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Der unterstrichene Autor wurde als Vortragender angemeldet

Public lecture

Kampf der Geschlechter im Genom: Epigenetik und die Bedeutung der elterlichen Prägung in Biologie und Medizin

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Alle Gene des menschlichen und anderer Säugertiergenome sind jetzt bekannt. Eine der wichtigen Fragen für die biologische und medizinische Forschung der nächsten Zukunft ist wie Gene während der Embryonalentwicklung und später in bestimmten Organen an- und abgeschaltet werden. Hier nimmt die Epigenetik eine zentrale Bedeutung ein, die sich mit Modifikationen der DNA und des Chromatins beschäftigt. Diese Modifikationen etablieren ein Signalsystem, durch welches Gene an- und abgeschaltet werden, und besonders wie ein aktivierter oder reprimierter Zustand von Genaktivität von der Zelle erinnert wird, und deshalb zur Stabilität der Gewebedifferenzierung beiträgt. Wir beschäftigen uns besonders mit elterlich geprägten Genen, bei denen die epigenetische Markierung dazu führt, dass Gene von Vater oder Mutter im Gewebe des Kindes unterschiedlich aktiv sind. Dies beruht nach einer vor einiger Zeit formulierten Hypothese auf einem Geschlechterkampf im Genom bei dem es um mütterliche Nahrungsresourcen geht. Außerdem interessiert uns die epigenetische Reprogrammierung, bei der reife Modifikationen wieder rückgängig gemacht werden, um eine weite Entwicklungspotenz des Genoms zu erreichen. Die Mechanismen der Reprogrammierung sind wichtig für die Entwicklung von Stammzelltherapien.

Keynote lecture

Proteomics, systems biology and molecular medicine

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New molecular medicine initiatives operate at the interface between clinical and basic research. A bi-directional translational research mode is inspired by unmet medical problems identified in the clinics as well as by opportunities arising by new tools and insights from basic research. Biology relies on the concerted action of a number of molecular interactions of gene products and metabolites operationally organized in so-called pathways. Impairment of pathway flow or connections can lead to pathology. The majority of targets of current therapeutics cluster in a limited number of these cellular pathways. However, current appreciation of the “wiring diagram” or “molecular maps” of these pathways is scanty. Through an integrated proteomics approach it is now possible to obtain physical and functional maps of entire human disease pathways (Nature Cell Biology 6, 97-105, 2004). Moreover, it has become feasible to map active compounds back on the pathways by identifying the protein interactors of the immobilized compounds. The mode-of-action of novel and existing clinical drugs, but also of pathologically-relevant gene products, typically identified by genetics and genomics, can thus be determined, linked to biological processes via these interdisciplinary systems-biology strategies and implemented into novel therapeutic and diagnostic approaches. Such a “systems biology” approach promises to create important synergies between the different research avenues and inaugurate a truly “postgenomic” molecular medicine era (Drug Discovery Today 8, 1067-77, 2003).

Plenary Session

Plenary Session 01

Energy-dependent nucleosome remodelling
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The term 'nucleosome remodelling' subsumes a number of alterations of canonical nucleosome structure that render nucleosomal DNA accessible to regulatory factors. A common denominator of all nucleosome remodelling enzymes is that they convert chemical energy (provided by ATP hydrolysis) into conformational changes that disrupt histone-DNA interactions. Depending on the nature of the enzyme, this action may lead to a different outcomes ranging from the sliding of intact histone octamers on DNA and the displacement of DNA segments from the histone octamer to partial or complete disassembly of the histone octamer itself. Although the phenomenology of nucleosome remodelling is diverse, recent findings suggest a common mechanistic principle. Accordingly, remodelling enzymes function as histone-anchored DNA translocases.
Nucleosome remodelling factors are crucial for all known nuclear functions requiring chromatin substrates, such as the transcription of genes, the replication of the genome, the recombination of chromosomes as well as the repair of DNA damage. How the multitude of different factors are integrated into these various physiological processes and how the remodelling activities themselves are regulated are challenging questions at the forefront of recent research. pbecker@med.uni-muenchen.de

Plenary Session 02

Sites of differential DNA methylation identified by genomic profiling of DNA methylation in normal and transformed human cells
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Cytosine methylation is a major epigenetic modification, required for mammalian development and often perturbed in human cancer. In order to identify sites of differential DNA methylation in the human genome we devised a novel approach for the unbiased isolation of methylated DNA and combined it with subsequent detection on DNA microