specific transcripts have been initiated. In a second phase, we plan to determine sequence variants in the RPE-specific genes in a large, well-characterized AMD patient group by high-throughput exon-scanning technology.

P1-10 27

Identification of genes preferentially expressed in the human retina using an expressed sequenced tag (EST) approach

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The retina is a highly specialized tissue composed of more than 50 cell types. Many active genes, especially those with retina-specific expression are required to establish the structure and maintain the integrity of this tissue. Studies of the etiology of more than 80 single-gene retinopathies have shown that mutations in the genes abundantly expressed in retina are responsible for many retinal disorders. Because the genetic causes of more than 70 retinopathies are as yet unknown, we aim to identify novel genes preferentially expressed in the retina. Moreover, a catalogue of genes preferentially expressed in the retina would facilitate the study of age-related macular degeneration (AMD). This multifactorial disorder, currently affecting 11 million people worldwide, accounts for most cases of vision impairment in the elderly population.
In order to identify retina-specific or retina-abundant genes we performed an expression profiling of the EST clusters assembled in the human UniGene database. From the 6190 UniGene clusters containing at least one retina EST, 2201 represent known genes and were not further analyzed. We then selected all clusters containing only retina ESTs (673 clusters) and those that had at least a 30% retina EST content (568 clusters). Exhaustive bioinformatical analyses of these clusters were carried out to further prioritize for in vitro expression analyses. Subsequent RT-PCR of the 248 chosen clusters led to the

identification of 41 retina-specific or -abundant and 41 neuronal EST clusters. Thus far, we have completed the cloning and characterization of a total of 18 genes and full-length cloning of the remaining transcripts is currently in progress. In addition, we are designing an interactive Retina Gene Expression Database. This information system will provide easy and integrated access to pertinent information about the genes identified in our study as well as other known genes expressed in the retina and will represent an important resource for ophthalmogenetic research.

P1-10 28

Functional analysis of gene and protein networks in Drosophila with microarray based genome expression studies

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The complete genomes of several dozen bacteria, Caenorhabditis elegans, Drosophila melanogaster and man have been sequenced. The most challenging task arising from this „digital" sequence data will be to decipher the role and function of the identified genes and their corresponding protein products in the context of an entire organism. To tackle this challenge, we combine DNA microarray based gene expression analyses with in vivo protein function studies to dissect gene and protein networks in Drosophila.
We generated a new annotation of the Drosophila melanogaster genome that identified 21.396 putative ORFs. Specific primer pairs were designed to generate PCR fragments representing all ORFs. So far we could successfully produce products for 20.903 ORFs (98%). The first version of our Drosophila microarray is available since May 8th 2002 and contains two copies of the 20.903 PCR-products, 70 nucleotide oligonucleotides for smaller ORFs, and 64 different controls (summarized, it contains 46.848 baits). Analyses based on this microarray will allow identification of genes whose activities are required for the execution of complex developmental gene networks and signal transduction pathways. In a first series of experiments, we will use mRNA pools from every stage of Drosophila development (e.g. 0-4 h old embryos, 4-8 h old embryos, etc.) to establish a gene expression profile of Drosophila development. This expression profile will serve as a reference for all of our other microarray gene expression analyses and in addition may provide first clues towards how many of the additional genes present in our annotation are expressed during the Drosophila life cycle. The results of this study will be presented at the meeting.

P1-10 29

Transcriptional profiling on Neurospora crassa employing spotted and in situ synthesized microarrays

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The filamentous fungus Neurospora crassa has been used as a model organism in basic science for more than 60 years.
Until now, only a fraction of the estimated 10-13,000 genes of N. crassa was characterised. To overcome this limitation to the understanding of the fungus' biology, a genome initiative was established. Whole genome shotgun sequencing was started in 2000 at the Genome Research Center of the Whitehead Institute, Massachussetts, USA. In february 2002 an assembly of 821 contigs >2kb containing 38,044,343 bp was made publicly available. In addition, a set of 10.082 putative genes was annotated.
For transcriptional profiling analyses on N. crassa, we started with a non-normalised cDNA library containing 4800 EST-clones (Nelson et al., 1997). After amplifying the inserts of the clones by PCR, high density microarrays were produced by spotting the PCR-products on poly-L-lysine-coated glass slides.
Having the genome sequence as well as annotated genes, expression analyses by using microarrays based on oligonucleotides became possible. Therefore a subset of 25 genes known to be light-regulated and another 30 selected genes were investigated employing oligonucleotide chips. The oligonucleotide chips were either fabricated by spotting pre-synthesized 70mers or by using in situ oligonucleotide synthesis on febits GeniomÒ technology.
Oligonucleotide probes of 20-30 bp in length were calculated using 3 different algorithms from 3 different groups for comparison.
Results on performance and concordance of the different approaches will be presented.

P1-10 30

Studies on the gene expression profiles in patients with abdominal aortic aneurysm

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Abdominal aortic aneurysm (AAA), a localized abnormal dilatation of aorta, is a life-threatening condition affecting 4-9% of population with a risk increasing with age. The results of the studies that has been done so far on the AAA indicate as the reason for an aneurysm development the loss of the arteries walls resistance caused by degradation of their structural elements (e.g. elastin). Risk factors include: hypertension, atherosclerosis, cigarette smoking and also familial occurrence indicating genetic factors to be involved. Since 1999 in our research group the genetic studies on the determination of the features favorable to intensification of degeneration processes within the aortic wall had been carried out. DNA and RNA isolated from a group of 30 AAA patients were collected. Here, we present the preliminary results of the studies on the gene expression level in AAA patients using DNA macroarray system.

P1-10 31

'Functional Genomics of Osteoarthritis' - from gene expression to function and application

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Osteoarthritis, the degenerative disease of articular cartilage, is the leading cause of disability with a very substantial economic impact of direct and indirect cost, but without causative treatment. Since three years a consortium of 12 industrial, academic, biotech and clinical partners is engaged in a functional genomics approach to identify and validate genes and proteins to enable the development of disease modifying drugs. In a first step an array of 4.500 cartilage specific genes was employed to detect genes differentially expressed in human articular cartilage. In a second step, the initial analysis was refined with a further microarray containing 6.800 preselected genes and 20 human tissue samples for each group to be analysed: macroscopically intact, early and late osteoarthritic human knee cartilage. Bioinformatics analysis tools included text mining tools to collect interaction of gene expression candidates with contexts defined by expert lists of relevant factors for OA. Furthermore, network models generated from databases and literature mining are used as further evidence for gene candidates based on their pathway/signalling contexts and the expression of neighboring genes in the resulting biochemical networks. As the first drug targets have entered the Aventis drug discovery pipeline, a more refined validation is now the focus of the interaction of the consortium.
The validation process includes further expression profiling and in situ hybridisation of human and murine tissues, protein-protein interaction analyses and the study of target genes in model organisms.
So far comparative expression analyses during skeletal development have proved to be very helpful for generation of concepts to study gene function in vitro and in vivo.

P1-10 32

Expression analysis of the dynamin related protein OPA1 in rat tissues

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Autosomal dominant optic atrophy (ADOA) is the most prevalent hereditary optic neuropathy characterized by an insidious onset of optic atrophy in the early childhood with moderate to severe decrease of visual acuity. Mutations in the OPA1 gene are responsible for ADOA. This OPA1 gene

encodes a 1015 amino acid polypeptide (approximately 110 kDa) with similarities to specific GTP-binding proteins of the dynamin protein family. The presence of typical sequence features (i.e. basic leader, MPP and MIP cleavage sites) at the N-terminus suggests that the OPA1 polypeptide is imported into the mitochondria. OPA1 may represent the human ortholog of the S. cerevisiae Mgml and the S. pombe Mspl proteins, which are important for mitochondrial inheritance and maintenance in these organisms. Northern Blot analyses show that OPA1 is expressed in all tissues examined, with the highest transcript level in the retina. Newly generated polyclonal antibodies raised against a carboxyterminal peptide of OPA1 were used for Western Blot analyses and immunhistochemical studies. The OPA1 protein was detectable in all probed tissues. In liver several bands between 70 and 110 kDa were detected, which may represent different processed forms of the OPA1 protein. Some of these processed forms are specific for different cellular fractions. In addition, we performed immunhistochemical stainings to examine the spatial localization of OPA1 in neuronal tissues. Studies in rat retinal sections revealed a highly localized staining of specific cells in the ganglion cell layer (GCL), the inner plexiform layer (IPL) and the inner nuclear layer (INL).

P1-10 33

RZPD-Toolbox for Gene Expression Profiling

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The complexity of any biological system is ultimately determined by its genes - their mere number and composition as well as their interactions and regulation. Gene expression profiling is the method of choice for analyzing expression levels of genes in given tissues. Detection of changes in gene expression provides a vantage point for in-depth analysis of mechanisms relevant for tissue and organ development, carcinogenesis and its treatment.Within the last two years RZPD developed a broad expertise in gene expression profiling based on an integrated portfolio of products and services available to RZPD's customers. RZPD is one out of only six Affymetrix Service Providers world-wide. As an alternative and/or complementary approach, RZPD also offers an Expression Profiling Service based on our proprietary Unigene Clone Collection for human and mouse. The Human Unigene Set - RZPD III comprises 40.000 and the Mouse Unigene Set - RZPD II 35.000 sequence verified clones. Our clone selection procedure makes them ideally suited for hybridization experiments on micro-/macro-arrays. Genes represented on Affymetrix GeneChipsÔ are bioinformatically linked with clones from RZPD's Unigene Sets. Therefore, after a first genome-wide screen - based on Unigene Sets and/or Affymetrix GeneChipsÔ - indication specific subsets can be assembled from RZPD's Unigene Clone Collections applying RZPD's proprietary Re-arraying technology. Re-arrayed subsets are used for the fabrication of custom-made microarrays that provide an economic way for the analysis of large sample sets e.g. from patient cohorts. Currently, RZPD provides two predefined „Indication Arrays", the ImmunoArray - RZPD I and the OncoArray - RZPD I. Both microarrays have been designed in collaboration with outstanding experts in the field. They represent 1,200 genes of immunological and 1,600 genes of oncological relevance, respectively. Additional indication specific arrays are in preparation.

P1-11 01

A New Syndrome of Cardiac, Cerebral, Renal, Gastrointestinal, and Skeletal Malformation and Intrauterine Death

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We present three fetal sibs, who died in 24th, 25th, and 14 th gestational week.
The first fetus, male, was small for gestational age. Autopsy revealed hydrocephalus, cystic dysplastic kidneys, fusion of cervical and thoracic vertebral bodies, and mesenterium commune. A second pregnancy ended in the uneventful delivery of a healthy girl which is now five years old. Fetus 2, female, had prosencephaly, a hypoplastic left ventricle and endocardial fibroelastosis, and cystic dysplastic kidneys. Fetus 3, female, had a complex cardiac malformation, intestinal malrotation, and cystic dysplastic kidneys. Cerebral anomalies were suspected in ultrasound studies. Cytogenetic examinations were normal. Both parents are of Turkish origin and are unaware of any consanguinity between them. We were unable to match the malformation pattern to a known syndrome. We propose a new syndrome for which autosomal recessive inheritance is likely.

P1-14 01

PEDMST, a New Program for IBD Pattern Enumeration and Identity CoefficientsCalculation

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Currently, no specific algorithm has been built to list all possible IBD patterns between the genes at a given autosomal locus for individuals in a pedigree. We describe a labeled tree structure to enumerate such patterns and develop a recursive algorithm to construct this tree. When the construction is done, the patterns can be easily listed using the path searching on the tree. Based on these ideas, We develop a Visual C++ program, PEDMST (short for Pedigree Master) which implements the three major functionalities. First, the function of pedigree operation allows the user to construct the pedigree or import the pedigree from a text file used by other software such as PFIDDLER. A special graph-drawing algorithm based on the generations of the individuals is designed and implemented for import. Second, the function of kinship and identity coefficients calculation gives the user not only numerical results but also graphic results in terms of detailed or condensed identity states. Generalized kinship coefficients are used to compute the identity coefficients. Third, the function of IBD pattern enumeration allows the user to view all possible IBD patterns between the input genes whose maternal or paternal source is specifiable. The maximal number of the genes currently is set to 10, but can be expanded easily later according to the large capacity of the tree model we use. We have received positive user feedback on features such as an instant view of the pedigree while editing, graphic output of the calculation results, the ability to represent the pedigree and do the computing on the same screen, and the unique function of IBD pattern enumeration.

P1-14 02

Haplotype Estimation and Linkage Disequilibrium for phase-unknown SNP genotypes

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An extension of the expectation-maximization (EM) algorithms for the haplotype estimation for phase-unknown SNP genotypes applied to samples of single individuals or of nuclear families has been used to carry out pairwise and multilocus Linkage Disequilibrium (LD) estimations.
It can be shown, that for the identification of LD blocks along the genome a series of successive pairwise linkage disequilibria sometimes may underestimate LD blocks and should therefore be supplemented by multilocus LD estimates or the whole matrix of pairwise LD.
The effect of different LD measures to identify LD blocks and the power to detect them, has been investigated by simulation studies, information to support the process of finding haplotype and/or LD blocks along the genome.

P1-14 03

Calculating precise genetic distances for common markers in and around the DMD Gen

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DMD (and BMD) are the most common X-linked lethal muscle dystrophies in man, affecting ~1 in 3000 (~1 in 14000) live born males. Problems in genetic counseling occur in sporadic cases without living index patients or in some cases with female carriers. In these families a multipoint linkage analysis may be one possibility to define the haplotype at risk. About 60% of the affected boys have a deletion and 40% a point mutation (neglecting duplications). According to our former publications the sex ratio of mutation rates is different for deletions (μdel ~ 0,2 vdel) and point mutations (μpm ~ 4,6 vpm). The a priori probability of a patient's mother of being carrier will be 108/55 μ for deletions and 112/55 μ for point mutations. When these data are used in Bayesian risk calculations together with informative DNA markers carrier detection is much more precise than using the simpler model of equal mutation rates. We have analysed the data of 455 pedigrees which were tested in our department from 1982 to 2001. After remodelling and selecting 21 specific markers in and around the dystrophin gene we re-calculated the genetic distances in cM between markers. Our results were compared to previous linkage studies. The application of these new data in multipoint linkage analysis will result in more precise risk assessments for genetic counseling.

P1-14 04

SNP-typing experiments for molecular epidemiological studies on flexible, in situ synthesised oligonucleotide microarrays

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The investigation of gene-environment and gene-gene interactions in complex diseases requires large, epidemiologically well characterised populations and flexible, high-throughput genotyping technologies. Important for the study of context-specific single base polymorphism (SNP) combinations is a flexible chip design.

For the analysis of SNPs associated with the existence of lymphoid neoplasms, such a microarray has been established. Initial aim of this project is the comparison of epidemiological data obtained from a case-control study of 600 patients and 600 controls with molecular information on some 100 appropriate SNPs. While a set of evaluated polymorphisms (in cytokine genes, cytokine receptor genes and other genes of relevance to the immune system) already exists, more are currently selected. To this end, the Geniom system is used, which permits on-site production and use of oligonucleotide microarrays of entirely flexible design. Controlled by a mask-free, light-induced synthesis process, up to 40,000 different oligonucleotides can be synthesised on a single microarray. Also the detection unit is contained in the system, permitting quality control during synthesis and, subsequently, quantification of signal intensities upon the hybridisation of DNA-targets. The complete freedom in the choice of oligomer sequences is elementary for the evaluation and selection of informative SNPs. Currently, 24 oligonucleotides are being used for each SNP, 12 for either DNA-strand. The PCR-products of all studied SNPs are analysed simultaneously in a single hybridisation.

Results from the analysis and the various steps leading from the identification of potentially interesting SNPs for epidemiological studies to a practical genotyping chip will be discussed.

P1-14 05

Descriptive methods in large scale association studies involving many candidate genes

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In association studies for complex diseases many markers are accumulated in the process of investigating candidate genes. A systematic and comprehensive analysis of associations of single alleles and allele combinations (ACs) requires proper statistical tools. We present descriptive and inferential statistics to meet these requirements. Several measures of association (odds ratio, normalized odds ratio to measure epistatic effects, p-values of individual allele combinations) are used to rank the importance of alleles and combinations thereof. Graphical representations allow for convenient evaluation of two way interactions. ACs can be ranked according to several criteria, thereby selecting the most relevant ACs. Analysis of typing data concerning Multiple Sclerosis illustrates the relevance of the methods.

P1-16 01

The German cDNA Consortium

Wiemann, S., Weil, B., Wellenreuther, R., Gassenhuber, J., Glassl, S., Ansorge, W., Bocher, M., Blocker, H., Bauersachs, S., Blum, H., Lauber, J., Dusterhoft, A., Beyer, A., Kohrer, K., Strack, N., Mewes, H. W., Ottenwalder, B., Obermaier, B., Tampe

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The most successful German cDNA Consortium, which had been established in the first phase of the DHGP as the second project worldwide, has been extended to continue with the isolation of full-length cDNAs of novel human genes. In the first phase of the project, 7.87 Mb of high quality sequence were generated, equaling 3,300 cDNAs. In the reported period of the second phase, another 924 cDNAs (total ? 3,285,985 bp) have been finished. The total achieved in the second funding period is 14.4 Mb finished sequence. The average insert size of the cDNAs was pushed from 2.4 kb to over 3.5 kb.The first publication of the consortium activities has been published (Wiemann et al., 2001. Genome Res. 11, 422-435). The cDNAs analysed by the consortium have been basis for a number of publications, both systematic functional genomic?s approaches and projects dealing with individual cDNAs. The international importance of the consortium is reflected by two notes that have been published in Nature and Science, respectively.Wiemann, S., Weil, B., Wellenreuther, R., Gassenhuber, J., Glassl, S., Ansorge, W., Bocher, M., Blocker, H., Bauersachs, S., Blum, H., Lauber, J., Dusterhoft, A., Beyer, A., Kohrer, K., Strack, N., Mewes, H. W., Ottenwalder, B., Obermaier, B., Tampe, J., Heubner, D., Wambutt, R., Korn, B., Klein, M. & Poustka, A. (2001) Genome Res 11, 422-435.

P1-16 02

Antimicrobial peptides on human chr. 8p22-p21

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Defensins (DEF) are small cationic peptides contributing to innate host defense of higher organisms against microorganisms. Depending on the pattern of disulfide bridges, three branches are known, designated a- and b-defensins (DEFA, DEFB), and the cyclized species, rhesus-theta-defensin (RTD). In the course of our genomic sequence efforts to characterise disease-linked regions on human chr. 8 we have made two contigs of 0.7 and 0.3 Mb in 8p22-p21 available (http://genome.imb-jena.de), where 5 a-(DEFA1-4, HE2/EP2) and 4 b-defensins (DEFB1-4), and pseudogenes with 92% identity to RTD are clustered. The genomic approach has identified an new highly cationic b-defensin (DEFB3) in 8p22-p21, which was experimentally characterized (1, 2).

With the exception of the arrangement of the disulfides and the property mediated by positively charged amino acids, the defensins show little sequence conservation reflecting the variation of the antimicrobicidal potency and salt-sensitivity of the individual defensins. The broad-spectrum antimicrobial activity is mediated presumably by binding to and permeabilizing cell membranes. To dissect the structure-function-relationship we use a recombinant system for the DEFB3 expression (2)and the isolation of variants thereof, generated by random- and site-directed mutagenesis. The variants are tested for salt-dependent antimicrobial activity towards E. coli, Pseudomonas aeruginosa, Salmonella spec., and Staphylococcus aureus. It is aim to isolate DEFB3 variants with differential high specifity against bacterial targets.

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P1-16 03

Mouse models for deafness and inner ear development

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Within the Munich ENU-Mouse-Mutagenesis Screen deaf mutants and mutants showing head tossing or head shaking behavior indicating defects in the inner ear have been detected. A European deafness consortium was launched to analyse these valuable mutants in more detail. Two mouse lines will be presented: Beethoven (Bth) is a semidominant mouse model for progressive hearing loss. Beethoven was mapped to mouse chromosome 19 to a 4.4-cM region. As a candidate gene Tmc1, the mouse ortholog of the known human deafness gene TMC1, was sequenced. A T /A transversion in exon 13 was detected and identified as causative for the Beethoven phenotype. Bth provides deeper insight into molecular factors affecting age related progressive hearing loss.

A second mutant, headturner (Htu), is a dominant mutant that displays missing posterior and sometimes anterior ampullae, structures that house the sensory cristae. Headturner mutants also demonstrate a significant reduction in the numbers of outer hair cells in the organ of corti. Htu was mapped to a 6.6 cM region on mouse Chromosome 2. A missense mutation at position 1134 in the Jag1 gene (notch ligand) was identified to cause the Htu phenotype. Although there existed already Jag1 knock outs, only via the

headturner allele a new function in inner ear development could be assigned to the Jag1 gene.
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P1-16 04

Sequence Variation and Haplotype Analysis in Target Genes for CNS Active Therapeutics in a Representative Sample of European Population

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Knowledge about DNA sequence variation among individuals and their respective population frequencies is important for a wide range of biomedical applications. In our project, a gene based SNP detection approach is under way in a representative sample (n=96) of the European population. In this panel, coding regions and exon-intron boundaries of 102 candidate genes are being re-sequenced, which are potential targets for CNS-active therapeutics. Candidate genes are selected according to their known or assumed biological function. A semi-automated analysis pipeline was established for large scale polymorphism detection and analysis. The results obtained so far show, that 71% of SNPs detected in the representative European sample of individuals, are still unknown in public databases. In our sample, 22% of all detected SNPs are frequent (minor allele frequency >= 0.2), 22% are infrequent (minor allele frequency between 0.05 and 0.2), 30% of SNPs are rare (minor allele frequency < 0.05) and 26% are private (minor allele occurring only once). About 58% of SNPs from public databases, annotated to the genes examined in the present study, could not be detected in our European samples. The rate of non-synonymous substitutions is 20%. Our results not only represent knowledge about variations in potential targets of CNS-active drugs, but also enable computational estimation of gene based haplotypes. This dimension of our project is also important for the analysis of genetically complex diseases, since knowledge on the complete pattern of haplotype variation in a certain population can guide SNP selection in LD-mapping studies.

P1-16 05

Isolation and sequence analysis of cosmid clones mapping to the rhesus major histocompatibility complex (MHC)

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The major histocompatibility complex (MHC) plays a major role in graft rejection and controlls susceptibility to many diseases mostly of autoimmune or infectious nature. Among the 120 expressed genes of the 3.8 Mb encompassing human MHC, the HLA complex, the most characteristic ones are the highly polymorphic class I and class II genes, whose gene products control specific immune responsiveness.
The rhesus macaque (Macaca mulatta) serves as an animal model for several human infectious diseases, e. g. AIDS and susceptibility to viral infection is mainly controlled by class I molecules. However, knowledge of rhesus macaque class I genes is based on cDNA sequence information and the genomic structure of the rhesus MHC is largely unknown. Thus, we have screened a cosmid library with a probe derived from the highly conserved exon 4 of the rhesus macaque MHC class I gene Mamu-A, that should crossreact with most, if not all rhesus class I genes. A total of 122 class I gene-carrying clones could be isolated. So far, for 95 clones end sequences have been obtained. These rhesus macaque sequences are aligned to the human MHC sequence, selected clones are/will be sequenced. Furthermore, the sequences of two cosmids have been completely determined and sequencing of a third clone is in progress, two overlapping cosmids that map to the so called extended MHC class II region and contain the SACM2L and KE4 genes as well as a cosmid that contains the gene coding for myelin oligodendrocyte glycoprotein (MOG), the major autoantigen involved in multiple sclerosis.

P1-16 06

Disease gene-oriented genomic sequence analysis in man and mouse

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Large-scale genomic sequence analysis is an indispensable tool for modern biosciences and their application. Together with complementing technologies like cDNA and SNP analysis, genomic sequence analysis will lay the ground for life sciences over the next decades. In the frame of the Human Genome Project the Germam human genome sequencing consortium is part of the worldwide public „Bermuda“ initiative, which will guarantee free access to the genomic sequence data not only for man but also for the sequence of other model organisms.
During the first phase of the German Human Genome project (DHGP1) 7,1 Mb of human genomic sequence were generated in highest quality (finished sequence) at the MPIMG. The main focus of our centre was the generation and analysis of the finished sequence of the entire chromosome 21 based on gapless sequence-ready maps constructed by the group of M.-L. Yaspo at the Max-Planck-Institute in Berlin. This project was done as a collaboration of two Japanese groups (RIKEN Sagamihara, Keio University Tokyo) and the three German sequencing centres (GBF Braunschweig, IMB Jena and MPIMG Berlin). Up to now, chromosome 21 is one of four chromosomes declared to be finished. Other main targets during the first phase of the DHGP were chromosome and X, where we sequenced selected medically important regions.
Based on the results and achieved experience of the first phase of DHGP we proposed to sequence medically important regions of human chromosomes 1p, 3q, and 17p in highest quality (finished sequence). In addition to the clinical relevance, this effort represents a contribution to the generation of the finished sequence of the entire human genome, which is scheduled to be achieved in April 2003. Additional sequencing targets of the mouse genome comprise regions on chromosome 2 and 9. In total, we proposed to generate approx. 3,3 Mb of genomic sequence in highest quality.
In the first 16 month (April 2002) of the project we initiated the genomic sequencing of all proposed regions in human and mouse but concentrated on human chromosomes. We generated 1,9 Mb of finished and 1,2 Mb of working draft sequence within this period. In collaborations with clinical groups we are involved in disease gene identification projects for Hailey-Hailey disease (HHD), nephronophtisis (NPHP3), Malignant Hyperthermia (MHS4) and Myotonic Dystrophy (DM2). So far, we were successful in the identification of the HHD and the Bartter syndrome underlying mutations.

P1-22 01

Candidate gene testing for Emery-Dreifuss muscular dystrophy

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Until now STA and LMNA, have been assigned to Emery-Dreifuss muscular dystrophy (EDMD). Scanning 93 patients suffering EDMD or associated phenotypes at our unit revealed that mutations in STA and LMNA together account only for 36 % of the patients. Thus, further genes are likely to be involved in EDMD. Forced by the lack of families, suitable for a classic positional cloning approach, we started a candidate gene approach. Emerin and lamin A/C are components of the inner nuclear membrane and the nuclear lamina. Thus it seems very likely, that defects in other genes encoding functionally related peptides to or interacting with emerin and lamin A/C could cause EDMD. Thus we considered lamin B1 (LMNB1) and B2 (LMNB2), lamin B receptor (LBR), lamina-associated polypeptides 1 (LAP1) and 2 (LAP2), nurim (NRM), integral inner nuclear membrane protein (MAN1) barrier to autointegration factor (BAF), DEAD/H-Asp-Glu-Ala-Asp/His-box polypeptide 16 (DDX16) and proteasome activator subunit 3 (PSME3) as candidates for EDMD. Additionally, we considered genes, which are expressed specifically in heart and skeletal muscle such as FLNC encoding filamin C and SMPX encoding a small muscular protein. The candidate genes were scanned for DNA variations by heteroduplex analysis in 54 mostly German patients who were excluded to carry mutations in STA or LMNA. In 126 exons of the candidate genes. 39 unique aberrant heteroduplex patterns have been found so far and 30 DNA variations were validated by sequencing. The frequency of the DNA-variations has been estimated in approx. 400 chromosomes of a German reference population. So, seven novel single nucleotide polymorphisms (SNPs) in FLNC, three in LAP2, two in DDX16, LMNB1 and MAN1, respectively, and one in NRM were identified. whereas SMPX, LMNB2 and LAP1 were proven to be monomorphic. Until now, no disease causing mutations have been found. However, the novel SNPs provide tools to study association or linkage of the respective genes with other disease phenotypes. The candiurther genes encoding components of the recently more precisely defined nuclear envelope proteome.

P1-22 02

Congenital myasthenic syndrome with episodic apnea in patients homozygous for a ChAT missense mutation

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Impairment of neuromuscular transmission can be either acquired or inherited. Congenital myasthenic syndromes (CMS) are due to gene mutations in proteins located in the presynaptic, synaptic or postsynaptic part of the neuromuscular junction. The presynaptic syndrome of congenital myasthenia with episodic apnea (CMS-EA) presents usually in the neonatal period with hypotonia, ptosis, dysphagia and respiratory insufficiency with apnea. If the patient survives this initial phase the condition improves, but recurrent crises with the possibility of sudden death or anoxic brain damage can occur due to infections, fever, or overexertion. CMS-EA can be caused by mutations in the gene coding for the enzyme choline acetyltransferase (ChAT). ChAT catalyses the synthesis of the acetylcholine from acetyl CoA and choline in neuronal cells. Direct sequencing of ChAT coding exons identified a previously unknown missense mutation affecting a highly conserved amino acid residue (I336T) in a consanguineous Turkish family. The high degree of conservation in different species strongly suggests that I336 is a functionally important amino acid residue. The absence of I336T from a large control sample further supports a pathogenic role in CMS-EA. This is the second report of ChAT mutations causing presynaptic CMS. Interestingly, none of the CMS-EA patients reported so far was homozygous for a null-mutation. The residual ChAT activity is probably the reason why CMS-EA patients, despite the important function of ChAT in brain, have no signs of central cholinergic dysfunction. (Kraner et al., submitted)

P1-22 03

A new CHRNA4 mutation in familial frontal lobe epilepsy

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The neuronal nicotinic acetylcholine receptors (nAChRs) are involved in signal transduction by fast synaptic transmission, axo-axonic transmission, and in the modulation of presynaptic transmitter release. Presynaptic nAChRs can increase the release of excitatory as well as of inhibitory transmitters, and can thereby control neuronal excitability. Thus, the genes coding for the different nAChR subunits are likely candidates for neurological disorders. Since 1995, three CHRNA4 (S248F, S252F, 776ins3) and two CHRNB2 (V287M, V287L) mutations were found in families with autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE). ADNFLE was the first partial epilepsy found to be segregating as a single gene disorder. It is characterised by nocturnal motor seizures, which often occur several times a night. We have now identified the fourth CHRNA4 mutation, a T265I amino acid exchange located at the extracellular end of the second transmembrane domain. The mutation was found in all seven affected individuals from a four-generation German ADNFLE family, but not in a sample of 158 control chromosomes. Most of the affected individuals had a mild ADNFLE phenotype, and in several of them the nocturnal seizures were misinterpreted as nocturnal motor activity or hyperactivity. The mutated threonine is highly conserved in CHRNA4 subunits from different species as well as in homologous nAChR proteins. Electrophysiological experiments in Xenopus oocytes showed that the T265I amino acid exchange increases the receptors apparent affinity for ACh. The mutation identified here further supports the role of AChRs in idiopathic epilepsy.

P1-22 04

LGI1 is mutated in familialtemporal lobe epilepsy characterized by aphasic seizures

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Sporadic and familial temporal lobe epilepsies can be divided into two main categories according to the seizure semiology, one with medial temporal lobe symptoms and one with lateral symptoms. The symptoms of sporadic and familial lateral temporal lobe epilepsy usually consist of simple partial seizures with mainly acoustic and / or even visual hallucinations. Autosomal dominant lateral temporal lobe epilepsy (ADLTE) has previously been linked to chromosome 10q22-q24, and recently mutations in the LGI1 gene (leucine-rich, glioma inactivated 1) have been found in some ADLTE families. The LGI1 gene is a putative transmembrane protein characterized by an extracellular leucine-rich repeat (LRR) domain, which is flanked by conserved cysteine clusters. We have now identified a missense mutation C46R affecting one of the conserved cysteine residues in the extracellular region of the LGI1 protein. This mutation is absent in 104 Norwegian and 212 German control chromosomes. The C46R mutation is the first point mutation reported affecting a conserved extracellular residue in the LGI1 protein. The associated ADLTE in a large Norwegian family shows unusual clinical features like short lasting sensory aphasia and auditory symptoms. Immunohistological experiments demonstrated that LGI1-immunoreactive neurons are found in all layers of the frontal and temporal cortex with strongest labeling intensities in layer II/III. LGI1 presented the first evidence that a gene not apparently coding for an ion channe l can cause idiopathic epilepsy. We also identified three formerly unknown LGI-like genes (LGI2, LGI3, and LGI4) and characterized their genomic localization and expression pattern. The four LGI genes belong to a new gene family and are possible candidates for epilepsy and some malignant diseases . - (Gu et al., Annals of Neurology, in press; Gu et al,. FEBS Letters, in press).

P1-22 05

Extended phenotype of pontocerebellar hypoplasia with infantile spinal muscular atrophy (PCH-1)

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Pontocerebellar hypoplasia (PCH) is rarely associated with anterior horn cell disease and designated as PCH-1. This phenotype is characterized by severe muscle weakness and hypotonia starting prenatally or at birth with a life span not exceeding a few months in most cases. Milder disease courses with later onset and longer survival are normally not diagnosed as PCH-1. We herewith describe the clinical and neuroradiological findings in nine patients out of six sibships with evidence of cerebellar defects and early onset spinal muscular atrophy (SMA), representing a broad spectrum of clinical variability. In all patients, the diagnosis of SMA (Werdnig-Hoffmann disease) was made on the basis of electrophysiological data and muscle biopsy, however, genetic testing failed to confirm the diagnosis of infantile SMA with a gene defect on chromosome 5q and resulted in clinical re-evaluation. Age at onset was after a normal period in the first months of life in three sibships and pre- and postnatally in the other three families. Life span was 2-4 years in patients with later onset, and age at death occurred after birth or within months in the more severe group. Two sibships showed discordant ages at death despite similar treatment.

In contrast to the previous definition of PCH-1, our observations suggest the existence of milder phenotypes with pontocerebellar hypoplasia or olivopontocerebellar atrophy in combination with anterior horn cell loss. A pontine involvement is not necessarily seen by neuroimaging methods. The genetic basis of PCH-1 remains to be determined, the gene locus for infantile SMA on chromosome 5q could be excluded by linkage studies. Parental consanguinity and affected sibs make autosomal recessive inheritance most likely.

P1-22 06

Mutation analysis of 14-3-3 zeta and two homologues genes in German Parkinson's disease patients

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14-3-3 proteins participate in diverse biological processes, including neuronal development and cell growth control. 14-3-3 is abundant in brain, comprising approximately 1% of its total soluble protein, and has been found in the neurofibrillary tangles in patients with Alzheimer's disease and in Lewy bodies of Parkinson`s disease patients. 14-3-3 functions as a protein kinase-dependent activator of tyrosine and tryptophan hydroxylases. It therefore regulates the synthesis and

excretion of bioamines as dopamine. Recently, it has been shown that synuclein shares physical and functional homology with 14-3-3 proteins and binds to 14-3-3 proteins and to its ligands. Mutations in alpha-synuclein have been identified in some rare families with autosomal dominant Parkinson's disease (PD). We therefore investigated whether 14-3-3 proteins are also involved in the pathogenesis of PD. We searched for mutations in the 14-3-3 zeta (YWHAZ) gene on human chromosome 2p25.2-p25.1 and two homologous genes on chromosome 10p12.33 and Xp11.4, respectively. The YWHAZ gene and its homologous genes consist of one coding exon and one untranslatated exon.
We performed a detailed mutation search of the coding region in 303 sporadic and familial PD patients by SSCP screening and sequence analysis.
We found no mutation in the 14-3-3 zeta gene, but analysis of the homologous gene on chromosome X revealed an A95G transition leading to a Gln32Arg exchange in a single PD patient. This mutation was not found in any of 226 examined controls. The functional implication of the A95G transition is currently being investigated.
Furthermore we identified a single-base pair deletion of G98 in the 14-3-3 homologous gene on chromosome 10 in one patient but in none of 360 unaffected control individuals. However, an affected sister of the index patient did not carry this mutation questioning whether the G98 deletion is causative for PD.

P1-22 07

Frequency and phenotypic variability of the GAG deletion of the DYT1 gene in an unselected group of dystonia patients

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Dystonia is a clinically and genetically heterogeneous movement disorder characterized by sustained muscle contractions affecting one or more sites of the body, frequently causing twisting and repetitive movements or abnormal postures. A 3-base-pair (GAG) deletion in the DYT1 gene is held responsible for most cases of early-onset primary generalized dystonia in Ashkenazi Jews as well as in non-Jewish patients. To investigate the prevalence of the GAG deletion in the DYT1 gene and the phenotypic variability in the general population, we tested patients with different subtypes of dystonia from four different movement disorders outpatient clinics in Germany. Six out of 256 patients did carry the GAG-deletion in the DYT1 gene. However, only two of the six mutation carriers present with features of early-onset primary generalized dystonia. The DYT1 mutation was also detected in two patients with multifocal dystonia, one of them showed involvement of cranial and cervical muscles, and in two patients with writer´s cramp of both hands with only slight progression. Our findings demonstrate that the mutation may be associated not only with generalized but also multifocal and segmental forms of dystonia and underline the wide range of phenotypic variability of this mutation. Therefore, a priori prediction of the mutation carrier status in dystonic patients and genetic counseling of affected families with respect to the clinical manifestation may prove difficult.

P1-22 08

Detection of repeat expansions in patients with PROMM by PCR and non-radioactive hybridization

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Proximal myotonic myopathy (PROMM, DM2) is a recently delineated multisystem disorder including dystrophic myopathy, myotonia and cataract, which is genetically distinct from myotonic dystrophy type 1 (DM1). PROMM is caused by a CCTG expansion in intron 1 of the zinc finger protein 9 (ZNF9) gene located on chromosome 3q21. ZNF9 is expressed in a broad variety of tissues, especially in heart and skeletal muscle. ZNF9 contains 7 zinc finger domains and is thought to be an RNA-binding protein.
The range of expanded allele sizes is large, spanning from 75 to ~11000 CCTG repeats with a mean of approximately 5000. Very large repeats, like in PROMM, are difficult to detect by standard PCR assays, therefore we performed nested PCR analyses followed by non-radioactive hybridization with a DIG-labeled oligonucleotide. Using this method we tested 80 samples from patients with a myotonic myopathy without repeat expansion in the DM1 gene. In 9 patients we detected CCTG expansions varying between hundred and a few thousand copies. 64 samples were heterozygous for wildtype alleles. The PCR based detection procedure reveals extreme mosaicism of the PROMM repeat expansion showing a broad variation of size in affected individuals.

P1-22 09

MUTATION ANALYSIS OF THE GFAP GENE IN GERMAN PATIENTS WITH ALEXANDER`S DISEASE

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Background: Alexander disease (AD) is a rare disorder of cerebral white matter due to a dysfunction of astrocytes. The most common infantile form presents as a sporadic, megalencephalic leukodystrophy. Affected infants show developmental delay, macrocephaly, seizures, spasticity and rapid deterioration. Juvenile and adult forms of AD are characterized by a more slowly progressive course. Neuroradiological features include various white matter changes, enlarged ventricles, and basal ganglia abnormalities. Neuropathological examination reveals megalencephaly, Rosenthal fibers, astrocytosis, and demyelination. Recently de novo mutations in the glial fibrillary acidic protein gene (GFAP) have been demonstrated to be associated with AD. Aims: Molecular genetic confirmation of clinical diagnosis of AD and delineation of phenotypic variability. Methods: Diagnosis of AD was based on clinical and neuroradiological criteria. DNA was extracted from blood lymphocytes. Fragments containing all coding exons of GFAP were PCR amplified and sequenced using the ABI dye terminator method. Detected mutations were confirmed by digest with suitable restriction enzymes. Results: We report heterozygous GFAP mutations in 6 patients with clinical and neuroradiological features of infantile AD, including a pair of monozygotic twins. Novel mutations were detected affecting nucleotides 304T->C (L97P) and 730G->C (R239P) in two other patients. None of the parents carried the mutations stressing dominant de novo mutations as the cause of infantile AD. The presence of an identical mutation 250G->A (R79H) in monozygotic twins with infantile AD points to the origin of these GFAP mutations in germ cells or very early postzygotic stages. Conclusions: Our current data confirm the association of GFAP mutations with AD. Molecular genetic analysis facilitates the diagnosis and should be provided in suspected Alexander`s disease.

P1-22 10

Gene targeting of Gemin2 in mice reveals a correlation between defects in the biogenesis of U snRNPs and motoneuron cell death

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Autosomal recessive spinal muscular atrophy (SMA) is one of the most frequent monogenic causes of death in infancy and childhood morbidity and characterized by progressive degeneration of motoneurons resulting in atrophy and weakness of voluntary muscles. The SMA causing gene (SMN) exists in two copies termed SMN1 and SMN2 on human chromosome 5q13. Whereas the SMN1 gene allows expression of a full-length protein, the major product of the SMN2 gene is a differentially spliced, truncated and non-functional protein. Patients with homozygous absence of SMN1 express only low levels of a functional SMN protein and therefore develop the disease phenotype. The SMN protein and its interacting partner Gemin2 are ubiquitously expressed and form part of a macromolecular complex (SMN-complex) that mediates assembly of spliceosomal U snRNPs. SMN has been shown to facilitate the formation of the Sm core domain of the snRNPs, most likely by regulating the proper binding of Sm proteins onto U snRNAs. A model arising from these data suggests that patients expressing only low levels of SMN, exhibit defects in the biogenesis of snRNPs, and hence suffering from SMA. Using mouse genetics we investigated the function of this complex in motoneuron maintenance. Reduced Smn/Gemin2 protein levels lead to disturbed U snRNP assembly as indicated by reduced nuclear accumulation of Sm proteins. This correlates with enhanced motoneuron degeneration in Gemin2+/-/Smn+/- mice. Our data provide the first in vivo evidence that impaired production of U snRNPs contributes to motoneuron degeneration.

P1-22 11

Identification of modifying pathways involved in the phenotypic variability of spinal muscular atrophy (SMA)

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Proximal spinal muscular atrophy (SMA) is a neuromuscular disorder, caused by homozygous mutations of the survival motor neuron gene 1 (SMN1). SMN1 is part of an 800 kDa protein complex (gems) with a crucial role in snRNP biogenesis, pre-mRNA splicing and a presumable function in neural transport. SMN2, a nearly identical copy of SMN1, predominately produces exon 7-skipped transcripts, whereas SMN1 mainly produces full-length transcripts. The SR-like splicing factor Htra2-beta1 was shown to interact with SMN and to restore full-length SMN2-mRNA in vivo.

In rare cases siblings with identical 5q13-homologs and homozygous absence of SMN1 show variable phenotypes, suggesting that SMA is modified by other, yet unknown factors. By analyzing seven discordant families with affected and unaffected siblings, we demonstrate that in EBV-transformed cell lines homozygously deleted unaffected individuals not only display significantly higher levels of SMN protein, but also exhibit high expression levels of Gemin2, Gemin3, and ZPR1 as compared with their affected siblings. In contrast, in primary fibroblast cultures no protein expression differences could be observed.

Additionally, Htra2-beta1 was found to be regulated similarly. Interestingly, other SMN-interacting proteins, which are not components of gems, such as p53, are not subject to a SMN-dependent regulation. Since we could not find any sequence or transcription differences among intrafamilial siblings, we postulate that the SMA phenotype is modified by a factor that directly influences the SMN complex, most probably acting upstream of Htra2-beta1, which for its part up-regulates SMN, which then up-regulates other „ gems"-complex members. Furthermore this modulating effect is tissue-specific.

P1-22 12

Facioscapulohumeral Muscular Dystrophy (FSHD) – clinical presentation in patients with borderline D4Z4 repeats

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FSHD is associated with a deletion of an integral number of D4Z4 repeats at 4q35. The role of the deletion in this non-coding region remains unclear. In healthy controls, the repeat number varies from 10 to 100 repeats (fragment sizes 38-300 kb). In FSHD only 1 to 9 repeats (< 35 kb) are left. A few cases with more than 10 repeats are reported.

To systematically assess the genotype-phenotype correlation of borderline D4Z4 repeat numbers (10-14) and to explore their validity for diagnostic cut-off, we re-examined 41 of our FSHD patients with 8 to 14 repeats.

On clinical grounds, the 41 patients were subdivided into four categories: 61% (n=25) with typical FSHD; 14.6% (n=6) with facial-sparing FSHD; 12.2% (n=5) with atypical features for FSHD; 12.2% (n=5) without FSHD.

Seven patients presented with typical FSHD and >9 repeats (>35kb), 6 patients classified as atypical or no FSHD within the diagnostic range of <9 repeats (<35 kb). Five patients with facial-sparing FSHD exhibited repeat numbers within the diagnostic range. Thus a clear diagnostic threshold was missing.

In the borderline region of 8 to 14 repeats, we find a high percentage of atypical clinical presentations. This could give rise to considerations of an expanded clinical heterogeneity in FSHD. On the other hand one may discuss diagnostic limitations of the test in this borderline region. Whether FSHD with facial sparing represents an own clinical entity or an abortive form of FSHD remains to be answered. Further data on the clinical and genetic features of the patients with borderline D4Z4 repeat numbers will help in diagnosis and genetic counselling, and might even contribute to the understanding of the genetic mechanisms in this disease.

P1-22 13

Lessons from 3 years european external quality assessment for Charcot-Marie-Tooth disease

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Charcot-Marie-Tooth disease (CMT) type 1A is usually caused by a 1.4-Mb tandem duplication in chromosome 17p11.2-12 comprising the peripheral myelin protein 22 (PMP22) gene. Patients show reduced nerve conduction velocities (NCV<38 m/s). Genetic testing for CMT1A is routinely offered and performed using a wide variety of methods like: detection of CMT1A junction fragments, STR and RFLP analysis to assess dosage or the presence of 3 alleles, FISH, quantitative PCR to detect PMP22 dosage and other techniques. The main benefit for the patient and clinician is to offer differential DNA diagnosis for CMT disease. In 1999, a pilot External Quality Assessment (EQA) scheme for CMT was offered by the European Molecular Genetics Quality Network (EMQN) for the first time. Fourteen laboratories from 12 European countries registered. Following this successful trial, the scheme was offered to a wider audience in the year 2000 with 20 laboratories from 11 countries participating. One genotyping error leading to a misdiagnosis was detected. Thirthy out of 31 laboratories from 15 countries reported for the 2001 CMT EQA. Two genotyping errors occurred. However, due to the use of multiple methods these errors did not lead to misdiagnosis of the CMT1A duplication. The obvious trend, a higher number of participating laboratories resulting in more genotyping errors, is an indicator for the necessity of not only EQA schemes, but also the construction of national CMT diagnostic networks to equalize the individual laboratory performance. The development and improvement of European guidelines for genetic diagnosis of CMT (www.emqn.org) may encourage more laboratories to participate in the EQA schemes in the future. Lessons drawn from the development of both the scheme and European guidelines should help in harmonising laboratory standards for the genetic diagnosis of CMT.

P1-22 14

Searching a genetic predisposition of Complex Regional Pain Syndrome

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Several clinical symptomes of the complex regional pain syndrome (CRPS) suggest „neurogenic" inflammation which coincides typical inflammation characteristics together with trophic changes and hyperalgesia. The controlled release of neuropeptides from afferents or molecules in their subsequent signalling pathway seemed to be impaired in CRPS. Our recent data show that substance P (SP) is more effective to induce edema in CRPS patients – independent from disease activity or affected body region - compared to normal controls. In a first step we therefore screened for mutations in functional candidate genes related to the SP signalling and neuropeptide degrading enzymes, as there are neurokinin 1-receptor NK1-R, neutral endopeptidase NEP and angiotensin-converting enzyme ACE. The patient collective consists of 6 familial cases and 28 sporadic patients who suffered from CRPS once or repeatedly. For the NK1-R gene we could not find any deviation from the wildtyp sequence when investigating the genomic DNA of 10 repeatedly affected CRPS patients. In the ACE gene we checked for the deletion-deletion D/D intronic polymorphism and we did neither find a significantly increased frequency of the (D/D) genotype as reported in a Japanese CRPS population nor a co-segregation of the D/D genotype with CRPS in familial cases. Finally, for NEP a Met8Val mutation could be identified for one of the familial CRPS patients and her yet unaffected mother. Her father is in the group of repeatedly affected CRPS patients. The Met8Val variation couldn't be verified for the patient's father. Furthermore, a dinucleotide repeat vaiation in intron 6 of the NEP gene was discovered. It is homozygous elongated compared to the wildtype sequence (TA)= 8 to (TA)= 13 in 13 out of 32 CRPS patients. This variation could have some influence on the gene expression. Quantitative PCR experiments will show whether CRPS is caused by altered expression levels of NEP, ACE and NK1-R.

P1-22 15

Recurrent parkin mutations: Hot spots or founders?

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Early-onset parkinsonism (EOP) is a neurodegenerative disorder and has been associated with mutations in the parkin gene (PARK2). Some mutations were found to be recurrent. Periquet and colleagues (2001) addressed the origin of a range of recurrent parkin mutations, concluding that exon rearrangements occurred independently, whereas some point mutations have a common founder.

We studied 182 EOP patients of different ethnic background and 350 healthy blood donors from South Tyrol for mutations in the parkin gene and found 33 different mutations, 13 of which were detected recurrently. Using 12 DNA markers for the PARK2 locus, we genotyped 44 mutated chromosomes with recurrent mutations. Allele frequencies were taken from databases (www.gdb.org) and generated by genotyping another 132 chromosomes from Central European controls.

A common founder is conceivable for four (Ex2del, Ex3-4del, Ex4del, Ex7del) of the six studied exon deletions, two (1072delT, 256delA) of three small deletions, and one (924C>T) of three point mutations but not for the duplication. Interestingly, we confirmed that one of the most common mutations (924C>T) seems to have a distant European founder, suggested by the presence of a rare allele (in 3 % of controls) at the marker D6S305 in all mutation carriers (10/10 published and 10/10 this study).

In conclusion, we demonstrate that both point mutations and exon rearrangements may be founder mutations.

P1-22 16

Candidate gene testing for Emery-Dreifuss muscular dystrophy

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Until now two genes, STA and LMNA, have been associated to Emery-Dreifuss muscular dystrophy (EDMD). Scanning 93 patients suffering EDMD or associated phenotypes revealed that mutations in STA and LMNA together account for only 36 % of the cases. Obviously, further genes are likely to be involved in EDMD. Forced by the lack of families suitable for a classic positional cloning approach, we started a candidate gene approach. Beside others we considered genes encoding factors interacting with emerin and/or lamins A/C candidate genes for EDMD. Thus, BAF (barrier to autointegration factor), DDX16 [DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 16] and PSME3 (proteasome activator subunit 3) were scanned for DNA-variations by using primers to amplify all exons including the exon/intron boundaries in 56 mainly sporadic German EMD patients who were excluded to carry mutations in STA or LMNA. Until now, we have analysed 33 exons excluding the 5‘ and 3‘UTR regions of the three genes mentioned above by PCR/heteroduplex analysis. By scanning the patients, nine unique aberrant heteroduplex patterns have been found so far in exons 1 and 2 of BAF, exons 2 and 18 and introns 5, 11, 18 and 19 of DDX16 and intron 2 of PSME3. Seven DNA variations have been validated by sequencing so far. Six sequence variations have been tested in 400 chromosomes of a German normal population. Two of them occurred with frequencies for the rare allele of 0,0053 and 0,0158, respectively, thus validating them as single nucleotide polymorphisms (SNPs). There was no association of these two novel SNPs to EDMD. Additionally, four different unique sequence variations have been identified in DDX16 of three unrelated sporadic patients, which did not occur in 400 chromosomes of a normal population. However, a segregation of these sequence variations with EDMD in the patient’s families could not be validated.

P1-22 17

Disruption of the serine threonine kinase 9 gene (STK9) as the cause of severe X-linked infantile spasms

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X-linked infantile spasms (ISSX, MIM # 308350)), sometimes called West-syndrome (WS) are characterised by onset of generalized seizures between 3 and 7 months of age, hypsarrhythmia on the electroencephalogram (EEG) and mental retardation (MR). In our study of disease-associated balanced chromosome rearrangements (DBCRs)we have acquired two patients with balanced translocations 46,X,t(X;6)(p22.3;q14)and 46,X,t(X;7)(p22.3;p15) and clinical features of severe ISSX/WS. Both patients had early onset of seizures at age 2-3 month, and developmental arrest with profound mental retardation. Cloning of the X-chromosome breakpoints revealed the serine threonine kinase 9 gene (STK9) to be interrupted by these rearrangements. Mutation screening of the 21 exons of the STK9 gene in three ISSX families was negative. In the meantime the ISSX/WS gene interval was refined on one Canadian family to ~7 Mb region in Xp21.3-Xp22.1 (Bruyere et al. Clin Genet 55:173, 1999); importantly, excluding the STK9 gene.

More recently, mutations in the Aristaless-related homeobox gene (ARX) have been found in four ISSX/WS families and in families with syndromic and nonspecific mental retardation (Stromme et al., Nat Genet 4:441, 2002, Bienvenu et al., Hum Mol Genet, 11:981, 2002). Based on the identical phenotype of our two patients with balanced X-autosome translocations we suggest, that there are at least two genes for ISSX on the human X-chromosome. We propose, that lack of a functional STK9 protein is responsible for a severe form of X-linked infantile spasms.

P1-22 18

Congenital prosopagnosia - indicative of simple autosomal dominant inheritance

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Prosopagnosia (PA), a term introduced by Bodamer (1947, Arch Psych Nerven 179:6ff), defines the inability to identify a person by it´s face alone. The specifity of this deficiency is supported by double dissociation between probands with impairment of e.g. object agnosia but not prosopagnosia and vice versa. Almost all reports (#259 citations in medlineR) are single casuistics or collections of unrelated patients who acquired prosopagnosia after brain injuries, strokes, or atrophy of at least the right occipito-temporal area. There are only a few reports of congenital PA - almost all of them sporadic cases (#5 citations in medlineR). The only hints of familial ecurrence in 2 generations are given by McConachie (1976, Cortex 12:76ff) and by Haan (1999, J Clin Ex Neuropsy 21:312ff). The family trees were not studied in any more detail and no entries were added to OMIM. We have found 4 distinct families of at least 5 persons each with congenital PA ascertained in 4 generations. We are also aware of several other unrelated families with 2 persons showing congenital PA. This indicates a much higher prevalence than represented by literature data. The cumulative segregation ratios are compatible with a simple autosomal dominant mode of inheritance. Evaluation of the prevalence in the general population is in progress. Linkage analyses in all 4 families have been started. By whole genome scan 75% of the genome could be excluded until now.

P1-22 19

Clinical features and neuropathology of autosomal dominant spinocerebellar ataxia (SCA17) caused by CAG expansion in the TATA-binding protein

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Autosomal dominant inherited spinocerebellar ataxia (SCA) is a neurodegenerative disorder clinically characterized by late-onset ataxia of gait, dysarthria, and in some patients with extrapyramidal dysfunctions. Several forms (SCA1, SCA2, SCA3, SCA6, SCA7) are caused by unstable CAG repeat expansions in the coding region of the responsible genes. Recently, CAG repeat expansions in the TATA-binding protein (TBP) have been found in a new form of SCA which has been assigned SCA17. We determined the frequency of SCA17 in a large sample of 1318 SCA patients of German origin for whom CAG repeat expansions in the SCA1, SCA2, SCA3, SCA6, SCA7, SCA8, SCA12 and Friedreich’s ataxia genes had been excluded. As controls we studied 150 people older than 60 years with no neurological signs, 250 patients with dystonia, 81 patients with genetically confirmed Huntingtons disease, and 198 SCA patients with known mutation.

In total, 3 autosomal dominant SCA families and two apparently sporadic patients with CAG repeat expansions in the TBP gene ranging from 45 to 54 repeats were identified. In 481 control individuals the largest allele detected consisted of 42 alleles and in the group of SCA patients with known mutation alleles of 43 CAG repeat

were found defining a small non-overlapping region between normal (43) and expanded (45) alleles. The clinical features of SCA17 differ from other SCA subtypes by manifestation with psychiatric detoriations in addition to neurological symptoms. Based on routine histological examination, the neuropathology of SCA17 can be classified as a „pure cerebellar" or „cerebellooliveary" form of ataxia. However, intranuclear neuronal inclusion bodies with immunoreactivity to anti-TBP and anti-polyglutamine were much more widely distributed throughout the brain gray matter than in other SCAs.

P1-22 20

Mutation analysis of genes involved in brain iron homeostasis in Parkinsonian patients

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In the pathogensis of Parkinsons disease a disturbance of iron metabolism at different levels including iron uptake, storage, intracellular metabolism and post-trancriptional control has been discussed for many years. Using transcranial ultrasound an accumulation of iron in the substantia nigra is being detected in about 90 % of Parkinsonian patients and there is accumulating evidence indicating that increased iron content of the brain may serve as a marker for nigrostriatal vulnerability. To investigate whether this ultrasound feature may be associated with mutations in genes encoding for proteins of the iron metabolism we performed mutation analysis in 180 patients with Parkinsons disease who displayed the feature of increased substantia nigra iron levels on transcranial ultrasound in comparison to 180 controls without this ultrasound feature. Mutation analyses were performed of the genes encoding for IRP2 and ceruloplasmin. IRP2 is one of the cytosolic iron regulatory proteins involved in the regulation of iron transporting and storing proteins, whereas ceruloplasmin seems to play a major role in cellular iron import and export. All 22 exons of the IRP2 gene and all 19 exons of the ceruloplasmin gene were amplified by polymerase chain reaction. Heteroduplex analysis was performed using the Wave-system(R), which forms a mixture of hetero- and homoduplexes with pooled probes in individuals with heterozygous or homozygous mutations. Exons of individuals with evidence of heteroduplex formation were amplified for sequence analysis. Comparison of mutations and polymorphisms in these genes in Parkinsonian patients and controls may help to elucidate the role of iron homeostasis in Parkinsons disease.

P1-22 21

False positive association findings in 45 hypothetical candidate genes for Alzheimer disease ?

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The genetic association between APOE e4 and late onset Alzheimer disease (AD) was discovered almost one decade ago. A cumulative review of epidemiological, family, twin, linkage, and theoretical studies allows to conclude that there exist risk alleles in several genomic loci in order to fully explain the proportion of AD attributable to genetic factors. However, up to now none of the numerous studies involving more than 100 candidate genes revealed convincing evidence for any predisdposing risk alleles in genes other than APOE. In the published literature there are more than 45 loci with positive association findings. Most of these findings (including these of our group) could not be confirmed. There are only few loci deserving further replication studies. This presentation briefly reviews possible reasons for this lack of success and proposes criteria for a more efficient selection of positional and functional candidate genes for AD. Supported by DFG, grant Fl 704/1-3.

P1-22 22

Analysis of association of Alzheimer disease with allelic variants of TFAM located on chromosome 10

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TFAM encodes mitochondrial transcription factor A that participates in mitochondrial genome replication and in activation of mitochondrial transcription. Both mechanisms are essential for mitochondrial function and integrity. Mitochondrial dysfunction may be involved in neurodegeneration observed in late-onset Alzheimer disease (AD). TFAM locates to chromosome 10q21, a region linked to AD[1] and plasma level of Ab42 in AD families.[2] Linkage to 10q21 was most evident in APOE e4 positive samples.[1] Therefore, TFAM represents both a positional and functional candidate gene for AD. We genotyped a polymorphism in evolutionary non-conserved codon 12 of TFAM (S12T) in 372 patients with AD and 295 nondemented controls. There was a trend towards an association between absence of T12 (T-) and AD (p=0.062). Conditional logistic regression analysis entering covariates age, gender, TFAM (T- vs. T+), APOE (e4+ vs. e4-), and interaction [APOE x gender x TFAM] revealed an interaction between the three covariates with an exp B („OR") of 3.39 (95% CI 1.93-5.94) in addition to significant associations with AD for APOE (OR 1.96, 95% CI 1.34-2.85) and TFAM (OR 0.74, 95% CI 0.59-0.92). These data suggest a possible association between absence of TFAM allelic variant T12 and AD which is influenced by gender and APOE genotype. Supported by DFG, grant Fl 704/1-3.

[1] Myers et al., Science 290:2305-2305, 2000
[2] Ertekin-Taner et al., Science 290:2303-2304, 2000

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Mutationsanalyse des Pank4-Gens in Parkinson Patienten

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Ätiologie und Pathogenese der Parkinson'schen Erkrankung sind weitgehend unklar. In einigen wenigen Familien konnten Mutationen im alpha-Synuclein-Gen und im Parkin-Gen nachgewiesen werden. Mittels Kopplungsanalysen wurden kürzlich 2 Genloci in der chromosomalen Region 1p35-36 identifiziert, die mit einer früh manifestierenden Parkinson Erkrankung einher gehen sollen. Durch den Nachweis von Mutationen in einem Pantothenat-Kinase-Gen (Pank2, Genlocus 20p13) konnte kürzlich die Ätiologie der Hallervorden-Spatz-Erkrankung geklärt werden. Diese Erkrankung geht mit einer Eisenakkumulation im Globus pallidum und parkinsonähnlichen Symptomen einher. Mittels Datenbanksuche gegen das menschliche Genom findet man eine hohe Sequenzhomologie zwischen Pank2 und Pank4, welches in die Region 1p36 kartiert. Wir untersuchten die DNA von 184 Parkinsonpatienten (AAO <= 50. LJ) auf das Vorliegen von Mutationen in Pank4. Nach PCR-Amplifikation der 19 Exons führten wir ein initiales Mutationsscreening mittels dHPLC (Transgenomic WAVE System) durch. Die dabei auffälligen Proben wurden anschließend sequenziert (Beckman Coulter CEQ 2000). Mittels dHPLC wiesen wir in den 184 Proben insgesamt 415 (zum Teil fragliche) Shifts nach, die zu 53 unterschiedlichen Shift-Gruppen zusammengefasst werden konnten. Bisher konnten 7 Gruppen sequenziert werden: in einem Fall zeigte sich bei einem fraglichen Shift eine Wildtypsequenz; 5 Shifts konnten als intronische Mutationen identifiziert werden; bei einem Shift (6 Proben) konnte eine Mutation in Exon 13 nachgewiesen werden (1640 C->T; 547 Ala->Val). Die optimierten Bedingungen des Screenings von Pank4 mittels dHPLC und die vollständigen Ergebnisse der Mutationsanalyse werden vorgestellt.

P1-22 24

QUANTITATIVE ANALYSIS OF SMN1 AND SMN2 COPY NUMBER USING TAQMAN TM TECHNOLOGY

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Objective: Spinal muscular atrophy (SMA) is usually caused by homozygous deletion of the SMN1 gene. The detection of heterozygotes for SMN1 deletion has many clinical implications. The number of SMN2 copies correlates statistically with the severity of SMA. The SMN2 copy number could be of interest in the view of possible future therapeutic trials. The quantitative SMN analysis, however, is difficult since the SMN1 and SMN2 genes differ in only a few base pairs. In order to allow a simple and reliable test to determine the number of SMN1 and SMN2 copies, we developed a quantitative test using the well known TaqManTM technology.
Methods: Minor groove binding (MGB) probes were used for the detection of single nucleotide polymorphism in order to differentiate between

SMN1 and SMN2. As a reference locus exon 3 of the factor VIII gene was used. Genomic DNA from 40 unrelated parents of SMA patients carrying one SMN1 copy and 100 normal controls were analysed. The number of SMN copies was determined by analysis of the multicopy markers AG1-CA and C212. The reproducibility was evaluated on the basis of 5 independent PCRs and revealed non overlapping results. The proposed method allowed a clear distinction between typical carriers (one SMN1 copy) and controls (two SMN1 copy) with ranges from 0.60 to 1.20 (carriers) and 1.60-2.20 (controls). Low copy numbers of SMN2 were precisely classified whereas the differentiation of very high numbers was more difficult.
Conclusion: The TaqManTM based quantitative analysis of SMN1 and SMN2 can be regarded as a reliable method to determine the SMN1 and SMN2 copy number. While the determination of the SMN1 copy number has numerous clinical implications, the SMN2 copy number is so far mainly of scientific interest.

P1-22 25

Clinical and molecular studies of Holoprosencephaly

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Holoprosencephaly (HPE) is a common congenital defect resulting from loss or incomplete formation of midline structures of the forebrain and face. The etiology of HPE is heterogeneous and includes environmental factors, non-random chromosomal aberrations as well as gene mutations. Currently, mutations in SHH (7q36), SIX3 (2p21), ZIC2 (13q32) and TGIF (18p11.3) account for appr. 16-20% of all HPE cases. Here we report the results of the analysis of HPE patients in the German population. Molecular studies include direct sequencing of the coding regions of SHH, SIX3 and ZIC2. DNA samples without identified mutation are further analyzed for mutations in additional HPE candidate genes in a collaborative research study (M. Muenke, NIH/Bethesda) after written consent of the patients or parents, respectively. Identified mutations include the previously reported SHH nonsense mutation Glu324(amber) as well as a new SHH missense mutation Gly27Ala, both occurring in dominant HPE families with solitary median maxillary central incisor (SMMCI) and wide intrafamiliar clinical variability. A new SIX3 missense mutation Arg218Pro was observed in the highly conserved homeo domain in a 3 year old girl with semilobar HPE and profound developmental delay. Analysis of the parents unexpectedly revealed the clinically healthy mother to be a mosaic carrier. The low grade mosaicism in maternal genomic DNA prepared from peripheral blood was only detected by RFLP analysis using a restriction enzyme exclusively cutting the normal allele. A new truncating ZIC2 mutation del A in codon 221 was identified in a 7 year old boy with alobar HPE and relatively mild facial features. Further analysis of the phenotype genotype correlation in HPE patients will improve genetic counseling of affected families and our understanding of the genetic control of normal and abnormal brain development.

P1-22 26

Mutations in the ganglioside-induced differentiation-associated protein-1 (GDAP1) gene in autosomal recessive Charcot-Marie-Tooth neuropathy

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Mutations in the gene for the ganglioside-induced differentiation-associated protein-1 (GDAP1) on 8q21 were recently reported to be responsible for about 25% of cases with autosomal recessive Charcot-Marie-Tooth sensorimotor neuropathy (CMT). Neurophysiology and nerve pathology were heterogeneous in these cases: a subset of GDAP1 mutations was associated with peripheral nerve demyelination while others resulted in axonal degeneration. In the present study on autosomal recessive CMT including 11 families, we identified two families with novel mutations disrupting the GDAP1 reading frame. Homozygosity for a single basepair insertion in exon 3 (c.349_350insT) was observed in two affected children from a Turkish inbred pedigree. The other novel allele detected in a German child from unrelated parents was a homozygous mutation of the exon 4 donor splice site (c.579+1G>A). Our patients with GDAP1 mutations displayed severe, early childhood-onset CMT neuropathy with prominent pes equinovarus deformity and impairment of hand muscles. Nerve conduction velocities (NCV) were between 25 and 35 m/s and peripheral nerve pathology showed axonal as well as demyelinating changes. There was no specific morphological feature that allowed to distinguish our patients from other types of CMT. Our findings underline the role of GDAP1 for the pathogenesis of CMT and allow further insight into the mechanisms vital for axonal integrity and Schwann cell properties. Identification of GDAP1 as the causative gene defect allows direct mutation analysis in individual patients also from small, non-consanguineous families. A molecular genetic diagnosis is important for accurate genetic counselling of affected families.

P1-22 27

Molecular genetic approach in families with possible autosomal recessive Charcot-Marie-Tooth neuropathy (ARCMT)

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While the majority of CMT cases is autosomal dominantly or X-linked dominantly inherited, autosomal recessive CMT (ARCMT) is much rarer and highly heterogeneous. The distribution of weakness and sensory deficits is similar to the dominant forms, but the clinical course is generally more severe with an earlier age of onset. At least ten loci responsible for ARCMT and six genes have been identified so far. Thus, a routine molecular genetic analysis is not available yet. We have developed a stepwise molecular genetic approach in cases suspected of having ARCMT. First, we exclude a mutation in the PMP22, Cx32, MPZ, and EGR2 genes. The next step depends on the size and structure of the family under investigation: Linkage studies are performed in extended pedigrees, homozygosity mapping in case of parental consanguinity, and direct mutation analysis of known ARCMT genes in isolated patients. In case a family is compatible with one of the ARCMT loci, haplotype analysis is followed by direct sequencing of the respective gene (if available). Thus far, two truncating mutations in GDAP1 and one frameshift mutation in PRX could be identified. Linkage has been established in two ARCMT multiplex families to loci on chromosome 5q32 and 11p15 respectively. Linkage analyses as well as haplotype sharing will help to further narrow down candidate regions if the responsible gene is not yet known. Appropriate candidate genes are currently under investigation. The identification of gene defects in ARCMT is important for genetic counselling of affected families and for a better understanding of the different pathomechanisms underlying peripheral nerve dysfunction. To further pursue these studies we are highly interested in additional families with possible ARCMT.

P1-22 28

Charcot-Marie-Tooth disease: A novel Tyr145Ser mutation in the Myelin Protein Zero (MPZ, P0) gene causes different phenotypes in homozygous and heterozygous carriers

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Mutations in the MPZ gene cause Charcot-Marie-Tooth type 1B (CMT1B), a demyelinating hereditary motor and sensory neuropathy (HMSN). Mutations in MPZ have been related also with axonal neuropathy (CMT2), particularly due to a T124M mutation in exon 3. Patients who carry this mutation are additionally affected with Argyll Robertson-like pupils and frequently with deafness and dysphagia. Here we present a Costa Rican family affected mainly with CMT1B; the parents presented a mild neuropathy with a late age of onset (50 years), whereas the two children presented an earlier age of onset (late 30 years) and a more severe neuropathy. Clinical signs are distal sensory deficits and absent ankle jerks. Pupillary abnormalities, distal weakness and atrophies are restricted to the children. Electrophysiological studies revealed axonal degeneration and demyelination. A sural nerve biopsy from one of the children showed signs of axonal degeneration as well as demyelination. Both in the maternal and the paternal family histories other patients with this type of peripheral neuropathy exist. After sequencing of all 6 coding MPZ exons for the parents and children, a Y145S mutation in exon 3 was found; the parents are heterozygous for this mutation, whereas the children are homozygous carriers. In contrast to the T124M mutation, only the homozygous Y145S carriers are affected with pupillary anomalies, and the Y145S heterozygous patients are less affected than the T124M

heterozygous. We propose that by the exchange of similar side chains (uncharged polar) the slightly altered protein conformation has only low impact on the protein function; this could explain the mild phenotype of heterozygote carriers. However, a homozygous change could be sufficient to cause a neuropathy associated with pupillary anomalies.

P1-22 29

Investigations into the Molecular Mechanisms of Brain Function: Disruption of a Serine-threonine Kinase is Associated with Motor and Cognitive Deficits in a Patient with a t(Y;4) Translocation

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Numerous genes, coding for proteins with a wide variety of functions, play some role in normal cognitive development. It is estimated that in a significant percentage of cognitive disorders, there is a direct link between genotype and disease phenotype, and recent advances on the X chromosome provide the framework within which molecular cascades important for cognitive function can be elucidated. We have utilised data from routine chromosome analyses in patients with early onset, non-syndromic forms of mental retardation and established cell lines from patients for which balanced chromosome rearrangements provide likely explanations for the phenotype. Here we report the results of a study involving a patient carrying a translocation t(Y;4)(q11.2;q21) and presenting with a severe neurodegenerative disorder, characterised by a progressive loss of speech and other motor activities that was first observed at the age of thirteen months. Motor degeneration was accompanied by severe mental retardation and a seizure disorder which persisted despite administration of classical anti-seizure medication. We have determined that, while the breakpoint on chromosome Y lies within a highly repetitive region that likely contains no gene, the breakpoint on chromosome 4 disrupts a serine-threonine kinase that plays an established role in apoptosis and stress response, and based on its restricted expression pattern, is likely involved in CNS development and function. Further, we have confirmed that the truncated protein is expressed in the patient, suggesting that the underlying molecular mechanism is not one of simple haplo-insufficiency. Current studies aim to determine the role that this mutant protein plays in the patient disorder, specifically, to understand how its presence may affect the normal signaling cascade within which the wild type protein functions.

P1-22 30

Identification of a <5Mb interval on proximal Xp playing a major role in non-syndromic X-linked mental retardation NS-XLMR and large-scale mutation screening of all relevant genes

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X-linked gene defects may account for one third of all idiopathic forms of mental retardation in males, and most of these cases are non-syndromic. Causative mutations in 12 different genes as well as linkage studies have documented that NS-XLMR is highly heterogeneous, and until recently it was believed that up to 100 X-linked genes might be involved. However, as judged from the recent finding that up to 20 percent of all patients with this condition have mutations in the ARX gene (Stromme et al., Nat. Genet. 2002; Bienvenu et al., Hum. Mol. Genet. 2002), the total number of genes may be much lower. As a prerequisite for identifying other major genes in the etiology of NS-XLMR, we have analyzed all available linkage data from the European XLMR Consortium and from other sources to estimate the distribution of gene defects underlying NS-XLMR along the length of the X-chromosome. Apart from several peaks corresponding to ARX at Xp22 and other known genes for NS-XLMR, a <5Mb interval on proximal Xp was identified which seems to harbor (one or several) novel important XLMR genes. An integrated, functionally annotated map of this interval was constructed, and DHPLC-based mutation screening of all brain-expressed genes in this region is in progress.

P1-22 31

NMDA RECEPTOR POLYMORPHISMS IN ALCOHOLIC PATIENTS

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Alcohol dependence is a clinically heterogeneous syndrome caused by complex interactions of genetic and environmental factors. The estimated genetic effects from twin studies account for 40-60% of the variance in the liability to develop alcoholism. The ionotropic glutamatergic N-methyl-D-aspartate receptor is known to be reduced acutely by physiologically relevant concentrations of ethanol. After chronic ethanol exposure tolerance develops, and NMDAR function increases. In this study we tested, if recently found polymorphisms in the genes of the NMDAR1 (G2108A) and NMDAR2B (C2664T) subunits are associated with ethanol dependence. Genotyping was done using both FRET-probes in a PCR/melting curve analysis, and by conventional RFLP analysis. The study included 367 alcohol dependent individuals and 335 control individuals of German origin for the case control study, and 72 trios of Polish origin for the family based study. For NMDAR1, there was a significant difference in the genotype distribution between alcoholic patients and controls. Furthermore, patients with the homozygote mutation had a significantly less ethanol intake than those bearing a G allele. For NMDAR2B the T allele prevalence was significantly reduced in patients with an early age at onset, in patients with vegetative withdrawal syndrome, and in Cloninger type 2 alcoholics. Furthermore, patients carrying the C-allele showed higher rates in the personality trait subscale of impulsivity compared to those lacking it. Our family based study revealed a preferred transmission of the C allele by fathers to their affected offspring.

P1-22 32

Splice mutations of the LIS1- gene leading to severe classical lissencephaly

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Classical lissencephaly is a severe brain malformation with absent (agyria) or poor sulcation (pachygyria) because of an impaired neuronal migration. It occurs isolated or associated with other symptoms. The most common form is present as Miller-Dieker Syndrome with its characteristic facial dysmorphic features. Clinically isolated lissencephalies (IL) are associated with refractory epilepsy, severe mental retardation and poor life expectancy. Two genes are associated with isolated (classical) lissencephalies. Autosomal dominant lissencephalies are linked to alterations of the LIS1 gene on chromosome 17 and X-linked lissencephalies to mutations of the DCX gene, respectively. The brain malformations due to LIS1 mutations present predominantly with occipital agyria, whereas DCX mutations lead to frontal predominant agyria. Most common mutations are submicroscopic deletions of the LIS1 gene. This microdeletions may be detected by FISH in up to 40% of the cases. But also intragenic mutations of the LIS1 gene are reported and detectable in approximately 12% of ILs. While missense mutations of the LIS1 gene might lead to milder forms of lissencephalic brain malformations, nonsense mutations are associated with more severe grades of lissencephaly. Here, we present our results of mutation analysis of the LIS1 gene and the detection of splice mutations in two patients with severe form of lissencephaly.

P1-22 33

Filamin 1 mutation in a female patient with periventricular nodular heterotopia (PNH)

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Periventricular nodular heterotopia (PNH) is a heritable neuronal migration disorder with defective migration of neuroblasts from their origin, the subventricular zone to the neocortex. The brain malformation is characterized by nodules of heterotopic neurons resting along the lateral ventricles due to a defect in the initiation of the neuronal migration. PNH is mostly inherited in an X-chromosomal dominant manner and is linked to mutations in the X-linked filamin 1 gene (FLN1). While mutations in the X-linked filamin 1 gene (FLN1) at Xq28 cause PNH in heterozygeous females, in hemizygeous male embryos filamin 1 mutations usually lead prenatally to death. Families with filamin mutations are characterized by an excess of female family members and a high rate of abortions. This neuronal migration disorder leads to epilepsy, vascular abnormalities and less frequent to moderate mental retardation. In patients with symptomatic epilepsy this disorder is diagnosed more frequently in the last years due to improved MR-imaging techniques. Here, we present a female patient with bilateral PNH suffering from epilepsy with seizures refractory to antiepileptic treatment. She underwent epilepsy surgery due to an epileptic focus of the left parietal lobe. The mutation analysis revealed a novel 2 bp insertion in the 9 exon of FLN1 gene leading to a frame shift and premature stop codon. This is the first description of an FLN1-insertion mutation leading to PNH.

P1-22 34

Mutation analysis of the DCX gene in Lissencephaly / Subcortical Band Heterotopia Spectrum

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Lissencephaly and subcortical band heterotopia (SBH) (synonyma: Double Cortex Syndrome) are heritable neuronal migration disorders. The occurence of lissencephaly and SBH within the same pedigree, even within the same patient uncovered their relationship and lead to the term „Lissencephaly / Subcortikal Band Heterotopia Spectrum". This spectrum range hereby from complete absence of gyri (agyria) to broad gyri with reduced sulci (pachygyria) and merges with subcortical band heterotopia. Genetically these diseases are heterogeneous with different modes of inheritance. X-linked lissencephaly and subcortical band heterotopia are allelic disorders caused by mutations in the doublecortin gene (DCX or XLIS) at Xq22.3-q23. Mutations in hemizygeous male patients usually lead to lissencephaly with severe mental retardation, epilepsy and poor life expectation. In heterozygeous female patients with DCX mutations, subcortical band heterotopia is associated with normal development or moderate mental retardation and epileptic seizures. Here we demonstrate a large series of familial and sporadic cases with X-linked lissencephaly and/or Double Cortex Syndrome analized for mutations in the DCX gene. We have identified 13 different mutations, including 9 novel nonsense- and missense mutations and 1 insertion. Our analysis revealed that the subcortical band heterotopia is characterized by high rate of somatic mosaicism and incomplete penetrance. In addition we detected a somatic mosaic mutation in the DCX gene in a male patient leading to subcortical band heterotopia.

P1-22 35

Congenital myasthenia in Brahman calves caused by homozygosity for a CHRNE truncating mutation

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Congenital myasthenic syndromes (CMS) are inherited disorders in which the safety margin of neuromuscular transmission is compromised by presynaptic, synaptic or postsynaptic defects. Most CMS patients have a history since infancy or early childhood of fatiguable weakness involving ocular, bulbar and limb muscles. There are some reports of inherited myasthenic weakness in domestic animals but so far the molecular basis in these cases has remained elusive. The analysis of such spontaneous animal models can contribute to our understanding of the pathophysiological mechanisms underlying CMS in humans, too. We were now able to identify a homozygous 20bp deletion within exon 5 of the acetylcholine receptor e-subunit (bovCHRNE) in Brahman calves affected by CMS. The 470del20 mutation causes a frame shift in the predicted bovCHRNE protein after 129 codons, substituting 342 wild-type amino acids residues by 40 aberrant amino acids followed by a stop codon. The frame shift occurs 90 amino acids residues N-terminal of the first transmembrane region. Thus, the bovCHRNE mutation reported here leads to a non-functional allele, which is likely to be the primary cause for myasthenia in the affected calves. Interestingly, all four calves were severely affected at a very young age. This is in contrast to human myasthenic patients with homozygous truncating CHRNE mutations, most of them showing a less severe course of the disease. ? (Kraner et al., Neurogenetics, in press)

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P2-01 01

Patient's Views on Ethical Issues; Surveys in USA, Germany, France

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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, and 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 (US 53%, Germany 42%, France 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. C. Privacy. There was universal distrust of insurers and employers. 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. 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. E. Disability. Most (78-90%) would respects parents' wishes to refuse a lifesaving 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. About 30% fewer in US would abort for each of 24 genetic conditions than in Germany. On most issues, patients' views differed from geneticists in their own country. It is time to examine reasons for these differences.

P2-01 02

Does Knowledge influence attitudes towards psychiatric genetics?

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In the framework of the German Human Genome Project we conducted a study to assess the attitudes towards psychiatric genetic research and testing and to identify factors influencing them in a representative sample of 3077 persons of the German general population. One factor assumed to influence the attitudes is knowledge about psychiatric disorders and genetics, which was assessed by questions about psychiatric illnesses and genetics.
Our results show that a higher knowledge concerning psychiatric illnesses and genetics is correlated with more critical -but still positive- attitudes towards psychiatric genetic research (r = .200, p =.000), predictive testing(r = .125, p = .000), prenatal testing (r = .255, p = .000) and access to genetic information (r = .323, p = .000). Further analyzes concerning the means of the investigated scales, show significant differences between the different knowledge groups. For a further analyzes of the influence of knowledge on attitudes in the field of psychiatric genetics, knowledge about schizophrenia and ethical standards in the application of psychiatric genetics was tested in 88 medical students before and after a short teaching program. Before the intervention the knowledge about schizophrenia was alarmingly low and misbeliefs with

respect to clinical symptoms and course of schizophrenia widespread. Although knowledge could be significantly increased common misbeliefs showed to persist in a substantial part of the students.
Our findings show that knowledge influences attitudes and furthermore indicate that specific training programs are warranted to fight widespread misbeliefs.

P2-01 03

Defining and understanding disease in the genomics era
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The term 'genetic disease' is used on a daily basis, but the concept of genetic disease seems to be far from clear. Most human diseases have a complex aetiology, involving genetic, and among others physiological, psychological and life style factors on their causal pathway. Genetic explanations of a disease are highly context dependent (state of knowledge, background of non-genetic factors, study population). They have been discussed as a reflection of increased technological capacities.
Even with a complete knowledge of aetiology a classification of diseases in terms of cause would not be satisfactory. Diseases have to be described in terms of aetiology as well as in the pathology and pathophysiology which results.
Six groups of students of medicine differing in their level of clinical and theoretical experience (1st, 2nd, 3rd and 6th year of medical education, 300 from the regular curriculum, more than 200 from the reformed curriculum) were interviewed by a structured questionnaire focussing on their concept of disease in general and genetic disease in particular, the latter understood as a causally oriented classification on the one hand and as an assignment to the specialty human genetics within the realm of medicine on the other.
Answers of study participants reflected the present prominence of human genetics for the diagnosis of monogenetic diseases and diseases with a well established mode of inheritance; they highly overestimated the importance of human genetics in the diagnosis of multifactorial diseases and in therapy and prevention of disease with a known genetic aetiology. A condition has a significant genetic or environmental component only relative to a range of genes or a range of environments. In the teaching of medicine more emphasis has to be placed on the development of a realistic estimation of human genetics for medical practice in general.

P2-01 04

Human genetics and the meaning of 'prevention' - unprecise concept causes confusion in contemplation and application
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Within the specialities of medicine human genetics is one of the youngest. The introduction of its concepts and its molecular methods has an influence on medical thinking and the use of basic medical concepts. Genetic research is highly interdisciplinary and collaborative which makes necessary the communication between geneticists, various academic disciplines and the public. The informational content of technical terms, which have a (nearly) clear meaning in the realm of medicine in general, is liable to be garbled when transferred to and uncritically used within human genetics. One of the technical terms we focussed on is 'prevention', which as primary prevention keeps disease from occurring at all by removing risk factors but in human genetics especially in prenatal diagnosis is mainly understood as prevention of the ill.
Fourteen scenarios were developed from genetic counselling before conception to abortion of a child with trisomy 21. Three groups of students of medicine differing in their level of clinical and theoretical experience (1st, 3rd and 6th years of medical education) were interviewed using these scenarios in a structured questionnaire. Confounders, concepts of disease and assessment of human genetics in the realm of medicine were recorded.
About 50% of the study group made a distinction between prevention of a disease and 'prevention' of a (genetically)diseased human being, while on the other hand rating human genetics highly responsible for the prevention of genetically caused diseases. The judgement of the different scenarios by the participants was done without a clear concept of 'prevention.

P2-01 05

Attitudes of Medical Personnel to Ethical
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A WHO meeting of experts in genetics was convened in Geneva, Switzerland, in december 1997, to review proposed international guidelines on ethical issues in medical genetics and genetic services. The medical application of genetic knowledge must be carried out with due regard to the general principles of medical ethics; Autonomy, Beneficience, Non-malficience, Proportionality and Justice.
Human genetics with its advances, specially in the last two decades, has created new ethical, legal and penal issues.
This study was carried out with the purpose of obtaining points of view of 756 physicians, nurses, midwives and common people, with regard to ethical principles in medical genetics. The study was performed by questionary method and the descriptive and analytical assesment was accomplished on the results. The results showed that the application of these ethical principles in health care, have been observed with different views.
According to these views, the principles were categorized based on priority of acceptance:
1- Proportionality; 2- Beneficience; 3- Justice; 4- Autonomy and 5- Non-maleficience.
Analytical assesments suggest that a number of personal, cultural and social variables were taken in to account and the relationship between negative views and the variables were assessed. Autonomy, with age and marriage statues, have shown statistical significance. More adults than middle aged, and more singles than married individuals, responded negatively. For beneficience, the variable of profession has shown statistical significance. Non-malficience and proportionality showed to be not statistically significant by the variables. Residents of Tehran produced more negative responses to justice than those living in other cities.

P2-02 01

Tissue- rather than gene-specific models help to elucidate regulatory networks via promoter sequence analysis
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The aim of our group is the description of regulatory promoter elements for identification of gene regulatory networks. For this purpose, we collected promoter sequences of genes related to the monogenic disorders of MODY type 1 to 6 (maturity onset diabetes of the young). The MODY syndromes are a subtype of non-insulin dependent diabetes mellitus type II (NIDDM) with autosomal dominant inheritance. Our in silico modeling techniques (GEMS Launcher, Genomatix) allowed us to identify conserved transcription factor binding sites in the genes for the MODY-associated transcription factors HNF1alpha (MODY 3), HNF1beta (MODY5), HNF4alpha (MODY 1), PDX1 (MODY 4), NeuroD1/BETA2 (MODY 6) and HNF3beta (pancreatic network key regulator) and to reconstruct part of the the MODY-associated regulatory network. A model derived from promoters of insulin genes from human, mouse, rat and apes proved to be too specific for identifying sufficient co-regulated genes. Solving the problem of overly specific models, we developed a general method for detecting tissue-associated genes. Our tool „LILIAN" (literature linkage analysis) was used to identify potentially pancreas-associated transcription factors. These data were used to refocus the following in silico promoter modeling to the most important transcription factors and associated genes. Thereby, we were able to apply more generalized promoter models for our database searches. The new models were more tissue- than gene specific and allowed us to identify several more potentially co-regulated genes. Thus we could enlarge the existing molecular regulatory and metabolic network related to MODY.

P2-02 02

Interaktives Patienteninformationssystem für Humangenetische Institute und Praxen
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Alle bekannten Patientenverwaltungsprogramme sind ausschließlich auf einzelne Patienten zugeschnitten und erfüllen die im Fach Humangenetik erforderliche Verwaltung von Familiendaten nicht adäquat. Daher wurde am Institut für Humangenetik an der Universität Würzburg eine Datenbank unter der Microsoft Access Entwicklungsumgebung entworfen. Die schon bestehende Datenbankversion unter Dbase wurde hierbei als Grundlage genommen, die vorhandenen Daten aller Patienten übernommen und um die hinzugekommenen technischen und logistischen Anforderungen erweitert. Seit Anfang 2000 wird das gesamte Patientenaufkommen des Instituts für Humangenetik mit der neuen Anwendung in Access verwaltet. Die Erfassung der Daten erfolgt in drei Schritten. Die Eingabe der Familie mit allen relevanten Personen, die jew-

eiligen Patientenstammdaten mit dem Untersuchungsfortschritt und die Ein- und Ausgabe der Untersuchungsaufträge für die einzelnen Labore. Alle drei Bereiche sind auf eigens dafür entworfene Eingabemasken verteilt, sodass dem Benutzer eine klare Struktur vorliegt, die einfach zu bedienen ist und alle erforderlichen Eingaben auf einen Blick erkennen lässt. Durch die grafische Benutzerführung werden fehlende Informationen sofort erkannt und Eingabefehler weitestgehend vermieden. Da Access netzwerkfähig ist, kann von jedem an ein Netzwerk angeschlossenen Computer die Daten- Abfrage und Eingabe erfolgen. Ein besonderer Vorteil ist der kontinuierlich abrufbare Untersuchungsstand jedes einzelnen Patienten. Damit ist ein effizientes Monitoring auch bei hohen Patientenzahlen gewährleistet. Abfragen des Datenbestandes können für alle Anforderungen mit geringem Aufwand erstellt werden. Hierzu gehörten unter anderem Auftragslisten für die jeweiligen Laboruntersuchungen wie auch Wiedervorlagelisten, z.B. für schwangere Patientinnen um eine möglichst schnelle Diagnostik zu gewährleisten. Diese Listen können für Laborbesprechungen unter Angabe der gewünschten Kriterien auf einer Bildschirmmaske mittels weniger Mausklicks erstellten in Relation zueinander zu setzen und miteinander zu vergleichen. Das System kann mit relativ geringem Lernaufwand selbst erweitert und gewartet werden. Die Abhängigkeit zu teuren Wartungsverträgen kann entfallen. Die Erfassung der erbrachten Leistungen ist durch das Programm weitgehend automatisiert.

P2-02 03

Statistical Service Unit at the Genome Mapping Center of the MDC

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It is already a fairly long time that the dissection of complex genetic traits is high on the agenda in the analysis of complex genetic traits, with its still unresolved practical and theoretical problems as genetic heterogeneity and multifactorial etiology. An undissputed prerequisite to overcome these problems lies in recruitment and genotyping of larger samples (using microsatellites and more and more SNP).
The high number of data, the use of different types of genetic markers, the rapidly upcoming new genotyping methods and lab equipment as well as the need for compatibility to other gene mapping centers, requires a high amount of flexibility in the management of the laboratory information management system in order to check, control and monitor the different work flows through the GMC.
In the analysis of the data the focus has shifted from conventional family based linkage studies to population based studies with their special problems in quality checking, Hardy-Weinberg Equilibrium, Linkage Disequilibrium, haplotype estimations and subsequent association studies of haplotypes to qualitative and quantitative traits, as well as identification of haplotype (linkage disequilibrium) blocks along the genome.

P2-02 04

Bioinformatics Support within the DHGP Community

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Bioinformatics is a major requirement for most areas of biological and biomedical research. The growing complexity of data and bioinformatics tools requires well-trained scientists with knowledge in the underlying biological concepts and the different computing methods. Often it is difficult and time consuming for the researcher to select the correct combination of applications and databases. Therefore, in order to bridge the considerable gap between large-scale data-collection and its interpretation we have developed a task system that allows the integration of applications and methods to create tailor-made analysis. This can be used in high throughput analysis e.g. genome annotation, cDNA mapping, gene and protein domain function assignment, gene structure prediction, protein secondary structure prediction, array oligos design.
In collaboration with a DHGP research group we developed a task which allows the semi-automatical analysis of EST sequences supporting the search of functional annotations of novel transcript sequences. In this task, repeats, vector parts and low quality sequences are masked within the input DNA. A further analysis is than performed into successive steps, firstly by the identification of already known transcripts present within human mRNA and genomic DNA reference database. Secondly tools for the clustering of `anonymous` ESTs and for further database searches on the protein level are comprised. Finally the relevant results are presented in a descriptive summary.
Our comprehensive services include hot-line, regular news, introductory courses and advanced workshops on specialized topics: sequence comparison, multiple alignment, database searching, profiles and HMMs, promoter and gene prediction, phylogeny and protein prediction.
Support and free registration: phone 06221/42-2349 or email genome@dkfz-heidelberg.de, Home page: http://genome.dkfz-heidelberg.de

P2-02 05

Statistical analysis of tissue-annotation derived from EST libraries

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The huge number of expressed sequence tags (ESTs) in public databases represents a major part of the entire set of human genes. Gene indices are clusters of ESTs related to the same gene. They are used to reconstruct the sequence of transcripts by aligning ESTs. Additional information about the expression of those transcripts is covered in the sequence annotations.
Most ESTs are annotated with information about tissue, tumor, or developmental stage from which they are derived. However, the annotation of these features is often inconsistent, thus complicating the assignment of ESTs to specific tissues or stages. The high rate of misannotation further decreases the reliability of this data.
Based on our GeneNest gene index database we analyze the tissue distribution of ESTs related to the same gene. We assign a tissue to every EST semiautomatically. The statistical evaluation of this tissue information leads to the detection of potential tissue-specifically expressed genes.

P2-02 06

Exploring Sequence Space from Genome to Proteome

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The SYSTERS protein sequence cluster set provides an automatically generated classification of protein sequences into disjoint protein family and superfamily clusters. For the remaining layer in the hierarchy, the domain level, we rely on one of the currently established domain databases, namely the Pfam collection of protein domains. The underlying data set consists of all sequences from the SWISS-PROT, TrEMBL, and PIR databases as well as currently available protein sets of fully sequenced organisms. The SYSTERS cluster set is available at http://systers.molgen.mpg.de/. It is possible to enter the hierarchy at any layer, e.g., by searching for a keyword, choosing a certain taxonomic composition, or selecting a domain. For each family cluster a consensus sequence is generated. All consensus sequences together build a database searchable by BLAST. SYSTERS family clusters are linked to our GeneNest database based on precomputed sequence similarities. GeneNest is a database of gene indices derived from ESTs and mRNAs.The sequences of a GeneNest cluster are assembled and consensus sequences build a searchable sequence database. The GeneNest cluster set is available at http://genenest.molgen.mpg.de/. The visualization provides further information about the sequences, the represented genes and open reading frames. The integration of SYSTERS and GeneNest into one framework permits an over-all exploration of the whole sequence space covering protein, mRNA and EST sequences, as well as genomic DNA.

P2-02 07

MouseExpress: Repository and data mining of expression patterns in mouse mutants from the Munich ENU Mutagenesis Screen

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We developed a repository to store, preprocess and statistically evaluate expression data of the mouse mutants from the ENU Mutagenesis Screen in high-throughput.

The Oracle-based storage conforms recent standards (e.g. MAGE) and organizes data in distinct annotation spaces. Modularized web-interfaces for upload, re-annotation, quality control, and online statistics are located at http://mips.gsf.de/proj/mouseExpress/ME.html. The privacy of datasets is controlled via an authentication module. Preprocessing routines include flag handling, various normalizations, and genomic as well as functional annotation.
With the help of advanced clustering and biclustering tools as well as sophisticated statistical tests we are looking for significant differentially expressed genes of the mutant relative to the wild type lines and are investigating the coherent change of the expression patterns due to the mutation. For a functional analysis of the expression data we partition the data in existing annotation schemes and are using supervised and semi-supervised approaches to score experiments (different mutations) and significant co—expression.

P2-02 08

Identification of proteins containing a Methyl-CpG-binding domain (MBD)
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MeCP2 is a transcriptional repressor binding to single CpGs by a domain called MBD (Methyl-CpG-binding Domain). Mutations in this gene lead to the Rett syndrome phenotype, a kind of mental retardation with an onset at the age of 6-18 month after an apparently normal period of growth and development. Rett syndrome clinically presents with regression, loss of speech and purposeful hand use. Other features include autism, ataxia, and stereotypic hand movements such as hand-washing and wringing.
The disease is almost exclusively found in females and with an incidence of 1 in 10000-15000, it is one of the most common causes of mental retardation in females.
Although MeCP2 is ubiquitously expressed, mutations in this gene lead to an exclusive neuronal phenotype. This might be explained by compensation of its repressional activity in other tissues by different proteins also containing a MBD. So far four more proteins with a MBD (MBD1, MBD2, MBD3, MBD4) were known and studied intensively.
We performed blast and database searches and could identify several new MBD proteins in human, mouse, rat, Xenopus laevis, C. elegans genomes as well as in different plants. Further studies on these proteins might lead to the understanding of the neuron-specific phenotype of MeCP2-mutants as well as a better insight into the cause of the Rett syndrome.

P2-02 09

Mutual Information Mapping
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Microarray technologies make it possible to monitor the expression of many thousands of genes simultaneously. Analysis of experiments in which a test sample is compared against a control generally involves ranking genes according to their degree of up- or down-regulation. The analysis of experiments involving a series of hybridizations at different times following a stimulus or during a developmental time course is more complicated, and often will attempt to arrange genes into groups, or clusters, of genes displaying similarities in their expression patterns.
Many methods are available to analyze microarray time-course data including hierarchical clustering, k-means clustering, and self-organizing maps, and there is as yet no clear-cut way of determining which clustering method is best suited for a given data set.
We have developed a novel adaptation of Kohonen's self-organizing map (SOM) algorithm using a mutual information content (MIC) metric for the analysis of microarray data, and implemented it as a Java application.
Shannon's definition of the entropy of a discrete random variable can be thought of as a measure of the uncertainty of a random variable; MIC, in turn, refers to the amount of information shared by two random variables. Our implementation of the SOM algorithm involves transforming the real-valued gene expression data into discrete bins, and using a mutual information (coherence) metric to determine the winning SOM nodes (most implementations of the SOM algorithm use a Euclidean or Pearson metric for this step). Preliminary testing indicates that our algorithm „learns" to classify genes into related clusters such that the average coherence between individual genes and the node of the SOM cluster increases to over 90% of the maximum possible value. Algorithmic details and results of clustering on several publicly available microarray data sets will be presented.

P2-02 10

A systematic search for unique sequences on the X-chromosome
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By using the REPuter program we have performed a systematic search for large DNA sequences without repetitive structures of different regions on the X-chromosome. The program allows an efficient and complete search of various types of repeats as well as the selection of unique sequences within a chromosome band or of the whole chromosome. We have investigated the genomic organization of the DMD gene (Duchenne muscular dystrophy) on Xp21, the XIST (X inactive-specific transcript) locus on Xq13 and the FMR-1 (fragile X mental retardation) gene on Xq27. These sequences are publicly available in the database. As REPuter allows the setting of different parameters for the computation, we were able to visualize all repeated substrings in the regions of interest according to our own criteria. This led us to the observation of „blank" regions, i.e., without any repeated substring. To determine the usefulness of these unique sequences, we have designed primers and amplified several DNA fragments within these subsequences and used them in Fluorescence in situ hybridization (FISH) experiments. In addition, we have selected BAC and PAC clones of these specific regions and established them as high quality FISH probes. Here we present a promising strategy for the identification of unique sequences on the X-chromosome by combining this specific software program with an experimental approach.

P2-02 11

Architecture for Information Retrieval and Analysis of Integrated Molecular Data
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Due to the increasing number of internet databases and information systems with information concerning mechanisms and substances involved in inborn errors of metabolism, we created the prototyp of an integrated information system of molecular and clinical data. It integrates various databases representing the current knowledge of enzymes and their catalyzed biochemical reactions (BRENDA), biochemical pathways (KEGG) and transcription factors (Transfac), using the idea of federated database systems. Additionally, medical knowledge (OMIM) and clinical data (RAMEDIS) is integrated, too. Based on the integrated data and prepared in a well structured and uniform way, case-based search algorithms are performed to retrieve new information to detect inborn errors of metabolism. As a second step methods for integration of tools, simulating gene controlled networks will enhance this system and lead to an integrated server for gene controlled biochemical networks. This work is part of the project „Modelling of gene regulatory networks for linking genotype-phenotype information" and is supported by the German Ministry of Education and Research in the German Human Genome Project.

P2-02 12

Modeling the Architecture of Signal Transduction Networks: Insulin Receptor Signaling Pathways
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Modeling the integral activity of large regulatory networks is a great challenge of modern molecular biology and bioinformatics. We focus on modeling the architecture of signal transduction networks which represents causal connections between network elements and is a logical skeleton of each regulatory system. To model such an architecture, we have applied a special formalism that treats processes of different complexity as multiple conditional events and is based on propositional logic. By using this formalism, regulatory pathways could be expressed in an algebraic form suitable for storage and computer-assisted analysis. While representing the status of pathways, we evaluate the robustness of the corresponding constructions. The robustness is the measure of the logical redundancy of network compositions and reflects the ability of a system to continue functioning in face of substantial changes (mutational damage) of its components. An important advantage of our modeling approach is its simplicity. Our models are basically static and do not need to simulate step by step the corresponding processes. The approach proposed enables a quantitative evaluation of the architectural role of individual elements in signal transduction pathways. This

might be particularly useful for modeling effects of pathologically relevant mutations in distinct components of signal transduction and transcription systems, as well as for protein target finding. The approach has been applied to insulin receptor signaling pathways as they are provided by the TRANSPATH database on signal transduction. Particular attention has been paid to pathways regulating the activity of transcription factors. The pathways are displayed according to their hierarchical organization, e.g., as a combination of many paths. Each path is represented as a sequence of steps, each of which, in its turn, can be decomposed into 'reactions' and 'molecules' of the TRANSPATH database.

P2-02 13

Construction of a Relational Database Management System (RDBMS) for the analysis of RPE-enriched expressed sequence tags

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Age-related macular degeneration (AMD) is the most common cause of legal blindness in industrialized countries and predominantly affects the elderly population over 75 years of age. Although the primary events in AMD pathogenesis are not well understood, it is thought that the deterioration of the highly metabolic retinal pigment epithelium (RPE) is involved in the degenerative processes.
Our aim is to identify and characterize genes specifically or abundantly expressed in the RPE. Towards this end, a bovine cDNA library enriched for RPE was constructed in-house using a PCR-based suppression subtractive hybridization technique, which normalizes sequence abundance and achieves high enrichment for differentially expressed genes. The individual sequences of 1002 cDNAs were analyzed and querried against sequences deposited in the GenBank and dbEST databases. A RDBMS was designed and developed to organize the storage and retrieval of the sequencing and blast searching information. RDBMS leads to more efficient use of queries, forms, and reports, increases the reliability of the extracted information, and can expand as database information requirements grow and change. Our results demonstrate that 64% (642) of the clones are derived from known genes, while 36% (360) of clones showed no matches in dbEST. In silico expression analysis of the known RPE genes revealed that 7.5% are RPE-specific, 4.2% are retina-specific, and 4.2% are expressed in both retina and brain. Extrapolation of these data to the EST clusters with no significant homolgy to known genes suggests that the number of novel RPE/Retina-specific genes to be around 10-15. Characterization of the full length human orthologs of the bovine RPE/Retina-specific transcripts have been initiated. In a second phase, we plan to determine sequence variants in the RPE-specific genes in a large, well-characterized AMD patient group by high-through put exon-scanning technology.

P2-02 14

Cooperative Research Project: Network Modeling for Genotype-Phenotype Correlations

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Within the last 2 and a half years, the project attempted to achieve a bioinformatics description and characterization of regulatory and metabolic networks and their clinically relevant aberrations. These descriptions of the molecular causes of certain diseases were to be connected with proper representations of clinical phenotype descriptions as they were contributed by physicians. In the course of the project, a number of databases on transcriptional regulation (TRANSFAC, PathoDB), signal transduction (TRANSPATH), enzymatic reaction (BRENDA) and metabolic diseases (METAGENE, MDDB, RAMEDIS) were successfully adapted and integrated at different levels, i. e. within the individual subprojects, bilaterally, and in a generic system. A new system has been developed to bridge phenotype and genotype information (PheGe). Enhanced data acquisition has been initiated by newly developed text mining routines. To enable computation with regulatory and metabolic networks, methods for formal descriptions of these networks were developed. Boolean functions have been applied to represent the architecture of regulatory networks and to estimate their robustness. The tools developed have been combined with existing ones to analyze genomic sequences for regulatory signals and to apply this information to the identification of new candidate genes that may be relevant in a certain disease area. The first comprehensive application example was MODY (Maturity Onset Diabetes of the Young) where we could identify new target genes.

P2-02 15

RAMEDIS - Collecting clinical and molecular genetic data of inborn metabolic diseases via the internet

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An internet portal (www.ramedis.de) for the electronic publication of patients with rare metabolic diseases has been established. The aim of the patient database is to provide information about the clinical, biochemical and molecular genetic data of patients with rare metabolic diseases. In addition, follow up of treated patients is possible.
The home page describes the intention and function of RAMEDIS, which runs with version of 11th Jan 2002 on an internet server at the Institute of Technical and Business Information Systems at the OvG-University of Magdeburg.
The data can be accessed in a read only modus (analysis tool) or with the input and editing tool respectively. The following data are documented:

Main - general information, e.g.. diagnosis, ethnic origin, author, abstract etc.
Symptoms - clinical symptoms in a hierarchical manner.
Laboratory - laboratory data, ordered alphabetically with SI-units and qualitative ranking which enables extended graphical presentation.
Pictures - possibility to include figures as in articles.
Molecular Genetics - molecular genetics using the terminology of S.E. Antonarakis.
Therapy/Development - general therapy and growth parameters
Diet/Drugs - special dietary and drug therapy

A statistic function is integrated, to show the content of RAMEDIS.
So far (Mai 2002), 375 cases have been submitted from different centres. First studies (e.g. concerning extended newborn metabolic screening) have been performed.
To use RAMEDIS in prospective studies and to improve the performance of the data acquisition, a form based input tool will be developed.
In summary, an anonymous electronic documentation system for patients with genetic metabolic diseases has been developed which is accessible on the internet.

P2-02 16

DNA sequence-based virtual human chromosomes

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The human genome can be analyzed morphologically with microscopic and chemically with molecular genetic means. At present, it is not possible to directly compare, correlate, exchange, or jointly analyze data that are generated the one or the other way within a single platform. One prerequisite for such an integrated approach is the transformation of the descriptive cytogenetic and molecular cytogenetic data into a sequence format. The availability of the human sequence (http://genome.ucsc.edu/) enabled now for the first time a direct evaluation of the relationship between the sequence and the chromosomal banding pattern. Our reconstruction of the human chromosomes with electronic means revealed an astonishing concordance between these two banding patterns. This observation proofs that the „large-scale" chromosomal banding solely reflects the DNA sequence and that it is hardly modified by epigenetic factors. However, virtual chromosomes will provide also a unique basis for the joint processing of cytogenetic, FISH, and molecular genetic data within a single DNA sequence-based framework. The accurate, scale-independent, and highly region-specific banding pattern can be easily adapted to that of natural chromosomes irrespective of their state of condensation. In combination with the possibility to electronically reconstruct any chromosome rearrangement with a hitherto unknown molecular precision, this may provide the basis for novel cytogenetic pattern recognition systems. Moreover, we are also able to map and display the chromosomal position of any sequence or set of sequences, including the distribution of the currently available approximately 14.000 genes. The superimposition of such a graphic interface onto molecular genetic databases will thus enable the visualization of molecular events in a chromosomal fashion. In addition to virtual gene mapping, such a tool will enable the display of micro array-de-

rived DNA and gene expression profiles. It also remains possible to integrate more fuzzy-defined ined with conventional cytogenetics and with a variety of FISH methods.

P2-06 01

The candidate genes TGFA and MTHFR in a German CL/P population

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Non-syndromic cleft lip with and without cleft palate (CL/P) are complex disorders involving several genes and environmental factors. Association studies of CL/P have implicated a number of candidate genes but have proved difficult to replicate. Here, we report the findings of two candidate genes, MTHFR and TGFA, in a German CL/P population.
Maternal folic acid supplementation during early pregnancy has been suggested to play a role in the prevention of oral clefts. Several study groups tested the 677C®T polymorphism of the gene for 5,10-methylenetetrahydrofolate reductase (MTHFR) in their CL/P population. Some investigators reported about an association of the 677TT genotype to CL/P, whereas others could not support these findings. In order to contribute to this discussion we examined this MTHFR polymorhism in a control population (n=233) and in CL/P patients (n=72). The case-control study does not reveal a significant difference in C- and T-allele frequencies or in genotype distributions (Cochran-Armitage trend tests P=0.13). Therefore it is questionable if this polymorphism plays an important role in the susceptibility for CL/P in the German population.
In contrast to this, we were able to demonstrate in agreement with case-control studies from other countries, that there is a statistically significant association between the D4 bp polymorphism of the transforming growth factor a (TGFA) and CL/P (Cochran Armitage trend tests P = 0.03). Therefore, the results of our case-control study support TGFA as a modifier gene in CL/P. Biological support for a role of TGFA arises from its presence at high levels in the epithelial tissue of the medial edge of the palatal shelves at the time of shelf fusion in mice.

P2-06 03

Further Fine Mapping Within The Psoriasis Susceptibility Region On Chromosome 19 In German Trios

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Previously we performed a genome wide linkage scan for psoriasis and identified a further susceptibility locus at the pericentromeric region of chromosome 19. In a follow-up study using an independent cohort of 210 Falk-Rubinstein-trios, we scanned this 40 cM region for association using a dense set of 45 microsatellites. We obtained a positive association for the markers D19S922 (p = 0.008) and D19S916 (p = 0.016) corresponding to the linkage peak. We also found two other regions showing negative association with psoriasis, at D19S917 on 19p13.1 (p = 0.0034), and at D19S425 (p = 0.0005), compatible with the hypothesis of protective loci. We explored these two novel regions in more details using novel in silico identified microsatellite markers at an average distance of 100 kb and identified further associated microsatellites in both regions. Analysis of SNPs from these refined regions will eventually allow identification of the underlying susceptibility alleles.
In addition, we investigated SNPs in candidate genes at two further psoriasis susceptibility loci. While we could confirm a strong association in our cohort for SNPs within the HCR gene on 6p (p = 0.000058), we failed to confirm association to the recently presented candidate SLC12A8 on 3q.

P2-06 04

Developmental genes as candidates in cases with neural tube defects (NTD)

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Little is known about the identity of genetic factors involved in the complex aetiology of neural tube defects (NTD). Reasonable human NTD candidate genes are the human homologues of developmental genes in mice for which a functional role in neural tube closure can be assumed. In mice, Twist, Bmp4 and its specific inhibitor Noggin are expressed in tissues adjacent to the developing neural tube. Twist knock-out mice fail to close the neural tube in the cephalic region, whereas in Noggin knock-out mice a failure of neural tube closure can be observed in cranial and in lumbar regions. We therefore tested the three genes as candidates that might be involved in the aetiology of human NTD. The coding sequences were screened for mutations in 200 NTD patients (anencephaly, encephalocele, spina bifida aperta) using single strand conformational analysis (SSCA). In TWIST, no sequence alterations could be detected. In the single exon of NOG we found a point mutation in one spina bifida aperta patient (SBA) that leads to a change of the predicted amino acid sequence at an evolutionaryly conserved position (G92E). In BMP4 four different missense mutations in four unrelated SBA patients were found: three mutations were located in the propeptide region of the protein (S91C, T225A, R226W) and one in the carboxy-terminal domain characteristic for TGFß2-family (S367T). All sequence alterations change amino acids which are highly conserved among mammals and thus may influence the stability or function of the protein. In all cases (BMP4 and NOG) the mutation was inherited from one parent.
The present study displays new genetic variants in patients with neural tube defects which, together with other factors, may have contributed to the patients' phenotypes in these individual cases. Further investigations are necessary to support these observations.

P2-06 05

Periodic catatonia: confirmation of linkage to chromosome 15 and further evidence for genetic heterogeneity

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We earlier reported on significant evidence for linkage on chromosome 15q15 in periodic catatonia, a sub-phenotype of the schizophrenic psychoses. The disorder is characterized by qualitative hyperkinetic and akinetic psychomotor disturbances through acute psychotic episodes, and debilitating symptoms in the long term with psychomotor weakness, grimacing facial movements, and apathy. Here, we confirm mapping of a major gene locus on chromosome 15q15 in a second genome scan in a new set of four multiplex families. Non-parametric multipoint linkage analyses identified a broad region with a maximum peak of Zall = 3.91 (p =0.0063) and Zlr = 3.04 at D15S1234 (p = 0.0013), satisfying conventional criteria for confirmed linkage. Parametric affected-only analyses gave maximum HLOD score of 1.65 (D15S1234) with an estimated 47% of families linked. Analysis of individual families showed that one large family showed linkage while two other could be clearly excluded, confirming genetic heterogeneity. No other locus reached suggestive levels of significance. Haplotype analysis on chromosome 15 in this and previously linked families placed the susceptibility region to a 11 cM interval between marker D15S1042 and D15S659. Periodic catatonia is the first sub-phenotype of the schizophrenic psychoses with confirmed linkage despite existence of considerable genetic heterogeneity.

P2-06 06

Evaluation of two voltage-gated potassium channel subunits in idiopathic forms of epilepsy

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Idiopathic generalized epilepsy (IGE) affects about 1% of the population worldwide. Common subtypes of IGE including childhood absence epilepsy (CAE), juvenile absence epilepsy (JAE), juvenile myoclonic epilepsy (JME), and epilepsy with grand mal seizures on awakening (EGMA) are inherited as genetically complex traits and defined by recurrent seizures presenting with characteristic clinical and electroencephalographic features in the absence of any detectable brain lesion. Results of a recent genome wide search (Sander et al., 2000) provided suggestive evidence for the existence of two novel IGE susceptibility loci: on chromosome 14q23 (ZNPL = 3.28 at D14S63; P = 0.000566) and on

chromosome 2q36 (ZNPL = 2.98 at D2S1371; P = 0.000535). In these regions we identified two potential candidate genes both coding for subunits of voltage-gated potassium channels: KCNE4 (alternate symbol: MIRP3) on chromosome 2q36 which belongs to the Isk-related subfamily of voltage-gated potassium channels and KCNH5 (alternate symbol: Eag2) on chromosome 14q23 which belongs to the subfamily H of eag-related potassium channels. We systematically searched for mutations in 32 IGE patients. We identified two common polyorphisms (KCNH5: G2233A; KCNE4: G435T) which were further analyzed in a family-based association study including 120 parent-child-trios. However, TDT analyses did not reveal a significant deviation between alleles transmitted and non-transmitted, and we thus conclude that genetic variation in both genes does not contribute to an increased IGE risk.

P2-06 07

Genetic background of the TNFa mRNA expression in patients with coronary atherosclerosis

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The TNFa is an important cytokine in the complex signalling pathway involved in the development of atherosclerosis. Methods: We studied the interaction of the TNFa mRNA expression (competitive RT-PCR), a promoter polymorphism (PM) of TNFa (G-238A; SSCP) and their effect on coronary atherosclerosis. In this study 256 patients with angiographically confirmed diagnosis were involved: 128 patients with severe coronary atherosclerosis (CAD; 81 males, average age: 50.3y) and 128 patients without any coronary symptoms (WAS; 83 males, average age: 50.4y) as controls. Results: As proposed by recent studies the mRNA expression of TNFa was shown to be elevated in the CAD compared to the WAS group (7.4 vs. 6.7ag/cell; n.s.). In order to prove the role of the genetic background we examined the dependence of the mRNA expression on the G-238A PM: Whereas the mRNA expression in WAS patients was only slightly increased in dependence on the genotype (AG+AA: 7.4 vs. GG: 6.6ag/cell) the mutation carriers among the CAD patients showed significantly higher expression than wildtype carriers (AG+AA: 12.6 vs. GG: 6.9ag/cell, p<0.012). An analysis of the genotype distribution of the G-238A PM revealed no significant differences in the frequency of AG+AA-carriers (CAD: 0.09 vs. WAS: 0.12). However the evaluation of the importance of this PM for the early development of severe CAD described by an early age of onset (<45y) revealed significant changes in genotype distribution. The CAD patients with an early age of onset were significantly more often mutation carriers than CAD patients who exhibit coronary symptoms at higher age (<45y vs. >45y: 0.18 vs. 0.02; p<0.012). This result underlines the importance of the mutant genotypes AG+AA of the TNFa G-238A PM for having a more pronounced mRNA expression and also for the development of coronary symptoms at younger age (<45y). Conclusions: Investigating the role of the message expression of TNFa in the development of coronary atherosclerosis the importance of the genetic background always be taking into consideration.

P2-06 08

RNomics: identification and function of imprinted, brain-specific small non-messenger RNA genes in the Prader-Willi Syndrome region

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In our quest to identify novel non-messenger RNAs (snmRNAs) in model organisms (an approach for which we coined the term RNomics), we have identified three brain-specific snmRNAs, designated as HBII-13, HBII-52 and HBII-85 from a mouse brain cDNA library. We have isolated the human orthologues of the three snmRNAs and mapped them between the SNRPN and UBE3A genes on chromosome 15q11-q13. Thereby, we have sequenced 180 kb encompassing the HBII-52 locus. The region containing the three snmRNA genes has been implicated in the etiology of the Prader-Willi syndrome (PWS), a neurogenetic disease resulting from a deficiency of paternal gene expression. Two of the snmRNA genes, HBII-52 and HBII-85, are encoded in tandemly repeated arrays of 47 or 27 units, respectively. Interestingly, these RNAs were absent from a PWS patient cortex and from a PWS mouse model, demonstrating their paternal imprinting status and pointing to their potential role in the etiology of PWS. By a bioinformatical approach, we were able to identify three additional snmRNA genes within the same locus, designated as HBII-436, HBII-438A and HBII-438B, which are also subject to imprinting and are predominately expressed in the brain.
Due to conserved sequence and structure motifs, the six snmRNAs can be assigned to the class of small nucleolar RNAs (snoRNAs). This class of RNA molecules has been shown to target ribosomal RNAs by a short antisense element located within snoRNAs. By this mechanism, ribosomal RNAs are targeted for modification at the site of complementarity to snoRNAs. All six brain-specific snoRNAs from the PWS region lack complementarity to ribosomal RNAs. In one case, the HBII-52 snoRNA, we could show a potential interaction with a brain-specific mRNA, the serotonin receptor mRNA 5-HT2C at a site where the mRNA is regulated by alternative splicing and editing. This implies (a) novel function(s) of this class of snmRNAs, namely the regulation of gene expression.

P2-06 09

Study of Toll-Like Receptor Genes in Sarcoidosis.

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Sarcoidosis is a multifactorial disease of granulomatous inflammation, affecting a variety of organs, primarily the lung and lymph system. The aetiology of sarcoidosis is unknown and presumably complex. Current concepts assume interactions of one or more environmental triggers with an inherited susceptibility of the patient. According to this hypothesis are genes involved in innate immunity candidate susceptibility genes in sarcoidosis. Toll like receptors (TLR) are a main component of innate immunity. TLR comprise a group of transmembrane molecules with an extracellular leucine-rich repeat domain and a cytoplasmic interleukin-1 receptor like region. Different TLR respond selectively to specific bacterial components, and lead to enhanced activity of proinflammatory cytokines, that are involved in the typical sarcoid immune reaction. So far, eight members of the TLR gene family have been characterised in more detail. Two of them, TLR4 and TLR9 reside at chromosomal regions on 9q and 3p that exhibited moderate non-parametric linkage peaks (p < 0.05) at a previous genome-wide link-age scan in sarcoidosis families. We have studied the TLR gene loci using close microsatellite markers and intragenic polymorphisms in an extended panel of patients and families suffering from sarcoidosis.

P2-06 10

No evidence for involvement of the promoter polymorphism -866 G/A of the UCP2 gene in early-onset obesity in humans

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Recently, association between obesity and the G-allele of the -866 G/A polymorphism in the promoter region of uncoupling protein-2 gene (UCP2) was reported (Esterbauer et al., Nat. Genet. 28: 178-83, 2001). Both allele frequencies and genotype distributions for this polymorphism differed between obese individuals and never-obese controls. We attempted to confirm this finding. Genotyping was performed by polymerase chain reaction with subsequent restriction fragment length polymorphism analysis (PCR-RFLP). We analyzed transmission disequilibrium of the (wild type) G-allele for 200 extremely obese children and adolescents from 93 concordant sib pair families using the pedigree transmission disequilibrium test. Additionally, using a one-sided asymptotic Pearson's chi-square-test, we tested, if the G-allele occurs more frequently in 277 extremely obese children and adolescents (including the 93 index patients of the concordant sib pairs) than in 188 never-obese controls. The one-sided asymptotic Cochran Armitage trend test was used to determine differences in genotype frequencies between extremely obese and healthy underweight individuals. The PDT analysis revealed no evidence for transmission disequilibrium in obesity. Allele and genotype frequencies did not differ between the extremely obese and never-obese subjects. In conclusion, we cannot confirm the results of Esterbauer et al. in our young sample.

P2-06 11

Polymorphism of genes involved in bone metabolism and association with osteoporosis in Poland

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Osteoporosis is a common disease characterized by decrease in bone mineral density (BMD) and microarchitectural deterioration of the bone structure leading to higher susceptibility to fractures. Development of osteoporosis is multifactorial process in which environmental and genetic factors play an important role. Recent studies have indicated that the majority (up to 80%) of the variability in bone mass and density is genetically determined. Molecular genetic basis of osteoporosis remains difficult to define because the bone mass, a major determinant of osteoporosis fracture risk, is quantitative trait, influenced by interaction between many genes and environmental factors. Several candidate genes are investigated in search for association of polymorphisms or mutations with this disease. We studied polymorphisms of osteoprotegerin gene (OPG), transforming growth factor b1 gene (TGF-b1), vitamin D receptor gene (VDR), estrogen receptor 1 gene (ER1), collagen type 1a1 gene (COLIA1) and analysis of 4 polymorphisms of ANKH gene (craniometaphyseal dysplasia gene) was performed. Statistical analysis of all single polymorphisms and analysis of interaction between these polymorphisms were performed. For majority of polymorphisms no statistically significant influence on osteoporosis was observed. Only analysis of OPG 9G/C polymorphism showed statistically significant results. Genotypes with allele C were over-represented in patients with severe osteoporosis as compared with Polish population group ($p<0.02$). A tendency for influence on development of osteoporosis was observed for allele G in ANKH exon 5 -51A/G polymorphism, allele A in ANKH exon 5 -52A/G polymorphism, allele C in TGF-b1 29T/C polymorphism and allele f in VDR FokI polymorphism.

P2-06 12

Involvement of a Melanin-concentrating hormone receptor 1 allele in human obesity

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The orexigenic neuropeptide melanin-concentrating hormone (MCH) is involved in regulation of food intake and energy balance. MCH is the endogenous ligand of the receptor MCHR1 which through coupling to multiple G proteins mediates diverse intracellular signalling pathways. Presently, there are two MCHR1 mRNA entries in databases leading to conflicting gene models. mRNA 1 is composed of two exons, whereas mRNA 2 was deduced from genomic DNA and corresponds to exon 2 of mRNA 1 but is elongated upstream. Comparison of allele and genotype frequencies of the silent SNP rs133073 C/T located in exon 1 of MCHR1 indicated association of the C-allele with obesity. Transmission disequilibrium tests (TDT) confirmed a preferential transmission of the C-allele. As no functional significance of rs133073 is evident, we sequenced MCHR1 and 4.6 kb of its upstream region in nine obese children and adolescents homozygous for C at rs133073 and who had contributed to the positive TDT, as well as in ten obese children and adolescents homozygous for T. These two genotype based groups should be most divergent for functionally relevant SNPs. We identified 18 SNPs 12 of which contribute to two ancestral haplotypes. Since the majority of the SNPs is located upstream of the transcribed region, promoter fragments representing the two haplotypes are functionally tested in luciferase reporter gene assays.

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P2-06 13

Sequence diversity of KIAA0027/MLC1: are megalencephalic leukoencephalopathy and schizophrenia allelic disorders?

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The aim of the study is to validate the etiological role of KIAA0027/MLC1 in childhood-onset megalencephalic leukoencephalopathy with subcortical cysts (MLC) and in schizophrenia, particularly the catatonic subtype, which were reported to be allelic diseases. Among a series of five patients with MLC, four mutant alleles were detected: one case of compound heterozygosity for a splice site mutation and a 6 base-pair inframe deletion, one patient with a homozygous frameshifting insertion-deletion, and a further case heterozygous for a A157E substitution. A systematic mutation screening in 140 index cases with schizophrenia revealed 13 different single nucleotide polymorphisms (SNPs): one SNP in the 5'-UTR, seven SNPs in intronic regions, two synonymous codon variants (T52, Y199), and three coding variants. Two of them, C171F and N218K, were observed in controls at a significant frequency, and the L309M variant -that was previously supposed to be the causative factor for chromosome 22qtel linked-periodic catatonia- was found non-segregating in a further multiplex pedigree. Furthermore, a 33 bp insertion/deletion polymorphism in the 3'-end of exon 11 of MLC1 was found at equal frequency among schizophrenic patients and controls. In summary, our study provides further evidence for allelic heterogeneity in megalencephalic leukoencephalopathy, exclude MLC1 as a susceptibility locus for schizophrenia, and thereby rule out that MLC and schizophrenia are allelic disorders.

P2-06 14

DIFFERENTIAL EXPRESSION OF ADIPOSE-TISSUE GENES IN OBESITY-ASSOCIATED HYPERTENSION IN MAN USING cDNA MICROARRAYS

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Obesity is the principal risk factor for the development of hypertension, but the molecular basis for this relationship remains poorly understood. Obesity is characterized by an enormous growth of adipose tissue, and despite an increasing body of knowledge on the endocrine and paracrine role of adipocytes, the pathophysiological role of adipose tissue in obesity-related hypertension has not been investigated in depth. In this study, we examine differential regulation of adipose-tissue genes in lean and obese normotensive and hypertensive subjects in across-sectional study and follow those genes during dietary weight reduction and salt restriction, as these interventions lower blood pressure in obese subjects.

All volunteers are characterized by 24 hour ambulatory blood pressure and anthropometric measurements. Blood and 24 hour urine samples were taken for further hormonal characterization. Total RNA was isolated from adipose tissue obtained by subcutaneous needle biopsy. The complete RZPD2 Human UNIGENE Set (about 76,000 clones) was spotted on 6 nylon membranes. A subset of 29 adipose tissue RNA samples was hybridized to the complete library to obtain a list of differentially expressed clones. This subset of clones (about 3,000) is currently spotted on a smaller membrane that will be used to obtain gene expression data of the whole study population (cross-sectional and interventions).

90 adipose tissue biopsies have been performed in the cross-sectional study, and the weight reduction study is currently underway. Results on candidate gene expression (adiponectin, 11ß-HSD1 and 2) help to explain findings on low adiponectin plasma levels and increased local formation of cortisol in obese subjects. Examples for preliminary results from the use of cDNA arrays suggest increased expression of the forkhead transcription factor gene FOXP1 in hypertensive subjects irrespective of weight, and of the gene encoding fructose-1,6-bisphosphate aldolase B (ALDOB) in obese subjects irrespective of blood pressure.

P2-06 15

Differentially expressed genes in rat during development of cardiac hypertrophy

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To identify candidate genes contributing to the initiation or progression of cardiac hypertrophy, an adaptive response to chronic increased workload, we have screened differential gene expression in the heart of spontaneously hypertensive rats (SHR) at different stages of their development in comparison to the wild type Wistar-Kyoto rats (4, 12, 26 weeks pp; male). We established a subtractive hybridization system based

on suppression PCR and cDNA selection using mRNA from these stages resulting in identification of 145 different cDNA clones. Screening of these clones revealed 56 cDNAs as candidates for differentially expressed genes. Northern blot analysis of 42 cDNAs identified 16 upregulated and 4 downregulated genes in cardiac tissue of SHR. In silico analysis of these cDNA sequences identified several known genes which are being discussed already in the context with cardiomyopathy (g-sarcoglycan) or cardiac hypertrophy (acyl-coA dehydrogenase) and in addition 8 so far unknown genes. Validating our results of northern blot analysis we established quantitation of gene expression through real time PCR for our candidate genes. Using these method we could indicate an significant upregulation of g-sarcoglycane and an other so far unknown gene in cardiac tissue of SHR rats during development to cardiac hypertrophy. Mapping of these novel genes with respect to rat chromosome segments harboring known QTLs for cardiac hypertrophy and analysis of their expression pattern might be relevant to understand processes leading to cardiac hypertrophy in rat and man.

P2-06 16

Association analysis of CD86 gene polymorphisms with infantile atopic dermatitis and atopy

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Atopic dermatitis (AD) is a chronic inflammatory skin disease that is characterized by an intensely itchy rash with a typical morphology and distribution. Infantile atopic dermatitis is commonly the first clinical manifestation of allergic disease and the majority of affected children develop asthma or allergic rhinitis later in life. We have recently identified a susceptibility locus for atopic dermatitis and atopy on chromosome 3q21. CD86 is a type I membrane protein of the immunoglobin superfamily that has been mapped to this region. CD86 interacts with CD28 to provide co-stimulatory signals for T cell activation and has been implicated in the activation of Th2 cells which are thought to play a pivotal role in mediating allergic inflammation. Furthermore, overexpression of CD86 on B cells of AD patients has been demonstrated. CD86 therefore represents an interesting positional and functional candidate gene for atopic dermatitis and atopy.

To test whether CD86 may be a susceptibility gene for infantile atopic dermatitis or atopy, we have screened the coding region of the CD86 gene and have identified five polymorphisms in Exons 1, 2, 4, 5, and 8. The coding variants of the CD86 gene were genotyped using mini-sequencing protocols. We used the study group that showed strong linkage to chromosome 3q21 consisting of 199 families with at least two children affected with atopic dermatitis. Transmission disequilibrium revealed no association of the mutant allele of coding variants with either atopic dermatitis or allergic sensitization.

P2-06 17

Expression of HERV-K on protein- and RNA-level both in rheumatic and in normal synovial tissue

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Rheumatoid arthritis is the most common autoimmunologic disease in man. Although clinically well defined, primary pathologic mechanisms are still unclear. Besides genetic, infectious, and unknown endogenous factors also the involvement of human endogenous retroviruses (HERVs) has been discussed as increased levels of anti-HERV- antibodies have been found in rheumatic patients in several studies. While most HERVs are defective, a nearly intact member of the HERV-K(HML-2) family was identified on chromosome 7. Moreover mosaic-trisomy 7 is frequently found in synovial tissue from rheumatoid arthritis, osteoarthritis but rarely in normal controls. To identify a trisomy 7 related expression of HERV-K(HML-2) sequences on mRNA and protein level we performed RT-PCR-analyses for HERV-K-cORF-RNA and immunofluorescence labeling of the cORF protein in 28 primary synovial cell cultures from patients with autoimmune rheumatic disease (RA), osteoarthritis (OA), and normal synovia. In all cases the percentage of trisomy 7 was investigated by FISH. A significant correlation between trisomy 7 rate and HERV-K expression, however, was not obvious. Unexpectedly cORF-Protein was detected in all normal synovial cultures. The same, but on a reduced level, holds true for the majority of RA and OA cases. By RT-PCR likewise expression of HERV-K specific mRNA could be proven in all cases of normal synovia and OA, and in 90% of RA cases. Additionally several different HERV-K specific transcripts were identified presenting a variant expression pattern between normal and rheumatic synovial cells. By sequence analysis the expressed sequences could be assigned to several chromosomal loci. The reduction of HERV-K expression and the change of the expression- pattern might be a specific genetic marker in rheumatic disease.

P2-06 18

The GENICA Study: Assessment of Breast Cancer Risk Factors

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There are about 46,000 incident breast cancer cases each year in Germany most of which are unexplained with respect to genetic origin. We foster the hypothesis that most breast cancers may develop due to complex gene-gene and/or gene-environment interactions. To clarify this point we are currently conducting a population-based case-control study in an area of one million inhabitants in Germany (city of Bonn and neighboring districts of Siegburg and Euskirchen). The ultimate goal is to include 600 subjects for both the patient and control group by 06/2002, and to include a matched population-based control group from the KORA survey 2000 (Augsburg). So far, 530 patients with breast cancer and 550 population-based controls have been recruited between 08/2000 and 0/4 2002. All patients and controls are interviewed with a standard questionnaire to obtain information on potential risk factors, i.e. reproductive factors and hormone intake, nutrition and life style as well as others. DNA obtained from blood mononuclear cells is currently analyzed at polymorphic key players potentially involved in the metabolic pathways of the assessed exposures. Genotyping includes fragment analysis, Taqman®, MALDI-TOF, and Lightcycler technologies. Protein expression of selected prognostic and predictive markers in breast tissues is analyzed by immunohistochemistry. All data are currently collected in a MS ACCESS database for future statistical analysis including conditional logistic regression analysis. Our study aims to estimate women's relative risks to develop breast cancer under both genetic and environmental influences.

P2-06 19

FAMILY-BASED ASSOCIATION STUDIES OF TRACE AMINE RECEPTOR GENES ON 6Q23.3 IN BIPOLAR AFFECTIVE DISORDER

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There is evidence suggesting a role of trace amines (TA) in the etiology of bipolar affective disorders (BPAD). A functional deficiency of TA has been proposed as a potential etiological factor in depression, increased levels of TA were found to be associated with manic phases of BPAD. The genes for two trace amine receptors, TA-1 and TA-4, are both located on chromosome 6q23.2. Interestingly, this particular chromosomal region has shown evidence for linkage to BPAD in a genome-wide screen (Cichon et al., 2001). This prompted us to search for genetic variants in the TA-1 and TA-4 genes and test them for association with BPAD. Systematic sequencing of the TA-1 and TA-4 genes in 96 control individuals identified two common SNPs in the TA-4 receptor gene (914A/G and 993A/G) and one in the TA-1 receptor gene (1212A/G). TDT analysis of the three SNPs was performed in 118 parent-offspring triads that were partially derived from the genome screen sample. No association between the SNP in the TA-1 receptor gene and BPAD was found. However, we observed a preferential transmission of the 914G allele of the TA-4 receptor gene (p=0.014) and a non-significant trend of the 993G allele (p=0.06). Replication is clearly necessary to support a possible role of the TA-4 receptor gene in BPAD.

P2-06 20

FAMILY-BASED ASSOCIATION STUDIES OF ALPHA-ADRENERGIC-RECEPTOR GENES IN REGIONS SHOWING LINKAGE TO BIPOLAR AFFECTIVE DISORDER

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Several lines of evidence suggest an involvement of the noradrenergic neurotransmitter system in the pathogenesis of bipolar affective disorder (BPAD). Drugs that enhance the activity of norepinephrine can precipiatate manic-like episodes in some individulals while precipitous withdrawal of these drugs can be associated with depressive symptoms. Three genes for alpha-adrenergic-receptors (ADRA) are located in chromosomal regions that showed evidence for linkage in a genome wide screen for linkage to BPAD in 75 families (Cichon et al., 2001): The ADRA-2A gene maps to chromosome 10q25, the ADRA-2C gene is located on chromosome 4p16, and the ADRA-1A-gene maps to 8p21. We analysed a Cys492Arg variant in exon 2 of the ADRA-1A gene, a -1291G/C variant in the 5´UTR of the ADRA-2A gene, and STR marker adra2c1 in the 5´UTR of the ADRA-2C-gene. 117 parent-offspring triads with BPAD partially derived from our genome screen sample (n=53) were genotyped and analysed using the Transmission Disequilibrium Test (TDT). No significant differences in transmissions were observed for any of the three ADRA-genes. Thus, our data do not support the hypothesis of an involvement of the three studied adrenergic-receptor gene variants in the development of BPAD.

P2-06 21

LINKAGE DISEQUILIBRIUM ANALYSIS IN A SUSCEPTIBILITY REGION FOR BIPOLAR AFFECTIVE DISORDER ON 8Q24

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A genome wide screen for linkage to bipolar affective disorder (BPAD) has suggested a new susceptibility locus on 8q24 (Cichon et al., 2001). Marker D8S514 gave a two-point LOD score of 3.62 and a GENEHUNTER-NPL score of 3.56 (p=0.00029). The positive linkage region is large and extends over 30 cM. In an attempt to narrow down the linked region, we have performed linkage disequilibrium (LD) analysis in a sample of 119 parent-offspring trios with BPAD using the Transmission Disequilibrium Test (TDT). Part of the triads (53) were extracted from the genome screen families, the other 66 triads were independent. In a first step, eight microsatellite markers were tested for LD that are located in the highest linked region (about 5 Mb). Individual markers and two-marker haplotypes were tested for LD. TDT analysis revealed two regions of interest: One is characterized by preferential transmission of two particular two-marker haplotypes (markers A-C, p=0.008; markers B-C, p=0.035). A second region is located more centromeric. A particular allele of maker D (p=0.01) and the two-marker haplotype D-E (p=0.001) were preferentially transmitted. To follow up this finding, we have isolated new polymorphic STR markers in the region which are currently being analyzed by TDT.

P2-06 22

Testing of GRID2 and TACR3 as candidate genes for migraine with aura

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Migraine with aura (MA) is a common neurological disease with complex inheritance. Very recently a first genome wide scan for MA has been published describing a highly significant locus on chromosome 4q24. We therefore analyzed this susceptibility region and were able to identify two genes which proposed function made them attractive candidates for MA. GRID2 encodes the delta-2 subunit of the ionotropic glutamate receptor and is predominantly expressed in cerebellar Purkinje cells, where it is supposed to be involved in the regulation of neuronal excitability. TACR3 codes for a tachykinin receptor, which is thought to be involved in pain perception in the central nervous system. We elucidated the genomic structures of both genes and established a mutation screening strategy to amplify all coding exons and adjacent splice sites from genomic DNA. GRID2 is composed of 17 exons and TACR3 consists of 5 exons. Direct sequencing of PCR products from index patients from 24 multiplex families with MA revealed one common silent polymorphism and one intronic polymorphism in GRID2, whereas no alteration was found in TACR3. We are currently performing association studies in a case-control sample with these two novel SNPs. In parallel, further candidate genes from this susceptibility locus are investigated to identify the MA susceptibility gene on chromosome 4q24.

P2-06 23

Genetic dissection of LDL and HDL-cholesterol concentrations: Role of Cholesterol ester transfer protein (CETP)

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Introduction: Cholesteryl ester transfer protein (CETP) mediates the exchange of cholesteryl esters and triglycerides between apoprotein-B-(chylomicrons, VLDL, LDL) and apoprotein-containing lipoproteins (HDL) in the plasma. The association of family-derived haplotypes based on six common SNPs in the CETP gene with HDL and LDL-cholesterol was studied in 286 individuals. A cladistic approach, allowing for grouping of haplotypes based on evolution, was applied. Results: Two major groups of haplotypes could be distinguished in a cladogram: The putative „wild type" haplotype and those haplotypes resulting from mutation and recombination events in the population („variant haplotypes"). The haplotype frequencies did not much differ between male and female persons. The variant haplotype group was associated with higher HDL and lower LDL levels compared to the wild types. This effect was observed only in males, not in females. The association was more pronounced in individuals with lower triglyceride concentration compared to those with higher triglycerides. Discussion: These data support the following hypothesis: 1) When CETP is saturated by its substrates (triglycerides and cholesterol esters), the plasma concentration of LDL and HDL- cholesterol is not responsive to the effect of genetic variants within the CETP gene. 2) The results suggest an interaction between gender, genetic variation within the CETP gene and the plasma cholesterol concentration. 3) SNP derived haplotypes appear to be better markers for functional alleles, since the effects of SNPs were much less significant.

P2-06 24

Genome-Wide Scan for Childhood and Adolescent Obesity Genes in German Families

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Objective: Up to now, several genome scans have been performed for adult obesity. Single formal genetic studies suggest a higher heritability of body weight in adolescence. In addition, genes influencing body weight in adulthood might not be the same as those relevant in childhood and adolescence. We therefore performed a whole genome scan for childhood and adult obesity genes in german families.

Design: The genome scan based on 89 families with two or more obese children (sample I). The mean age of the index patients was 13.63 ± 2.75 years. 369 individuals were initially genotyped for 437 microsatellite markers. A second sample of 76 families was genotyped using microsatellite markers that localize to regions for which maximum likelihood binominal (MLB) LOD scores upon use of the concordant sib-pair approach exceeded 0.7 in sample I.

Results: Regions/loci on chromosomes 1, 2, 4, 8, 9, 10, 11, and 19 fulfilled these criteria in sample I; MLB LOD scores on chromosomes 8p and 19p exceeded 1.5. In sample II MLB LOD scores of 0.68 and 0.71 were observed for loci on chromosomes 10 and 11. respectively.

Conclusion: In conclusion, whereas our scan did not reveal MLB LOD scores ^{3} 2, we nevertheless consider the fact that several of the previously

identified peaks also gave a signal in this scan as promising.

P2-06 25

Heritability of left ventricular mass and related echocardiographic parameters

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Left ventricular hypertophy (LVH), i.e. a left ventricular mass (LV mass) exceeding clinically defined threshold levels, is an important risk factor for cardiovascular morbidity and mortality. Age, blood pressure, and body weight are known determinants of left ventricular mass. Interestingly, the variability of blood pressure and body mass index has substantial genetic components. In addition, as shown previously by our group, there is growing evidence for independent genetic factors influencing LV mass and hypertrophy. Specifically, in comparison matched population controls, siblings of LVH patients display an increased risk (odds ratio 1.7) for the condition as well, even when adjusted for blood pressure and body mass index. We now estimated the strength of the genetic component, i.e. the heritability of left ventricular mass and related echocardiographic parameters by analysing sibling correlation and by variance component analysis (using the program SOLAR).

The study population consisted of 212 individuals with LVH and their siblings. The probands were ascertained as a subgroup of individuals participating in the World Health Organization MONICA project in the city of Augsburg and two adjacent counties in Southern Germany. We found a substantial heritability of LV mass ($h2 = 0.36$, $p=0.004$) and LV end systolic diameter (LVESD) ($h2 = 0.48$, $p<0.00001$), which represents a measure of cardiac contractility. Wall thickness of the posterior wall and septum showed lower heritabilities of ($h2 = 0.16$, $p=0.07$ and $h2 = 0.14$, $p=0.05$). Whereas part of the heritability of LV mass is due to genes influencing body weight and blood pressure (which determine about 0.20 of the variance), LVESD seems to be more independent from these parameters. The relatively high heritability renders a search for genes involved in the determination of left ventricular mass and contractility (as measured by LVESD) promising. Knowledge of genes underlying these traits would be of great interest, since it could eventually lead to better preventive and therapeutical management of cardiovascular disease.

P2-06 26

Role of HADHA gene encoding the a-subunit of trifunctional protein as candidate gene for HELLP syndrome

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The HELLP syndrome (HELLP) is a multi systemic disorder with high maternal and perinatal morbidity and mortality. It belongs to hypertensive diseases in pregnancy (HDP), which complicate 5-7% of all life births. Due to familial clustering a strong genetic influence in the aetiology of HDP has been established. In the pathogenesis of HDP diverse mechanisms, like abnormal placentation, immunological maladaptation, endothelial dysfunction and disorder of lipoid metabolism, have been proposed. Therefore, the analysis of certain candidate genes, in patients diagnosed with HDP, is a strategy to identify genetic risk factors. In a systematic study with at least 250 families with HDP we started among other strategies to screen for mutations in a couple of candidate genes. One attractive candidate gene is the HADHA gene encoding the a-subunit of the trifunctional protein (TFP) which is responsible for the long-chain 3-hydroxy acyl-CoA dehydrogenase deficiency (LCHAD). Recently an association between maternal HELLP and LCHAD deficiency has been proposed. The majority (69,4%) of LCHAD-deficient children are homozygous carriers of a G1528C mutation in the HADHA-gene.

Mutational analysis of the HADHA was performed in 112 mothers with HELLP syndrome 90 of their children, 22 fathers and 110 women with uncomplicated pregnancies the women with HELLP by SSCP and, if necessary, by direct sequencing. The G1528C M was tested in all individuals by using PCR and RFLP analysis.

None of the women with HELLP showed a M in the coding region resulting in an amino acid exchange. One of 103 patients showed a homozygote G/T M at position -1, which was not detectable in 109 controls. Whether this mutation causes a shifting of splice site is now investigated. An incorrect original genomic sequence in intron 9, 11 and 15 was identified. In intron 15 we additionally found a polymorphism (T/G) which showed almost identical allele frequency in patients and controls. None of the studied individuals showed a G1528C M.

The HADHA gene dos not have any relevant impact on the pathogenesis of HELLP syndrome.

P2-06 27

Breakpoints in balanced constitutional chromosome aberrations as indicators of candidate gene loci associated with complex genetic diseases

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Classically, the first step for positional cloning of genes associated with complex genetic diseases, e.g. chronic inflammatory diseases (CID), involves linkage studies of affected families. A less explored approach is the study of balanced constitutional chromosomal aberrations. Prospective cytogenetic studies are hampered by the low incidence of chromosome aberrations, which (excluding Robertsonian translocations and the inv(9)-polymorphism) is only ~0.5%. Therefore, we retrospectively interviewed 161 adult carriers of balanced constitutional chromosome aberrations for the occurrence of CID by means of a self-administered standardized questionnaire. Cytogenetic studies had been performed to elucidate abnormal results in prenatal diagnosis or to unravel reasons for infertility or recurrent miscarriages. Of the 72 (45%) questionnaires returned so far, 19 probands (27%) reported to suffer from CID (Crohn's disease/ulcerative colitis: 5; psoriasis: 5; diabetes/pancreatitis: 4; periodontitis: 5) and 6 probands (8%) described to have relatives affected with CID (Crohn's disease/ulcerative colitis: 1; psoriasis: 4; asthma: 1). Interestingly, two patients with colitis ulcerosa and psoriasis, respectively, presented alterations in regions of chromosomes 10 and 16 which were suggested by recent linkage studies to contain candidate loci for CID. For delineation of breakpoints we used a sequential FISH approach applying bacterial artificial chromosome (BAC) clones with sequence information available. In this way, we identified clones spanning the breakpoints in chromosome 10. Additional analyses are underway to determine the breakpoint on chromosome 16 and to investigate whether the breakpoint regions indeed contain genes associated with CID.

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P2-08 01

The Overexpression of the Insl3 in Female Mice Causes Descent of the Ovaries

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Developmental impairment of the gubernaculum in male mice lacking the insulin-like 3 factor (Insl3) leads to disruption of testicular descent. Results derived from in vitro experiments demonstrated that Insl3 and androgens induce outgrowth of the gubernacular primordia. To provide in vivo evidence that the Insl3 mediated activity is responsible for induction of the gubernaculum development in an androgen-independent manner, we generated transgenic male and female mice that overexpressed Insl3 in the pancreas during fetal and postnatal life. Functional complementation with the transgene restored the cryptorchid phenotype of the Insl3-deficient male, suggesting that the regulatory secretory pathway present in islet ß-cells efficiently processed the Insl3 to the functional hormone. All transgenic females displayed bilateral inguinal hernia. The processus vaginalis developed and contained peritoneal contents with intestinal loops. The Müllerian derivatives developed into oviduct, uterus and upper vagina, and Wolffian duct derivatives were absent, indicating the loss of the androgen- and anti-Müllerian hormone-mediated activities in transgenic females. The ovaries descended into a position over the bladder and attached to the abdominal wall via the well developed cranial suspensory ligament (CSL) and the gubernaculum. Administration of dihydrotestosteron during the prenatal development suppressed the development of the CSL and thereby the descent of the ovaries into the processus vaginalis. These results suggest a crucial role of the Insl3-mediated activity in the

induction of gubernaculum development and exclude a potential role of androgen in this process. Furthermore the transgenic females exhibit reduced fertility, which is due to fetal death during midgestation.

P2-08 02

Simultaneous statement of two clustered ADAM familiy genes, Testase 2a and Testase 2b, during development of mouse germ cells

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Testase 2 (also known as ADAM 25) is a member of ADAM (A Disintegrin And Metalloprotease) family of proteins.The ADAM family presents the best characterized candidates for mediating gamete interaction and membrane fusion in mammals. As previously reported and confirmed in this study this gene show testis specific expression. During experiments we found two different restriction patterns of subcloned fragments of the gene, suggesting presence of two transcripts. Further experiments and Celera database search revealed that these two transcripts are the products of two separate genes, which show high similarity to the published testase 2. Testase 2a and testase 2b as we called them are similar to published testase 2 in 87,8% and 95,4% respectively, and in 87,4% to each other. Both genes are located on chromosome 8 (as well as ADAM 3, ADAM 5, ADAM 9) in close distance of 24 kb. Genomic structure of testase 2a and b differs from that of other ADAM family members, like cyritestin or fertilin which contain around 20 short exons, they are composed of only two exons. First one is very short, about 85 bp while second is over 2.5 kb long. Both genes demonstrate the same temporal and spatial expression pattern during testicular germ cell development with onset of expression in haploid stages. Further functional analysis of these molecules will contribute to a better understanding of the molecular mechanisms underlying mammalian sperm-egg fusion.

P2-08 03

Differential expression of fork head genes in the stomach mucous gland

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The stomach mucosa is a proliferating tissue with a high potential of regeneration. But the high number of patients with ulcer or stomac cancer shows that the maintenance of the functioning tissue can be easily disturbed. There is not much known about the genetic background of the stomach regeneration and differentiation of the mucosal stem cells. Therefore we are interested in genes which are involved in the regeneration process.
Fork head genes are transcription factors which play important roles in biological processes and are involved in embryonic development, cell cycle regulation, regulation of tissue-specific gene expression and cell signalling. Some fork head genes are expressed in the stomach and we were interested, in which cell types these genes are expressed and if they could play a role in the stem cell differentiation.
To answer this question we performed in situ hybridizations on sections of adult mouse stomach. Using probes for the fork head genes Foxa1, Foxa2, Foxa3 and Foxq1 we found expression of all four genes specifically in the pepsinogen producing zymogenic cells, but in different stages of differentiation. While Foxa1 and Foxa3 are expressed in pre-neck- and neck cells (which will become zymogenic cells later), Foxq1 expression starts in the pre-zymogenic stage. Foxa2 is only expressed in the completely differentiated zymogenic cells. We postulate that the different fork head genes are not sufficient for the initiation of the differentiation, but they play an important role in the differentiation of zymogenic cells.

P2-08 04

Multifactorial infertility in proacrosin deficient mice

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In human, male and female partners contribute more or less equally to the infertility problem. In about 20 % of infertile couples the concurrence of male and female factors is suggested to be responsible for infertility. Neither any of these factors are known nor a model system to prove this assumption is established. We present such a model system in the mouse, in which the lack of acrosin in the male and modifications of the zona pellucida in the female result in a significant reduction of fertilization rate in vitro. We have generated mice carrying a deletion in the prolin-rich domain (PRR) of the proacrosin gene which results in the absence of proacrosin in the homozygous PRR -/- male mouse. Modifications of the zona pellucida of oocytes after superovulation were achieved by their treatment with dimethylsulfoxide (DMSO) and aroclor-1254 and by in vitro ageing. It is known that under these conditions a time-dependent hardening of the zona pellucida occurs. The rate of fertilization in vitro of treated and aged oocytes, respectively, using spermatozoa from PRR -/- mice was found to be significantly reduced when compared to that rate reached with wild-type sperm. The relevance of the acrosin as well as of the zona pellucida for fertilization success is further substantiated by the result that fertilization rate depends on the thickness of the zona pellucida. Our results demonstrate that the lack of acrosin in spermatozoa in combination with modifications of the zona pellucida can affect fertility and can be a model for unexplained infertility in human couples in which male and female derived factors are suggested to be the underlying causes.

P2-08 05

Sertoli cell- germ cell interaction: expression studies and functional analysis of the murine calgizzarin gene

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The familiy of S100 proteins is a large familiy of calcium binding proteins, with various functions in extracellular and intracellular processes, such as: apoptosis, cellular growth and differentiation, enzyme-modulating activities. Calgizzarin is a member of the S100 familiy of proteins, it has two calcium- binding domains and it is highly conserved in human, mouse, rat and chicken. Calgizzarin is thought to be involved in Sertoli cell-germ cell interaction, being expressed in Sertoli cell culture and in coculture of Sertoli cell-spermatid,spermatocyte fractions.
However, a high mRNA level of this gene is maintained until day 15 of postnatal development in testis (when pachytene spermatocytes appear) and then declines sharply, as opposed to ovary, where the expression pattern is constantly high. In ovary, the calgizzarin expression is maintained highly during all the five stages of the oestrus cycle.
Calgizzarin is expressed in Sertoli cells (testis) and granulosa cells (ovary). In order to establish the role played by calgizzarin gene in Sertoli cell-germ cell interaction, we carried out expression studies, using prenatal and postnatal testis and ovary tissues in different stages of development. We also generated a knock-out model, using PTK Neo as the vector back bone of the knock-out construct. The process of obtaining null mutants is on-going. Further studies are needed in order to assess the role of calgizzarin gene in this kind of testis cell-cell interaction.

P2-08 06

Polycystin-2 is required for left-right axis determination in mice

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The establishment of left-right asymmetry can be divided into (1) initial breakage of symmetry, (2) establishment of asymmetrical gene expression and (3) transfer of this positional information to the developing organs. Molecular events involved in the first and third step are hitherto largely unknown, but much progress has been made in elucidating the establishment of asymmetrical gene expression. Embryological and genetic experiments revealed a conserved asymmetric signaling cascade, which during early embryogenesis transmits asymmetric cues from the embryonic midline to the lateral plate mesoderm and the forming organs. The central players in this scheme are four asymmetrically expressed genes: nodal, Lefty1 and Lefty2, and Pitx2c. In mouse monocilia on ventral cells of the node are required upstream of the nodal cascade; in chick and frog gap junctions are essential prior to node formation. It was hypothesised that differential activity of ion channels results in unidirectional transfer through gap junctions, resulting in asymmetric gene expression.
Recently we have generated a Pkd2 knockout mouse. Pkd2 encodes polycystin-2, an intracellular Ca- release channel which is expressed abundantly in the endoplasmic reticulum membrane. In humans mutations in PKD2 account for 15 % of polycystic kidney disease.
Surprisingly, Pkd2 knockout mouse embryos displayed left-right positional defects: embryonic turning, heart looping and placement of abdominal organs were randomized and the lung showed right pulmonary isomerism. Lefty1, Lefty2 and nodal were not expressed in the left lateral plate mesoderm and Pitx2 was absent from heart and lung primordia and bilaterally expressed in body wall, mid and hindgut. The em-

bryonic midline, however, was present and normal levels of hnf3beta and shh were expressed. We suggest that in mouse the ion channel polycystin-2 acts upstream of the nodal cascade in left-right axis determination and Pkd2 might be one of the master genes of the body plan.

P2-08 07

Screening for retinal degeneration mutations in the mouse, Mus musculus

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To identify genes involved in retinal degeneration we examined mice of different strains with a high throughput electroretinography (ERG) method. This non-invasive screening method allows a clear separation of affected from unaffected individuals. The animals (between 3 weeks and 3 months old) were dark-adapted for at least 12 hours and anaesthetized. After pupil dilation, individual mice were fixed on a sledge (Steinbeiß-Transferzentrum, Tübingen, Germany) and ERGs were recorded in a Ganzfeld stimulator (Espion, Cambridge, UK) with two illumination intensities (500 or 12,500 cd/m2).
We have established the wild type baseline for several mouse strains (101, 129/SvJ, AKR, BALB/c, C57BL/6, CBA, CD1, DBA, JF1); as a positive control for hereditary retinal degeneration we used C3H/El mice. During these investigations, we identified several animals with lowered b-wave amplitudes in ERG among the CD1 out-bred mice as well as among the 129/SvJ and the JF1 inbred mice. Histological analysis of some of these mice revealed a good correlation between the lowered electrophysiological response and the retinal morphology. In 129/SvJ we detected the same retroviral insertion associated with the Pde6b/rd1 allele that is known to be causative for the retinal degeneration in C3H mice. In contrast, molecular analysis of CD1 and JF1 mice excluded this retroviral insertion. Genetic confirmation of these variations is in progress. The method is now being used to screen for hereditary retinal degenerations among the offspring of ENU-treated C57BL/6J males.
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P2-08 08

The X-linked non-specific mental retardation gene Arhgef6 is specifically and highly expressed in the fetal placenta

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According to our working hypothesis the genes for X-linked non-specific mental retardation, MRX, are the best candidates for human speciation genes. The specific trait developed during human speciation is general cognitive ability. This trait gets introduced simultaneously with reproductive isolation which exerts its effect in the testis as fertility gene and/or in the placenta as growth gene. Therefore it is expected that speciation genes should affect the function of cognitive abilities and the function of the testis and/or placenta simultaneously. During this study the expression pattern of Arhgef6, a guanine nucleotide exchange factor for Rho GTPases, was analyzed during murine development. In the embryo proper and especially the brain of the embryo no specific expression was observed. Beginning from day 7.5 p.c. a highly specific signal was observed in the placenta. The major function of the placenta is to enable the fetus and the mother to interact for the promotion of fetal growth and viability. To identify the cells expressing Arhgef6 coybridizations were performed with the marker genes, Mest (mesodermis specific transcript), for endothelial cells of the syncytiotrophoblast, 4311 for the spongiotrophoblast, and PL-1 (placental lactogen), for the trophoblast giant cells. Arhgef6 is specifically expressed in the decidua basalis and in the trophoblast giant cells. In the junctional zone, high levels of Arhgef6 mRNA are observed in the spongiotrophoblast and in the trophoblastic cells of the labyrinth.
In his pioneering thesis on X-linked mental retardation R.G. Lehrke has already pointed out that impaired intrauterine development may be a contributing side effect of X-linked mental retardation genes.

P2-08 09

Expression of mouse Tbx22 supports its role in palatogenesis and glossogenesis

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We have investigated two X-chromosomal deletions at Xq21 by Inter-Alu array CGH and PCR and found TBX22 to be deleted in one of the two studied cases. TBX22 codes for a transcription factor of the T-box gene family, mutated in patients with cleft palate and ankyloglossia (CPX), and our male patient had been earlier described with a cleft lip and palate. Although is had been assumed that TBX22 is a human-specific gene, we were successful in identifying mouse Tbx22, which allowed us to study its expression during embryogenesis by RT-PCR and in situ hybridization. Tbx22 transcripts were found in distinct areas of the embryonic head during the time of palate development. Its expression pattern points to a yet unknown, primary role of the nasal septum in secondary palate development and explains the ankyloglossia phenotype in CPX. To learn more about the regulation of TBX22 gene expression, we have identified positionally conserved transcription factor binding sites by comparing genomic sequences of mouse, rat and human TBX22. In this way, strong upstream-acting candidates were found, one of which has been implicated previously in facial clefting.

P2-08 10

The Ror2 Knockout Mouse as a Model for the Development of Spondylocostal Dysostosis in Autosomal Recessive Robinow Syndrome

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Robinow syndrome (RS) is a short-limbed dwarfism characterized by abnormal morphogenesis of the face and external genitalia, and vertebral segmentation. Homozygous mutations in the receptor tyrosine kinase ROR2 are responsible for autosomal recessive RS by a loss of function mechanism. Ror2-/- mice, exhibiting the typical RS features, serve as a model to analyze the development of the underlying vertebral malformations in RS. During development the structure of the vertebrate axial skeleton is based on the metameric structure of the somites. The somites are formed in a dynamic segmentation process that is subject to an oscillatory mechanism in the presomitic mesoderm (PSM). Using alcian blue, alizarin red staining Ror2-/- mice exhibit hemivertebrae and rib fusions and a shortened tail. Whole mount in situ hybridizations of embryos of stages E9.5 and E10.5 with probes for Paraxis, Myf5 and Uncx 4.1 reveal that the pattern of somite boundaries is disturbed. Hybridization with probes for Notch1, Delta1 and Delta 3 show that the PSM is strongly reduced in size. Expression of Pax1, Pax9 and mCer1 suggest that the somite pattern is disturbed in the presumptive somites or while a newly formed somite separates from the PSM. Currently the expression of oscillatory genes is under investigation, in order to better characterize the role of Ror2 in vertebral development and RS.

P2-08 11

The Runx Genes: Roles in Cartilage Development and Evolutionary Implications

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The three mammalian Runx genes belong to a small family of transcription factors. Runx Proteins play pivotal roles in various developmental processes such as hematopoiesis (Runx1), osteoblast differentiation (Runx2) and intestinal development (Runx3). We demonstrate Runx2 and Runx3 expression in dynamic patterns during early and late chondrocyte development. We used overexpression experiments in chicken embryos and a transgenic mouse approach to assess the role of Runx genes in cartilage differentiation. We show that Runx2 is a positive regulator of chondrocyte condensation and differentiation and that Runx2 and Runx3 act cooperatively during chondrocyte maturation. Furtermore we demonstrate that Runx2 is, albeit essential, not sufficient to induce osteoblast differentiation and thus bone formation.
Features such as hematopoiesis and cartilage have newly emerged during vertebrate evolution. The development of these features might have been promoted by the stepwise duplication of a single founder Runx gene. We cloned the Runx gene of the primitive chordate Branchiostoma lanceolatum. We found that Branchiostoma has

only one Runx gene (Br-runx) which shows the closest homology to mammalian Runx3. Br-runx is expressed in the intestinal tract. This is in good accordance to recent reports that show a function for C. elegans Runx in intestinal development and involvement of human RUNX3 in gastric cancer. Thus it is likely that Runx3 has conserved ist original function in intestinal development and that the newly emerged paralogs Runx1 and Runx2 acquired new functions during craniota evolution.

P2-08 12

NBS1 founder mutation and Darwinian fitness

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The NBS1 founder mutation is present in individuals of eastern, western and southern slavic origin. Thus, the original mutational event occurred while the slavic people were still together in their homeland, the Pripet swamp, more than 1,500 years ago. We found an unexpectedly high carrier frequency of this mutation in newborns of five different slavic populations: Czech Republic (8/1234), Poland (18/3569), Ukraine (Lvov, 5/908), Bulgaria (9/1002), and the Sorbs (family studies, 8/326). This frequency rate is much higher than the one found in Berlin (1/900). If one excludes that this independent increase in allelic frequency is simply due to chance, it must result from a different reproduction rate of the gene carriers. We have therefore performed a cohort study on the reproductive history of persons from 18 pedigrees from the Czech Republic. Each individual was personally interviewed, and blood samples were analysed for the NBS1 founder mutation. We found that the normal homozygous female probands (n=45) suffered a total of 9 miscarriages, while in the heterozygous group (n=39) only 2 were registered. In addition, the female carriers of the founder mutation gave birth to a total of 90 children compared to 78 children for the normal homozygotes. Thus, the total number of pregnancies per woman of 2,3 in the heterozygotes and only 1,7 in the normal homozygotes showed a clear difference. These findings were confirmed in principle by comparing the number of offspring between normal homozygous and heterozygous sibs from the same generation. In principle, a higher number of offspring from NBS heterozygous mothers could explain the high frequency of this deleterious founder mutation in the slavic population. Our preliminary data suggest that the higher fertility might be due to a lower miscarriage rate. Since about 50% of all spontaneous abortions are due to aneuploidy, this would implicate that the NBS heterozygotes show a lower non-disjunction rate compared to normal homozygous females.

P2-08 13

Alternative splicing of the MID1 gene, mutated in Opitz BBB/G syndrome, leads to the introduction of premature termination codons: implications for regulatory mechanisms of MID1 gene expression

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Mutations in the ubiquitously expressed MID1 gene cause Opitz BBB/G syndrome, a specific defect of the developing ventral midline. Although we have acquired detailed knowledge of the protein function of the MID1 gene product during the last few years, the basic question of how mutations in the ubiquitously expressed MID1 gene can produce a specific ventral midline phenotype, remains open. Tissue specific splicing is an attractive model to answer. NIX analysis of the genomic sequence of the human, murine and Fugu MID1 gene predicted several exons in addition to the published MID1 sequence. By RT-PCR most of them could be shown to be transcribed and spliced into the MID1 cDNA in a tissue dependent manner. Interestingly, all splice variants found, introduced Stop codons into the MID1 coding sequence. These exons might be fundamental for two different mechanisms regulating MID1 expression and function: Some of them, that use the polyA+ tail of the MID1 gene, are carrying premature termination codons and lead to nonsense mediated mRNA decay of the respective MID1 transcripts. Others, comprising own polyA+ tails might code for C-terminally truncated MID1 proteins. This goes in line with proteins of 25kD, 30kD, 35kD and 45kD that can specifically be detected on Western blots using an antibody against the N-terminus of MID1. We have shown previously that C-terminally truncated MID1 proteins have dominant negative effects on the MID1 protein function.

P2-09 01

Iler-Gerolg syndrome - the clinical findings in a 4years old boy

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Baller-Gerold syndrome (BGS) is a rare disorder presumably autosomal recessive in nature (MIM 218600). The diagnosis of BGS is based on the presence of craniosynostosis, and preaxial upper limb reduction defects. Different skeletal, oral-facial, cardiac, genitourinary, anal malformations, along with mental and motor retardation have also been described in a few patients. We present the clinical findings of a 4-years-old boy affected with BGS. The patient is the third child of healthy consanguineous parents, his two older sisters are healthy. The mother had two prior first trimester miscarriages. The pregnancy was uneventful, birth weight was 2025 g, length 47 cm. First examination of the newborn boy detected brachycephaly with flat occiput and prominent forehead, ptosis, clubhands, hypoplasia of the thumbs, ambiguous genitalia, bilateral clubfoot. Further investigations at the age of 2 months showed atrial septal defect, mild hydrocephaly and slight hip dislocation. Reexamination at the age of 4 years showed moderate motor and mental retardation, turribrachycephaly, midface hypoplasia, ptosis, beaked nose, high palate, microstomia, low-set dysplastic ears, short and broad neck, hypoplasia of the radius bones and thumbs, severe hypoplasia of the penis and scrotum, cryptorchidismus. The karyotype was 46,XY, 15ps+, without increased spontaneous chromosome breakage. At the time of examination no evidence for Fanconi anemia was found. Our patient confirms the clinical variability of BGS, autosoma l recessive inheritance is supported by parental consanguinity. Several authors have questioned whether BGS is a distinct entity. The wide variability of the BGS phenotype and its overlap with other disorders will be discussed.

P2-09 02

De novo distal deletion (6) (q23q25) with mild phenotype in a two year old boy

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Distal deletions of chromosome 6 are associated with growth and mental retardation, microcephaly, short neck, retinal abnormalities, skeletal, heart, anal and other variable defects.
We present a two year old boy with mild clinical features. He was born after an uneventful pregnancy at term (body weight 2770 g [3. percentile], body length 52 cm [25. percentile], head circumference 35 cm [25.-50.percentile]).
Our propositus presented after birth mainly healthy, only breathing gave suspicion for an aspiration. The physical examination showed a slightly dystrophic male newborn with inconspicuous genitals. Only the right testis was not palpable. Later on some mild clinical features such as a tall and thin stature, low-set ears, a high forehead, antimongoloid lidaxes, a receding chin, deep nasolabial creases and muscular hypotonia led to a cytogenetic analysis because of suspicion for a syndrome.
The cytogenetic and FISH-analysis exhibited a distal deletion (6) (q23q25) for the propositus.
Our case report gives an overview concerning the development of the now two year old boy and reviews the known literature.
Although our propositus has only a mild phenotype and now at the age of two years mainly motorical and sensomotorical deficits without any known abnormalities of inner organs, especially heart and brain, a mental retardation can not be excluded.

P2-09 03

Overgrowth and Transient Mental Retardation in a Family: Sotos Syndrome ?

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Intellectual impairment is common reason for a child to be referred to a genetic clinic. To diagnose a syndrome which includes mental retardation more specific features are necessary. These may be discrete or remain unconsidered. Overgrowth narrows the diagnosis to relatively few possibilities. We present family, a 37 year old woman, her ten year old son and her 15 months old daughter with transient mental retardation, extreme overgrowth, cardiac defects, and a pe-

culiar face. Diabetes I is present in the mother and may be an additional feature.
We consider Sotos Syndrome to be the likely diagnosis. It is quite uncommon though, that more than one person in a kindred is affected by the Sotos syndrome and that mental retardation settles before puberty.
Other diagnoses will be discussed.

P2-09 04

A novel mutation that causes Bardet-Biedl syndrome 4 (BBS)

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Bardet-Biedl syndrome (BBS) is a rare, multisystem disorder with an autosomal recessive mode of inheritance. The cardinal features are central obesity, rod-cone dystrophy, postaxial polydactyly, learning difficulties, hypogenitalism in males and renal dysplasia. The population prevalence ranges from 1:13 500 livebirths among the Bedouin of Kuwait to approximately 1:160 000 in Western-Europe. BBS is geneticall y heterogeneous with at least six different chromosome loci linked to the disease: BBS1 on 11q13, BBS2 on 16q21, BBS3, on 3p12-p13, BBS4 on 15q22.3-q23, BBS5 on 2q31 and BBS6 on 20p12. To date, three BBS genes ? MKKS, BBS2, BBS4 ? have been identified. The BBS4 gene product comprises 519 amino acid residues and shows strongest homology to O-linked N-acetyl-glucosamine transferase (OGT) from several species, including humans. Here, we report the identification of a novel c.1247 delG mutation in exon 14 of BBS4, homozygous in an Egyptian BBS patient, who presented with the typical clinical features of the syndrome. The deletion causes a frameshift resulting in a truncated protein of presumably 467 amino acid residues. This is the first report of a homozygous single base deletion causing a truncated BBS4 gene product.

P2-09 05

Hypodontia as a cardinal symptom in Kabuki syndrome

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In 1988 Niikawa et al. described a new syndrome with mental retardation, postnatal dwarfism and a peculiar facies characterized by long palpebral fissures with eversion of the lateral third of the lower eylids, high-arched eyebrows with lateral thinning, broad and depressed nasal tip, large prominent earlobes, a cleft or high-arched palate, scoliosis, persistence of fingerpads, radiographic anomalies as dislocation of hip joints and recurrent otitis media in infancy. Later, other authors described ectodermal abnormalities as hypoplastic nails, abnormal tooth crown shape, developmental defect of enamel, premature breast development and breast enlargement in girls. In 1997, Lerone et al. reported a girl with Kabuki syndrome and ectodermal abnormalities like hypodontia, hypoplastic nails and brittle hair. These findings were confirmed by Courtens et al. (2000) and Matsune et al. (2001). The latter found missing permanent teeth in three of six patients with Kabuki syndrome.
Here we report a 14y old boy with scoliosis, dislocations of hips and typical facies of Kabuki syndrome, however, without postnatal growth retardation (the body length corresponds to the 25th centile) but with ectodermal abnormalities like hypoplastic nails and oligodontia. The following dental germs were missing: 13, 12, 22, 23, 33, 32, 31, 41, 42, and 43. Yet, serration was anticipated in the 2nd month of life. This agree well with descriptions in the literature. In addition to mental retardation he shows behaviour disorders like unsociable behaviour, thumbsucking, jactatio capitis nocturna, and fits of laughing. The spectrum of symptoms in Kabuki syndrome may be larger than assumed so far. Ectodermal abnormalities and behavioural disorders are cardinal symptoms in Kabuki syndrome, too.

P2-09 06

Fetal valproate embryopathy in monozygotic twins

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The use of valproic acid periconceptionally and during pregnancy is associated with adverse fetal outcome including intrauterine growth retardation, prematurity, abnormalities of the central nervous system and of the cardiovascular system as well as musculosceletal abnormalities. Other organ systems are affected less frequently. The risk for stillbirth or perinatal death is increased in children exposed to valproic acid during pregnancy. Fetal valproate syndrome is characterized by typical dysmorphic features such as high and broad forehead, bitemporal narrowing, hypertelorism, short palpebral fissures, epicanthal folds, flat and broad nasal bridge, shallow philtrum, hypoplastic midface, small ears, hypoplastic nails, clinodactyly and camptodactyly and often mental retardation. We present female monozygotic twins who were exposed to 2.4 g of valproic acid through all over pregnancy. We saw the girls first at the age of 11 months and later of two years. The twins were delivered spontaneously in the 37th week of gestation. Body length, weight and head circumference are at the lower normal limit. The girls show almost identical dysmorphic features (high and broad forehead, hypertelorism, infraorbital groove, flat and broad nasal bridge, small posteriorly rotated ears, shallow philtrum, thin upper lip, camptodactyly, broad distal phalanx of fingers). A hearing loss is treated with hearing aids. The girls have VSD and pulmonary stenosis. They show developmental delay and feeding difficulties. Surprisingly identical clinical signs are produced by a drug in genetically identical individuals. A high recurrence risk of valproate embryopathy is known in sibships. Possibly the metabolism of valproic acid either in the mother or in the fetuses is responsible here.

P2-09 07

Small microdeletion 1p 36 in a 12 year old boy with a mild clinical phenotype but sudden lethal course

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Monosomy 1p36 is a recently delineated contiguous gene syndrome, which is now considered to be one of the most common subtelomeric microdeletion syndromes. The phentype usually consists of psychomotor and growth retardation, microcephaly, seizures and typical craniofacial anomalies. The incidence is estimated to be about 1: 10000.
We report a 12 year old patient born to healthy German parents. He had mild dysmorphic features: strabismus on both sides, mild bilateral ptosis, slightly deep set eyes, mild progenia; there was a fusion of the right canine tooth with the neighboured molar tooth, alopecia areata occipital (2X2cm diameter). The body length of our patient was 138 cm (P3), his weight 34 (25 percentile) and head circumference 53.3 cm (25-50 percentile). He walked at 2 ½ years. At the age of 3 years generalized seizures occured and he developed hyperphagia. Under low calorie diet obesity could be avoided. Speech development was delayed. At 12 years he was moderately retarded. He could speek whole sentences but was not able to read. He knew some figures. He showed stereotypic hand movements. One month after presentation the child became ill with pneumonia, he suddenly suffered an asystolia requiring resuscitation. A causal status epilepticus could be excluded. The boy developed severe cerebral edema resulting in an apallic syndrome. He died within 8 weeks.
Monosomy 1p36 was considered clinically. FISH analysis was performed and confirmed the suspicion. A microdeletion 1p36.3 could be identified: ish del(1) (pterp36.33-36.32)([TelVysion 1p]-,CD2L1-,HKR3+) The deletion is less than 3-4 Mbp. Chromosome analysis demonstrated a normal karyotype 46, XY. FISH analysis of the mother did not reveal a microdeletion 1p36.3. The father was not available for testing

P2-09 08

Mesoaxial syndactyly with cataract: A distinct syndrome

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Syndactylies are a heterogeneous group of anomalies of the hands and feet and occur either as an isolated congenital malformation or as part of a syndrome. In 1993, Pavone et al. reported on a boy with mesoaxial syndactyly, cataracts, brachycephaly, mild facial anomalies, mild generalized hirsutism, thinning of the lower legs, and mental retardation.
The authors suggested that the findings in t his patient were unique and differ from cases previously reported. Here we report on a second case with mesoaxial syndactyly of hands and feet and

congenital cataract, however, with apparently normal mental and motor development. The girl is the second child born to healthy consanguineous parents of Turkish origin. She has brachydactyly on both hands with partial cutaneous syndactyly between fingers II-IV on the left, and osseous syndactyly between fingers III-IV and minimal cutaneous syndactyly between fingers II and III on the right hand.
There is complete cutaneous syndactyly between toes II and III and partial cutaneous syndactyly between toes III-V bilaterally. On examination at age 4 month a left-sided microphthalmos, microcornea, and nuclear cataract was diagnosed which was considered to be congenital. In addition, there are mild facial anomalies and a low hairline on the back. Although the girl seems to be less severe affected than Pavone's case, this second case may support the suggestion that this pattern of malformation represents an unique condition of apparently autosomal recessive inheritance. The findings are very similar to the cataract-webbed Peromyscus maniculatus autosomal recessive mouse mutant described by Huestis [1951] and Anderson and Bruns [1979].

P2-09 09

Microcephaly-chorioretinal dysplasia
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The combination of microcephaly, mental retardation and chorioretinal dysplasia was first described by Tenconi et al. in 1981. Until now, only 4 further families were reported. We here describe a mother and her daughter, supporting the supposed autosomal dominant inheritance of the syndrome and showing the great variability. At the age of 2 years the index patient had a weight of 6,7 kg (3kg <3P), height of 80,3 cm (1 cm <3P) and a head circumference of 38 cm (8 cm < 3P). Ophthalmologic examination showed right-sided mild microphthalmia, and a fibrovascular membrane with a densely pigmented retinal periphery. On the left eye she had a retinitis pigmentosa. Psychomotor development was severely retarded.
The mother had a head circumference of 50 cm (2,2 cm <3P), a weight of 77 kg (2 kg >97P) and a length of 160 cm (25-50P). Fundoscopic examination revealed chorioatrophic regions, hyperpigmented areals, retinal folds and optic atrophy. She had a normal psychomotor development.
Fryns et al, (1995) argued that microcephaly-lymphedema, microcephaly-chorioretinal dysplasia and the cases, reported by Jamas et al. (1981) with microcephaly and retinal folds all have the same condition. Our
two cases may support this hypothesis.
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P2-09 10

Microdeletion del (14)(q11.2q13.1) with severe phenotype
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Microdeletions of the proximal segment of the long arm of chromosome 14 are rare. Here we describe such a chromosomal aberration that occurred de novo in the second child of a healthy unrelated Austrian couple. Already at birth (10 days after date), following an uneventfull pregnancy, several phenotype anomalies were noticed. Most obviously a pronounced microcephaly with an occipito-frontal cirumference (OFC) of 32.5 cm (< 3 centile) was noticed, whereas weight of 4435 g and length of 55 cm were both > 90 centile. MRT of the brain performed at an age of 9 month did not reveal any obvious anomaly of cerebral structural but just enlarged ventricles. At one year of age he shows an even more pronounced microcephaly (<< 3 centile) with weight and length at the 50 centile, a severe psychomotor retardation, a bilateral optic atrophy, several distinct facial dysmorphic features, like a long philtrum and genitourinary anomalies as a hypospadia III and a bilateral hydronephrosis II-III.
High resolution banding allowed to confirm the microdeletion 14q, which was suspected already from standard cytogenetic preparations leading to the karyotype 46,XY,del(14)(q11.2q13.1)de novo. Subsequently performed FISH analysis using region specific BAC clones allowed to further define chromosomal breakpoints. Additional microsatellite and BAC contig analysis narrowed down the deleted segment to 5 Mb and the number of potential candidate genes related to the phenotype anomalies already mapped to that chromosoaml segment is currently less than 20. Therefor this case could be helpful in identifing one gene or several genes that are related to severe phenotype anomalies. This should improve our understanding on the genetic factors involved in the regular and altered formation of craniofacial and genitourinary structures.

P2-09 11

Seckel syndrome with central nervous system anomalies
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Seckel syndrome is a rare, heterogeneous form of primordial dwarfism. The clinical delineation of this disorder has been inconsistent, using even Seckel´s original criteria. There are only few cases with a detailed description of the central nervous system anomalies.
We report on a newborn boy with Seckel syndrome showing marked intrauterine dwarfism, severe microcephaly with holoprosencephaly, facial anomalies including a receding forehead and chin, large beaked nose and bulging eyes. There was no known parental consanguinity, family histories were unremarkable.
Imaging studies of the central nervous system in Seckel syndrome have only been described in three other reports: A 2 month-old boy demonstrated hypoplastic cerebrum and cerebellum resulting in a largely empty intracranial space. In a 28 years-old woman dysgenetic appearance of the cerebral cortex with a midline interhemispheric cerebral cyst was diagnosed. A newborn girl showed agenesis of corpus callosum, hypoplasia of the cerebellar vermis and a dysgenetic cerebrum with pachygyria and a medially located dorsal cyst.
The genetic heterogeneity and the phenotypic variability of the Seckel syndrome is discussed.

P2-12 01

Coping strategies of pregnant women after so-called „Triple-Diagnostic" and those of their partners
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Objective: To compare coping strategies of pregnant women after triple diagnostic, genetic counselling and prenatal diagnosis and those of their partners.
Background: Triple diagnostic has become a prenatal screening method by combination of three serum parameters (alpha-feto-protein, unconjugated estriol, human chorionic gonadotropin) with personal parameters to define the individual's probability to bear a child with a chromosomal aberration, especially trisomy 21. The screening often takes place without sufficient information and so a „bad result" is synonymous with a handicapped child.
Methods: Our investigations were carried out by questionnaires. Coping strategies, mood states and attitudes of 92 women and 52 partners were evaluated.
Results: Only 65% of the women were informed about this screening method by their gynaecologist. Their partners were informed to about 27% by their wives and to about 23% by the gynaecologist. After having been informed of a elevated risk, 87% of the women and 72% of their partners felt depressed and a little part felt activated (2% and 10%). After genetic counselling, there is a trend to activated mood (women: only 37% feel depressed and 46% feel activated, partners: only 45% feel depressed and 50% feel activated). After genetic counselling, negative coping strategies dropped down (women: 22% to 3,5%; partners: 13% to 5%) in favour of active coping. A quarter of the pregnant women rejected offered prenatal chromosomal analysis by invasive methods.
Conclusions: Genetic counselling holds up an important position in development of positive coping strategies. It should give not only information but also therapeutic support for pregnant women and their partners.

P2-12 02

Social, medical and pedagogic care in patients with Klinefelter´s syndrome
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Klinefelter´s syndrome is the most common gonosomal aberration in males. However, little is known on how adult patients and parents of affected boys cope with the disease. In order to evaluate coping strategies we sent out questionnaires to adult patients with Klinefelter´s syndrome and parents of affected boys focussing

on familial, professional, and social aspects. In addition, data on medical care were analyzed. 129 uestionnaires - 70 from adult patients and 59 from parents of affected boys under the age of 18 years - were evaluated. The karyotype was 47,XXY in 102 cases (79,1%), mosaic in 7 cases (5,4%), 48,XXYY in 3 cases (2,3%), 48,XXXY and 49,XXXXY in 2 cases (1,6%), respectively. For the remaining cases of Klinefelter´s syndrome the karyotype was unknown. Among the group of affected boys and juveniles the diagnosis was made prenatally in 66,1%. This high rate can be explained by a low termination rate and the mild phenotype, especially in childhood. Only 15% of the parents and 17% of the adult patients felt to be adequately informed on the disease and counselled, but most of the parents (69,5%) and adult patients (74,3%) were satisfied with their medical care. Among adult patients 91,4% completed one or more professional trainings, of those 17,2% had a university degree. Altogether 86,8% enjoyed their profession. 30% of the parents and 49% of the adult patients felt to be disadvantaged by the disease. Our results may be helpful in genetic counselling of patients with Klinefelter´s syndrome and of parents faced with the prenatal diagnosis of Klinefelter´s syndrome.

P2-13 01

The social situation, family relations and medical support of families suffering from heredoataxias in Germany

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The aim of the current study is to evaluate the social situation, family relations, medical and social support in German families suffering from heredoataxias. The study is carried out by questionnaires and interviews. Both, atactic persons and their partners, were asked.
Only a minority of the patients received the information about their disease from their doctors. Most gathered their knowledge from relatives or through own searches. Lack of information is the most common problem. All patients still take part in a wide spectrum of physiotherapy, logopedics and ergotherapy. Both, patients and their partners, consider these therapies suitable to help them. However, the numbers of sessions are not sufficiently as necessary. In general, the unaffected partners see more problems in their partnership than the affected persons. The main problems are the limitation of their freedom in the spare time and the inability of their affected partner to cope with the handicap. There are more problems with sexuality than for the patients. On the other hand, the affected partners are more reserved in sexual contacts but do not feel less attractive for their partner. Most have no problems in relation to their family members. However, they reported problems with friends since first atactic symptoms had been seen.
As a result, we found some resources to give certainty to the patients and their partners, especially concerning the progress of the disorder. The knowledge what progress stands for in everyday life is very important for the partnership in order to cope in a better way. Especially the medical professions should be better informed about heredoataxias in order to inform their patients and the relatives.

P2-13 02

The situation and attitudes of parents with children suffering from a hereditary disease

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The aim of our study was to investigate the situation and attitudes of parents with children suffering from mental retardation (like Down's syndrome or fragile X syndrome; group A) and myopathies (like Duchenne's progressive muscular dystrophy; group B). We wanted to find out, whether due to heredity there are specific features in the process of coping. Points of interest were effects on partnership, lifestyle, family planning and attitudes to prenatal diagnosis. Method: The current study was carried out questionnaires. To find out the coping strategies we used standardized questionnaires. Results: 42% of the parents from group A and a quarter of group B had to restrict their occupational life. 44% of group A and 20% of group B felt confronted with more social exclusion. About 48% parents of group A and only 7% of group B had conflicts with family members. Quite different are the effects on partnership: Roughly half of group A reported conflicts with their partners and in 19% the partnership was dissolved. In group B 72% of the parents held the view that difficulties of their situation had strengthened the partnership and only about a quarter had conflicts with their partners. Similar are the opinions about family planning and towards prenatal diagnosis: 23% of the parents planned further children. About 90% wished to undergo prenatal DNA diagnosis. About two thirds thought that termination of pregnancy after positive prenatal diagnosis is justified. Conclusion: For parents with children suffering from hereditary diseases the doctor is important not only for giving information and treatment, but also for supporting the process of coping with the affected child in order to strengthen relationships in the family.

P2-13 03

The dialectics of the genotype-phenotype talk and genetic discrimination

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This historical study is concerned with innovation in genetics, the genetic conceptualisation of organisms and a genetic concept of „Ganzheit“ that re-establishes the primacy of nature over nurture. It casts some light on actual demands in genomics towards the integration of epigenetic and environmental conditions. The case study deals with a debate between geneticists, gynaecologists, and radiologist around 1930 in Germany. It will be shown that the conflict was mainly one about the claim of geneticists to the primacy of their methods and the genetic knowledge: Genetic findings about mutability can be transferred to the usage of X-rays in medicine. Analogous reasoning and the validity of animal models should be investigated thoroughly in epistemological and systematic terms. In this epistemological perspective modelling has to be understood as a process of production. Historically it has been connected to remarkable trials within the „higher mendelism“ 1930 to come to a „Ganzheitsauffassung“ (Timoféeff-Ressovsky) of organisms and to describe gene action in terms of the „norm of reaction“. However, it turns out that these considerations did not restrict the claim of genetics to primacy but widened it. At the same time, the inherent conception of nature and nurture functioned as the basis of discriminating health policy - even though the self-image of geneticists and human geneticists was mostly not political. Considering this historical experience it seems possible that recent consideration of environment in genomics will end in a description of human beings in terms of genetic risk. Genetic discrimination, then, may not be the matter of ethics but the outcome of the factual constraints of genetic reduction.

P2-15 01

Chromosomal factors of infertility and recurrent pregnancy loss

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Chromosomal abnormalities are one of the known factors of infertiliy and recurrent pregnancy loss. Cytogenetic screening was done in candidate couples for assisted reproduction and exploration of semen alteration (Infertility group) and couples who had more than 2 spontaneous fetal losses (Abortion group).
In our retrospective study (1988-2000) we compared frequency of chromosomal abnormalities in these indication groups. In Infertility group cytogenetic diagnosis of 982 candidates were performed, 403 female (41%) and 579 male (59%). The chromosomal aberration rate was in total 6,9 % (n=68), 5,4% female (n=22) and 7,9% male (n=46). Structural aberrations were found in 2,1% (n=21), 24% female (n=5) and 76% male (n=16). In the Abortion Group a total of 1877 candidates was tested, 1129 female (60%) and 748 male (40%). In 4,2% (n=79) aberrant karyotypes were found, 4,9% female (n=56) and 3% male (n=23). Structural aberrations were found in 2,6% (n=49), 62% female (n=30) and 38% male (n=19).By detailed aberration analysis in both indication groups we try to define chromosomal factors of infertility.
An overall increased frequency of chromosomal aberrations was found, that confirms that it is necessary to perform genetic counseling and cytogentic investigation of both partners in assisted reproduction and exploration of recurrent pregnancy loss.

P2-15 02

Cytogenetic findings in patients prior to assisted reproduction

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Approximately 10 to 15% of Caucasian couples experience temporary or permanent fertility problems. The heterogeneous etiology includes an increased frequency of chromosomal aberrations in both males and females. In order to identify individual genetic risk situations, a thorough examination of the medical and family history of both partners should be performed prior to assisted reproduction. In addition, depending

upon the obtained clinical and anamnestic data karyotyping and/or additional gene analysis might be indicated. Here we report the cytogenetic findings in 1401 male and female patients after genetic counseling and prior to assisted reproduction (7/2000 to 4/2002). All cases were analyzed by GTG banding and additional special staining techniques, if necessary. At least 12 metaphase spreads were examined per patient and 20 metaphases in case of a single abnormal metaphase. In patients with two or more metaphases with identical aberrations or at least 3 different cell lines 30 to 50 cells were tested by conventional analysis and/or additional 100 metaphases by FISH (wcp). 673 of the analyzed patients had a primary indication for ICSI. The second group (728) comprised patients, where initial genetic counseling had identified other risk factors in a patient and/or their close relatives, which required exclusion of chromosomal aberrations („non-ICSI"). In total, 53 (3.8%) aberrant karyotypes were identified (ICSI group: 27/673 = 4.0%; non-ICSI group: 26/728 = 3.6%). In the ICSI-group 11 autosomal and 1 gonosomal structural aberrations were observed; 4 patients were found to carry constitutional gonosomal aneuploidies and additional 11 patients gonosomal mosaicism. Aberrant karyotypes in the non-ICSI group included 5 autosomal and 1 gonosomal structural aberration, 1 constitutional gonosomal aneuploidy and 19 patients with gonosomal mosaicism. Our data further confirm not only couples prior to ICSI to be at higher risk for chromosomal aberrations but also a subgroup of couples employing other tech

P2-15 03

Phenotype in patients with two CFTR mutations identified prior to TESE

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Homozygosity or compound heterozygosity for CFTR mutations is thought to be the major genetic cause for obstructive azoospermia or severe oligozoospermia. Here we report the clinical data of 5 patients, each with two identified CFTR mutations and the current status of their fertility treatment. Only one of them had previously been diagnosed as a CF patient. Mutational screening for the most common 31 CFTR alleles in the Caucasian population and analysis of the polythymidine tract in intron 8 (IVS8) were performed after genetic counseling. For one of these patients thorough interview during genetic counseling revealed recurrent episodic salt wastage with dehydration, at one point requiring hospitalization for i.v. liquid substitution. After a positive sweat test direct sequencing of the CFTR gene confirmed two CFTR mutations occurring with low frequency in the Caucasian population: I336K and 3359delCT, which had been missed during routine CFTR screening. A serious pancreatitis at the age of 4 years was reported from another patient (compound heterozygous R347P/5T). His maternal grandfather had died of pancreatic cancer after a long history of recurrent unexplained serious abdominal pain attacks, which were also reported from the mother of the index patient carrying the CFTR 5T allele on both chromosomes. Subsequently all patients with two known pathogenic mutations and/or clinical CF minor signs were referred to a CF ambulance for adults for clinical work up and counseling. Sufficient amounts of spermatozoa were retrieved by testicular biopsy in 4 of the 5 patients; 11 IVF/ICSI cycles so far resulted in 2 completed pregnancies. This report further underlines the importance of genetic counseling prior to ICSI which should include the search for atypical CF phenotypes in „healthy" men with oligo- or azoospermia.

P2-18 02

Gain-of-function screen for a systematic functional analysis of X chromosomal genes in Drosophila melanogaster

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Conventional genetic screens are aimed at generating mutations that reduce or eliminate gene functions. On the other hand, it is known from Drosophila and other model organisms that knock-outs do not produce any obvious loss-of-function phenotype in over 60% of the genes. We perform, therefore, a gain-of-function screen: over- or misexpression of genes might identify roles for the products of otherwise phenotypically silent genes.

The basic scheme for our gain-of-function screen is adapted from P. Rorth?s (1996) modular system, combining random P element insertional mutagenesis with GAL4-regulated gene expression. The P vector used is P{MaeUAS.6.11} (Crisp and Merriam, 1997) which carries at the 5? end binding sites for the yeast transcription factor GAL4 close to a basal promoter. This scenario allows for the ectopic expression of genomic sequences adjacent to its 5? end when a second transgene containing an activated GAL4 gene is introduced into the fly genome.

Starting-point of the screen is the mobilization of a single P element that is inserted on a dominantly marked autosome. Currently, more than 15,000 such crosses were performed resulting in over 1,000 lines with an X chromosomal P{MaeUAS.6.11} insertion. Most of these lines were already tested for misexpression phenotypes by introducing a ubiquitously expressed GAL4 gene, P{Act5C-GAL4}. This combination results in detectable phenotypes, e.g. lethality, in over 30% of the cases. Up to now, the insertion sites for about 900 lines are molecularly determined by inverse PCR and hence the overexpressed genes are identified.

The insertions lines serve as starting points for the identification of novel gene functions in Drosophila. Since genes as well as regulatory circuitries are molecularly conserved throughout the animal kingdom this will, eventually, help to unravel the corresponding human gene functions.

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P2-18 03

Comparative Primate Genomics

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Although humans and their closest evolutionary relatives, the chimpanzees differ only in a minute amount of their nucleotide sequence, they are unlike in many morphological, behavioral and cognitive aspects. Here, we explore the underlying molecular principles of those differences in an extensive chimpanzee human omparison project that addresses the question using three different approaches. In a first approach we compared human and chimpanzee genomic orthologous sequences to get an insight into the patterns and dependencies of differences across the genome. 0.1 % of the chimpanzee genome was sequenced in a shotgun approach and was compared to the corresponding human sequence. The average nucleotide divergence was determined as 1.27 %. However the substitutions appear not to be distributed uniformly across the chromosomes. The sex chromosomes differ clearly in their nucleotide divergence from the autosomes, with the X-chromosome howing the least substitutions and the Y-chromosome showing the most between the two species. Further, we found evidence that the distribution of substitutions among the autosomes is not random, suggesting chromosome specific influences on the mutation rates. Second, in order to explore evolutionary patterns in genes, we compared transcribed sequences of the chimpanzee to the corresponding sequences in humans. For this purpose ESTs were generated from chimpanzee testis and brain cDNA libraries for comparison with their human orthologous sequences. Comparing the variation within humans and between humans and chimps, it is striking that the 5'UTR within humans is relatively more conserved than the between humans. Apart from the observation of general patterns of substitutions, we also screened for evolutionarily distinctive genes with an excess of non-synonymous over synonymous substitutions. With our approach we found twenty candidate genes for positive selection out of 1266 coding region alignments screened. Third, in addition to pure sequence analysis we investigated chann the expression of individual genes among primates. Therefore, we compared the transcriptome in blood leukocytes, liver and brain of humans, chimpanzees and macaques using cDNA arrays. With our approach we identified a total of 158 genes that differ significantly in their expression pattern between at least two of the species. Furthermore, while the rates of transcriptome change in liver and blood were generally similar in chimpanzees and humans, it is almost fourfold accelerated in the human brain. The comparison of genomic sequences, EST data and gene expression levels between chimpanzees and humans provides an insight into general evolutionary patterns of primates as and represents a powerful tool to pinpoint genes of importance for either chimpanzee or human specific traits.

P2-18 04

How do imprinted genes correlate with placental dysplasia in mouse (Mus) interspecies hybrids?

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Interspecific hybridisation in mammals results in several consistent hybrid dysgenesis (HD) effects, most notably male sterility and abnormal growth. It can be assumed that other, less obvious HD effects occur. Indeed, in those interspecific hybrids where this has been assessed to date, abnormal placental development was observed. Interspecific hybrid placental dysplasia (IHPD) was analysed in some detail in the two rodent genera Peromyscus (1) and Mus (2) and strong similarities were detected between these groups. Reciprocal placental phenotypes, hypoplasia or hyperplasia, appeared in reciprocal matings; the placental tissue that was mainly affected was the spongiotrophoblast; and placental dysplasia exhibited genetic linkage to the X-chromosome. In Peromyscus only, involvement of the autosomal imprinted, paternally expressed gene Peg3 in the generation of placental hyperplasia was demonstrated (3). The pronounced similarities between Peromyscus and Mus hybrid placental dysplasias suggested that Peg3, or at least loci linked to Peg3, should also be involved in placental hyperplasia in Mus. However, our study, which combined BC analysis, allelic expression (loss-of-imprinting) analysis, and the use of BC males heterozygous at the Peg3 locus, does not support a major role of this imprinted gene in murine IHPD. This finding suggests that different molecular mechanisms have been recruited in comparatively closely related rodent groups to produce indiscernible HD phenotypes. However, our BC analysis has shown that another imprinted chromosomal region exhibits linkage to placental hyperplasia.(1) Rogers, J.F. & Dawson, W.D., J. Reprod. Fertil. 21, 255-262 (1970).(2) Zechner, U. et al., Nature Genet. 12, 398-403 (1996). (3) Vrana, P. et al., Nature Genet. 25, 120-124 (2000)

P2-18 05

Meiotic chromosome behavior in relation to the microtubule cytoskeleton in yeast

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Chromosome pairing is a prerequisite for homologue segregation at meiosis I and contributes to gametogenesis and sexual reproduction. Perinuclear telomere clustering (bouquet formation) occurs at leptotene/zygotene during first meiotic prophase and is thought to facilitate homologue alignment and pairing. We have recently established that the bouquet motif is part of the meiotic pathway in the model organism Saccharomyces cerevisiae. In search for factors that are involved in meiotic telomere movements and since it has been observed that microtubule (MT) poisons adversely affect chromosome movements and pairing in plant and mammalian meiosis, we investigated the course of chromosomal events in the presence of the MT-disrupting drugs benomyl, nocodazole and colchicine. While we failed to detect inhibition of meiosis-specific telomere redistribution, MT disruption inhibited centromere redistribution and homologous chromosome pairing. In support, meiotic telomere movements occurred in a haploid meiosis-competent yeast strain despite the deletion of the telomere component Sir3p and the kinesin-like Kar3p motor protein that has been implicated as a meiotic telomere motor. Our results opt for a role of cytoskeletal components other than microtubules as promoters of meiotic telomeric movements. Finally, the KAR3 disruption rendered less cells entering meiosis, which suggests that Kar3p may be important for other than bouquet functions - possibly in the premeiotic division.

P2-18 06

Phenotypic analysis and chromosomal mapping of ENU-induced mouse mutants with alterations of the immune system

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Analysis of gene function in vivo has mostly been performed by transgenic insertion, inactivation of the respective gene by homologous recombination in embryonic stem cells and gene trapping. We have complemented this approach by isolation of mutant mouse lines with phenotypic alterations of the immune system from ENU-mutagenized mice.
In this phenotypic approach C3HeB/FeJ were randomly mutagenized in two central facilities in Munich using the potent mutagen ethylnitrosourea (ENU). ENU is most potent during spermatogenesis and consequently mutagenesis was performed on male mice which were subsequently mated to female WT mice. Progeny thereof were analyzed for inherited alterations of the immune system using a panel of immunological parameters. Both, F1 heterozygous mice carrying one mutated allele of a given gene (dominant screen) as well as Generation 3 (G3) mice harboring identical mutations in both alleles of a given gene (recessive screen) have been used to establish mutant mouse lines with alterations of the immune system.
With this approach we aim at the discovery and functional characterization of novel genes and pathways which are relevant for the prevention, diagnosis and therapy of human immune deficiencies and auto-immune diseases. At the same time, the mutant mouse lines used to identify these genes and pathways are supposed to serve as mouse models for corresponding human diseases.
Out of more than 10.000 F1 animals and more than 2500 G3 mice analyzed thus far we have established more than 50 mutant mouse lines with dominant and more than 25 mutant mouse lines with recessive phenotypes. Many of those resemble known human diseases like severe combined immune deficiency (SCID), hyper IgM, selective IgA deficiency and different aspects of auto-immunity.
Of these we have analyzed selected lines in more detail and mapped the corresponding mutation. As of today we were able to map the responsible mutation in four lines. The intervals for the respective mutations could be narrowed down to 1-4cM. In one case the mutation could already be identified at the molecular level. Data from these studies will be presented.

P2-18 07

Environmental factors influencing postcoital genetic selection. The depressed sex ratio following the cigarette smoke exposure in C57BL congenic mice differing in alleles of ahr gene occurs in the ahr bb progeny of ahr bb mothers sired by the younger ahr bd males

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The cigarette smoke (CS) induced changes in the fetal resorption rates and sex ratio were assessed in the series of pairings differing in ahr alleles. The frequency of resorptions in standard conditions was higher in females ahr dd (5/30 = 0,17), as compared to females ahr bd and ahr dd (7/61 = 0,11). Following the exposure to cigarette smoke (CS) the number of resorptions was found increased in the groups of ahr bb and ahr bd females up to the frequency 0,17 (7/44), that is equal to the values in the group of unexposed ahr dd females. The CS exposition did not increase the number of resorptions in the group of ahr dd females. This indicates that the low activity ahr d allele does confer resistance to the cigarette smoke induced increase in fetal resorptions.
In the series of pairings ahr bb (female) x ahr bd (male), in which the increased frequency of fetal resorptions was found after CS exposure during the period of pairing and the early pregnancy, we have noted also the decrease in sex ratio (M/all) in the ahr bb progeny as compared to the increased sex ratio in the ahr bd progeny. The greater effect of CS exposure on the sex ratios in progeny differing in ahr genotype was seen in the exposed group sired by the younger males. These effects may be related to the decrease in sex ratio noted among children of the fathers exposed to dioxin when young, as described by Mocarelli et.al. (Lancet, 2000).

P2-18 08

Doppel-transgene Tiermodelle zur Analyse von a-Synuclein, UbcM4 und Elk1 in der Pathogenese des Morbus Parkinson

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alpha-Synuclein spielt eine zentrale Rolle in der Pathogenese der Parkinson'schen Erkrankung (PD). So ist alpha-Synuclein eine Hauptkomponente der intrazellulären Proteinaggregationen bei betroffenen Patienten. Mutationen im alpha-Synuclein-kodierenden Gen führen zu einer seltenen autosomal dominant vererbten Form der Erkrankung. Die normale Funktion von alpha-Synuclein, wie auch die Ursache des Zelltods dopaminerger Neurone in der Substantia nigra pars compacta bei PD-Patienten, sind weitgehend ungeklärt. In vitro wurden Proteinaggregate von alpha-Synuclein identifiziert, die u.a. die MAP Kinase ERK2 und dessen Substrat, den Transkriptionsfaktor Elk1 enthalten.

Wir wollen nun neueste Pathogenesemodelle anhand von doppel-transgenen Tiermodellen (Maus) in vivo überprüfen. Zum einen soll mit Hilfe von alpha-Synuclein(-/-)/Elk1(-/-) doppelknock-out Tieren analysiert werden, in wie weit der MAP-Kinase Weg und nachfolgende Stoffwechselwege speziell des Dopaminstoffwechsels beeinträchtigt sind. Dies könnte wichtige Hinweise auf eine physiologische Funktion beider Gene im Dopaminstoffwechsel ergeben. Bei den jeweiligen einzel-knock-out-Tieren ist ein Verlust dopaminerger Neurone zunächst nicht offensichtlich, jedoch zeigen alpha-Synuclein(-/-) knock-out Mäuse eine gestörte Dopaminfreisetzung. alpha-Synuclein und der Transkriptionsfaktor Elk1 wiederum interagieren mit der MAP Kinase ERK2, so dass eine Beteiligung der durch Elk1 initiierten Genexpressionskaskade im Dopaminstoffwechsel zu untersuchen ist. Andererseits gibt es bisher keine Untersuchungen, ob sich Elk1 wie alpha-Synuclein in den Lewy Körperchen ablagert und damit den MAP-Kinaseweg bei der PD beeinträchtigt.

Wir haben daher alpha-Synuclein-überexprimierende transgene Mäuse mit Tieren mit reduzierter Proteindegradation (UbcM4/UbcH7(-/-)) gekreuzt. Die zu erwartende Komplexität der beeinträchtigten Stoffwechselwege bei den doppel-transgenen Tieren wird gegenwärtig mit Hilfe der Transkriptom- und Proteomanalyse (DNA-Microarrays und MALDI) weitestgehend charakterisiert.

P2-18 09

Phenotyping and linkage analysis of a new mouse line: SMA002

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SMA002 is a dominant mutant mouse line developed from the Munich ENU-mouse-mutagenesis-screen (see also Mammalian Genome Volume 11, July 2000) which is bred on the genetic background of inbred C3HeB/FeJ-mice. From days 8 to12 pp on heterozygous mutants show growth retardation and reduced body weight gain as well as abnormal increased cleaning and scratching behavior. These characteristics make this mutant an interesting model for diseases associated with pruritus in humans, like allergological, auto-immune or nervous disorders. Generally, injuries to the skin through scratching and nibbling do not occur. Histopathological examination of the skin gave no evidence of allergic reaction such as an increased number or degranulation of mast cells. Also the total plasma IgE level was not elevated. Blood samples were collected from 3-month-old mutants and wild-type littermates and were analyzed for clinical chemical, immunological and hematological parameters. The mutants show significant changes in clinical-chemical parameters, such as reduced plasma cholesterol and triglyceride levels. For chromosomal mapping, an outcross was performed by mating male mutant mice to wild-type C57BL/6JIco females. From these crosses, mutant F1 hybrid progeny were backcrossed to wild-type C57BL/6JIco animals. Tail clips from the N2 descendants were taken for isolation of genomic DNA. Twenty DNA samples each from carriers (mutants) and non-carriers (wild-type) were pooled and used for chromosome screening. 60 different microsatellites (polymorphic between C57BL/6JIco and C3HeB/FeJ) were amplified from these pools by polymerase chain reaction (PCR) and analyzed by agarose gel electrophoresis. The SMA002 mutation was mapped to chromosome 13 to the locus between the microsatellites D13Mit20 (35cM) and D13Mit253 (37cM). In this region, Madh5 (Smad5) and Neurod3 are promising candidate genes which are currently being analyzed.

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The Munich ENU-mouse-mutagenesis project: Achievements of the clinical-chemical screen - an update

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The clinical-chemical screen detects new mouse mutants with clinically relevant phenotypes within the offspring of ENU mutagenized founder animals by a combination of clinical, clinical-chemical and hematological investigations. Mice are weighed every other week and examined clinically. Routine diagnostic procedures are utilized to measure hematological and 17 clinical-chemical parameters, including plasma enzyme activities, specific substrates and electrolytes. Additionally we started to analyze spot urine samples for qualitative protein excretion by SDS polyacrylamide gel electrophoresis at the end of 2001. A detailed description of the screening protocol was published in Mammalian Genome Vol 11, No 7, pp 543-546. Until April 2002 about 12000 F1 offspring from mutagenized founder animals were screened for dominant mutations. Among these 285 mice were identified carrying a stable abnormal phenotype. The phenotype was transmitted to their offspring by 62 of these animals, and 58 new mutant mouse lines were established. Four phenotypes got lost due to low penetrance or breeding problems. In the recessive screen 3523 G3 offspring belonging to 153 pedigrees were screened and 64 variants were identified and tested for a mutation by confirmation crossing. Out of these 13 new recessive mutant mouse lines were established. The urinary protein screen identified six mice with abnormal patterns of protein excretion in urine. Two mice died without having produced any offspring. The other four cases proofed not to be inherited. The poster will present an overview of the actual screening achievements, the phenotypes of the lines established and the collaborations set up. More detailed information on two of these lines is presented on separate posters (see Howaldt et al. and Tran et al.).

P2-18 11

Conditional Expression of Human alpha-Synuclein in Mice

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alpha-synuclein, a protein enriched in presynaptic nerve terminals, may have an important role in the development of Parkinson´s disease, dementia with Lewy-bodies and other neurodegenerative diseases, also known as synucleinopathies. To elucidate its involvement in these diseases, we have used the tet-off transactivator system to generate conditional transgenic mice. The tet system allows conditional expression of human alpha-synuclein as a function of oral administration of tetracycline analogs.

To control the expression of human alpha-synuclein in mice and to direct the expression to the brain, we used a tetracycline controlled transactivator (tTA) driven by the PrP gene control elements and a tTA-responsive promotor (PhCMV*-1) linked to the human alpha-synuclein gene.

To analyze the conditional expression of human alpha-synuclein in transgenic mice, we performed Western blot analysis with protein extracts from several tissues and immunostaining of paraffin embedded brains.

We showed that double transgenic mice express human alpha-synuclein in a brain-specific manner, whereas little or no alpha-synuclein protein was detectet in mice carrying only the alpha-synuclein construct. The alpha-synuclein expression in double-transgenic mice was abrogated to basal levels upon administration of doxycycline for 20 days due to the binding of doxycycline to tTA. We currently investigate, wether double-transgenic mice show a neuropathological or behavioral phenotype. The conditional expression of human alpha-synuclein in mice may help to gain insight to the pathogenesis of synucleinopathies. A future challenge is to investigate, if downregulation of gene expression could slow down or stop the progression of the neuropathology. This could facilitate the development of pharmacotherapeutics

P2-18 12

Investigations into the mechanism of retinal degeneration in a mouse model for X-linked juvenile retinoschisis

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Deleterious mutations in RS1 encoding retinoschisin are associated with X-linked juvenile retinoschisis (RS), a common form of macular degeneration in males. Retinoschisin is a retina-specific polypeptide of 24 kDa that is secreted as a disulfide-linked oligomeric protein complex from both the rod and cone photoreceptors and the bipolar cells. The protein consists almost exclusively of a discoidin-like domain that has been implicated in cell adhesion and cell-cell interaction. To gain further insight into the function of retinoschisin and its role in the cellular pathology of RS, we have generated a knock-out mouse deficient in Rs1h, the murine ortholog of RS1. In histologic murine eye sections, there is marked splitting of the inner nuclear layer, overall disorganization of the retinal cell layers with irregular displacement of cells, and later degeneration of the photoreceptors which appears more pronounced in cones. The observed distortion of the retinal layers could be explained by the loss of cell-cell and/or cell matrix interactions, both of which are thought to be mediated by the discoidin domain. We are now interested to investigate whether apoptosis is the final pathway of photoreceptor cell death and currently pursue the developmental time course of the degeneration. In addition, in the Rs1h-/Y retinae we observed disturbed vectorial transport of PSD-95 to the outer and inner plexiform layers, where Rs1h is ordinarily present in high amounts. PSD-95 is a synapse-associated protein and plays a crucial role in the targeting and clustering of ligand- and/or voltage-gated ion channels at synaptic junctions. This has prompted further investigations into developmental aspects of the Rs1h-/Y retina with specific regard to retinal synaptogenesis.

P2-18 13

Generation of a mouse model for Best vitelliform macular dystrophy

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Best vitelliform macular dystrophy (BMD) is an autosomal dominant disorder of the macula characterized by an accumulation of lipofuscin-like material within the retinal pigment epithelium (RPE) and by a progressive loss of central vision. A unique electrodiagnostic feature of BMD is an abnormal electrooculogram indicating that the RPE is the primary site of pathogenesis. The disease-causing gene, VMD2, encodes a 585 amino acid residue protein, named bestrophin, which has 4 putative membrane-spanning domains. To further elucidate the molecular pathogenesis of mutant bestrophin, we directed our efforts towards generating a knock-in mouse model targeting the murine Vmd2 locus. The initial step included identifcation and characterization of the orthologous murine gene. We established the genomic organization of the Vmd2 gene and fully sequenced a 8.2 kb region from exon 3 to the 3'-UTR. The N-terminus of the deduced amino acid sequence is highly conserved between human and mouse (83% identity), while the sequence identity deviates substantially in the C-terminal region (29% identity). Via site-directed mutagenesis, the missense mutation Tyr227Asn was introduced into the murine Vmd2 gene. This mutation segregates in a large Best disease family and is located in an evolutionary highly conserved region. As selection marker for the homologous recombination event, the neor gene was engineered in close proximity to the mutation. The final Vmd2 knock-in targeting construct is composed of a 1.8 kb 5'-fragment from exon 3 to intron 6 containing the mutation, a neor cassette and a 4.2 kb 3'-fragment from intron 6 to intron 9. Prior to electroporation into WW6 murine embryonic stem cells, the construct was verified by restriction enzyme digestions and linearized with XmaI. Currently we are screening for ES colonies harboring the homologous recombination event (Vmd2+/Tyr227Asn). Further steps will include microinjection of positive clones into recipient mouse blastocysts, determination of chimeras and breeding of offspring to test for germline transmission.

P2-18 14

Phenotypic characterization of an ENU-induced mouse mutant: HST001

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The dominant mouse mutant HST001 was identified within the clinical-chemical screen of the Munich ENU-mouse-mutagenesis project by elevated plasma urea concentrations in 3-month-old animals. Since this mutant might be an interesting model for diseases associated with azotemia, a more detailed clinical and pathological characterization was performed. The body weight gain of wild-type mice in comparison with mutant mice is characterized by a significantly steeper slope of the growth curve from day 21 to day 180. The body weights of the wild-type mice are significantly higher than those of the mutant mice, which show progressive emaciation with increasing age. Mutant mice are characterized by progressively increasing plasma urea concentrations and elevated plasma total protein levels, but significantly reduced plasma glucose and triglyceride levels. At the age of 4.5 and 6 months the hemoglobin concentration and the number of erythrocytes are reduced in mutant vs. wild-type mice. Mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and hematocrit (HCT) are also decreased in mutant mice. In mutant mice, the plasma urea concentrations correlate negatively with the hemoglobin concentrations and the numbers of erythrocytes. The histopathological investigation of the kidneys did not show any sign of chronic kidney disease. Azotemia can be caused by reduced urea excretion or increased urea production due to an elevated protein catabolism and is frequently associated with anemia. Therefore the HST001 mouse line is an interesting model to study the causes and consequences of chronic azotemia and the pathogenetic connections of azotemia and anemia. A backcross was set up to map the HST001 mutation by microsatellite linkage analysis.

P2-18 15

Characterization of mouse models for bone and cartilage related diseases from the Munich ENU-Mouse-Mutagenesis Screen

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Within the Munich ENU-Mouse-Mutagenesis Screen several mutants with defects in bone or cartilage development have been detected. In some of these mutants, we performed an in depth phenotypic characterization and high resolution genetic mapping.
Two mutant lines, ALI14 and ALI18 exhibit a swollen limb phenotype at adult stage. In ALI18 and ALI14 heterozygotes become red initially then swollen around 10 weeks of age. Homozygous ALI18 mutants exhibit a more severe phenotype than the heterozygous mice. The swollen hind feet phenotype in homozygotes can be observed earlier, at 4-8 weeks of age, and subsequently severe swelling deforms toes.
In ALI14 homozygotes are sterile. Different genetic backgrounds can repress the phenotype completely.
The recessive mutant KTA041 shows a complex phenotype. Homozygous KTA041 mice are smaller than wild type mice. They have a shorter tail whereas the number of vertebrae is not changed. In most animals the tail is kinky, sometimes quite severe. Some rib vertebrae have abnormal shape. Strongly affected animals have rib fusions in the corresponding ribs.
Mutants are kept on a pure C3HeB/FeJ background, mapping of dominant mutants is carried out via an outcross back cross breeding strategy with C57BL/6Jico mice. Recessive mutants are mapped by outcross-intercross. ALI18 was mapped to mouse chromosome 4. High resolution mapping is in progress. KTA041 was mapped to chromosome 17.
Candidate gene approaches are currently under investigation. The mouse mutant lines ALI14 and ALI18 could serve as models for arthritis in human. Further experiments to prove this hypothesis are underway.

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Disruption of the meiotic chromosome core alters telomere dynamics

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Meiosis is a specialized division type that mediates chromosome pairing, recombination and independent assortment of homologues - vital aspects of sexual reproduction. During meiotic prophase telomeres attach to the nuclear enve-

lope (NE) via a specialized thickening of the ends of the axial cores that run along replicated sister chromatids. This attachment plate likely reinforces chromosome connections to the NE during a prophase stage where telomeres move along the inner nuclear membrane and homologues engage in pairing. Here, we have tested by analysis of Scp3 knockout spermatocytes the role of the axial core in telomere clustering, morphogenesis of the attachment plaque and homologue pairing. Using three-dimensional immunocytology and FISH we show that SCP3-/- telomeres attach to the NE and perform meiosis-specific movements that involve the formation of a chromosomal bouquet. The timing of the latter is altered in that nuclei with clustered telomeres accumulate in mutant meiosis. EM analysis disclosed an altered morphology of the attachment plaques with the conical thickening pointing towards the NE missing at Scp3-/- telomeres. FISH with chromosome-specific probes revealed a defect in homologue pairing. However, when homologues were regionally paired this involved the formation of short stretches of SC. Immunofluorescent staining of meiosis-specific cohesin axes suggests that cohesin cores are not sufficient to generate meiosis-specific chromosome cores and their termini.

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Data Management and Bioinformatics of the Munich ENU-Mouse-Mutagenesis Project

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The ENU Mouse Mutagenesis Screen has been set up as a large scale mutant production, phenotyping and mapping project. It encompasses two animal breeding facilities and a number of screening groups located within the general area of Munich.

In order to guarantee multi-user access, completeness and consistency of the data generated in a such a large-scale project, a professional database management system is a necessity. Our system is based on Sybase Adaptive Server Enterprise 12.5. running on a Hewlett-Packard cluster (2 servers) on HP-UX 11.0. We have developed and implemented the ENU-Screen software system which is called „MouseNet©". It enables database access to the screeners participating in the ENU project via a Graphical User Interfa

ce based on common WebBrowsers and an ApacheWebServer (using Java applet or servlet techniques). MouseNet© consists of three major modules:

1. The first module, the Animal Management System (AMS), records all animals, location of their cages and other data necessary for maintaining a large mouse colony. It enables the animal caretakers to record any changes immediately on-line on the database. The database itself initiates actions by automatically generating work lists.

2. The Result Documentation System (RDS) stores the data generated by the various screens. The screeners are able to display results for selected groups of mice, lines, individuals or their entire genealogy. Depending on the values of the measurements, screeners can post requests for animal stock breeding, additional samples of blood/tissue or confirmation crosses from the core facilities.

3. Within the Sample Tracking System (STS), sample tracking lists are produced and modified. In addition, the storage of samples (e.g. tail clips for DNA extraction, frozen sperm) is being recorded. For example, if a query shows no living animal of the desired phenotype within the animal facilities, information on archived spermata will be displayed instead.

MouseNet© is an essential tool for functional annotation of the data generated in the project. It enables us to link data in appropriate formats to other genomedata resources.

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P2-18 18

A systematic, phenotype-driven mutagenesis for gene function studies: Recent results of the Munich ENU-mouse-mutagenesis screen

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With the completion of the human genome sequence and the prospect of a complete mouse sequence within the near future, a major challange is the systematic determination of gene function in mammals. The growing ENU mouse mutant resource provides a powerful entry point to gene function studies.

Here, we give an update of one of the largest ENU mutagenesis programs in Europe, the Munich ENU Mouse Mutagenesis Project. Since proof-of-principle of a large scale ENU mutagenesis program has been demonstrated during the first phase of the project by focusing on dominant traits, we put our main efforts during the last year on the recessive screen by producing 100 micropedigrees (with 20 G3 offspring/micropedigree) per year. In parallel, we continue to produce about 2000 F1 animals to further isolate novel dominant alleles of known and new genes.

Currently, more than 30.000 mice have been investigated for dysmorphology and blood based parameters. To date, more than 400 mutant lines have been isolated. Novel dominant or recessive phenotypes have been identified with specific abnormalities comprising congenital malformations, biochemical alterations, immunological defects and complex traits such as behaviour or predispositions to allergies.

Mutants of clinical relevance for inherited diseases in human have been further analysed by backcross mapping and genome-wide microsatellite typing. Many mutant lines deriving from this ENU Screen are under detailed phenotypic characterisation and have been proceeded for fine mapping and positional cloning in order to isolate the causative mutation (Kiernan et al. 2001, Vreugde et al. 2002, Graw et al. 2001). Recent mapping data of new mutant phenotypes will be presented.

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P2-19 01

FISH for carrier detection of large deletions in the Factor VIII gene

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Haemophilia A is a common congenital bleeding disorder caused by a deficiency of coagulation factor VIII. The disease is inherited in a sex-linked recessive pattern with an estimated incidence of 1 per 5000-10000 males. The gene encoding factor VIII is large (186 kb) and is located about 1 MB from the telomere in Xq28. Asymptomatic female carriers transmit haemophilia A to 50 % of their male descendants. Large deletions comprise 3 % of the molecular defects in severe haemophilia A. While they can easily be detected in males by PCR and Southern Blot it is more demanding work to determine the presence of a large deletion in a female. The second intact X-chromosome complicates the results of PCR based methods and at a best semi-quantitative results e.g. by Southern Blotting can be obtained. In order to unequivocally assign the status of a potential female carrier of a large deletion we have established FISH (fluorescence in situ hybridisation) for individual F-VIII exons. PCR products containing individual exons and part of their adjacent introns were amplified to generate probes of about 5 kb. After labelling with fluorescent nucleotides the probes were hybridised to metaphase chromosomes of four mothers of patients with known deletions. An X-chromosomal probe of the centromeric region was used as an internal control. All four women turned out to be carriers of the familial deletion. With probes of the deleted exon only one of the two X-chromosomes was labelled. In contrast probes of flanking exons showed signals on both X-chromosomes. The extend of the deletion (as known

from PCR analysis in the patient) could be confirmed by FISH in all cases. In conclusion the FISH-technique is well suited for direct visualisation of a large deletion in a female carrier.

P2-19 02

Suspension fluorescence in situ hybridization (S-FISH) - a versatile technique for interphase analyses

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FISH on human chromosomes in meta- and interphase is a well established technique in clinical cytogenetics as well as for studies of evolutional and interphase architecture. However, all published FISH approaches are based on the airdrying procedure of chromosome preparation. This procedure leads to well spread metaphases on the slide surface, if an adequate humidity in the air is present, and to a flattening of the originally spherical interphase nuclei. It is well-suited for nearly all FISH approaches, however, when interphase architecture shall be studied the flattening of the nuclei may lead to questionable results. Here we present a technique, where the whole FISH-procedure is performed on cell suspension and the cells are brought on a concave polished slide as the final step of the procedure, just before the evaluation. We call this procedure suspension-FISH (S-FISH) and show that it is possible to do 3-D-analyses on totally spherical interphase nuclei or even on three-dimensional metaphases. The S-FISH approach has been tested successfully on 10 different chromosomal suspensions using up to two-color FISH experiments. Evaluation was done by a Zeiss-Axioplan 2 or by a Zeiss Laser Scanning Microscope 510 META. It could be demonstrated (i) that it is possible to perform the complete FISH procedure in suspension, (ii) that it is not necessary for the evaluation of the interphase nuclei that those are fixed (and flattened) on the slide surface, and (iii) that even metaphases can be analyzed in principle three dimensionally by S-FISH. At present we are working on the adaptation of the method for five color FISH experiments. Supported by the Herbert Quandt Stiftung der VARTA AG, the Wilhelm Sander-Stiftung (99.105.1) and the EU (ICA2-CT-2000-10012 and QLRT-1999-31590).

P2-19 03

Characterization of two small supernumerary marker chromosomes by acro/cenM-FISH - first case with partial hexasomy 15pter->15q13

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Cytogenetic analysis performed in a three year old girl resulted in a karyotype 48,XX, +2mar [25/25]. She had the severe mental retardation, microcephaly, postaxial hexadactyly at both hands and at the right foot plus a pachygyry. Such small SMCs often are uneasy to characterize in standard cytogenetic or molecular cytogenetic approaches. 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., 2001, Hum Genet, 108, 199-204). The technique, called cenM-FISH has been extended by the introduction of an additional probe specific for the short arm of all human acrocentric chromosomes called midi54 (described in Mrasek et al., 2001, Cytogenet Cell Genet, 93, 242-248). This acro/cenM-FISH probe set revealed in the present case, that the both SMC were identical derivatives of chromosomes 15 with two specific signals for the centromere 15 specific and the midi54 probe. Additional FISH experiments using the high resolution multicolor banding (MCB) technique and probes specific for the Prader-Willi/Angelman syndrome region (SNRPN and D15S10), respectively, characterized the derivative chromosomes as dicentric iso-chromosomes i(15)(pter->q13::q13->pter). To the best of our knowledge, this is the first case with partial hexasomy 15pter->15q13. Studies to clarify the origin of the derivatives (UPD-analysis) are in progress. In summary, acro/cenM-FISH is a very useful approach for the one step identification of all human chromosomes by their centromeres and acrocentric p-arms. Supported by the Herbert Quandt Stiftung der VARTA AG, the Wilhelm Sander-Stiftung (99.105.1) and the EU (ICA2-CT-2000-10012 and QLRT-1999-31590).

P2-19 04

Genomic instability of distal 9p in families with BRCA2 mutation carriers

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About 5-10% of breast cancer cases are associated with a genetic predisposition to the disease. BRCA2 is one of the most commonly mutated genes in familial breast cancer. The majority of germline mutations are predicted to inactivate the BRCA2 protein. Recent studies show that the BRCA2 protein is involved in homologous recombination and DNA repair. Identical BRCA2 mutations can display different cancer phenotypes and in comparison to population-based studies there is a greater penetrance in high risk families. Thus the individual cancer risk may be determined by the type of mutation and the environmental factors but it is also modified by additional genetic factors. FISH analysis of lymphocytes of families with BRCA2 mutation carriers showed constitutional chromosomal alterations of 9p23-24 including duplications, amplifications and inversions. The 9p rearrangements are complex in all BRCA2 mutation carriers of the families tested. This indicates that this chromosomal region has suffered a number of intrachromosomal recombinations. In the 3 families analyzed the rearrangements identify an overlapping region of recombinations ranging from D9S144 to D9S269. These alterations of 9p were not detectable in a control group of individuals without BRCA2 mutations. Taken together these results suggest an association of BRCA2 mutations with genomic instability in 9p23-24 in at least a number of BRCA2 mutation carriers.
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P2-19 05

A set of high quality FISH probes for the detection of human chromosome imbalances

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Conventional fluorescence in situ hybridization (FISH) is a useful tool for the detection of human chromosomal abnormalities in prenatal and postnatal diagnostics. The quality of DNA probes contributes to signal detection and is important for a confident interpretation of every FISH result. We have developed high quality FISH probes suitable for the identification of major trisomies (21, 13, 18) as well as common structural rearrangements such as deletion of 22q11. Most of our BAC clones were isolated by using the REPuter program which allows to visualize distributions of exact and degenerate repeats with a minimal length of 20 bp. Regions with high gene content and relatively low repeat density were selected and primers were designed for the identification of BAC clones. Furthermore, for improvement of the FISH signals we have assembled clone contigs and used these as complex probe mixtures. These BAC clones were mapped to different regions of chromosome 21 including the centromeric region of 21q11, the Down syndrome critical regions on 21q22, and subtelomeric region of 21q22.3. Several DNA probes were isolated in the region of 13q32, 18p/qtel, 22q11 and subtelomeric region of 22q13. The probe set extremely facilitates the detection of specific chromosome imbalances on uncultured amniotic fluid cells and metaphase chromosomes and is greatly useful for the identification of partial trisomies and partial monosomies.

P2-19 06

Prenatal diagnosis and molecular cytogenetic characterisation of a de novo interstitial duplication 16q11.2~13 - a case report

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We describe the first prenatally detected case of a de novo interstitial duplication of chromosome 16q. This chromosomal aberration is extremely rare. There are only five comparable postnatal reports of duplications involving this chromosomal region. These patients present with little associated dysmorphic features but significant neurodevelopmental delay. Amniocentesis was indicated by advanced maternal age. During pregnancy, ultrasound examinations of the fetus showed no abnormalities. Conventional and molecular cytogenetic analyses on cultured amniocytes by comparative genomic hybridisation (CGH) and fluorescence in situ hybridisation (FISH) using partial chromosome paints and a lo-

cus specific YAC clone revealed a de novo direct duplication of the chromosomal region 16q11. 2q12~13 leading to a partial trisomy 16q (46,XX, dup(16)(q11.2q12~13).ish dir dup(16) (q12)(YAC 744e11++)). After genetic counseling the parents opted for the termination of pregnancy. Postmortem examination showed slight facial dysmorphic signs, minor dysgenesis of the ovaries and an atypical origin of the arteria thyreoida ima.

P2-19 07

Detection of chromosomal methylation patterns using bisulfite treatment and fluorescence in situ hybridisation (FISH)

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Recently, additionally to genetic DNA changes, epigenetic alterations have gained major attention in studies of human diseases including cancer. Epigenetic phenomenon such as differential replicating timing, heritable chromatin structures and foremost cytosine methylation do not affect the base pair sequence but nevertheless regulate the expression of a steadily growing number of genes including many, who are linked to tumorigenesis, i.e. cancer stimulating genes (oncogenes) and inhibiting genes (p16, p53). Methylation of cytosine within CpG islands in gene promotors has been shown not only to be jointly responsible for silencing of genes but also induce mutational events in early tumor progression. The appearance of altered methylation patterns in cancer such as promotor hypermethylation and genome wide hypomethylation may serve as markers in tumorigenesis and enables prediction of the clinical behavior to improve the treatment of individual patients. Different methods have been developed to examine the methylation profile of single gene sequences or to measure the overall content of 5-methylcytosine but none enables to describe the extent and chromosomal location of altered methylation patterns of transformed tissues. In our study, we performed a combination of sodium bisulfite modification of genomic DNA and a subsequent fluorescence in situ hybridisation. For that DNA isolated from human lymphocytes and a colon cancer cell line was treated under conditions whereby unmethylated cytosine are converted to uracil but 5-methylcytosine remains nonreactive. After hybridisation of the purified and nick-translation labelled probes to human lymphocyte metaphases a hybridisation signal of converted DNA can be seen especially in the heterochromatic region of chromosome 1, 9 and 16. After further improvements this technique may provide detailed information about individual tumor characteristics and may enable improved diagnostics and treatment of cancer.

P2-19 08

Mosaic del(22)/r(22): characterization of the derivative chromosomes by multicolor banding (MCB) and region specific probes

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We report on a case of mosaicism for two structural aberrations of chromosome 22 in a 1 2/12 year old girl. The child of healthy, unrelated parents was referred to chromosome analysis because of mild hyperplasia of the left lower leg, mild macrocephaly and mild facial dysmorphisms. Body weight and size were normal. At age of 2 years the right lower leg developed the same mild and slowly progressive hyperplasia as the left one. There is no retardation in mental development.
GTG-banding on chromosomes of the peripheral blood revealed a robertsonian translocation t(14;21) in all 15 cells analyzed and the presence of two clones with different derivative chromosomes 22. The t(14;21) was inherited from the mother, while the chromosomes 22 of both parents were inconspicuous. A detailed characterization of the aberration in the child was done by the multicolor banding (MCB) approach. Thus, the two derivative chromosomes 22 could be characterized as del(22)(q13.2)[26] and r(22)inv dup(p10q13.2)[4], which has been confirmed by FISH-analysis using region-specific single copy probes. This is the second report on mosaicism for structural aberrations of chromosome 22 involving a chromosomal deletion and an inverted duplication combined with a ring chromosome formation, even though the breakpoint in 22q of the presented case is more distal than that reported previously (Bergman and Blenow, Europ J Hum Genet 2000; 8: 801-804).
Supported by the the Willhelm Sander-Stiftung (99.105.1) and the EU (QLRT-1999-31590).

P2-19 09

Aimed manipulation of Breast cancer Cell lines by micro cell mediated chromosome 8 transfer (MMCT) results in suppression of tumorigeneicity

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Cytogenetic and molecular evidence support the existence of a breast tumour suppressor gene(s) on human chromosome 8. To identify a functional role for this chromosome in breast cancer, we introduced a chromosome 8 into the MDA-MB-231 breast cancer cell line. The stable transfer of chromosome 8 resulted in suppression of tumourigenicity in nude mice and reversion of other neoplastic properties of MDA-MB-231 hybrids. The transfer and changes of chromosome material were controlled by micro satellite markers and a wide spectra of cytogenetic techniques like SKY, CGH, FISH-MD, and reverse painting. FISH with labelled mouse DNA exclude the presence of mouse chromosomes in cells after transfer. This procedure explains the extent of transferred chromosome 8 as a complete one, and the complete cytogenetic situation before and after transfer and infection in nude mice. A reduced representation of 8pter-p21 in MDA-MB-231 has compensated by the transferred chromosome 8. Thus, the suppression of growing of MDA-MB-231 correlates with the diploid presence of 8pter-p21. These results provide functional evidence that a breast cancer tumour suppressor gene (s) resides on human chromosome 8.

P2-19 10

Segmental duplication of the region flanking the proximal NF1-LCR in Pongo pygmaeus

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Sequence analysis of the human genome revealed an unexpected high degree of recent segmental duplications, which have accumulated during primate evolution. The dynamic nature of these duplications most probably contributed to variations of intra- and interspecies genomic architecture. Segmental intra- or interchromosomal duplications are implicated in the generation of new or mosaic transcripts, thus contributing to gene evolution. Recent analysis of the entire genome suggests that interchromosomal duplications are enriched in pericentromeric and subtelomeric regions. Aberrant recombination between duplicated genomic segments termed duplicons or Low-Copy-Repeats (LCRs) is the molecular basis of microdeletions or duplications of the genomic region between these repeats in several genetic diseases. In about 5-10% of NF1 patients, interstitial 17q11.2 microdeletions are caused by nonhomologous recombination between 60kb duplicons separated by ~1.5Mb. FISH analysis has shown that the duplicons flanking the NF1 gene region are already present in the genome of the great apes. In the orangutan (Pongo pygmaeus), however, we observed an additional segmental duplication of about 40kb flanking the proximal NF1-LCR. This segmental duplication contains at least two genes and has been transposed into the short arm of the orangutan chromosome 19 (PPY19), which is homologous to HSA17. These findings allude to the dynamic nature of specific segments of the NF1 gene region during primate evolution. Comparative FISH analysis on great ape chromosomes with subregional probes flanking the paracentric inversion of PPY19 revealed that PPY19 is very similar to the ancestral form of this chromosome in hominoids. Therefore, the segmental duplication in PPY 19p might also be ancestral and has been progressively eliminated during the genome evolution of the African great apes and human. This will be investigated by molecular characterization of this segmental duplication and comparative FISH analysis in gibbons and Old World mo

P2-19 11

The Azoospermia region AZFa: An evolutionary view

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The human Y chromosome is unique in its bipartite structure and function. At the telomeres it presents the pseudoautosomal regions (PARs), that undergo meiotic recombination with the X chromosome. These PARs differ from the much larger non-recombining region on the Y (NRY). Besides the testis determining gene SRY, the NRY contains other genes and gene families that play an important role in spermatogenesis. Deletions detected on the Y chromosomes of infertile men gave rise to the definition of three re-

gions, AZFa, b and c, that contain azoospermia factors. Because of the inhibition of recombination, the NRY has evolved into a patchwork of segments that show different stages of sequence homology to the X chromosome, and additional segments that are Y-specific. Boundaries of these X-Y homologous segments may be defined by evolutionary breakpoints, that are marked by differences in the X-Y sequence similarity (Lahn & Page 1999).
Via fluorescence in situ hybridisation (FISH) we have analysed the AZFa-region, important for human spermatogenesis. Its location was defined by a few infertile patients with deletions in Yq11.21. The variability in the extent of these deletions determines its maximal length of approximately 1.4 Mb.
We have mapped a human Y chromosomal PAC contig spanning 2.8 Mb, that includes AZFa by FISH analysis. The hybridisation results for the PAC clones of the classical AZFa differ from surrounding clones due to a lower conservation of the X/Y homology. This allows a new, evolutionary definition of AZFa with a size of about 1Mb. Comparative mapping of the PAC contig clones that form this evolutionary defined human AZFa region to higher primates may result in novel information on the conservation emphazising the importance of AZFa for spermatogenesis.

P2-19 12

Fiber-FISH mapping of MHC genes in the rhesus monkey

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The human MHC-region that spans about 4Mb on the short arm of chromosome 6 in 6p21.3, is essential for the immune system. Its hallmark is the extraordinary polymorphic structure and evolution of this region. The MHC is among the most gene dense regions of the human genome annotated so far. A first complete gene map of the human MHC was published by the MHC-sequencing consortium (1999). From centromere to telomere the human MHC is divided into three regions: MHC class II (1 Mb), class III (1 Mb) and class I (2 Mb), (Campbell and Trowsdale 1993). The region contains more than 220 gene loci of which 128 are predicted to be expressed. In comparison to the human MHC little is known about the organization of the MHC of the rhesus monkey (Macaca mulatta), the primate species most extensively used in laboratory research. As a non-human primate its importance especially increased as it is a valuable model for investigating human diseases such as AIDS. In the present study we tried to shed light on the chromosomal position and genomic arrangement of MHC genes in the rhesus monkey. Using fluorescence in situ hybridization (FISH) we could unequivocally show that the rhesus MHC-region maps to rhesus monkey chromosome 6 in 6q2.4, the orthologous region to human 6p21.3.Furthermore we used fiber-FISH to physically map five genes of the rhesus MHC on chromatin fibers released from cultivated rhesus blood lymphocytes. Thereby we were able to orientate the rhesus MHC and to clear up the internal order of these MHC genes of the rhesus monkey.

P2-19 13

Rad51 protects cells in S/G2 phase from radiation-induced chromosome rearrangements

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In order to assess the effects of Rad51-mediated recombinational repair on chromosome stability, both chromosome-type and chromatid-type aberrations were scored in constitutively Rad51-overexpressing rat fibroblasts and parental controls following treatment with different doses of gamma irradiation. The number of chromosome breaks (gaps, deletions and rings) was the same in the two populations. However, the number of chromatid breaks (gaps and fragments) and exchanges (triradials), which result from double-strand breaks (DSBs) generated during or after replication, was significantly reduced in Rad51-overexpressiong cells.
In a conceptually related experiment, synchronized chick embryo fibroblasts (CEF) were transiently transfected with human Rad51 gene activity or control vector. The frequency of radiation-induced translocations between macrochromosomes was measured by spectral karyotype analysis. After irradiation of S or G2 phase cells with a dose of 6 Gy, the macrochromosome translocation frequenceny was significantly lower in the Rad51-transfected cultures, compared to controls. However, the transfected Rad51 had no protective effect in cells that were irradiated during G1 phase. Collectively, our experiments suggest an increased recombination and repair function of Rad51 in S/G2 phase. Homologous recombination plays an important role in DSB repair in replicating mammalian cells.
In CEF cells the microchromosomes are usually distributed throughout the entire nucleus and, thus, in close physical contact with the macochromosomes, whereas in chicken DT40 cells the microchromosomes are sequestered in the center of the nucleus. The frequency of radiation-induced translocations between microchromosomes and macrochromosomes was significantly higher in CEF than in DT40. Evidently, higher-order nuclear organization provides the structural basis for the formation of cell-type specific chromosome rearrangements.

P2-20 01

Alternative to Southern/Northern Blotting: HPLC plus PCR based technique amenable to full automation

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We have begun to utilize an alternative method for the diagnosis of myotonic dystrophy (MD) that is based on HPLC separation of restriction fragments and subsequent PCR of collected fractions. Mutated MD alleles show expansion of CTG-triplet repeats with up to thousands of added repeats. Normal PCR analysis for repeat expansion is not always possible for this disorder because PCR can amplify only low copy numbers CTG-triplets as it is the case in non-affected healthy probands but not the high copy numbers occurring in MD-patients.
In contrast to the regular PCR protocol with primers spanning the repeats, our PCR primers amplify a 200 bp region located 3´ to the CTG-repeat-stretch. Restriction enzymes are chosen to cut 5`to the repeats and 3` to the PCR region. Thus, both the repeat and the PCR region are on the same restriction fragment. In case of a repeat expansion, the fragment yielded by this restriction-digest is of increased size as opposed to normal alleles. Thereafter the DNA is size separated by HPLC and the expanded fragment is isolated from the wild type allele. The subsequent PCR reaction of all fractions with our 3´primers yields positive products only in the collected fraction consistent with the WT allele and in those fractions containing much higher fragment lengths due to expanded triplet repeats . As added control, the WT allele fragment can be analyzed by the regular PCR reaction that spans the repeats. Conclusion: by using automated HPLC and a laboratory robot for the pipetting steps, all Southern/Northern blotting applications can be replaced by fully automated HPLC plus PCR based kits.

P2-20 02

Preparation and charaterisation of a protein expression library for mouse Th1 long stimulated cells

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In order to make a expression library for mouse TH1 cells, we have directionally cloned the cDNA into a pQE-32-NSTattB vector, which permits IPTG-inducible expression of His6-tagged recombinant proteins. The library was then transformed by electroporation into Escherichia coli SCS1 cells carrying the plasmid pSE111 with the lacIQ repressor and an argU gene, which codes for a rare arginine tRNA. The transformed cDNA library was plated on 22x22cm agar plates and the clones are transferred into 384 well plates by a picking robot. To date, over 80,000 clones have been picked. The average insert size of this cDNA library, as determined by PCR, is 1.5kb. In order to identify clones with an insert in the correct reading frame, the His6-tag expression was examined. First the clones were spotted by a robot onto PVDF membranes. Then, after overnight growth on LB agar, protein expression was induced by IPTG. The membranes were next screened with an anti-His antibody. Aproximately 17% of the total clones in the library were found to express recombinant proteins. These expression clones have been rearrayed to create a protein expression subset.
We have characterised the protein expression library using a number of monoclonal antibodies against proteins expressed in T helper cells. The results of the antibody screening have also been confirmed with both Western immunoblot analysis of individual clones and DNA hybridisation of DNA arrays made from this library, and will be presented (Gutjahr et al., in prep.).

P2-20 03

Distribution of 13 polymorphic sites in the SLC7A9 gene indicates an

involvement of silent variants in the etiology of cystinuria

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Cystinuria is a hereditary disorder of cystine and dibasic amino acid transport across the luminal membrane of renal tubule and intestine resulting in recurrent nephrolithiasis. While mutations in the SLC3A1 gene cause type I cystinuria, patients with non-type I cystinuria carry mutations in the SLC7A9 gene. Both gene products form the renal amino acid transporter rBAT/b0,+AT affected in cystinuria.
In the present study a total of 59 patients with different ethnic background were screened for sequence variations in SLC7A9, out of these 32 were of German origin. For determination of allele frequencies of detected polymorphisms 58 healthy German controls were investigated. Molecular genetic analysis was performed using single strand comformation polymorphism analysis, restriction assays and sequencing. Allele frequencies were analyzed statistically for the detected polymorphisms.
In addition to the 6 already known variants we identified 7 new polymorphisms. Statistical analyses showed a significantly different distribution of alleles between German patients and German controls in case of the polymorphisms c.147C>T (exon 2), c.386C>T (exon 3), IVS3 +22T>G, c.584C>T (exon 4), c.610T>C (exon 4), c.692C>T (exon 5), c.852C>A (exon 6) and c.872C>T (exon 6). These results corroborate previously published data on different frequencies of SLC7A9 alleles in cystinurics and controls and indicate that silent variants in the SLC7A9 gene are involved in the etiology of cystinuria.

P2-20 04

Analysis of components of the GH/IGF axis in Silver-Russell syndrome patients

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Silver-Russell syndrome (SRS) is a heterogeneous clinical syndrome with evidence for a substantial role of genetic factors in its aetiology. Apart from other clinical key features, severe intrauterine and postnatal growth retardation are the dominant characteristics of SRS. Therefore, studies on the genetic basis of the disease aimed on genes involved in growth and its regulation. Another key for the identification of (a) SRS gene(s) is the finding of disturbances of chromosome 7, i.e. structural rearrangements and uniparental disomies, in nearly 10% of patients; a putative SRS gene on chromosome 7 can therefore be assumed.
In approximately 5-10% of SRS patients, a growth hormone (GH) deficiency can be observed, one third of patients response to GH treatment.
Considering these different observations, we decided to perform an exhaustive analysis of members of the GH/insulin-like growth factor (IGF) axis, mutations in which should cause growth disturbances and GH excretion abnormalities. In a group of more than 40 SRS patients, we analysed the genes for GHRH, GHRHR, GH and further members of the GH cluster including CSH1, IGF2, IGFBP1, IGFBP3, IGF1R, IRS1, GRB2 and GRB10 for genetic variants. Additionally, in case of GHRHR, GH, IGF2, IGFBP1, IGFBP3, IGF1R and GRB10 the chromosomal localisation on chromosome 7 or other candidate regions further indicates an involvement in the etiology of growth disturbances.
However, apart from polymorphic sites and hemizygosity of CSH1, we did not detect relevant pathogenic variations in the investigated genes. It can therefore be concluded that mutations in further, so far unknown factors, are involved in growth regulation and the etiology of SRS. Investigations on such genes are still in progress.

P2-20 05

Cystinuria in children: spectrum of mutations in SLC3A1 and SLC7A9

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Cystinuria is a common inherited disorder of defective renal reabsorption of cystine, ornithine, lysine and arginine leading to nephrolithiasis. Two responsible genes have been identified so far: Mutations in the SLC3A1 gene encoding the heavy chain rbAT of the renal cystine transport system rbAT/b0,+AT cause cystinuria type I, while variants in SLC7A9, the gene of its light chain b0,+AT, have been demonstrated in non-type I cystinuria. In this study, we searched for mutations in both genes in a cohort of children with cystinuria.
We analysed 21 cystinuric children from 16 families by mutational analysis of the genes SLC3A1 and the SLC7A9. The patients were classified by the urinary amino acid excretion profile of their parents. Additionally, we screened 10 unclassified patients for genomic variants. The screening techniques included single strand conformation polymorphism analysis, restriction assays and direct sequencing.
Two novel mutations were identified in SLC3A1 and three in SLC7A9; three were missense mutations and two frameshift mutations. In the pediatric patients, we could demonstrate mutations in 54% of type I (SLC3A1) and in 25% of non-type I (SLC7A9) chromosomes. For this group of patients a total detection rate of 46,6% for mutations in both genes could be delineated. In the cohort of unclassified 10 patients, 70% of mutations could be determined. M467T and G105R were the preponderant mutations in SLC3A1 and SLC7A9, respectively; T216M was the major mutation in Turkey and Greece.
Interestingly, the detection rate for mutations in SLC3A1 and SLC7A9 in classified cystinuric children was lower than that in 10 further patients with a nonclassified cystinuria, although the clinical characterisation in the first group was more stringent; additionally, different spectrums of mutations were observed. The lack of detectable mutations in many patients indicate the possibility of other yet unidentified genes involved in cystinuria. In contrast to recent publications, we could not correlate the severity of the disease to the type of cystinuria in the pediatric patients.

P2-20 06

The LGI1 gene involved in lateral temporal lobe epilepsy belongs to a new subfamily of leucine-rich repeat proteins

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Recently mutations in the LGI1 (leucine-rich, glioma inactivated 1) gene have been found in human temporal lobe epilepsy. LGI1 presented the first evidence that genes not apparently coding for ion channels can cause idiopathic epilepsy. We have now identified three formerly unknown LGI-like genes (LGI2, LGI3, and LGI4). Hydropathy plots and pattern analysis showed that LGI genes code for proteins with an N-terminal extra- and an C-terminal intracellular domain connected by a single transmembrane region. The phylogenetic analysis demonstrated that the four human LGI peptides and their murine counterparts are closely related to each other, while other leucine rich repeat-containing proteins were placed more distantly on the evolutionary scale. The branching pattern of the dendrogram strongly suggested that the four LGI proteins belong to a formerly unknown subfamily of LRR proteins. The distribution patterns of LGI-mRNA expression in different tissues were studied by semi-quantitative PCR with cDNA from different tissues. All four LGI genes are expressed in brain as well as in several other tissues. In silico mapping and radiation hybrid experiments assigned LGI2, LGI3, and LGI4 to different chromosomal regions (4p15.2, 8p21.3, 19q13.11). Besides the involvement in epileptogenesis LGI1 is suspected to act as a tumor-suppressor gene. It was therefore interesting to see if the homologous genes hLGI2-hLGI4 are located in candidate regions for epilepsy genes and/or in regions showing loss of heterozygosity in different malignant diseases. - (Gu et al., FEBS Letters, in press)

P2-20 07

Refinement of the Mapping Region and Further Delineation of the Wittwer Syndrome

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A XLMR-syndrome mapping in Xp22.3 was previously described by Wittwer et al. (Am J Med Genet 64: 42-49). The spectrum of clinical symptoms includes prenatal and severe postnatal growth retardation, blindness due to microphtalmia or optic atrophy, moderate to severe hearing loss, dysmorphic features, epilepsy, and severe mental retardation with absence of speech, as well as anomalies of the urogenital system, gut and lungs. This family was reexamined for a more exact mapping of the underlying gene. To the previously found clinical symptoms progressive skeletal lesions with osteoplastic and osteoclastic changes were identified in the proximal

femora as well as vertebras. Haplotype analysis using 23 microsatellite markers on Xp22 localized the disease locus between DXS8095 and DXS7108 comprising 3.9 - 6.1 Mb. Within this interval, at least one MRX locus involved in contiguous gene-deletion syndromes has been suggested. Because the possibility of a contiguous gene syndrome has to be considered in Wittwer syndrome, PCR analysis of the known genes and ESTs in the critical interval was performed. No deletion was detected, suggesting that a contiguous gene deletion is rather unlikely in Wittwer syndrome.

P2-20 08

Homozygosity Mapping of a Second Gene Locus for Hereditary Combined Deficiency of Vitamin K-Dependent Clotting Factors (FMFD) to Chromosome 16

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Familial multiple coagulation factor deficiency (FMFD) of factors II, VII, IX, X, protein C and protein S is a very rare bleeding disorder with autosomal recessive inheritance. The phenotypic presentation is variable with respect to the residual activities of the affected proteins, its response to oral administration of vitamin K and to the involvement of skeletal abnormalities. The disease may result either from a defective resorption/transport of vitamin K to the liver, or from a mutation in one of the genes encoding g-carboxylase or other proteins of the vitamin K cycle. We have recently presented clinical details of a Lebanese and a German family with ten and four individuals, respectively, where we proposed autosomal recessive inheritance of the FMFD phenotype. Direct sequencing of gamma-carboxylase and microsomal epoxide hydrolase revealed no mutation. Biochemical investigations of vitamin K components in patients' serum showed a significantly increased level of vitamin K epoxide, thus, suggesting a defect in one of the subunits of the vitamin K 2,3-epoxidase reductase (VKOR) complex. We now have performed a genome wide linkage analysis and found significant linkage of FMFD to chromosome 16. A total maximum two-point LOD score of 3.4 at theta = 0 was obtained in the interval of 3 cM. In both families patients were autozygous for more than 20 markers in this region, suggesting inheritance by descent(IBD).

P2-20 09

Comparison of the Human Genome Sequence Data to Genetic and Physical Maps of a 20 Mb Interval on Chromosome 16

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The competing efforts to sequence the entire human genome have resulted in two draft sequences generated by the Human Genome Project (HGP) and Celera Genomics (CEL), respectively. Upon global comparison, the two sequence-based physical maps are similar in size and quality, predict a similar number of genes, and are in good agreement with the existing genetic maps. In the course of a positional cloning approach, we have examined in more detail a 20 Mb region of chromosome 16 and found significant discrepancies. We have compared a YAC-based physical map covering this chromosomal region with the draft sequences of HGP and CEL, the genetic map of Marshfield and the computed metrical map of the Location Database (LDB). With respect to locus order, the YAC map was in good agreement with the Marshfield map, whereas both sequence drafts required some „inversions" for adjustment. Many discrepancies were found in the LDB map. Possible reasons for these disagreements are problems with assembly in the physical maps, duplications that are known to occur frequently on chromosome 16 or errors in genetic and combined maps, respectively. In particular, the generation of secondary maps by computational integration of primary data appears to be error-prone. If a similar level of accuracy were to apply to other chromosomal regions it will still take some time to establish „the definitive map" of the human genome.

P2-20 10

Homogeneity and distinctiveness of Polish paternal lineages revealed by Y chromosome microsatellite haplotype analysis

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Different regional populations from Poland were studied in order to assess the genetic heterogeneity within Poland, investigate their genetic relationship with other European populations and provide a population-specific reference database for anthropological and forensic studies. Nine Y-chromosomal microsatellites were analysed in a total of 919 unrelated males from six regions of Poland as well as in 1273 male individuals from nine other European populations. AMOVA revealed that all of the molecular variation in the Polish dataset is due to variation within populations, and no variation was detected among populations from different regions of Poland. However, in the non-Polish European dataset 9.3% ($P < 0.0001$) of the total variation was due to differences among populations.Consequently, differences in pairwise RST-values between all Polish populations were notstatistically significant, whereas significant differences were observed in nearly all comparisons of Polish and non-Polish European populations. Phylogenetic analyses demonstrated tight clustering of Polish populations separated from non-Polish groups. Population clustering based on Y-STR haplotypes generally correlates well with the geography and history of the region. Thus, our data are consistent with the assumption of homogeneity of present-day paternal lineages within Poland and their distinctiveness from other parts of Europe, at least in respect to their Y-STR haplotypes.

P2-20 11

Molecular diagnostics in transcobalamin II deficiency

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Transcobalamin II (TC II) deficiency is an autosomal recessively inherited rare disorder of cobalamin metabolism. Most patients present with combined methylmalonic aciduria and homocystinuria. Here, we report on two patients of turkish and lebanese origin and a consanguineous background. Both patients presented during the first weeks of life with failure to thrive, vomiting, muscular hypotonia, and also pancytopenia. Treatment with parenteral cobalamin resolved all symptoms and signs.
Using data bank analysis (GenBank), the entire structure of the TC II gene including all exons with their flanking regions was elucidated. Hereby, the diagnosis of TC II deficiency could be confirmed by genetic analysis of cDNA and gDNA in the two patients. In the lebanese patient, a 2148 bp deletion was detected by gDNA sequence analysis. This mutation causes a loss of exon 7 of the TC II transcript. The turkish patient showed the mutation Ivs 4-175 A>T. This mutation results in a new 5' splice site, leading to an insertion of 87 bp in the TC II transcript and premature termination.
Until now, only 3 patients with 5 mutations of the TC II gene were described. Since the exact gene structure was not known, found deletions could not be determined precisely. The complete characterization of the entire TC II gene structure will facilitate molecular diagnostics in the future.

P2-20 12

Methylation sensitive-PCR improves PCR testing of myoclonus epilepsy - type Unverricht Lundborg

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The Unverricht-Lundborg type of progressive myoclonus epilepsy (EPM1; MIM 254800) is an autosomal recessive disorder. It is caused by the functional impairment of the cystatin B gene due to various point mutations or an expansion of a dodecamer repeat within the untranslated 5'-region of the gene. Both expanded and mutated alleles can occur in a homozygous form or may concur in compound heterozygotes. The status of the dodecamer repeat is usually determined by Southern blot and PCR analyses, the latter of which is, however, significantly hampered by the high CG content of the respective repeat region. We have developed a MS-PCR strategy to assess the EPM1- methylation and expansion patterns in the cystatin B promoter and repeat regions. We show that the promoter of the cystatin B gene is unmethylated in both normal and expanded alleles and, therefore, exclude that de novo methylation is part of the disease process. In contrast to the FRAX syndrome, methylation patterns can therefore also not be exploited for

diagnostic procedures. However, as expected, we found that DNA deamination significantly improves the PCR conditions for the amplification of the GC-rich repeat region. Thus, we were able to reliably amplify fully expanded alleles in a series of homo- as well as heterozygous individuals. We therefore conclude that our MS-PCR approach is a simple and fast alternative that can replace the laborious Southern-blot analysis for the evaluation of the EPM1-associated dodecamer repeat expansion.

P2-20 13

Random X-inactivation in sporadic and hereditary Medullary Thyroid Carcinoma (MTC)

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We have set up a methylation sensitive PCR assay for determination of X-skewing using two different microsatellite markers. FMR1 CGG repeat and HUMARA CAG repeat which are located on Xq27.3 and Xq11.2-q12, respectively have been used for dignostic as well as clonality and X-inactivation studies within different approaches using standard PCR –amplification. Based on bisulfite deamination of DNA we have designed a unique quantitiative PCR assay to amplify both unmethylated and methylated alleles within single reactions for both repeat markers. Quanitification of MS-PCR products using both markers allows us to determine the ratios of X-skewing from almost all cases informative either for one of both markers. To elucidate the effect of germline mutations of the RET protooncogene, predisposing for hereditary forms of medullary thyroid cancer (MEN2, FMTC), with regard to clonal selection and expression of disease in patients we investigated the X-inactivation patterns in different groups of female patients suffering from hereditary and sporadic medullary thyroid (sMTC). In addition we investigated X- skewing in unaffected RET-mutation carriers, predisposing for hereditary disease and healthy non-carriers. By our approach we could only find some females with sligthly X-skewing, without any significant difference between hereditary and sporadic MTC patients as well as mutation carriers and non-carriers. Thus we conclude that there is no clonal selcetion with respect to the mutator-phenotype in blood of patients.

P2-20 14

Comparative genomics of the TWIST region in human and Fugu rubripes

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One of the most common craniosynostosis syndromes, Saethre-Chotzen syndrome (SCS, MIM 101400), is caused generally by intragenic mutations of TWIST. However, several cases of SCS are associated with chromosomal rearrangements that leave the TWIST gene intact. Furthermore, patients with SCS and significant learning disability, are described to have a chromosomal microdeletion in 7p21 of approx. 2.4 Mb including the TWIST locus.
To identify long-range regulatory elements around human and Fugu rubripes transcription factor gene TWIST we compared 2.4 Mb and 60 kb of sequence around human and F. rubripes TWIST, respectively. Besides TWIST, we identified only six and five protein coding genes in the human and Fugu sequences, respectively. The Fugu Twist showes all common features of the TWIST protein family, i.e. the basic-helix-loop-helix-, and WR-domain, respectively.
TWIST and three additional genes of F. rubripes have been mapped on human chromosome 7p21.1, whereas two genes were localised on human chromosome 3p25 in the same direction and orientation. All genes located on 7p15 showed high conservation in their exon-intron structure. Most of the coding exons are identical in size, whilst most of the introns are dramatically compressed in Fugu. The order of genes between human and Fugu is nearly conserved with the exception of HDAC9 which is missing in the analysed Fugu genomic region and a mirrowed position of LOC168829. By sequence comparison between human, mouse and Fugu, we identified conserved non-coding sequences in the 3´-end of human TWIST which give evidence for cis-regulatory elements.
Ch.Kosan is a fellow of the GRK767 supported by the Deutsche Forschungsgemeinschaft

P2-20 15

Identification of a new missense mutation in WFS1 in a family with low frequency sensorineural hearing impairment

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We have previously reported a three generation German family with an autosomal dominant low frequency sensorineural hearing impairment (LFSNHI) linked between the genetic markers pter-D4S431-D4S432-cen (Brodwolf et al. 2001) to the non-overlapping interval DFNA6 and DFNA14 (Lesperance et al. 1995; Van Camp et al. 1999) on chromosome 4p16. The audiograms show consistently a hearing threshold of 50 ± 20 db hearing loss (HL) between 250 Hz and 1-2 kHz. Normal hearing level is reached between 3 and 6 kHz in all examined children. Adult patients show an additional hearing impairment (HI) in the mid and higher frequencies that seems to differ from presbyacusis. The HI is always bilateral and symmetrical. Recently, missense mutations were identified in the WFS1 gene, which was found to map to this chromosomal interval, segregating in three families with LFSNHI (Bespalova et al., 2001, Young et al., 2001). Consequent screening of the WFS1 gene in our family resulted in the identification of a new heterozygous missense mutation 2285G>C resulting in an amino acid exchange K705N. This sequence variant was not found in 100 control chromosomes.
The gene WFS1 is known to be responsible for Wolfram syndrome type 1 or DIDMOAD, an autosomal recessive disorder characterized by diabetes mellitus, diabetes insipidus, optic atrophy, and deafness (Inoue et al. 1998, Strom et al. 1998). Based on only a few data it is remarkably, the hearing impairment in WFS1 patients affects especially the high frequencies (Higashi et al., 1991), but it has been reported that in families with Wolfram syndrome an increased risk of sensorineural hearing loss is shown in heterozygous carriers. Functional studies are in progress to determine the role of WOLFRAMIN during the hearing process. The upcoming results should clarify the pathogenic effect of mutations in this protein.
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P2-20 16

Mutations in the TYR and P gene in individuals with oculocutaneous albinism

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Oculocutaneous albinism (OCA) is a genetically heterogeneous disorder with autosomal recessive inheritance. Caused by phenotypic variability clinical diagnosis of the albinism type is difficult. In contrast, genetic analysis allows precise classification and may be helpful for counselling of the patients.
Here, we report the mutation profile detected by molecular genetic analyses of the genes responsible for OCA1 and OCA2. OCA1 is caused by mutations in the tyrosinase gene (TYR) encoding the key enzyme in melanin biosynthesis. We investigated the 5 exons of the TYR gene of 176 unrelated patients by PCR and SSCP and found variations in 66 individuals (47 patients with 1 variation, 17 patients with 2, and 2 in 3 patients). OCA2 resulting from mutations in the human homologue of the mouse pink eye gene is the most common type of albinism world-wide. Analysing exons 2 to 25 of the P gene by PCR and SSCP aberrant signals could be detected in 36 persons (34x1, 1x2, 1x3 variations). In 11 patients conformational deviations were detected by SSCP in both the TYR and the P gene. In 85 patients (48.3%) no mutations were found. In our sample of German origin about 37.5% of the patients show SSCP changes in the TYR gene and 20.5% in the P gene suggesting that mutations at the latter locus are possibly underrepresented in Caucasians. The mutation profile presented here was detected by sequence analysis following SSCP screen.

P2-20 17

Three members of a new gene family of low-copy repeats in Xp22.3 expressed exclusively in testis: implications for recombinations in this region

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Illegitime recombinations between low-copy repetitive elements (LCR) have been implicated in the pathogenesis of various chromosomal rearrangements. In Xp22.3, two such duplicons have been reported previously, the CRI-S232 elements, involved in the generation of deletions in the steroidsulfatase gene, and five members of the G1.3 (DXF22S) repetitive sequence family. By molecular characterization of an Xp22/10q24 in a female patients translocation, we identified a duplicon of the G1.3 family in the breakpoint region in Xp22.3. We show that G1.3 harbours at least three expressed genes,

FAM9A, FAM9B, and FAM9C, and three putative pseudogenes, all mapped to Xp22.32-p22.31. The open reading frames of the three genes show about 80% pairwise identity and 46-65% identity at the amino acid level. FAM9A, FAM9B, and FAM9C are expressed exclusively in testis. By transient transfections of EGFP-FAM9 constructs, we found that all proteins were located to the nucleus, whereas FAM9A to the nucleolus, in COS7 cells. Each of the proteins encoded by members of this novel gene family show about 30% identity to SYCP3, a component of the synaptonemal complex located along the paired chromosomes during meiosis. Thus, although the function of the proteins is not yet known, they might play a role in meiosis. It has been speculated that, in addition to the high sequence homology of LCRs, other recombination promoting factors may also be present within duplicons, e.g. genes/pseudogenes expressed in germ cells. Indeed, FAM9B was found in the X-chromosomal breakpoint region of the Xp22/10q24 translocation. It is tempting to speculate that active transcription of FAM9B results in an open chromatin structure stimulating recombination in meiotic germ cells of the patient's father that might have promoted the recombination event resulting in a translocation between chromosomes X and 10.

P2-20 18

X-linked mental retardation with isolated growth hormone deficiency

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The aim of the project is to identify the underlying gene defect(s) in two families with X-linked mental retardation (XLMR) and isolated growth hormone deficiency (IGHD). Linkage studies narrowed the interval to Xq24-Xq27.3 and Xq22-Xq27.1 (Hamel et al., 1996, Am. J. Med. Genet. 64:35, Raynaud et al., 1998, Am. J. Med. Genet. 76:255). We first established a complete contig of the Xq26.1-Xq26.3 region by using GenBank and Celera sequence informations. This region was chosen as a starting point because a duplication in Xq25-q26 and Xq26-q27 is present in two families with a similar phenotype to IGHD, X-linked panhypopituitary (XPH) dwarfism (Lagerström-Fermer et al., 1997, Am. J. Hum. Genet. 60:910; Hol et al., 2000, Genomics. 69:174). Subsequently, candidate genes of the Xq26.1-Xq26.3 region were analysed for their expression in pituitary and hypothalamus RNAs. Genes expressed in these tissues were further analysed by mutation analysis and semi-quantitative RT-PCR. With the latter technique, one gene was found to be overexpressed in the lymphoblastoid cell line of a patient with XLMR and IGHD. Expression studies in other affected and non-affected family members are in progress.

P2-20 19

PREVALENCE OF SMALL REARRANGEMENTS IN THE FACTOR VIII GENE

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Haemophilia A is a common X-linked bleeding disorder caused by various types of mutations in the factor VIII gene. The most common intron 22-inversion is responsible for about 40% of the severe haemophilia A cases while large deletions, point mutations and small (less than 100 bp) deletions or insertions are responsible for the disease in the rest of patients.

We report on nine novel (6 deletions, two indels and one partial duplication) and five recurrent small rearrangements identified in 15 German patients with severe haemophilia A, negative for the intron 22-inversion. c.2208-2214delTTATTAC / c.2207-2215insCTCTT and c.4665-4678del / c.4664-4678insAAGGAA identified in the present study are the first small indels described in the factor VIII gene. Our analyses suggest that the prevalence of this type of mutations (predominantly located in exon 14) among patients with severe phenotype and negative for the common intron 22-inversion, is about 30%. The correlation between these molecular defects and formation of factor VIII inhibitors as well as the parental origin of the de novo mutations are evaluated. Finally we show that denaturing HPLC (DHPLC) and classic heteroduplex analysis (HA) are able to detect these sequence alterations on 100% and could be preferred as a screening approach when analysing for mutations in factor VIII in severely affected patients.

P2-20 20

Genotyping of allelic variants of the human hemoglobin beta gene (HBB) by MALDI-TOF mass spectrometry.

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Allelic variants of the hemoglobin beta gene (HBB) are involved in different human disorders and are highly prevalent especially in the Mediterranean population. While the nucleotide substitution FS6 A—>T leads to sickle cell anemia, absence or reduced amounts of beta chain due to various frame shift mutations or nucleotide substitutions cause b-thalassemia. Due to the high prevalence of sickle cell anemia and b-thalassemia, the HBB alleles represent important clinical targets for high throughput screening. In this context, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) based genotyping offers a promising technical alternative to common electrophoretic and chromatographic techniques. Following PCR amplification using genomic DNA as a template, allelic variants of the HBB gene were determined by allele specific elongation and termination of extension primers. Primer extension products were analyzed by MALDI-TOF-MS. The nucleotide substitutions, deletions and insertions of the HBB gene were genotyped by this approach. Using MALDI-TOF-MS, the allelic variants were unequivocally and reliably detectable either alone or in combination (multiplex). MALDI-TOF-MS based genotyping results were confirmed by conventional sequence analysis. Due to its accuracy and molecular resolution, this procedure can serve as an alternative method for clinical genotyping of HBB alleles in the first screening round.

P2-20 21

TFNR is a subunit of TFIIIB and interacts with a novel zink- finger protein

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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. 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 poymerase 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, cerebral cortex 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.

The N-terminal part of TFNR reveals a strong protein interaction to a novel zinc-finger protein identified by yeast two hybrid screens. The zinc-finger protein interacts with the first 640 aa of TFNR. It encodes a protein that contains three zinkfinger domains (N-terminus) and one POZ/BTB domain (C-terminus). Northern blots revealed a transcript length of ~ 5,5 kb in all tested human tissues. An antibody generated in rabbit shows a stronger expression in human brain and cerebellum on western blots. In immunofluorescence stainings a colocalisation with tubulin stained structures can be seen. Coimmunoprecipitation and functional analysis of the novel interactor are in progress.

Human and mouse antibodies from the N- and C-terminus of TFNR were established and will allow ontogenetic and functional analysis of TFNR.

P2-20 22

Multiple cases of sporadic brest and ovarian carcinomas in BRCA-positive families

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Aim: Since 1997, 221 families with hereditary breats and/or ovarian cancer were testet for mutations in the BRCA1 and 2 gene in our center. In high risk families, each patient was tested. The aim of this study was to analyse, if families with high penetrances also show multiple cases of sporadic breast and ovarian carcinomas indicating modifying factors.

Material/Methods: DNA of patients from 221 families was analysed using PCR reaction followed by the DHPLC method (Denaturing High Pressure Liquid Chromatography) or direct sequencing. In high risk families (* 2 patients < 50y.

or patients with breast and ovarian cancer in one family), if possible all index cases were analysed. Results: In 221 tested families, 41 pathological mutations and 33 unclassified variants were identified. 34/41 pathological mutations were found in high risk families. However, in five of these families, additional non-carrier patients were identified. The age of onset was 34, 39 and 42y. in three cases of breast carcinomas and 39 and 46y. in two cases of ovarian carcinomas, respectively.
Conclusions: In 14,7 % of high risk families with mutations non-carrier patients were identified with a striking low age of onset and the occurence of ovarian carcinoma cases.
We postulate, that in these families modifying factors exist, which result in a higher rate of „sporadic" breast and ovarian carcinomas. Therefore, these families are an excellent tool to identify modifying factors who may also be involved in sporadic breast and ovarian carcinogenesis.

P2-20 23

Molecular evolution of FOXP2, a gene involved in the development of speech and language

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Language is a uniquely human trait likely to represent a prerequisite for the development of human culture. It has become clear in recent years that aspects of speech and language development are likely to be influenced by genetic factors. Furthermore, the ability to develop articulate speech relies on capabilities, such as fine motoric control of the larynx and mouth1, which are absent in chimpanzees and other great apes. Recently, FOXP2, the first gene of relevance for the human ability to develop language, has been described2. A point mutation in FOXP2 cosegregates with a disorder in a large family in which half of the members have severe articulation difficulties, accompanied by linguistic and grammatical impairment3. In addition, this gene is directly disrupted by a translocation in an unrelated individual who has a similar disorder. Thus, two functional copies of FOXP2 appear to be required for acquisition of normal spoken language. In order to investigate the evolution of FOXP2, we sequenced the cDNAs that encode the FOXP2 protein in chimpanzee, gorilla, orangutan, rhesus macaque and mouse, and compared them to the human cDNA. In addition, we investigated the intraspecific variation of the FOXP2 gene in humans. The results strongly suggest that FOXP2 has been the target of selection during recent human evolution.

P2-20 24

Molecular characterization of a chromosome 1;22 translocation in a patient with Costello syndrome: implication for the genes of the PDGF and PDGFR families in the trait

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Costello syndrome is a rare congenital disorder characterized by postnatal growth deficiency, mental retardation, „coarse" face, loose skin of the neck, hands, and feet, cardiomyopathy, and nasal papillomata. Autosomal dominant inheritence of the trait with de novo mutations has been suggested whereas the genetic basis of the syndrome is yet unknown. We are investigating a female Costello patient carrying an apparently balanced translocation t(1;22)(q25;q13.1). By FISH analysis, cosmid LL22NC03-10C3 was found to overlap the breakpoint on chromosome 22. Sequence analysis revealed that the gene encoding the beta-subunit of platelet-derived growth factor, PDGFB, is located on the insert of the cosmid. By RACE- and RT-PCR, we identified three different PDGFB-fusion transcripts in the patient containing either the complete PDGFB coding region or only part of it suggesting that the patient is a mosaic and that the PDGFB gene is disrupted in a portion of her cells.
PDGFB belongs to a family including PDGFA, PDGFC, and PDGFD, that have important functions as paracrine growth factors in development. The various platelet-derived growth factor isoforms exert their effects on target cells by activating two structurally related protein tyrosine kinase receptors, alpha and beta, encoded by PDGFRA and PDGFRB. Thus, PDGFB as well as all other ligands and receptors of the PDGF family have been considered candidate proteins/genes for Costello syndrome. Mutation screening in all a.m. PDGF(R) genes in 18 Costello patients has identified so far an amino acid change (Val722Phe) in PDGFRB in one patient, and another one (Val1028Ala) in PDGFRA in a second patient. Although neither of the changes has been found in 100 controls their pathogenetic relevance remains to be determined. In conclusion, our data suggest that the PDGF and PDGFR genes are not the major genes involved in Costello syndrome.

P2-20 25

A case from a routine CF diagnostic lab: Complex deletion/insertion involving exon 1 of the CFTR gene in a patient with paternal uniparental isodisomy of chromosome 7

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Large deletions may account for some cases with cystic fibrosis (CF) in whom the underlying mutation of the CFTR gene cannot be identified. Here, we report on a girl severely affected by a pancreatic insufficient form of CF whose sample was provided for routine molecular genetic testing of the CFTR gene. Mutations were not detected by screening for the 33 most common CFTR gene mutations or by sequencing exons 2-24 of the CFTR gene. Exon 1, however, could not be amplified by the commonly used exon-flanking PCR primers. Narrowing the critical region with several other primer pairs revealed an unexpected longer PCR product instead of the predicted exon 1 amplicon. Sequence analysis identified a substitution of 120 bp, including the whole exon 1 except for the start codon, by a sequence of 300 bp from a reverted portion of the downstream intron. This unusual mutation, termed 135del120ins300, may have been generated by incorrect DNA repair. While the patient appeared to be homozygous for this allele, analysis of her parents confirmed this mutation in the father but not in the mother. Haplotype analyses with 13 different microsatellite markers throughout chromosome 7 demonstrated a paternal uniparental isodisomy of chromosome 7 in the patient. Two consecutive rare events thus explain the homozygous appearance of a probably rare deletion/insertion mutation in the absence of consanguinity. Cases like this may be taken into consideration when routine diagnostics fails to identify CFTR gene mutations in either a clinically confirmed CF patient or one of the parents.

P2-20 26

A novel type of mutation causes a splicing defect in ATM

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Disease-causing splicing mutations described in the literature are mainly concerned with changes in splice sites and, to a lesser extent, with variations in exonic regulatory sequences such as the enhancer elements. In the ATM cDNA of an affected patient we have identified a 65 bp aberrant exonic inclusion caused by deletion of four internal nucleotides in intron 20. Surprisingly, these four bases were located 12 bp downstream and 53 bp upstream from the borders of the cryptic exon, respectively. Analysis of the splicing defect using a hybrid minigene system led to the identification of a novel Intronic Splicing Processing Element (ISPE) complementary to U1 snRNA. This element exerts a positive effect for accurate intron processing and interacts specifically with U1 snRNP particle. The deletion completely abolished this interaction, causing the activation of the cryptic exon. In conclusion, a new type of intronic U1 snRNP binding site performs an essential function for accurate intron removal. Deletion of this sequence is directly involved in a splicing processing defect in a human disease.

P2-20 27

Molybdenum cofactor-deficient mice resemble the phenotype of human patients and a suitable therapy model

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The molybdenum cofactor (MoCo) is essential for the function of the molybdoenzymes sulfite oxidase, xanthine dehydrogenase, and aldehyde oxidase. Therefore, mutations of genes involved in MoCo biosynthesis lead to the inactivation of molybdoenzymes. In humans, MoCo deficiency is a rare and devastating autosomal-recessive disease for which no therapy is known. Affected patients show neurological abnormalities such as attenuated brain growth and neonatal seizures. Neurological damage due to sulfite toxicity, sulfate deficiency, or a combination of both, is irreversible, and many patients die shortly after birth. Because most patients harbor mutations in the MOCS1 gene, we generated mice with a targeted disruption of the murine MOCS1 gene by homologous recombination. A targeting vector was constructed to delete exon 3 which encodes a highly conserved domain from bacteria to man. As in humans, heterozygous mice display no symptoms, but homozygous animals die between days 1 to 10 after birth. Homozygous knock-out mice appear externally normal, but are smaller and have a hyperextended posture as compared to their littermates. Biochemical analysis shows that they do not possess any molydopterin nor active MoCo and sulfite oxidase activity is undetectable. The knowledge gained about the genetics of MoCo deficiency suggests that a small amount of MoCo activity is sufficient for a normal phenotype. The described animals therefore are suitable animal models to try biochemical and/or genetic therapy approaches.

P2-20 28

Stearoyl Co-A desaturase gene family: candidates for a sever mouse mutation associated with hair loss

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Stearoyl Co-A desaturase (SCD) is a microsomal key enzyme required for the biosynthesis of oleate and palmitoleate, which are the major monounsaturated fatty acids of membrane phospholipids, triglycerides and cholesterol esters. Alteration of the ratio of saturated to monounsaturated fatty acids is involved in various diseases,such as: non-insulin-dependent diabetes mellitus, neurological disorders, immune diseases, cancer and obesity. There are three isoforms of SCD in the mouse genome, which are highly conserved in their coding regions. However, the expression pattern of these genes is tissue-specific, SCD1 gene being highly expressed in liver, SCD2 gene-in brain, and SCD3 gene-mainly in skin. SCD genes are localized as a cluster on mouse chromosome 19. Here, we report a severe spontaneous mutation, associated with hair loss. Linkage analysis has revealed that the DNA marker D19Mit 119 is closely linked to the SCD locus. This new mutation is named pelota naked (pn), as compared to asebia (ab) or scraggly (sgl) mutations, which are exhibiting skin and hair defects, and are also associated with mutations in SCD genes. The pelota naked mutation is inherited in an autosomal dominant manner, as opposed to the asebia and scraggly mutations. Northern blot studies on liver tissues from the homozygous pelota naked animals have shown that SCD1 transcript is lacking from this tissue, as opposed to the SCD2 transcript, which is normally expressed in the brain of these mutant animals. This result suggest that SCD1 is inactivated in the homozygous animals. To address the question if this gene is involved in the phenotype of the pelota naked mice, we are performing molecular analyses on SCD1 gene, along with anatomo-pathological studies, in order to detect any malformation at the tissue level (liver, pancreas, brain, skin). SCD1 gene is required for the normal function of skin and eyelid,being also involved in the cholesterol homeostasis and lipid metabolism. Therefore, any new mutation in the SCD1 gene might contribute to our knowledge about the fatty acids metabolism, and pelota naked mice could be a reliable animal model for this purpose.

P2-20 29

Missense substitutions differentially affect the function of ATM by dominant interference in patients with breast cancer

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Ataxia-telangiectasia (A-T) is characterised by hypersensitivity to ionizing radiation and an elevated risk of malignancy. Epidemiological data support an increased risk for breast and other cancers in A-T heterozygotes. It has been hypothesized that ATM missense mutations are implicated in breast cancer. To investigate the functional significance of these changes we have introduced missense substitutions, identified in either A-T or breast cancer patients, into ATM cDNA prior to establishing stable cell lines to determine effect on ATM function. Pathogenic missense mutations and neutral missense variants were initially distinguished by their capacity to correct the radiosensitive phenotype in A-T cells. Furthermore missense mutations abolished the radiation-induced kinase activity of ATM in normal control cells, caused chromosome instability and reduced cell viability in irradiated control cells, whereas polymorphic variants failed to do so. This approach represents a means of identifying genuine ATM mutations and addressing the significance of missense changes in the ATM gene in a variety of cancers including breast cancer.

P2-20 30

Pseudo-dominant inheritance of Mal de Meleda (MDM), or transgressive palmoplantar keratoderma of Siemens, with mutations in the gene for SLURP-1

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Mal de Meleda (MDM), or transgressive palmoplantar keratoderma of Siemens, is a hereditary skin disorder characterised by diffuse palmoplantar keratoderma (PPK) and transgressive keratosis. There is no involvement of other organs but a rather broad spectrum of clinical presentations with other features is characteristic. MDM was first described in patients from the isle of Mljet (Meleda) in Croatia where a founder effect was supposed to be responsible for MDM. Recently, mutations in the ARS (component B)-81/s gene on chromosome 8q24-qter were identified in patients with MDM. We have shown that a very similar phenotype of transgressive PPK is not linked to chromosome 8q24-qter in several families from the United Arab Emirates. Here we present further families with transgressive PPK. We identified four novel mutations in ARS (component B)-81/s in consanguineous families from Turkey, Palestine, and the United Arab Emirates: two different mutations both resulting in the amino acid change G86R, and a mutation that alters the translation initiation codon. In a German family without known consanguinity originally supposed to have a dominant form of transgressive PPK we recognized pseudo-dominant inheritance. Three children and their affected mother were homozygous for mutation W15R while the unaffected father was heterozygous. Pseudo-dominance was confirmed by analysis of several neighbouring microsatellites. Our findings show that the MDM type of transgressive PPK may be caused by SLURP-1 mutations in patients from various origins, and allelic heterogeneity was demonstrated for mutations in SLURP-1.

P2-20 31

NOVEL MUTATIONS IN LAMIN A/C GENE AND AUTOSOMAL DOMINANT EMERY-DREIFUSS MUSCULAR DYSTROPHY

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The LMNA gene on 1q21.2-q21.3 encodes nuclear lamina proteins lamin A and C. Mutations in the lamin A/C gene are responsible for 4 different disorders: autosomal dominant Emery-Dreifuss muscular dystrophy (EDMD); limb-girdle muscular dystrophy type 1B (LGMD1B); Dunnigan-type familial partial lipodistrophy (FPLD) and dilated cardiomyopathy and conduction-system disease (CMD1A). Surprisingly, mutations in these nuclear envelope proteins lead to muscular dystrophy.
Here we report 4 different mutations in the lamin A/C gene, associated with EDMD phenotype. Three of them are new ones: exon 3 583A—>G (Asn195Asp), exon 6 1067T—>G (Leu356Arg), exon 6 1144G—>C (Gly382Arg). The fourth mutation in exon 9 1583C—>A (Thr528Lys) was detected in 3 unrelated patients. The mutations in exon 3 and 6 fall in the helical rod domain of the protein and according to the published data, are expected to provoke dramatical aberrant localization of the lamin A/C in many cells. The mutation in exon 9 affects the globular tail of the protein and most probably disrupts protein-protein interactions.
Our present knowledge on mutations distribution along the LMNA gene and their effect on the protein and/or clinical phenotype support the

speculation that the lamins A/C do not play one simple role in the nuclear envelope, but most probably different parts of these proteins are engaged in structural and/or regulatory functions.

P2-20 32

Mutation analysis in autosomal recessive polycystic kidney disease (ARPKD/PKHD1)

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Recently two groups independently identified a so far unknown gene responsible for autosomal recessive polycystic kidney disease (ARPKD) on chromosome 6p. The gene which we named polyductin extends over at least 470 kb and includes a minimum of 86 exons that are assembled into a number of alternatively spliced transcripts. The longest continuous open reading frame encodes 4,074 amino acids. The gene products share structure homology with hepatocyte growth factor receptor and plexins which belong to a superfamily of proteins involved in the regulation of cell proliferation, adhesion and repulsion. However, the definite gene function has still to be unravelled.

Thus far, we screened 78 patients diagnosed with ARPKD for polyductin mutations and identified a total of 81 mutations making up a detection rate of about 50%. We were able to disclose two underlying mutations in 33 patients and only one mutation in 15 cases. Mutations were found in all areas of the gene but were three times as frequent in the 5' half of the gene compared with the 3' half. In detail the mutations were as follows: 37 missense mutations (46%), 24 nonsense mutations (30%), 18 frameshift mutations (22%), and two splice site mutations (2%). Thus, about half of the changes is predicted to truncate the protein. One recurrent missense mutation in exon 4 most probably represents a mutational hotspot. Two founder alleles were present in the Finnish population responsible for about 60% of mutations in this cohort. Genotype/phenotype correlations could be established for the type of mutations rather than for the individual mutation.

The mutation detection rate of about 50% is comparable to that achieved for other large multiexon genes showing the feasibility of direct genetic diagnosis in ARPKD. A more effective approach for mutation analysis of this huge gene is currently worked out with the future aim of offering prenatal diagnosis based on direct genotyping.

P2-20 33

Nucleotide diversity patterns within ten X-chromosomal genes in humans and chimpanzees

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Humans and chimpanzees differ in a number of cognitive capabilities, some of which may be of fundamental importance for the differences in culture between the two species. In order to take a first step towards studying genes that may be of relevance for these differences, we have investigated the pattern and extent of nucleotide diversity in 10 X-linked genes where mutations in humans are known to cause mental retardation in humans. In each gene, we sequenced about 3kb in 20 humans sampled world-wide and 10 chimpanzees representing two subspecies. Nucleotide variability within and between species was low, suggesting a high level of constraint at these loci. Overall nucleotide diversity is about two-fold lower in humans than in chimpanzees. In contrast to previous studies, we find no correlation between diversity and recombination at these loci. Strikingly, we find that a summary of the allele frequency spectrum is significantly correlated in humans and chimpanzees, perhaps reflecting very similar levels of constraints in the two species. A possible exception is FMR2, which shows more non-synonymous than synonymous substitutions on the human lineage, suggesting the action of positive selection.

P2-20 34

SALL1 mutation analysis in Townes-Brocks syndrome - an update

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Townes-Brocks syndrome (TBS, MIM 104780) is a rare autosomal dominantly inherited malformation syndrome characterized by anal, renal, limb, and ear anomalies. TBS has been shown to result from mutations in SALL1, a human gene related to the developmental regulator sal of Drosophila melanogaster. Studies of its chick homologue Csal1 suggest regulation of Sall1 expression by Sonic hedgehog, Wnt3A/7A, and FGF4/8 signaling pathways. The SALL1 gene product is a zinc finger protein thought to act as a transcription factor. It contains four highly conserved C2H2 double zinc finger domains which are evenly distributed. A single C2H2 motif is attached to the second domain, and at the amino terminus SALL1 contains a C2HC motif. The protein is exclusively found in the nucleus and localizes to pericentromeric heterochromatin, acting as a transcriptional repressor. Here we present 7 yet undescribed mutations of SALL1 and an updated genotype-phenotype correlation. One of the novel mutations is positioned at the 3' end of exon 2 and was found in a girl also mosaic for trisomy 8. Another mutation is strongly associated with eye defects in another family. Together, 28 out of 29 SALL1 mutations known to date are located in exon 2, 5' of the third double zinc finger encoding region. These are nonsense mutations, one of which causes TBS in nearly half of the sporadic cases, short insertions and short deletions as well as one gross intraexonic deletion. One mutation within intron 2 creates an aberrant splice site. All mutations lead to preterminal stop codons and are thought to cause the phenotype via haploinsufficiency. However, Sall1 knock out mice do not show the TBS phenotype but homozygotes die from kidney malformations commonly seen in TBS.

P2-20 35

Treacher Collins-Syndrome: Molecular Spectrum, Genotype-Phenotype Correlation in 42 Patients

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The Treacher Collins-Syndrome (TCS, Franceschetti-Syndrome, MIM #154500) is an autosomal dominant disorder of craniofacial development associated with mutations in TCOF1 on chromosome 5q32-q33.1. Phenotypic expression is variable and is characterized by downward slanting of the palpebral fissures, lower lid coloboma, hypoplasia of the zygomatic arch and mandible, external and middle ear anomalies, sensorineural hearing loss and cleft palate.

The TCOF1 codes for the 1411 amino acid protein treacle which is a nucleolar phosphoprotein. It is composed of a 213 residue N-terminus followed by 10 repeated units with potential phosphorylation sites and a C-terminus with multiple putative nuclear localization signals. To date, 51 distinct mutations have been reported. To explore the phenotypic spectrum associated with mutations in TCOF1, we performed mutational analysis in 42 patients with tentative diagnosis of TCS. We identified heterozygous mutations in 25 of 35 patients with an unequivocal clinical diagnosis of TCS, five of them being familial cases. In another seven patients with doubtful diagnosis of TCS we did not identify a mutation.

Of a total of 25 mutations, 23 are novel. The mutational spectrum comprises 12 frameshift, 5 nonsense, 5 splice site, one missense mutation and one insertion of a lysin. In addition, we identified a de novo base substitution affecting the start codon (M1I) in a patient with severe expression of TCS. The missense change is located in the first of the 10 repeated units and leads to a substitution of a serine for an asparagine (S266N). Both amino acids have uncharged polar side chains. However, serine contains a polar hydroxyl group that may serve as a phosphorylation site.

We did not observe any correlation between location and nature of the mutation and phenotypic expression. Moreover, mutation carriers within families showed variable expression ranging from mild to severe phenotype. In conclusion,

our data indicate that there is no obvious phenotype-genotype correlation.

P2-20 36

The pseudoautosomal region is a fast evolving region in primate genomes

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The pseudoautosomal regions are located at the tips of human X and Y chromosomes. They are identical on both chromosomes and recombine during male meiosis. Several human pseudoautosomal genes have paralogous genes located at different autosomes. Analysis of the genomic region of the pseudoautosomal protein phosphatase gene PPP2R3L revealed that this genomic region is highly polymorphic in human with 1 SNP in approx.
150 bp, whereas the autosomal PPP2R3 gene has less than the average 1 SNP in 1 kbp.
Analysis of PPP2R3 and PPP2R3L in gorilla, chimpanzee and orangutan revealed that exchanges during evolution are more frequent in the pseudoautosomal than in the autosomal gene. As this is true for both exons and introns we suggest that differences in selective pressure arenot the reason for these differences.
Due to the high GC content of pseudoautosomal genes DNA variability was expected to occur predominantly within CpG
dinucleotides. Analysis revealed that differences in C to T transitions are not sufficient to explain differences of variability. Further reasons may be the genomic localization together with the elevated recombination rate within the pseudoautosomal region.

P2-20 37

X-linked mental retardation with isolated growth hormone deficiency

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The aim of the project is to identify the underlying gene defect(s) in two families with X-linked mental retardation (XLMR) and isolated growth hormone deficiency (IGHD). Linkage studies narrowed the interval to Xq24-Xq27.3 and Xq22-Xq27.1 (Hamel et al., 1996, Am. J. Med. Genet. 64:35, Raynaud et al., 1998, Am. J. Med. Genet. 76:255). We first established a complete contig of the Xq26.1-Xq26.3 region by using GenBank and Celera sequence informations. This region was chosen as a starting point because a duplication in Xq25-q26 and Xq26-q27 is present in two families with a similar phenotype to IGHD, X-linked panhypopituitary (XPH) dwarfism (Lagerström-Fermer et al., 1997, Am. J. Hum. Genet. 60:910; Hol et al., 2000, Genomics. 69:174). Subsequently, candidate genes of the Xq26.1-Xq26.3 region were analysed for their expression in pituitary and hypothalamus RNAs. Genes expressed in these tissues were further analysed by mutation analysis and semi-quantitative RT-PCR. With the latter technique, one gene was found to be overexpressed in the lymphoblastoid cell line of a patient with XLMR and IGHD. Expression studies in other affected and non-affected family members are in progress.

P2-20 38

TGGE Screening in Marfan Syndrome and Related Fibrillinopathies: 53 FBN1 mutations

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The Marfan syndrome (MFS) is an autosomal dominant heritable disorder of connective tissue with highly variable clinical manifestations, caused by mutations in the gene for fibrillin-1 (FBN1). The cardinal features occur in the skeletal, ocular and cardiovascular system.
We screened 126 individuals with MFS, other type-1 fibrillinopathies, and other potentially related disorders of connective tissue for FBN1 mutations, by developing temperature-gradient gel electrophoresis (TGGE) assays for all 65 FBN1 exons. We identified a total of 53 mutations. Mutations were identified not only in persons with classic Marfan syndrome but also in individuals with neonatal Marfan syndrome, atypically severe Marfan syndrome, isolated aneurysm of the ascending aorta, predominant ectopia lentis, and several individuals with only skeletal and ocular involvement. The overall mutation detection rate of 42% is typical of that reported for studies in large heterogeneous patient populations.
Together with the 53 mutations characterized in our study, a total of 337 FBN1 mutations have been published to date. The mutations are spread over almost the entire FBN1 coding sequence, and most of the mutations have been identified in only one individual or family. The only well established genotype-phenotype correlation is the finding that almost all mutations in individuals with neonatal Marfan syndrome occur in exons 24-32, the so-called neonatal region of FBN1. We have analyzed our results together with other published results for potential genotype-phenotype correlations. Although the total number of mutations is still too low to allow statistically significant conclusions to be made, our analysis suggests correlations of subsets of mutations at the 5' and 3' ends of FBN1 with relatively mild clinical phenotypes lacking aortic dissection.

P2-20 39

Evidence for a novel locus for human malignant infantile osteopetrosis on chromosome 6q21

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Human malignant infantile osteopetrosis (OMIM #259700) is a severe autosomal recessive disease characterized by osteosclerosis and pancytopenia which become apparent during the first months of life. Causative mutations have recently been identified in the a3 subunit of the H+-ATPase or the voltage-gated chloride channel CLCN7, demonstrating that inadequate bone resorption by dysfunctional osteoclasts is the basis of this disorder in the majority of cases. In a small inbred family with malignant infantile osteopetrosis we could not find a mutation in either of the two known osteopetrosis genes. We therefore started a candidate locus approach to elucidate the causative gene defect in this family. By microsatellite analyses we excluded homozygosity for several candidate gene loci which cause osteopetrosis in knock-out mice, among them c-FOS on chromosome 14q24, c-SRC on 20q11, OPGL on 13q14, and CSF-1 on 1p13. In addition, we investigated a locus on chromosome 6q21, that shows conserved synteny to a region on mouse chromosome 10 to which the locus for the osteopetrotic mouse „grey-lethal" has been mapped. We could demonstrate homozygosity in a region spanning approximetly 15cM between markers D6S1717 and D6S287 in the affected child. In this region, several functionally attractive candidate genes are located, like e. g. Fyn-related-kinase (FRK), BET3, and MARCKS. Currently, we are performing a systematic mutation screening of candidate genes in this region. In summary, we provide evidence for a novel locus for human infantile malignant osteopetrosis on chromosome 6q21 which probably represents the human ortholog of the murine „grey-lethal" locus.

P2-20 40

DNA methylation analysis by MALDI-TOF

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Epigenetic phenomena - defined as any gene-regulating activity that doesn't involve changes to the DNA code and that can persist through one or more generations - are manifested in DNA methylation, histone acetylation or methylation and formation of matrix attachment regions.
The connection between DNA methylation and gene activity is widely recognized. Methylation of cytosine occurs mostly in a CG content. Aberrant DNA methylation of CpG sites is among the earliest and most frequent alterations in cancer. CpG methylation can be analyzed by transferring the 5 base code (including 5-methylcytosine 5mC) in to a 4 base code via a bisulfite treatment. During this treatment cytosine is deaminated while 5-methylcytosine stays unaffected. A subsequent desulfonating step leads to an uracil or an unchanged 5-methylcytosine, respectively. The information „methylated cytosine" or „not methylated cytosine" is transferred into a C/T(U) polymorphism. This polymorphism can be analyzed with standard SNP detection assays.
Here we present a MALDI-TOF based methylation assay based on the methylation specific hybridisation of PNA probes to complementary target sequences on standard glass slides. Glass slides offer the advantage that many samples can be attached by standard arraying techniques and are available for simultaneous analysis. Oligonucleotides representing bisulfite-converted DNA were covalently bound to different activated surfaces and subsequently probed with charge tagged PNA probes. Due to its uncharged backbone it is suitable for MALDI analysis. The modification with a tertiary ammonium function (charge tag) enables highly sensitive MALDI analysis in the positive ion mode. Charge tag PNA libraries containing up to six different

PNA´s were synthesized. After hybridisation of the entire library the PNA´s are bound to their complementary DNA sequences. Each charge tag PNA has an individual mass. Therefore, the methylation status of the probed DNA can be concluded from the mass of the cooresponding PNA detected by MALDI-TOF analysis.

P2-20 41

Characterization of a novel brain specific transcript on human chromosome 20q13

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The human chromosome 20q13 segment (HSA20q13) is known to correspond to distal mouse chromosome 2 (MMU2) as they show a conserved synteny of gene loci. Uniparental disomies for distal MMU2 result in different neonatal lethalities with opposite anomalous phenotypes strongly suggesting the presence of imprinted genes in this region and therefore on the syntenic HSA20q13 as well. We have identified a novel human gene within the region of interest whose expression appears to be restricted to the brain as Northern analysis of 8 tissues revealed expression of a 3.2 kb and a 3.0 kb transcript in brain only. The corresponding cDNA (AJ311122) contains a 1680 bp ORF distributed on 13 exons spanning a genomic region of approximately 250 kb. The homologous mouse cDNA (AK005136) contains a 1680 bp ORF either, and multiple tissue Northern analysis has revealed 3 major transcripts of 3.3 kb, 2.9 kb and 2.5 kb visible in brain only emphasizing the human expression pattern. During detailed expression analysis using tissue from 8 different brain regions a complex pattern has been detected concerning the quantity of every single transcript depending on analyzed tissue. A developmental specific expression pattern has been found during embryogenesis displaying weak signals from day 10 pc and strong signals from day 15 pc onwards to adult mice suggesting a function from late development. Characterizing possible gene function, database search using the translated human gene product revealed homology to 3 hypothetical human proteins of yet unknown function sharing a striking homology of approximately 120 aa at the C-terminal end suggesting the existence of a shared domain. This homology seems to be conserved down to D. melanogaster and C. elegans. This work was supported by a grant of the DHGP.

P2-20 42

Association of HPC2/ELAC2 genotypes and prostate cancer in the German population and screening for other potential tumor suppressor candidate genes

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A candidate prostate cancer (PCa) susceptibility gene on chromosome 17, HPC2/ELAC2, was described by Tavtigian et al. (2000). They described linkage to chromosome 17 in PCa families. Germline frameshift, nonconservative missense mutations and disease-associated common variants were found in this gene. However, in one family the disease did not segregate clearly with the mutation. Two common missense mutations S217L and A541T were identified and the authors demonstrated that the strongly linked L217/T541 variants occur more frequently in PCa cases than in age-matched controls. This association was confirmed in several studies, but no association was found in others. We have analyzed the allele frequencies in 219 German PCa patients and 195 controls. No association of the L217/T541 genotype with PCa was found in our study. In our search for a PCa tumor suppressor gene on chromosome 10 we have identified the smallest region of overlapping deletions. This region spans 3.2 Mbp and oligonucleotides were deduced from all predicted exons. These will be arrayed onto glass slides and hybridized with normal/tumor RNA pairs to identify genes that are turned off in PCa. In addition, expression of known genes located in this region was analyzed by RT-PCR using RNA extracted from paraffin embedded tumor samples. For this purpose, we designed primer sets resulting in short PCR products. Some of these genes were downregulated or shut off in tumors compared to adjacent normal tissue.

P2-20 43

Designing & performing genotyping assays with a flexible probing tool

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Having the sequence of the human genome finished and more than 500 sequencing projects ongoing, analysis of the genetic diversity represents one of the next frontier of genome research. With more than 60.000 estimated SNPs falling within exons the next step will be to identify those SNPs or combination of SNPs that have a strong correlation to genetic disorders. To attack that great challenge flexible probing tools are needed.
Employing febits Geniom technology that allows microarray fabrication, hybridisation, detection and analysis within one single benchtop instrument, genotyping assays are easily set up.
Designing customized SNP arrays starts directly from the gene sequences holding the SNP to be investigated that are found in public databases. Having selected of up to 250 SNPs that may be screened in parallel into a spreadsheet, software tools will generate an input file for the geniom instrument, which will perform the synthesis of the SNP-array. One of the current designs follows a tiling scheme where each SNP is represented by 24 oligonucleotide probes including screening for both the sense and the antisense strand.
Sample preparation starting from PCR fragments is performed by employing terminale transferase and Dnase for labelling and fragmentation. During transferase reaction a biotin moiety is incorporated with in the target sequences. Fragmentation of these products to appox. 150 bp in length results in best performance during hybridisation. Detection is achieved through a strepatavidine-fluorophore conjugate.
With the CCD based detection many fluorophores can be employed and two color detection permits the comparison of two samples within one experiment. After generation an intensity raw data file from the resulting fluorescence image, the sequence at the analysed SNP position is given together with additional statistics. We have applied this kind of SNP-typing scheme to the genetic analysis of various variants of the green fluorescence protein (GFP). Furthermore, a broad lymphoma case control study - 600 patients and 600 controls - was recently started in collaboration with the German Cancer Research Center (DKFZ), looking at some 100 appropriate SNPs in parallel.

P2-20 44

A new quantitative PCR Multiplex Assay for rapid analysis of HMSN / HNPP

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A 1.4-Mb duplication containing the gene for peripheral myelin protein 22 (PMP22) on chromosome 17p11.2-12 is responsible for 70 % of the cases of the demyelinating form of Charcot-Marie-Tooth disease (CMT1A/HMSN), whereas a reciprocal deletion of the same region causes the hereditary neuropathy with liability to pressure palsies (HNPP). The CMT1A duplication increases the gene dose from two to three, the deletion reduces the gene dose from two to one. Previously HMSN / HNPP patients were mainly diagnosed by analysis of polymorphic markers in-between the repetitive elements flanking the PMP22 gene and by fluorescence in situ hybridisation (FISH). We now developed a new real-time qantitative PCR analysis assay for rapid determination of gene dosage. The method involves a multiplex reaction using newly designed FAM labelled Taqman-Probe with TAMRA quencher derived from PMP22 exon 3 and a VIC labelled probe with non-fluorescent quencher from exon 12 of the Albumin gene as internal reference. Reaction was carried out in a single tube on a Taqman 7900HT. Copy number of the PMP22 gene was determined by the comparative threshold cycle method (DDCt). Each sample was run in quadruplicate and analysed at two different threshold levels. The level giving the smallest standard deviation was scored. We evaluated this method through the retrospective analysis of 250 HMSN patients with known genotype and could confirm the previous findings in all cases. Thus this method exhibits comparable sensitivity to microsatellite analysis for duplication and FISH for deletions while it has the advantage of being a fast and uniform assay for both HMSN / HNPP.

P2-20 45

Spectrum of mutations found in 92 cases with Noonan syndrome (NS)

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Noonan-syndrome (NS, MIM 163950) is a well recognized autosomal dominant multiple malformation syndrome with an estimated incidence of

1 in 1,000 to 2,500 live births. Affected individuals have proportionate short stature and a characteristic facial appearance with hypertelorism, ptosis, downward slanting palpebral fissures, and low set posteriorly rotated ears. In addition, cardiac involvement, most commonly pulmonary valve stenosis, is frequently seen. NS is genetically heterogenenous. Linkage to a 5 cM region on chromosome 12q24 has been reported previously and only recently, mutations in the protein tyrosine phosphatase gene PTPN11, have been described. In a small sample, missense mutations were found in 50% of NS cases (Tartaglia, M et al., Nat. Genet. 29: 465-468, 2001). We have screened PTPN11 for mutations in 92 clinically well characterized familial and sporadic NS cases identified 16 different missense mutations in 32 (35%). Six mutations, 4 in the N-SH2 domain and 2 in the PTP domain, are novel and have not been described before. In addition, we found 4 mutations (Asp61Gly, Tyr63Cys, Ala72Ser, Asn308Asp) repeatedly in apparently unrelated cases. Most mutations cluster in the SH2 domain at the N-terminus (N-SH2), which acts as a molecular switch between the inactive and active protein form. No PTPN11 mutations were detected in 5 patients with cardio-facio-cutaneous syndrome (CFC), which shares many phenotypic similarities with NS.

P2-20 46

Towards elucidation of the disease mechanism underlying Sorsby fundus dystrophy (SFD)

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SFD is a late onset maculopathy caused by mutations in the tissue inhibitor of metalloproteinases-3 (TIMP3). In contrast to the other members of the TIMP family TIMP1, 2 and 4, TIMP3 is insoluble and localised to the extracellular matrix (ECM). The protein consists of 211 amino acids with 12 cystein residues forming 6 intramolecular disulfid bridges. All known mutations introduce potentially unpaired cystein residues in the C-terminus of the protein resulting in the formation of higher molecular weight protein complexes. We have now generated knock-out as well as knock-in mice carrying a disease-related Ser156Cys mutation in the ortologous murine gene. To study the nature and content of the higher molecular weight protein complexes and their possible role in the etiology of SFD we have established immortalized fibroblast cell lines from the knock-out and knock-in mice. The composition of the protein complexes was analysed by mass spectrometry. In addition, Western blotting was used to investigate the turn-over rate of the mutant protein in the ECM. Further, we applied a lactic acid and other assays to study the metabolic changes due to the mutation. Immunocyto-chemical techniques were used to analyse cell morphology. To compare functional activities of the normal and mutant protein we used direct and reverse zymograms. Taken together, our study demonstrates a higher amount of TIMP3 in ECM from mutant fibroblasts compared to normal. Moreover, homozygous Ser156Cys fibroblasts have a decreased mitochondrial activity. These data are supported by the lactic acid assay revealing 1.5 times less lactic acid in the conditioned medium of the mutant cells. Mutant fibroblasts are amoeboid and flat. There are four times more cells per cm^2 in normal confluent fibroblast cultures as in homozygous mutants. We propose that the increased amount of mutant TIMP3 is due to the formation of protein complexes with a decreased turn-over rate. This may lead to an unbalanced activity of the metalloproteinases (MMP) and struc

P2-20 47

Activation of three different 5‘ cryptic splice sites in exon 16 of the NF1 gene caused by the same 5‘ splice site mutation in the following intron.

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Detection of NF1 gene mutations is still a challenge, and no single technique is able to detect the entire NF1 mutational spectrum. The NF1 gene spans approximately 350kb of genomic DNA comprising 60 exons. The open reading frame codes for 2867 amino acids including three alternatively spliced exons. It is ubiquitously expressed and encodes an 11 – 13kb mRNA. Most mutations of the NF1 gene present as base substitutions or other small lesions throughout the coding sequence and the splice sites. To date, several hundred distinct NF1 mutations have been identified.
cDNA-based analysis is able to detect the effects of several different kinds of mutation on the RNA level. Missense and in rare cases silent mutations can generate cryptic splice sites which are responsible for aberrant splicing of the transcript. In other cases mutations of the conserved intronic splice site cause activation of cryptic splice sites.
Exon 16 is one of the largest exons in the NF1 gene and one exonic 5‘ cryptic splice site has already been published to be acivated by a splice site mutation (IVS 16+1 G->A). We detected the same mutation in two unrelated patients and two further mutations of exon 16 in two other patients (IVS 16+1 ins TT and G2709A). When analysing the effects of the mutations on the mRNA transcript by cDNA sequencing, we noticed the activation of three different cryptic splice sites in exon 16. Both 5‘ splice site mutations activated the known cryptic splice site at cDNA position nt 2618, but in one of the patients with mutation IVS 16+1 G->A two additional splice sites were activated at nt 2707 and nt 2761 resulting in three different mutant transcripts. Interestingly, one of them was the same found in the patient with the silent mutation G2709A (V903V). These results underline the importance of cDNA analysis in understanding the effects of different mutations on mRNA splicing and their pathological consequences.

P2-20 48

Mutations in the caplain-3 gene cause the most common form of limb-girdle muscular dystrophy

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The limb-girdle muscular dystrophies (LGMD) are a genetically heterogeneous group of progressive myopathies. In an individual male patient, the diagnosis is often difficult since the clinical presentation largely overlaps with the common X-linked dystrophinopathies of the Duchenne and Becker types, respectively. In the majority of LGMD cases, the transmission mode is best explained by autosomal recessive inheritance while autosomal dominant transmission has been documented in a few pedigrees only.
To date, at least 10 genes have been shown to cause some of the subforms of LGMD. These include the sarcoglycanes (a to e), a group of sarcolemmal membrane proteins which are tightly attached to dystrophin, calpain-3, caveolin-3, dysferlin, telethonin and lamin A/C. Specific antibodies to most of these proteins are available and can be used for diagnostic screening on muscle tissues from biopsies.
Over the past 10 years we have applied an increasing panel of these antibodies for diagnostic screening on Western blots of over 500 muscular dystrophy patients. By far the most common cause of muscular dystrophies are complete or partial deficiencies of dystrophin which account for about 70 % of young patients (< 15 years at biopsy) and about 30 % of older cases (> 15 years at biopsy). Deficiencies of the sarcoglycanes were rare in both age groups at 2 % and 0 %, respectively. Defects in calpain-3 were observed in 9 % of the adult cases and 1 % of the younger patients, thus constituting the most frequent cause of LGMD.
Upon sequencing of the calpain-3 gene, we have identified to date 8 different mutations in 7 index cases. A recurrent single base deletion (550delA) was observed on several alleles.

P2-20 49

GNAT2 is the third gene implicated in autosomal recessive achromatopsia

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Achromatopsia is an autosomal recessively inherited visual disorder that features the absence of color discrimination from birth. We have recently found that mutations in CNGA3 and CNGB3 that encode the alpha- and the beta-subunit of the cone photoreceptor cGMP-gated channel can cause achromatopsia including Pingelap blindness (Kohl et al. 1998, Nat Genet 19:257-259; Kohl et al. 2000, Hum Mol Genet 9:2107-2116). Screening for CNGA3 and CNGB3 mutations in a large cohort of achromatopsia patients, however, revealed further genetic heterogeneity in about one third of cases. We now report the identification of five independent achromatopsia families that segregate protein truncation mutations in the GNAT2 gene located on chromosome 1p13. GNAT2 encodes the cone photoreceptor specific alpha-subunit of transducin, a G-protein of the phototransduction cascade that couples to the visual pigment(s). The identified mutations most probably represent functional null alleles of GNAT2, that prevent the formation of the trimeric G protein complex or its interaction with the excited photopigments. Notably, all genes now known to be involved in achromatopsia encode crucial components of the cone phototransduction cascade. Due to the lack of cone function in these patients, it is rea-

sonable to argue that, like the cone-specific cGMP-gated channel, all three different types of human cone photoreceptors utilize a common transducin alpha-subunit. Our results also indicate that achromatopsia is a genetically very heterogeneous condition. Whereas mutations in CNGA3, CNGB3 and GNAT2 together account for about 70% of the cases, the genetic etiology of the remainders has still to be discovered.

P2-20 50

Isolated Cytochrome C-Oxidase(COX)-Deficiency: genetic, clinical and neuroradiological heterogeneity

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COX-deficiency is the most commen cause of respiratory chain disorders. Combined COX-deficiency is found in mutations of mitochondrial (mt)DNA. In isolated COX-deficiency mutations of mtDNA-encoded structural genes and nuclear encoded assembling genes (SURF1, SCO2) are increasingly found. We compare molecular, clinical and MRI/MRS data in 6 patients with isolated COX-deficiency. SURF1-mutations result in „loss of SURF-protein“ and systemic COX-deficiency. Patients present from 2 months of age to 4 years with Leigh-Syndrome, encephalopathy, developmental delay and myopathy. MRI shows mainly lesions of the brainstem sometimes the basal ganglia.

SCO2-encodes a protein transporting Cu++ to COX. The E140K mutation is found in all patients either compound heterozygous or homozygous. All patients develop encephalopathy, myopathy and a live limiting cardiomyopathy (CM) with death during the first weeks in the hetero-zygous patients. Homozygous patients present later with encephalomyo-pathy, peripheral neuropathy and CM . MRI/MRS showed variably distributed lesions in brainstemm, basal ganglia and white matter.

A stop-mutation (W58X) in COIII was found in a patient with extremely reduced COX-activity (10%) in muscle. Presenting at age 12 years with a non-progressive mild myopathy his CNS is clinically and radiologically intact at 19 years suggesting isolated muscle involvement.

In conclusion there is no clear correlation between COX-activity in muscle and clinical severity. In SURF1-patients there is heterogeneity concerning symptoms and progression. In SCO2-patients E140K homo-zygous patients show considerable differences in MRI/MRS and clinical course. Surprisingly the COIII-mutation causes very low COX-activity with a mild clinical picture.

P2-20 51

Genomic organization and mutation screening of the GAS6 gene encoding an activating ligand for MERTK in patients with autosomal recessive retinitis pigmentosa

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The protein encoded by GAS6 (growth arrest-specific gene 6) is an activating ligand for the Tyro 3/Axl family of receptor tyrosine kinases that includes Axl, Rse, and Mer. Recent studies demonstrated that autosomal recessive retinal dystrophy in the RCS rat is due to a loss of function mutation in the gene (Mertk) encoding Mer. Similarly, mutations in the human ortholog (MERTK) were found in patients with autosomal recessive retinitis pigmentosa. We determined the exon-intron structure of human GAS6 by genome-walking using oligonucleotide primers corresponding to the GAS6 cDNA. We performed mutation screening using SSCP and sequence analysis of all GAS6 exons (except those corresponding to nucleotides c.1-389) and adjacent splice sites in 306 patients with autosomal recessive retinal dystrophy. The 2037 bp coding region of GAS6 is divided into (at least) 15 exons, ranging in size from 25 to 229 bp, with exon-intron junctions conforming to the AG/GT consensus rule. Five sequence variants (c.862 G>A, 1/712 alleles; c.1245G>A, 16/200 alleles; c.1332C>T, 48/712 alleles; c.1725C>T, 1/712 alleles; c.1734C>T; 44/712alleles) were identified in the patient population screened. The latter four are synonymous changes (Leu415, Cys444, His575, and Thr578), whereas c.862G>A effects a Val284Met substitution present in the patient in heterozygous form. Additional sequence variants identified in nearby intronic sequences are not predicted to affect splicing. Our results suggest that GAS6 may not harbour critical mutations responsible for autosomal recessive retinal dystrophy, or that such mutations may be rare, raising the possibility that alternative ligands may be involved in activating MERTK function necessary for phagocytosis by the retinal pigment epithelium.

P2-20 52

New STR marker panels for man, mouse and rat at the GMC

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The Gene Mapping Center (GMC) in Berlin is performing high-throughput genotyping for numerous cooperative mapping projects in human and animal models to identify the underlying gene loci in monogenic and complex diseases. We developed human, mouse, and rat short tandem repeat (STR) marker panels to meet the requirements of a broad range of project designs such as linkage analysis, homozygosity mapping, QTL mapping, and haplotype sharing analysis. All marker panels are subject to regular updates. Our current marker sets are as follows:

A) The human marker set consists of three parts that can be typed separately or in combination, as needed by the project. Part I refers to our routine mapping set based on a modified Weber9-Set (Marshfield Institute) and is used for standard genome scans (average density 10 cM, 411 markers, mainly tri- und tetranucleotid markers). Part II consists of 305 additional markers designed and tested at the GMC. Combination of parts I+II results in an average density of 4.46 cM. For some project designs such as haplotype sharing analysis in isolated populations, a higher density is needed. Therefore, 347 additional markers (Part III) were designed to close gaps in the combined set of parts I+II larger than 5.5 cM resulting in a genome wide average density of 3.4 cM.

B) To map quantitative trait loci (QTL) in mouse and rat strains it is necessary to analyse the genotypes of the cross progeny with a panel of markers spaced at 10 to 20 cM intervals. Allele size data for the STR markers are provided in the public database only for a small set of inbred strains and rarely for their different substrains. In order to obtain panels of highly polymorphic markers applicable to different mapping projects we have tested more than 300 microsatellite markers in 15 mouse inbred str
ains and about 150 markers in 16 rat inbred strains or substrains.

P2-20 53

A High Throughput Functional Screen For Novel Factors That Induce Apoptosis

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In the wake of the human genome effort, the search for new pharmaceutical targets has been driven primarily by computational biology and expression screening efforts. The functions of the genes identified need to be defined in subsequent experiments. Mammalian expression screening approaches have thus far been limited to pool transfections resulting in limited sensitivity. Heterologous expression screens e.g. in yeast have met with limited success. Single cDNA clone transfections offer clear advantages with respect to sensitivity and hits. Also, since the functional assay is the first step in this type of analysis, the downstream evaluation of the gene function is straightforward. So far, this approach has been limited to the use of rather small selections of cDNAs due to limits in throughput. We have set up a high-throughput expression screen for apoptosis where single cDNA clones are prepared on a robotics platform. Subsequently, an automated transfection and readout system completes the four robot cascade. Currently, the setup is capable of producing up to 150,000 complete functional assays and subsequent analysis for individual cDNA clones per month. This assay allows for the unbiased screens of cDNA collections and libraries. The results of a first screen with examples for novel apoptosis inducing genes with new disease associations will be discussed.

P2-20 54

Molecular genetic studies in autosomal recessive hypotrichosis simplex

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Hypotrichosis simplex is a rare form of isolated alopecia. A generalized form affecting all body hair is distinguished from a scalp-limited form (HSS). In both forms, patients are born with normal hair and show a gradual loss of hair beginning at the middle of the first decade leading to a complete loss of scalp or body hair by the third decade. All families described up to date follow an autosomal dominant mode of inheri-

tance. Here, we report preliminary data from a family with an autosomal recessive form of hypotrichosis simplex, with predominant affection of the scalp hair.
The family originates from Saudi Arabia. The parents are third cousins. Four out of ten children are affected, six are unaffected. The affected siblings presented with progressive hair loss and thinning of scalp hair since early childhood. Two patients are almost bald, two show diffuse thinning of scalp hair. Furthermore, their body hair is sparse. No other associated abnormalities are present.
We have excluded linkage to the following candidate regions: 18p11 (hypotrichosis simplex), 16q22.1 (CDH3), 12q13.11 (vitamin D receptor), and 12q13.11 (keratin gene cluster). We are currently performing linkage analysis for chromosomal regions 6p21.3 (HSS), 17q11.2 (keratin gene cluster), and 8p21-p22 (hairless). If no linkage can be detected with one of these loci, a total genome scan will be undertaken.
We expect that the identification of the disease-causing gene will provide important insights in the genetic, molecular and cellular pathways that control hair growth.

P2-20 55

Characterisation of the human RAB22A gene belonging to Rab small GTPases
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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. We have identified a novel gene in this region of interest which is located on a BAC RPCIB753L051096 proximal to GNAS1 on HSA 20q13. Cloning and sequencing the full-length cDNA revealed a novel isoform of the human RAB22 subfamily of small GTP-binding proteins located in distinct intracellular compartments and playing an important role in the regulation of vesicular trafficking. Based on the EST WI-12997 this new isoform was isolated containing 2517 nucleotides and is designated RAB22A. Structurally, the RAB22A encodes a polypeptide of 194 amino acids which has 97% identity to the canine rab22. Northern blot analysis revealed two transcripts of 2.7 and 2.3 kb adapted from two different poly-A sites of the corresponding cDNA sequence indicating alternative splicing. The genomic structure was completed by database analysis and sequencing of the isolated BAC clone RPCIB753L051096. The gene consists of 7 exons spanning about 50 kb of genomic sequence.
Physical and FISH mapping revealed that RAB22A is located on a BAC RPCIB753L051096 proximal to GNAS1 but downstream of PCK1 on human chromosome 20q13. Supported by a grant of the DHGP.

P2-20 56

A Mutation in the CDMP-1 Prodomain Causes a Recessive Form of Brachydactyly Type C
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Hereditary brachydactylies are relatively frequent limb malformations, characterized by shortening of phalanges and/or metacarpals. Isolated brachydactylies are classified by Bell's classification in seven distinct subforms. We here describe a large consanguinous turkish family with four individuals that show brachydactyly type C (BDC). They exhibit brachymesophalangy and hyperphalangy of the index and middle finger with marked phenotypic variability. Some but not all of the parents of affected individuals show minimal signs as camptodactyly or minor nail hypoplasia. Mutation analysis revealed that individuals affected with BDC carry a novel homozygous missense mutation 517A>G of the CDMP1 gene and parents exhibit a heterozygous genotype. The mutation is located within a highly conserved seven amino acid region of the prodomain of the gene and changes a methionine to valine at amino acid position 173. Unlike previously described heterozygous frameshift or nonsense mutations this is the first report of a homozygous CDMP1 mutation leading to BDC. Considering the prodomain's key function in processing and secretion of active CDMP1, the mutation presented here is likely to lead to a reduced availability of biologically active CDMP1. The recessive nature of the mutation points out the importance of inspection of minimal signs in kindreds with limb malformations.

P2-20 57

Improved screening for OPA1-mutations in patients with ADOA by cDNA analysis
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Autosomal dominant optic atrophy (ADOA) is the most prevalent hereditary optic neuropathy characterized by an insidious onset of optic atrophy in early childhood with moderate to severe decrease of visual acuity, blue-yellow dyschromatopsia, and centrocoecal scotoma of varying density. ADOA occurs with an estimated disease prevalence ranging between 1:10000 and 1:50000 in different populations. The predominant locus has been mapped to chromosome 3q28-q29 and we have previously identified the responsible gene, called OPA1, by means of positional cloning. The OPA1 gene encodes a dynamin-related GTPase targeted to mitochondria. Most of the presently known OPA1 mutations were found by the analysis of exons amplified from genomic DNA. In our study we have identified 32 OPA1 mutations in 97 unrelated ADOA patients (detection rate 31%). These mutations include missense (10/32) and nonsense (5/32) alterations, frameshift-causing deletions and insertions (11/32) and splice site mutations (6/32). Due to the complex structure of the OPA1 gene (31 exons), this approach is cumbersome and time consuming. Moreover it may fail to detect mutations in the undefined gene promoter as well as larger deletions. Thus we established a sensitive and practicable OPA1 screening procedure by cDNA analysis. Single stranded cDNA is synthesized from blood leukocytes by reverse transcription and four overlapping cDNA fragments are amplified for mutational detection by subsequent direct cDNA sequencing. Applying this technique we could detect three missense mutations, one nonsense mutation, three deletions and three splice site mutations previously found by genomic DNA analysis. Furthermore we were able to identify one novel deletion and three novel splice site mutations.

P2-20 58

Mutations of NEMO in female and male patients with incontinentia pigmenti type 2
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Incontinentia pigmenti type 2 (IP2) is a rare X-chromosomal dominant disorder that is usually lethal in utero in males. Affected females show highly variable abnormalities of the skin, hair, nails, teeth, eyes, and, in some cases, the central nervous system. The IP2 gene encodes the regulatory subunit of a multiprotein kinase complex required for activation of the NF-kappa B pathway (NF-kappa B essential modulator, NEMO). NEMO plays a central role in immune and inflammatory processes and prevents apoptosis. Indeed, about 98% of affected females show a non-random X-inactivation in peripheral blood lymphocytes due to apoptosis of the cells expressing the mutant NEMO allele. NEMO maps in Xq28 and consists of 10 exons. It was shown that a large genomic deletion (delta4-10) accounts for about 80% of IP2 cases, 10% carry point mutations or small rearrangements, whereas in the remaining 10% of the patients no mutation was found. Most of the small mutations result in a truncated NEMO protein. So far, no clear genotype-phenotype correlation has been established.
In a total of 80 apparently unrelated patients with IP, including 2 living males, we performed mutation analysis. Fifty seven patients (~80%), including the 2 males, carried delta4-10. Both males were shown to be somatic mosaic. In the remaining 23 patients, SSCP analysis of all coding exons is being performed. To date, 5 different mutations have been identified in single patients, including a missense (C400Y) and a nonsense (Q290X) mutation, a point mutation in the splice consensus sequence (IVS8-2a>g), as well as two smaller gene rearrangements (deltaK290 and c.1115delC). Cosegregation of the respective mutations with the disease phenotype and their absence in 50 controls suggest that the sequence changes found are the primary genetic defect responsible for the disease. Data of segregation analysis in 10 families with delta4-10 suggests that this mutation arose in 80% of the cases in the paternal meiosis. The high frequency of the delta4-10 deletion in the NEMO gene greatly facilitates diagnostic testing for IP and provides a valuable tool for genetic counselling of the families.

P2-20 59

Hints and pitfalls of fragile X carrier testing

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Mental retardation in fragile X syndrome is, in the majority of cases, caused by CGG trinucleotide amplification within the FMR1 gene. The syndrome is caused rarely by point mutations or deletions within or around the FMR1 gene. Here we describe a family with two boys and one girl affected by fragile X syndrome and two healthy girls asking for carrier analysis. The two girls revealed two normal sized alleles in PCR and Southern blot analysis. However, exact sizing of the PCR fragments brought up three different FMR1 alleles (31/32 CGGs; 32/10 CGGs) for them, with one allel (10 CGGs) that was neither found for the mother or the father respectively. By haplotype and sequence analysis, the 10 CGG allel turned out to have arisen by a deletion of the mutated allel inherited from the mother. The deletion allel did not show mosaicism for the expansion and the deletion and therefore would have been missed by the standard procedures for molecular fragile X diagnosis, which is PCR and Southern blot analysis. This is the first case of a false negative carrier analysis due to a complete deletion of the expanded repeat sequences. How frequently we have missed these in affected boys and girls and weather or nor not this allele is associated with an increased risk for affected children is subject of further studies. It is advisable however, to perform extended molecular analysis for carrier detection and for highly suspicious fragile X males.

P2-20 60

MYBPC3 not MYH7 is the predominant gene mutated in a Large Cohort of Unrelated Consecutive Patients with Hypertrophic Cardiomyopathy

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Defects in ten sarcomeric genes are known to cause hypertrophic cardiomyopathy (HCM). Mutation types and frequencies in large cohorts of consecutive and unrelated patients have not yet been determined. We therefore screened 108 unrelated and consecutive HCM patients for mutations in six sarcomeric genes: cardiac myosin-binding protein C (MYBPC3), ß-MHC (MYH7), Troponin T (TNNT2), a-Tropomyosin (TPM1), Troponin I (TNNI3), and Troponin C (TNNC1).
HCM was diagnosed by echocardiography (septum > 15 mm, septal/posterior wall >1.3), angiography or based on a state after myectomy. Single-strand conformational polymorphism (SSCP) analysis was used for mutation screening, followed by sequencing. Sensitivity was determined in comparison with denaturing high-performance liquid chromatography (DHPLC) and reached > 97 %. A total of 34 mutations in 36 out of 108 patients were identified: 18 mutations in MYBPC3 in 20 patients, two of them being founder mutations; 13 missense mutations in MYH7 in 14 patients, one of them being a founder mutation (R870H); one amino acid exchange in TPM1, TNNT2 and TNNI3, respectively. No disease-causing mutation was found in TNNC1. Twenty-eight of the 36 mutation carriers (78 %) reported a positive family history with at least one affected first-grade relative, only 8 mutations occurred sporadically (22 %).
MYBPC3 was the gene that most frequently caused HCM in our cohort of unrelated patients. Systematic mutation screening in large samples of HCM patients leads to a genetic diagnosis in about 30 % of unrelated index patients and in about 57 % of patients with a positive family history.

P2-20 61

Usherin mutations in patients with Usher syndrome type IIA and autosomal recessive non-sydromic retinitis pigmentosa

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Usher syndrome (USH) is a both clinically and genetically heterogeneous, autosomal recessive disorder characterized by sensorineural hearing loss and retinitis pigmentosa (RP). Based on the phenotype, the syndrome has been divided into three clinical types (USH1-3), whereas each type consists of several genetic subtypes. Remarkably, mutations of the 4 known USH1 genes (USH1B, C, D, and F) have been implicated not only in USH1 but also in cases of non-syndromic deafness. In the same way, mutations of the gene (USH2A) implicated in the most frequent form of USH2 may also cause non-syndromic RP. The USH2A gene contains 21 exons and encodes a protein (usherin) of 171.5 kD with similarity to a number of extracellular matrix proteins and receptors with laminin-like epidermal growth factor and fibronectin type III repeats. Using SSCP analysis, we screened USH2A for mutations in 38 and 110 German patients with USH2 and autosomal recessive non-syndromic RP (arRP), respectively. So far, most likely disease-related USH2A mutations have been identified in 10 of the 38 (26,3%) USH2 patients/families studied, with c.2299delG in exon 13 (7,9% of disease alleles) being the most frequent change. Other mutations detected include two missense mutations (C419F and L1378P) and two „private“ rearrangements (926insACCA (4% of disease alleles) and c.1416del26). In the group of arRP patients, two different usherin mutations were identified; c.2276C>T (C759F) was found in 3 patients (1,36% of all disease alleles), and c.2299delG in 2 patients (0,91% of all disease alleles). Our data suggest that mutations in USH2A are frequent (5/110, 4,5%) among patients with arRP and are in line with findings of other groups. Further studies are needed to explore the spectrum of USH2A mutations in patients with Usher syndrome and non-syndromic RP as well as the relationship between genotype and phenotype.

P2-20 62

PyroMeth: quantitative analysis of CpG methylation with Pyrosequencing

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Methylation of CpG dinucleotides is a key element of the epigenetic control of genomic information in mammals. It plays a crucial role in chromatin structure and gene expression, and aberrant DNA methylation, including hypo- as well as hypermethylation, is often associated with pathogenesis, such as tumorigenesis. Although a variety of methods are available to assess the methylation status in biological material, studying methylation is still limited by the low accuracy and/or the high consumption of time, material, and labor of current protocols. We describe a rapid, quantitative method to assess methylation levels at specific CpG sites using PCR products of bisulfite-treated genomic DNA. The new method takes advantage of the fact that bisulfite modification of genomic DNA creates artificial single nucleotide polymorphisms (SNPs), such as [C/T], at differentially methylated CpGs, which we call methylation-based (mb) SNPs. We utilized the sequencing-by-synthesis technique Pyrosequencing to analyse the percentage of methylation at a CpG supposed to be hypomethylated exclusively in pilocytic astrocytomas (PA). Analysis of about 100 brain tumors with the new technique, called PyroMeth, revealed unspecific hypomethylation in all types of gliomas as compared to normal brain tissue. Methylation of the tumors ranged between 20% and 90% while the control tissues showed consistent methylation between 80% and 90%. Data were confirmed by an independent method. We consider quantitative mbSNP analysis by Pyrosequencing a favourable alternative to existing high-throughput methylation assays such as Methy Light or microarray-based approaches. It combines single CpG analysis with accurate quantitation and is easily amenable to high throughput.

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Detection of a new mutation (L257X) by complete scanning of the hereditary hemochromatosis gene (HFE1) using the denaturing HPLC technology

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Background: Between 90-95% of German hereditary hemochromatosis probands are C282Y or H63D homozygotes or (compound) heterozygotes. 5-10% of the probands lack both of these common HFE1 mutations. By the use of denaturing HPLC we scanned 14 DNA samples, in which either one or none of the two common mutations were identified.
Methods: Analytical conditions for each coding exon were determined by a combination of computer melting profile predictions and experimental melting curves. We tested samples harboring the C282Y and H63D mutation as well as samples in which both chromosomes lack these common mutations.
Results: DHPLC detected all known HFE1 mutations as well as a striking heteroduplex in exon 4 in 2 unrelated patients. Sequence analysis detected a previously undescribed stop codon mutation at position 257.
Conclusions: Denaturing HPLC can be used to scan for the two common mutations in the HFE1 gene in hemochromatosis probands and to identify previously undescribed mutations.

P2-20 64

Genetic refinement of the locus for Osteopathia striata with cranial sclerosis (OSCS) to a 9cM interval on chromosome Xp11.4- p11.22

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Osteopathia striata with cranial sclerosis (OSCS) refers to the radiological appearance of longitudinal striations of the tubular bones and fan-shaped configurations of the ilia. The skull is abnormally dense, and the clavicles are not striated. Based on various family observations OSCS had initially been considered an autosomal dominant condition with complete penetrance and high clinical variability. Recently, however, we and others suggested X-linked segregation due to the observation of female preponderance and of severely affected sons from OSCS mothers. Because OSCS is a mosaic carrier manifestation in females, the non-random X-inactivation reported by Viot et al. 2002 in OSCS women is unlikely. Here, we studied a three generation family from German origin that included a severely affected male, his mother, aunt and grandmother with OSCS. Assessment of X-inactivation status in the three affected females with the CAG polymorphic marker in the androgen receptor gene revealed a random X-inactivation pattern in the grandmother. The mother and aunt unfortunately were not informative. Linkage analysis by PCR based microsatellite marker genotyping was used to identify the disease locus. Based on recombination breakpoint analysis, we have determined that a critical interval is lovalised between markers DXS993 and DXS1039, placing the disease locus within a 9cM region to Xp11.4 - p11.22. Therefore our results confirm the X linked inheritance of OSCS on a molecular basis. Our observation of random X-inactivation in a OSCS female is in line with the mosaic phenotype of striated bone patterns and contradicts the published non-random X-inactivation status. Further X-inactivation and linkage studies are ongoing in a second 3 generation family with 5 affected patients.

P2-20 65

Frequency of BMPR2 mutations in German patients with Primary Pulmonary Hypertension

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Study Objectives:Primary pulmonary hypertension (PPH) is a dominantly inherited disorder characterized by remodeling and loss of patency of the pulmonary arteries. Recently it has been shown that mutations in the bone morphogenetic protein receptor 2 (BMPR2) gene, which encodes a transforming growth factor ß type II receptor on 2q33, are the cause of PPH in a substantial proportion of familial and sporadic cases. We investigated the frequency of BMPR2 mutations in a German PPH cohort.
Design: 82 patients were investigated by DHPLC (WAVE) and sequence analysis of aberrant exons in the first laboratory and by direct sequencing in a second independent laboratory. The results of both laboratories were obtained in a blinded setting and compared afterwards.
Results: BMPR2 Mutations were found in only 4 of the 74 sporadic patients. In addition, we found 3 unclassified variants. In the group of familial PPH (n=8) patients we found one mutation and one UV. None of the groups had missed a mutation. In only two cases we noticed sequencing problems which were clarified by resequencing or endonuclease digests. The results always confirmed the DHPLC data.
Conclusion: We conclude that the frequency of BMPR2 mutations in sporadic PPH patients is lower than previously reported (5%-10% instead of 26%), at least in the German population.

P2-20 66

X-inactivation and fragile-X-full-mutation methylation in human placentas

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In female somatic cells X inactivation is associated with differential CpG methylation on the X chromosomes, e.g., the LINE-1 element of the DXS255 minisatellite is extensively methylated only on the active X whereas CpGs in the androgen-receptor (AR) and the fragile-X-mental-retardation gene (FMR1) are methylated only on the inactive X chromosome. Full expansion of the FMR1-CGG repeat in male and female fragile-X patients is usually associated with promotor hypermethylation. In first trimester chorionic villi of female placentas, X-inactivation methylation differs significantly from somatic cells. Also, differences among X inactivation and full mutation methylation of FMR1 have been reported. Our project includes a detailed study of CpG methylation of the FMR1 promotor and other X linked loci in chorionic villi from first trimester and full term female placentas of normal and fragile-X-full-mutation individuals, in order to establish the timing of methylation on both, normal-inactive and full-mutation-fragile, X chromosomes: In contrast to other X-linked loci and to somatic tissues, the FMR1 promotor is not differentially methylated among the active and inactive X chromosomes in the trophoblast of female placentas at any stage of development. FMR1 methylation in the trophoblast represents an abnormal feature of fragile X syndrome, is only found with full expansion of the CGG repeat, but occurs at variable times usually between the 10th and 13th week.

P2-20 67

A systematic screening approach for monogenic and polygenic diseases in human Xp21.1-Xp11.22

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Human Xp21.1 to Xp11.22 is of high medical relevance since several monogenic as well as polygenic traits have been mapped in this region. Apart from 15 diseases for which the underlying genes have already been identified, 7 syndromic forms of X-linked mental retardation including Prieto, Renpenning and XMRE and more than 20 non-syndromic MRX families including MRX18, MRX51 or MRX56 have been completely or partially mapped to this interval. Additionally, loci for diseases such as X-linked optic atrophy, X-linked congenital nystagmus and juvenile spinal muscular atrophy have also been localized there. Diabetes mellitus type 1 and Graves' disease are two polygenic disorders for which susceptibility genes have been linked to the region. In order to initiate a systematic mutation screening approach, we have established a detailed gene catalogue for this region. The interval, which is flanked by markers DXS1237 and DXS1204, encompasses approximately 18 Mb and is under investigation via mapping and genomic sequencing at both the Sanger Centre (UK) and within our group. Through exploration of the genomic data, we have identified 142 genes, including 81 known genes, 65 novel genes (including transcripts with unknown function, for example the KIAA series), spliced ESTs and genes based on exon prediction only. In addition 35 pseudogenes have been found.
Detailed expression studies, involving in silico as well as wetlab experiments, are in process. Immobilization of the genes on membranes and subsequent RNA-hybridization approaches will allow the establishment of expression profiles in a wide variety of tissues. To date, mutation screening on the genomic and/or cDNA is underway for 3 syndromic and one non-syndromic form of mental retardation, as well as for the polygenic trait Diabetes mellitus type I.

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Chemical mutagenesis and clone library generation of mouse embryonic stem cells

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Facing a large number of genes with unknown functions in model organisms, mutant collections are valuable resources for studying gene function. For the mouse, ES cell technology offers the possibility to manipulate the genome and select for mutations in vitro. Mutant mice can then be generated from clones of interest to study the phenotype of these animals. A major advantage of such gene-driven approaches is that the lesion in the genome of a particular mutant can be identified before the animal is generated.

We are manipulating the genome of ES cells chemically using several different mutagens, one being TMP (trimethyl psoralene). TMP is generally known to predominantly cause „small" deletions in the genome of C. elegans but has not been established as a mutagen in mammalian systems yet. We have characterized TMP as a mutagen for mouse embryonic stem cells regarding death rates, mutation rates, and mutation spectrum. The majority of lesions induced at the Hprt locus were point mutations rather than deletions at the genomic level.
Work is in progress to establish libraries of mutated ES cell clones and to develop screening procedures for the identification of mutations in genes of interest. Current results of our screening approaches will be presented at the meeting.
Acknowledgement of support: ES cell mutagenesis and characterization is carried out through funding by the German National Genome Research Network (NGFN).

P2-20 69

Two metaphysal chondrodysplasia type Schmid cases with novel mutations

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Schmid metaphyseal chondrodysplasia (SMCD) is an autosomal dominant skeletal disorder characterized by short stature, waddling gait, and coxa vara. The genetic cause of SMCD are mutations found in the COL10A1 gene. Collagen type X is the most abundant extracellular matrix component synthesized by hypertrophic chondrocytes of the growth plate during the transition from cartilage to bone in the process of enchondral ossification. Collagen type X is classified as a short-chain nonfibrillar collagen and the chains are composed of three structurally distinct domains: an amino-terminal globular domain (NC2), a short triple helical region, and a carboxyl-terminal globular domain (NC1). The chains are synthesized with an N-terminal signal peptide, which is proteolytically removed from the pre-a1(X) chains during biosynthesis.
We will present two new familial SMCD cases: (1) A 15-year-old girl presented with short limbed short stature, bowed legs and waddling gait. Radiological findings consisted of coxa vara and metaphyseal changes. She and her affected father carried an amino acid substitution in position 554 (F554L). (2) The symptoms of a 5,5-year-old boy and her equally affected mother included short stature, coxa vara and metaphyseal abnormalities. In both, COL10A1 analysis revealed a nonsense mutation in position 653 (Q653X) and in addition a point mutation in position 1729 G to C leading to an amino acid substitution glycine to arginine (G545R) previously described as polymorphism.
The two novel mutations are located in the C-terminal nonhelical domain (NC1) of the a1(X) chain. It is still under discussion, why - with exception of two cases - all known mutations cluster in this domain. The NC1 mutations were proposed to cause reduced mRNA stability and also to affect the initial stages of the folding and chain assembly of collagen X, hindering nucleation of the triple helix and, therefore, impairing secretion of collagen X trimers.

P2-20 70

Determination of CTG repeats: Unstable alleles in Myotonic Dystrophy locus

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Human genome dynamic mutations are a new class of gene mutation represented by unstable number of trinucleotide repeats and causing several human hereditary neurological and neurodegenerative diseases with extremely variable phenotypic manifestation.
We present our experiences with the determination of an unstable CTG repeat sequences located in the 3'untranslated region of the myotonin protein kinase gene MDPK [locus 19q13.3]. CTG expansions up to several thousand repeats are specific for a severe form of myotonic dystrophy [MD] autosomal dominant disease. The physiological copy number rangers from 5 to 3o repeats that is easy determined by PCR methods using primers complementary to the CTG sequence border. To reduce the time for final results of DNA analysis in cases of MD patients, we have introduced and modified fast and efficient methods a] triplet repeat primed fluorescent PCR [TP PCR] [1] and b] XL system for long PCR targets with partial substitution of 7-deaza-dGTP for dGTP [XLCTGPCR] [2,3] and so we have suggested two steps PCR molecular diagnostic protocol. Thos protocol was checked up on the group of MD positive control DNA samples provided us by references laboratories and on the group of our MD patients. Determined CTG pathological long alleles by PCR methods were confirmed with Southern blott analysis [SBA], too. Application of PCR protocols and determination of unstable trinucleotide CTG repeat alleles we demonstrate on the example of MD family pedigree with severe affected girl in the age of eight with normal female karyotype and clinical symptoms of myotonic syndrome. In this MD family we have proved by the two steps PCR protocol verified by SBA, the maternal origin of CTG instability, the amplification lenght of pathological allele and worse phenotype with early succession to the consequent generation.

P2-20 71

False positive cystic fibrosis test result due to a DNA-polymorphism

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Cystic fibrosis (CF), the most common metabolic disorder with autosomal recessive inheritance, has an incidence in the Caucasian population of 1:2500 and is characterized by a severe chronic respiratory tract disease and pancreatic dysfunction. The disease is highly variable in presentation and course.
Since the characterization of the gene in 1989 more than 900 mutations were identified. Delta F508 is the most common mutation with a frequency of about 72% in the Caucasian population. Some other specific mutations account for 2-15% of CF alleles, depending on the ethnic background of the patient studied, with the rest of the mutations being very rare.
In our laboratory the CF-OLA kit (Cystic Fibrosis Assay) is used to screen for the most common mutations in the Caucasian population. Surprisingly, homozygosity for the dF508 mutation was found in a healthy woman. As the homozygote dF508 genotype is not compatible with her healthy phenotype and as technical problems have been ruled out by repeating the analysis, additional studies were implemented to clarify the situation regarding the dF508 status. Heterozygosity for dF508 was identified by fragm ent lengths polymorphism analysis of exon 10 PCR products with subsequent verification by sequencing. In addition, the polymorphism IVS9-61A->G, present in heterozygous state, was detected. As the PCR primer of exon 10 spans the polymorphic site, only the allele not carrying this DNA variant will be amplified. In case of heterozygosity for the polymorphism only one single allele will be present as PCR product. This will imitate homozygosity for mutations in this genomic region. In consequence of this, the CF OLA kit will produce a false positive test result. In order to assess the relevance of this findings, we started rescreening of 200 CF samples. The results of this study will be presented and the data should help to evaluate the reliability of the CF-OLA kit.

P2-20 72

Mutation screening in the fibrinogen gamma chain gene in 100 patients with acute ischaemic stroke

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Dysfibrinogenemia is a rare defect of haemostasis. Clinical symptoms of dysfibrinogenemia vary from mild to moderate bleeding, recurrent abortions, venous and arterial thrombosis, and renal amyloidosis. About half of the patients show no clinical symptoms. Dysfibrinogenemia is mostly diagnosed by discrepant values for plasma fibrinogen concentration analysed by functional and antigenic tests, and a prolonged thrombin time. Nevertheless, some alterations in the fibrinogen molecule detectable on the DNA level may escape diagnosis by routine coagulation assays.
In 100 patients with ischaemic stroke we currently screen for mutations in the three genes coding for the fibrinogen molecule by horizontal PAGE based SSCP analysis. Here we present our results for the mutation analysis of the fibrinogen gamma chain gene.
In two unrelated patients with an ischaemic stroke we found a novel missense mutation in exon 4 (C->G at position 2525) of the gamma chain. As the family histories of the patients are not indicative of further strokes or thromboembolic events these findings suggest that the gamma chain A108G substitution may not act as a major risk factor for these conditions. A newly developed PCR-RFLP assay offers an appropriate procedure for rapid screening for this mutation. Using this method we could not detect this mutation in a control group of 100 healthy volunteers.
In a third patient we found a new mutation A->C two nucleotides after the stop codon. A recent study in yeast [O Namy et al. EMBO Rep 2001; 2: 787-93] showed that this location might have an important positional effect on readthrough ef-

ficiency. Screening for this mutation in a control group of 100 healthy volunteers is on the way. Our results indicate that mutations in the fibrinogen gamma chain gene are a rare finding in patients with ischaemic stroke.

P2-20 73

Detection of duplications of proteolipid protein gene (PLP) in Czech families with classical form of Pelizaeus Merzbacher Disease, high de-novo mutation rate

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Pelizaeus-Merzbacher disease (PMD) is an X-linked disorder od central myelination. Two most important clinical types, the more common and milder clasical type and the rarer and more severe connatal type are frequently described. Classical type is caused in most cases by a duplication of proteolipid protein gene (PLP). This duplication is variable in its extent but also in the location on X chromosome. De-novo PLP duplications origin in most cases as an intrachromosomal event in paternal meiosis. Various methods as intrephase FISH, quantitative PCR and intragenic markers are used for detection of PLP gene duplications. No cases with PLP duplication were reported from Czech Republic yet.
We use quantitative comparative fluorescence PCR (QCFPCR) in combination with an intragenic dinucleotide marker for detection of the most common mutation in Pelizaeus Merzbacher disease. Exon 4 of the PLP gene and exon 4 of the CFTR gene are simuntaneously amplified in one multiplex PCR and exon 4 of PLP gene together with the exon 48 of the DMD gene are amplified in a second multiplex PCR reaction. Resulted fluorescently labled PCR products are subsequently analyzed on the ABI310 Genetic Analyzer using the GeneScan software. In the presence of PLP duplication the product intensity ratio is shifted to the PLP peak. Intragenic dinucleotide marked is used for detection of the origin of the PLP duplication and for assesment of the carrier status in female in risk relatives.
6 PMD patients with PLP gene duplications from 5 families were detected using this combination of methods. All 5 mothers of these patients were also carriers of the PLP gene duplication. In four of the 5 families the PLP duplication originated de-novo in patients mathernal grandfather meiosis as an intrachromosomal event. Three of the patients were born in three subsequent years in Czech Republic which shows, that the incidence of PMD must be at least 1:90 000 in our country.
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P2-21 01

Candidate gene studies as a tool for genotype diagnosis in Long QT syndromes

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The Romano-Ward-Syndrome (RWS) is the most common form of the so called „Hereditary Long QT-Syndromes“ (LQTS) that involve cardiac arrhythmias which can lead to sudden death. These syndromes are characterized by prolonged QT intervals which are visible on an electrocardiogram (ECG) and often lead to juvenile cardiac arrhythmia. These events are based on mutations in genes encoding for cardiac ion channels leading to abnormalities in cardiac repolarization. To date six loci and five genes responsible for LQTS were described with KVLQT1 being the most frequently involved in RWS (45-50%). In our study, these loci were analysed by segregation analysis and direct sequencing in four Romano-Ward families. Mutations in KCNE1 and KCNE2 can be readily determined by sequencing, the genes KVLQT1, HERG, SCN5A and the locus LQT4 were studied by segregation analysis using different sets of fluorescent-labelled markers. Sequencing of KCNE1 and KCNE2 revealed several single nucleotide exchanges, which either were silent or polymorphisms (SNP), but no diagnostic mutation. One family with only two affected persons showed linkage to the LQT1 locus, but we detected no diagnostic mutation. These results suggest that the KVLQT1-gene, which is most frequently involved in RWS in the US and Western Europe, shows different mutation frequencies in Polish RWS-families. After establishing the marker sets, segregation analysis of candidate gene regions represents an efficient and reliable tool for genotype diagnosis of LQTS.

P2-21 02

The genetic distribution pattern of polymorphisms regarded to influence the development of atherosclerosis

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Atherosclerosis is a very complex disease influenced by a variety of environmental and genetic factors. Among the genetic factors which are involved in different atherosclerotic processes there are a lot of well investigated established risk markers like e.g. polymorphisms (PM's) in the ACE-, ApoE-, and E-selectin-gen as well as the Leiden-PM in the factor V-gen. Methods: We investigated the genotype distribution of the above-mentioned PM's in a group of 362 longstanding healthy blood donors without any coronary symptoms and coronary risk factors including hypertension, smoking, diabetes mellitus and drug treatment (more than 3y in medical monitoring; mean age: 41.9y; 61.3% male). All donors belong to the same Central German Caucasian origin. Results: For evaluation of the genotype distributions the DD- (ACE), the 3/4- (ApoE), the Arg/Arg- (E-selectin) as well as the AA-genotype (factor V) were considered as pathologically in terms of coronary atherosclerosis in common consent. We evaluated the score of the pathological genotypes taking the gender as well as the age (18-29 y vs. 30-39 y vs. 40-49 y vs. 50-70 y) into consideration. We could not detected any gender specific significant differences in the score within the group of healthy blood donors irrespective of age (p=0.542) even though it is known that there is a gender specific preference to the incidence of coronary afflictions. In addition one could imagine that with increasing age the number of these pathological genetic risk markers should be decreased considerably in the group of healthy blood donors because of the supposed cardiovascular susceptibility of persons carrying these genotypes. Surprisingly this expected decrease with age was not found among the healthy blood donors (p=0.337). Conclusions: Our results suggest that the investigated genetic markers are not predictors for the incidence of coronary atherosclerosis in general. But nevertheless these PM's could be markers for the progress and therefore for a practicable clinical therotic disease.

P2-21 03

Vascular pathology types associated with the low activity variants of MTHFR

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The low activity variants of MTHFR gene (677 C>T and 1298 A>C) are implicitly associated with mild hiperhomocysteinemia. This last parameter is considered as an important determinant of vascular pathology. Both variants of MTHFR were originally studied in patients with neural tube defects, but also in vascular diseases, in particular in early coronary disease.
In this study distribution of MTHFR gene alleles is compared in groups of patients displaying the specific types of vascular pathology diagnosed on the basis of clinical course (Tab.1).
In the abdominal aortic aneurism (AAA) group the mixed heterozygotes (MTHFR 677 T, 1298 C) occured at increased frequency ($p<0.05$) as compared to the control group.
The study will be extended to include the patients with the obliteration of iliac arteries (syndrom Leriche; 34 patients) and more patients with arterial hypertension. Finally these last patients will be divided into groups on the basis of clinical symptoms and spectrum of risk factors potentially contributing to the disease.
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P2-21 04

Mutation analysis of the spastin gene (SPG4) in patients with autosomal dominant hereditary spastic paraplegia in Germany

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Hereditary spastic paraplegias (HSP) comprise a genetically and clinically heterogeneous group of neurodegenerative disorders characterized by progressive spasticity and hyperreflexia of the lower limbs. Autosomal dominant hereditary spastic paraplegia 4 linked to chromosome 2p (SPG4) is the most common form of autosomal dominant hereditary spastic paraplegia. It is caused by mutations in the SPG4 gene encoding spastin, a member of the AAA protein family of ATPases. In this study the spastin gene of HSP patients from 70 apparently unrelated families in Germany was analysed. We identified mutations in 16 out of the 70 HSP families; 13 of

these mutations have not been described before and only two mutation were found in more than one family. Among the detected mutations are 9 frameshift, 3 nonsense and 2 missense mutations as well as 2 mutations that affect splicing. Most of the novel mutations are located in the conserved AAA cassette-encoding region of the spastin gene. The relative frequency of spastin gene mutations in an unselected group of German HSP patients is approximately 23 %. Frameshift mutations account for the majority of SPG4 mutations in this population. The proportion of splice mutations is considerably lower than reported elsewhere.

P2-21 05

IDENTIFICATION OF FVIII-INHIBITOR EPITOPES USING CELLULOSE BOUND FVIII-PEPTIDE LIBRARIES

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Hemophilia A is caused by the deficiency or dysfunction of Factor VIII (FVIII). The most serious complication of replacement therapy is the development of alloantibodies (inhibitors) towards substituted FVIII in about 30% of patients with severe haemophilia A thus neutralizing FVIII activity. Anti-FVIII antibodies (Abs) are mainly IgG-molecules of polyclonal origin.

Until now Ab-binding to epitopes is not yet known on the amino acid level. In our project we characterize anti-FVIII Ab epitopes of hemophilia A patients using solid phase bound FVIII-peptide libraries. Actually our library presents the primary sequence of FVIII (2351aa) divided up in 13meric oligopeptides, overlapping 10aa one another. FVIII inhibitors which are specifically associated to oligopeptides after incubation are detected by HRPO-conjugated secondary Abs and chemiluminescence. Before screening the library, anti-FVIII Abs must be purified from human blood plasma on Protein G followed by affinity-chromatography on a FVIII-matrix.

This technique allows a screen of linear antigenic sites and possibly discontinuous epitopes on FVIII in only one assay. We have investigated Ab-preparations of different patients with FVIII inhibitors. The results showed several potential epitopes including part-sequences of regions described as frequently appearing epitopes in the literature. Many signals were observed in different patients. The pattern of patient specific epitopes is actually compared with epitopes found in healthy persons. Epitope data will be further correlated to genotype/phenotype data in the consortium.

P2-21 06

Down-regulation of TFF Expression in Gastrointestinal Cell Lines by Interleukin-1

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Background and Aims: Proteins of the trefoil factor family (TFF1, TFF2 and TFF3) are acute phase proteins up-regulated in response to gastrointestinal mucosal damage. They promote cell migration, protect and heal the mucosa and suppress tumor growth. We assume them to be regulated by the proinflammatory cytokines interleukin-1beta (IL1 beta) and interleukin-6 (IL6), which trigger the transcriptional factors NF-kappa B and C/EBP beta. Methods: After IL1beta and IL6 stimulation, expression of TFF genes was analyzed by reporter gene assays using TFF promoter constructs and by quantitative real-time RT-PCR in gastrointestinal cell lines HT-29 and KATO III. NF-kappa B and C/EBP beta were transiently co-expressed.

Results: We have functionally identified transcription factors NF-kappa B and C/EBP beta to inhibit transcription of human TFF genes. Down-regulation of TFF transcription is also observed by IL1beta and IL6, suggesting crosstalk with or in response to the immune system. Down-regulation of reporter gene transcription of all three TFF genes was observed by both IL1 beta and IL6 as well as by NF-kappa B and C/EBP beta. IL1 beta and IL6 caused a 3 to 11-fold reduction in TFF mRNA expression. displayed in real-time PCR.

Conclusions: Down-regulation of intestinal trefoil factor TFF3 caused by transcriptional repression by IL1 beta and NF-kappa B as well as by IL6 and C/EBP beta activation in vitro may reflect the situation in vivo and may contribute to ulceration and decreased wound healing in inflammatory bowel disease. Down-regulation of TFF1 and TFF2 by transcriptional repression by the above-named factors may explain the mucosal damage in the stomach during chronic gastritis and may contribute to gastric carcinogenesis.

P2-21 07

WT1 mutations and downstream molecular pathomechanisms causing nephrotic syndrome

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Nephrotic syndrome in the first year of life may be caused by an inherited glomerular disease like diffuse mesangial sclerosis. In association with male pseudohermaphroditism and a high risk for Wilms tumor it is known as Denys-Drash syndrome caused by constitutional heterozygeous missense mutations in the WT1 gene. WT1 encodes a transcription factor thought to be essential in nephrogenesis and the homeostasis of glomerular podocytes.

To elucidate downstream molecular pathomechanisms by which WT1 mutations lead to nephrotic syndrome we have compared kidney specimens from healthy and diseased individuals using different techniques. RNA expression was determined after laser microdissecting glomeruli from frozen tissue samples, isolation and amplification of the RNA and hybridization to cDNA arrays containing 3600 different genes. Furthermore we have compared the expression profile of cultivated podocytes from healthy and diseased persons Protein expression of the same material was analysed by immunohistochemistry. The results show that the presence of a WT1 missense mutation leads to a complex misregulation of genes which may be the cause of massive proteinuria. These include basal membrane and slit membrane proteins as well as proteins of the podocyte foot processes which are known to be essential components of the glomerular filtration barrier. In addition we have found features of either dedifferentiation or arrest at an early differentiation stage which may explain the presence of rudimentary not fully developed glomeruli. In summary it can be postulated that a combination of deregulative processes causes this severe disease. Which of them are caused directly by a mutated WT1 protein and which are secondary has to be examined in the future.

P2-21 08

Mutational analysis of the MODY genes, hepatic nuclear factor-1alpha; (HNF-1alpha) and glucokinase (GCK), in diabetic children

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Maturity-onset diabetes of the young (MODY) is a clinically and genetically heterogeneous disorder characterized by nonketotic diabetes, onset before the age of 35 years, and autosomal dominant inheritance. Owing to its mild manifestation affected children are often undiagnosed. Correct diagnosis, however, is essential as children with MODY, unlike those with type 1 diabetes, often do not require insulin and respond to oral antidiabetics. In addition, early therapy will prevent microvascular morbidity due to chronic hyperglycemia. MODY can be caused by mutations in at least 6 genes (MODY1-MODY6) involved in insulin secretion. The aim of the study was to investigate the prevalence of mutations in the genes encoding HNF-1alpha (MODY2) and GCK (MODY3) by sequencing analysis.

8 out of 164 diabetic children (5%) treated in our clinic had a clinical history suggestive of MODY. Except for one all had at least one first grade relative with type 2 diabetes, gestational diabetes, or impaired glucose tolerance. In 5 out of 8 cases (63%) novel mutations were detected within HNF-1alpha (L382fsdelA) or within GCK (E399X, Y247X, IVS6nt-1G>A). All mutations were heterozygous except for the splice site mutation within the GCK-gene, which was found in 2 mildly affected siblings.

The molecular diagnosis of MODY can be established in a large proportion of carefully selected patients by mutational analysis of the HNF-1alpha - and GCK-genes. The identification of mutations has therapeutic as well as prognostic implications and may help in the identification of asymptomatic family members, thus preventing future complications of untreated chronic hyperglycemia.

P2-21 09

Functional characterization of human LETM1 - a gene deleted in Wolf-Hirschhorn syndrome

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Recently, we have identified a novel gene LETM1 which is deleted in almost all Wolf-Hirschhorn

syndrome (WHS) patients. WHS (MIM 194190) is caused by deletions in chromosome region 4p16.3 and is thought to be a true contiguous gene syndrome. Rauch et al. (2001) described the first patient with a small interstitial deletion mainly restricted to a previously defined 165 kb WHS critical region (WHSCR) which excludes LETM1. Since the patient shows a partial WHS phenotype without seizures and severe neuromuscular features, WHSCR-flanking genes (like LETM1) may contribute to the complete WHS phenotype. Here, we present novel data concerning the functional characterization of LETM1 and its role as a putative candidate gene contributing to the WHS. Database sequence comparisons revealed that LETM1 is evolutionary conserved and shows significant homology to proteins from different species including A. thaliana, C. elegans and D. melanogaster. Interestingly, all of these proteins exhibit a characteristic conserved domain structure which includes a transmembrane domain, 1-2 EF-hand motifs, a specific phosphorylation site and a novel SAF/SAP related domain. Using a „pull down assay" we could show that in contrast to a standard SAF/SAP domain the SAF/SAP related domain possesses no conventional SAR/MAR-binding properties. The function of the novel domain remains unknown and will be analyzed further. To determine the subcellular localization of human LETM1 we transfected COS7 cells with cDNA-fusion constructs with fluorescent protein EGFP either on the N- and C-terminal site of LETM1. Our results clearly demonstrate that the fusion protein is located in the mitochondria, indicating that LETM1 may play an important (evolutionary conserved) role in mitochondrial function. Since mitochondrial dysfunction has gained considerable interest as a potential cause of epileptic seizures and neuromuscular disorders, it is tempting to speculate that LETM1 represents a suitable candidate for these clinical features characteristic for WHS patients with the full phenotype.

P2-21 10

Systematic clinical, cytogenetic and molecular characterization of balanced chromosome rearrangements that are associated with disease

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Clinical and molecular characterization of disease-associated balanced chromosome rearrangements (DBCRs) is a very powerful approach for the identification of disease genes and the elucidation of their function. To date, the Mendelian Cytogenetic Network (MCN) consists of 305 laboratories from 51 countries worldwide and 2345 DBCRs have been submitted to the central database. As a prerequisite for fine-mapping of breakpoints, we established a unique set of >5000 FISH-mapped YAC and BAC/PAC clones which covers >65% of the human autosomes as well as the entire X-chromosome. During the past 30 months, the probe set was employed to map breakpoint regions in >70 patients with DBCRs, most of which were associated with mental retardation (MR). So far, more than 15 promising candidate genes for autosomal MR and related disorders, as well as 6 candidate genes for X-chromosomal MR could be identified. These genes code for protein kinases and phosphatases, synapse-associated proteins, transcription factors, neuronal cell surface proteins and axon guidance molecules. To prove the identity of candidate genes for X-linked MR, mutation screening in >350 unrelated families is in progress. For autosomal MR, however, this strategy cannot be used due to the enormous heterogeneity of this condition. Therefore, functional approaches will be employed to substantiate the role of these genes in MR.

P2-21 11

Deletion Analysis and Prenatal Diagnosis in Iranian SMA Patients Type, I- III

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Autosomal recessive spinal muscular athrophy (SMA) is after cystic fibrosis, the second most common fatal monogenic disorder and after Duchene muscular dystrophy is the first common severe neuromuscular disease in childhood. The disease is characterized by degeneration of anterior horn cells leading to progressive paralysis with muscular athrophy. Depending on the clinical type (Werdnig-Hoffmann=type I, intermediate form=type II and Kugelberg-welander=type III), SMA causes early death (type I) or increaing disability in childhood (type II,III). All three types of autosomal recessive SMA map to chromosome region 5q13.1. Homologous deletions in exon 7 and 8 of the survival motor neuron (SMN) gene have been described in >90-95% of SMA patients.
The aims of this study were to screen the deletions of SMA gene (exon 7 & 8) in iranian patients for prenatal diagnosis of SMA. We have studied 26 families with SMA types I-III, partly with their affected children and their chorion villus samples (CVS). DNA deletion genotypes were determined by PCR-RFLP analysis amplifying exons 7 and 8 of SMN. Results revealed the homozygous deletions of exon 7 and 8 of the SMN gene in 23/26 (90%).
The data support that homozygous absence of SMN exon 7 & 8 is strongly associated with SMA. The percentage of homozygous deletions in the study is almost as high as that reported in other investigations. This method is useful, fast and effective for gene diagnosis and prenatal diagnosis of SMA.

P2-21 12

Mutation Detection and Prenatal Diagnosis of Patients With Cystic Fibrosis (CF) in Iran

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Cystic fibrosis (CF), the most common severe lethal autosomal recessive disorder in whites, is caused by mutation in the CF transmembrane conductance regulator gene (CFTR) on chromosome 7q31. The carrier frequency among Caucasians is approximately 1 in 25, with an incidence of approximately 1 in 2500 live birth. The gene for CF spans approximately 250 kb and contains 27 exons. CF patients have two defective alleles and may either be heterozygous for different mutations, or homozygous for one of the mutations.
Since the identification of the gene responsible for CF, more than 900 mutations was described in CFTR gene of patients affected by cystic fibrosis, but the prevalance of the CF shows a geographical and ethnical variations in the world. The delta F508 mutation in CFTR gene accounts for over 70 % all mutant CFTR alleles in the Europeans to 20 % in the Asians.
The DNA samples of 24 individuals, who were carrier for CF, partly with their affected children and their chorion villus samples (CVS) from the pregnant women, have been tested for five common mutations: deltaF508, G551D, G542X, W1282X and N1303k. The study was performed by using the ARMS method for mutation detection of CF gene. The delta F508 mutation was found only in one couple with first cousin marriage and three times in other partners of related/non related couples (28%). One of these couples were heterozygous for two different mutations (delta F508 and G551D). Results from this study revealed the following frequencies: Delta F508: 28%, G551D: 5%, G542X: 5%, W1282X: 0%, N1303k: 0%.

P2-21 13

Biogenesis of cellular iron-sulfur proteins and its implication for human disease

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Iron-sulfur (Fe/S) clusters are important cofactors of numerous proteins involved in electron transfer, metabolic and regulatory processes. In eukaryotic cells, known Fe/S proteins are localised within mitochondria, the nucleus and the cytosol. Only recently, the molecular basis of the biogenesis of these proteins in a living cell has started to become elucidated. Mitochondria perform an essential function in the biosynthesis of cellular Fe/S proteins. The organelles harbour a complex „iron-sulfur cluster (ISC) assembly machinery" consisting of some ten proteins. The ISC proteins (including a cysteine desulfurase, a ferredoxin, and an Hsp70 chaperone) are highly conserved from bacteria to man. Their function is crucial for maturation of both mitochondrial and extra-mitochondrial Fe/S proteins. Recently, we identified frataxin located in the matrix as a novel component required for cellular Fe/S protein maturation. The human homologue is depleted in patients of Friedreich's ataxia. The precise role of mitochondria in the maturation of cytosolic Fe/S proteins is still unclear, but according to a current working model, an Fe/S cluster or a derivative thereof is assembled in the mitochondrial matrix and exported by the „ISC export machinery". Its first known components are the ABC transporter Atm1p of the mitochondrial inner membrane, the sulfhydryl oxidase Erv1p (human ALR) of the intermembrane space, and glutathione. Depletion of these proteins results in a specific defect in the maturation of extra-mitochondrial Fe/S proteins. Mutations in ABC7, the human orthologue of Atm1p, are causative of a form of sideroblastic anemia (XLSA/A) underlining the importance of Fe/S protein biogenesis for normal cell function.

P2-21 14

Genomic characterization of candidate genes from a chromosomal region associated with infantile benigne myoclonal epilepsy

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The infantile benigne myoclonal epilepsy (FBME) belongs to the idiopathic epilepsies representing 40% of all epileptic illnesses in children. To date, only few of these conditions have been linked to specific candidate genes: Mutations have been described specifically in genes that encode ion channel proteins like KCNQ2 or SCN1B. We analysed 11 FBME patients for mutations in the SCN1B gene, without detecting sequence alterations. One of these patients served as starting point to persue an extended FBME candidate gene search as this case showed a deletion of chromosomal region 11q23.3-24, thus probably linking FBME to this genomic area. Using a hybrid cell line of the aberrant chromosome 11 in a CHO background, we were able to localize three genes in this deletion that would meet the criteria for a FBME candidate gene. Two of them (KCNJ1, KCNJ5) encode potassium channels and are related to proteins that are involved in other forms of epilepsy. The third gene (GRIA4) encodes a glutamate receptor, whose rat homologue is involved in neurological disorders. To determine the role of these genes in FBME we identified the exon-intron structure and sequenced the coding regions in 11 patients. In three FBME DNAs we detected a C-to-T transition in exon 1 of KCNJ1. We analysed 58 control DNAs and found the C-to-T change in 14 cases, thus classifying this transition as polymorphism. Our further strategy now includes the analysis of the remaining exons in our FBME cohort and the identification of additional candidate genes. The genomic characterization of the three genes on 11q has also provided the tools to screen for mutations in other patient groups with neurological defects linked to this chromosomal region.

P2-24 01

Genotoxic effects of Oxazepam - mitotic abnormalities

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Genotoxic effects of Oxazepam(benzodiazepines anxiolitic)and its influence on the mitotic process in human lymphocytes in vitro and in Allium roots meristem cells, are presented in this report.
It has been established that OX in concentration of 0.5 - 50 microg/ml causes disturbances in the kinetics, separation, structure and chromosomal organization in every phase of lymphocyte division and in onion roots meristem cells.
OX induces lagging, vagrancy, dislocation, irregular grouping, anaphase and telophase chromosome bridges, stickness and C mitosis.
The established irregularities point to the conclusion that OX induces spindle abberations and aneuploidy.

P2-26 01

CF Diagnosis using Polar Bodies - Problems and pitfalls

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Introduction:Preimplantation genetic diagnosis (PGD) by testing one or two blastomeres for chromosomal or single gene disorders is an established technique. PGD offers families at risk to have known inherited diseases the possibility to avoid affected offspring without pregnancy termination with its ethical problems and psychological consequences. However, PGD is not compatible with the German embryo protecting law (ESchG). The prefertilization genetic diagnosis by testing only polar bodies instead of blastomeres, is not restricted by the ESchG and is in certain cases an alternative to PGD. When polar bodies for autosomal recessive disorders are analysed, only the alleles inherited by the mother can be indirectly studied. Statistically, 50% of the oocytes carrying the mutation are fertilised by sperms bearing the wild type allele and yield to unaffected children.
Experiments: Here we present our results after three cycles with polar body testing for cystic fibrosis.
Comparing the results after primer extension preamplification (PEP) with the results after multiplex PCR, we could show that multiplex PCR is more accurate and reliable than PEP for polar body testing.
By testing only first polar bodies we found a rate of heterozygosity of 54% for the deltaF508 mutation. In these cases no information about the oocyte, whether it is a carrier of the mutation or not, is available. Statistically, 50% of these heterozygous oocytes bear the wild type allele after the second meiotic division, but had to be discarded if only the first polar body could be tested.
In the third cycle we were able to test four second polar bodies. The results indicate for another possible pitfall if only the first polar body is tested. In one case the second polar body was heterozygous while the first polar body had presented with homozygosity.
Discussion: Prefertilisation genetic diagnosis by testing polar bodies is a possibility to perform preimplantation diagnosis. However, this alternative to PGD needs a great number of oocytes. A large number of oocytes have to be discarded, since the paternal allele can not be tested. Oocytes with heterozygous first polar bodies have also to be discarded. Only by testing the first and second polar body the number of oocytes available to be transferred can be increased. But analysis of the second polar body can not be performed in the same cycle. Therefore, oocytes have to be cryopreserved at the pronuclear stage, which will result subsequently in a lower pregnancy rate as compared to fresh cycles.

P2-26 02

Preimplantation diagnosis (PGD) in Germany - attitudes and prospective usage in genetic high-risk-couples and matched control-couples

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In our study we assessed the attitudes, ethical concerns and prospective usage of PGD in the light of other possible reproductive options (refrain from having more children, adoption, prenatal diagnosis(PD), pregnancy without PD) in a population of 162 couples with recessive and dominant genetic disorders (high risk sample) in their reproductive age. These are compared to responses of 149 couples, matched for age and number of children and controlled for social status. As expected, the reproductive history of the two populations differ significantly. The high risk sample reports more miscarriages and abortions. 30% of the high- risk population versus 15,2% of the control population had used PD in at least one pregnancy, 24,2% versus 10,8 % of the children were born after PD. The Odds Ratio for the high-risk group for refraining from having more children in reproductive age is 1,7 (CI 1,3-2,7) referred to the control group. For 17% of those high risk couples who opt for having more children, PGD is, although not allowed in Germany, the `most probable option´. The PGD group assesses the burden of having an(other) affected child and their subjective genetic risk significantly higher, has less healthy living children, and their desire to have an(other) child is stronger. An overwhelming majority of the whole sample considers the preimplantative embryo to be `my child´ or `quite my child´(77,1%). Only 4,4% say the embryo is `a cell cumulus´. 18% of the whole sample think the PGD should not be legalized in Germany, whereas 82 % think it should be legalized in a more or less restricted way. Our data allow a deep insight into the influences on the personal attitudes towards PGD and other reproductive options in a population of high vs. average genetic risks.

P2-26 03

Erste Erfahrungen in der Anwendung der Polkörperdiagnostik

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Chromosomale Veränderungen in der Eizelle (EZ) führen zu IVF-Versagen und zu Fehlgeburten. Ihr Auftreten ist abhängig vom mütterlichen Alter oder der mütterlichen Prädisposition. Die Polkörperdiagnostik (PKD) stellt eine Möglichkeit dar, chromosomale Fehlverteilungen bei EZ zu erkennen, bevor diese befruchtet werden.
Die Entnahme der Polkörper (PK) durch Mikromanipulation muss die Unversehrtheit der Eizellen garantieren, um eine ungestörte Befruchtung und Embryonalentwicklung zu gewährleisten. Zusätzlich ist die Vollständigkeit der PK für die nachfolgende Diagnostik mittels der Fluoreszenz-in situ-Hybridisierung (FISH) erforderlich. Der Vergleich mehrerer Methoden zur

Extraktion von PK (A: Extraktion mit spitzer Pipette; B: Extraktion nach Laser Zona Drilling (LZD) mit stumpfer Pipette; C: Extraktion ausschließlich mittels Laserlicht (Kombination aus LZD und Extraktion mittels Laserfalle)) zeigte einen eindeutigen Vorteil für den Einsatz von Laserlicht.
Bei über 200 PK von verworfenen EZ wurden mittels FISH einzelne Chromosomenabschnitte mit 2 oder 5 Sonden farblich markiert. In 86% ließen sich die PK nach der Präparation hybridisieren und in 66% auswerten. Die Aneuploidierate ergab sich bei Verwendung von 2 Sonden mit 26% und bei 5 Sonden mit 69%. In 44% der Fälle fanden sich mehrere Chromosomen aneuploid.
Seit Anfang diesen Jahres evaluieren wir die PKD in einer Studie. Bisher wurden 15 Patientinnen rekrutiert, davon 7 mit maternaler Translokation. Das Indikationsspektrum, die Besonderheiten bei der Stimulationsbehandlung und unsere Erfahrungen bei der Mikromanipulation und der FISH Untersuchung werden im Detail vorgestellt.

P2-27 01

Influence of ethnic origin on the level of „triple-screen" serum parameters with special consideration of a Turkish subpopulation in Germany

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A database including the results of 38539 routine risk assessments for chromosomal disorders and neural tube defects (so-called „Triple-Test") was screened for Turkish, Arabic, Persian, and South-East-Asian patients living in Germany. Their serum levels (Alpha-Fetoprotein=AFP, Human Choriongonadotropin=hCG, free Estriol= uE3) were compared with those of German patients.
After exclusion of all cases where the serum values might have been influenced by other factors than ethnic origin (affected pregnancies, abortion, gestosis, smoking, etc) 25377 cases were left for a comparative analysis. 3.7% patients were of Turkish origin and by far the most numerous subgroup besides the 94.3% Germans. As the body weight of Turkish (mean: 65.0kg) and German women (mean: 68.4kg) differed significantly only weight adjusted MoM values were compared. At a 1% alpha level there were no significant differences regarding the hCG and uE3 MoM values. A highly significant difference was stated for AFP though the AFP mean in the Turkish subgroup (0.96 MoM) was only slighly lower than in the German population (1.01 MoM). Because of only small differences in serum levels we conclude that adjustment of serum MoM values is not necessary for Turkish patients.

P2-27 02

Protein chip analysis to detect new protein markers in the maternal serum specific for trisomy 21 pregnancies

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Prenatal diagnosis mainly focusses on the detection of trisomie 21, which is the most common chromosomal abnormality. During the last decade a lot of ambitious efforts were undertaken to replace the risky fetal cell sampling techniques like amniocentesis with non-invasive procedures like maternal blood sampling. The analysis of fetal cells isolated from maternal blood is still not usefull under routine conditions. On the other hand maternal serum parameters like triple test and PAPP-A in combination with nuchal translucency (NT) measurement and maternal age are already used to calculate the probability for the occurance of trisomie 21. To detect new protein markers in serum of pregnant women we analysed 15 samples with the surface enhanced laser desorption ionisation technology (SELDI). This technology is based on protein chip arrays with solid phase chromatographic surfaces, which were examined by MALDI-TOF (Matrix associated laser desorption ionisation time of flight). SELDI allows the detection of nearly all proteins present in serum. For analysis serum samples were mixed with the chip type appropriate binding buffer and were directly spotted on the chip surface. After incubation and several washing steps an energy absorbing matrix (EAM) was added to ensure the energy transfer from laser to proteins. Then the chip was transferred to the SELDI system and analysed by an automated data collection protocol. The analysis of fifteen sera (12 - 16 weeks of gestation) from women carrying a fetus with trisomy 21 and fifteen controls from women with an inconspicuous pregnancy resulted in at least two significantly differentialy expressed proteins in the range of 5 to 15 kDa. These results indicate that beneath the already existing protein parameters other markers can be found in maternal blood to differentiate between normal and trisomy 21 pregnancies.

P2-27 03

Pitfalls in prenatal diagnosis of a supernumerary marker chromosome and exclusion of uniparental disomy

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Clinical relevance of supernumerary marker chromosomes (SMC) found in amniotic fluid depends on the amount of euchromatic material and might be a hint towards uniparental disomy (UPD) of the normal homologs. Problems associated with UPD include trisomy mosaicism, homozygosity of autosomal recessively inherited mutations, and genomic imprinting. In the case described here amniocentesis was performed in the 16th week of gestation due to advanced maternal age. Ultrasound evaluation was unremarkable. Cytogenetic analysis showed a SMC in about 48 % of metaphases. By Akro-M-FISH the marker was characterized to originate from chromosome 14 or 22 and no euchromatic material was detectable. Because of the advanced time and the risk of maternal or paternal UPD 14, which both are associated with growth retardation and dysmorphic features as well as in a proportion of cases with developmental delay and skeletal abnormalities, molecular investigations for UPD 14 were undertaken with a pannel of 12 micro satellite markers located on chromosome 14. One of these markers, which moreover was located on 14q32 a region where imprinted genes have been located recently, showed only one maternal allele indicating segmental maternal UPD 14. Therefore, a chromosome 22 centromer specific probe (22Z4; provided by Dr. M. Rocci, Bari, Italy) was used in parallel to the 14/22 probe. In 14 of 30 metaphases the marker was ascertained and to be a derivative chromosome 22. As a consequence the molecular result was considered as a new mutation in the paternal allele resulting in a homozygous or hemizygous status in the fetus. In conclusion, for a confident UPD diagnosis it is necessary to find at least two independent and informative loci indicating uniparental inheritance.

P2-27 04

Experiences of nuchal translucency screening to detect fetal aneuploidies

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OBJECTIVE: Measurement of nuchal translucency (NT) is a widely used screening method for chromosomal abnormalities. During fetal NT screening, ultrasound is used to assess for a fluid collection behind the fetal neck. Increased NT can identify fetal aneuploidies as e. g. trisomy 21 and is associated with major defects of the heart and great arteries and a wide range of genetic syndromes. In this study we evaluated the pregnancy outcome after NT screening.
METHODS: Fetal NT thickness and crown-rump length (CRL) were measured in 633 women with singleton and 51 with multiple pregnancies. The risk for trisomy 21 was estimated on the basis of maternal age and fetal NT thickness for CRL. The distribution of estimated risk was determined and the sensitivity of a cut-off of 1 in 300 was calculated. Fetal karyotyping was performed in 89 cases (12 %).
RESULTS: The median maternal age was 32 (range 17-44) years, the median gestation age at screening 12.7 (11-14) weeks and the median fetal CRL 61.2 (38-84) mm. An abnormal karyotype was found in 21 cases (2.9 %). The estimated risk calculated by the maternal age was 1 in 300 or greater in 262 (36 %) cases. The respective value based on maternal age and fetal NT thickness for CRL was ^{3} 1 in 300 in 85 (11,5 %) cases. These included 60 (9.2 %) of the normal fetuses, 11 of 12 (92 %) cases with trisomy 21, 5 (100 %) with trisomy 18, and 2 (100 %) with trisomy 13. With a cut-off of 1 in 300, the sensitivity was 95% at a false-positive rate of 8.2 %.
CONCLUSION: The combination of maternal age and fetal NT provides an effective screening method for chromosomal defects. Application of a cut-off of 1 in 300 is efficient for the screening of fetal aneuploidies.

P2-27 05

Prenatal diagnosis of rhizomelic chondrodysplasia punctata

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In the 19th week of the fourth pregnancy of a 28-year old woman a detailed sonographic investigation was performed to exclude fetal malformations. Her first 5-year old son had a very short stature, extremly short humeri, contractures in distal joints, delayed myelination, bilateral cataracts, seizures and severe developmental delay. A lot of efforts have been made to establish a diagnosis for him, but without success. Two further pregnancies ended in miscarriages in the first trimester. The woman and her husband were second degree relatives. The detailed sonographic investigation in the 19th week of pregnancy revealed no anomalies. Four weeks later, in the 23st week short humeri, an abnormal profile with hypertelorism, depressed nasal bridge, anteverted nostrils and flexion contractures of the 3rd and 4th fingers were detected. We suspected that this fetus has the same autosomal rezessive syndrome as the first child. Under the hypothesis that the son and the fetus might have a rizhimelic chondrodysplasia punctata efforts were made to confirm this diagnosis in the son. After these investigations the diagnosis of rhizomelic chondrodysplasia punctata was nearly certain. The mother decided to terminate the pregnancy. The fetus had the typical facial dysmorphism, short humeri and joint contractures. The X-ray showed the typical epiphyseal stippling confirming the diagnosis of rhizomelic chondrodysplasia punctata. Mutation analysis of the PEX7 gene is in progress. In the next pregnancy an earlier prenatal diagnosis can be offered with biochemical or DNA analysis.

P2-27 06

Prenatal diagnosis of oto-palato-digital syndrome type II

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In the 21st week of her second pregnancy a 28-year old woman was seen for detailed sonographic investigations. Her first pregnancy ended with a stillborn malformed male child in the 30th week. This child had shortened and curved limbs, malformed feet, flat profile with micrognathia. A campomelic dysplasia with a low recurrence risk was the given diagnosis. However no SOX-9 mutation could be detected. The anomalies detected sonographically in the 21st week of her second pregnancy were very similar: shortened limbs with very hypoplastic fibulae, malformed hands and feet and facial dysmorphism. These findings were compared with the photographs and the pathology report of the first child. The X-linked oto-palato-digital syndrome type II was suspected due to the combination of the striking anomalies of hands, feet, the facial dysmorphism and the very hypoplastic fibulae. The mother has mild facial dysmorphism with antimongoloid slant of palpebral fissures and hypertelorism, a sign, which was reported in other carriers of OPD syndrome type II which is localized on Xq28. It could be demonstrated that the mother has a skewed X-inactivation pattern, further confirming the diagnosis. In the next pregnancy an indirect DNA diagnostic can be offered.

P2-27 07

Reduced amplification efficiacy of KIAA0027/MLC1 alleles: Implications on the molecular diagnosis of MLC1

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Mutations in KIAA0027/MLC1 are causative for the chromosome 22qtel-linked autosomal recessive childhood-onset megalencephalic leukoencephalopathy (MLC). A high degree of allelic heterogeneity has been observed with 15 disease causing mutations. Exon 11 is a hot spot for mutations among patients suffering from MLC, and in multiplex pedigrees with schizophrenia occurred a rare coding variant L309M of unknown biological significance, which was observed segregating as well as non-segregating with the disease. Using DHPLC-analysis, reproduction of earlier findings on L309M revealed homoduplex resolution patterns among individuals, who had been described to be heterozygous for the variant, which was further confirmed by sequencing the respective PCR-products. The preferential amplification of specific alleles of exon 11 may be caused by a cumulative effect of high Tm domains, several incomplete intronic tandem-repeats of 68 bp (copynumber 6.3) and of 33 bp length (copynumber 9.5). Secondary folding structures due to tandem repeat elements, and a common polymorphism, 1165ins33bp, at the 5' -end of exon 11 may be causative for preferential amplification of mutant 1040A alleles. Consistent amplification was obtained only when we employed exonic primers directly adjacent to the L309M variant. We propose a differentiated proceeding for mutational screening of exon 11 of MLC1 gene among patients with the clinical phenotype of MLC.

P2-29 01

New possibilities for the gene therapy of neurological diseases

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To date, gene therapy approaches can be divided into those using viral vectors and those using plasmid DNA. While the viral vector strategies imply complex production protocols and serious risks for the patients, plasmid therapy protocols have the main drawback of a very low transfection efficiency. The use of protein translocation domains (PTDs) in fusion proteins encoded on a plasmid vector allows the expression of marker and therapeutic proteins in a transfected cell, which are subsequently exported from the producing cell and taken up by other cells. This biodistribution increases the number of cells reached by a therapeutic protein by orders of magnitude in comparison to the number of transfected cells. Use of the native or protein-engineered PTD from the HIV-TAT protein allows the distribution of large molecules, e.g. beta-galactosidase, including the transport across the blood-brain barrier.
This approach is especially suitable for the treatment of recessive neurological diseases, where the missing gene product can be delivered to the brain via the blood after, e.g., production in the liver, since hepatocytes can be transfected more efficiently than many other cell types including neurons. Examples will be shown from murine models, which illustrate that injection of marker DNA into the liver effectively results in 100% of positive hepatocytes and that the marker protein can be detected in the choroid plexus of the brain, where the blood-cerebrospinal barrier is crossed. Details of the vector requirements will be explained as well as suitable candidate diseases, whose treatment can be tested in murine models.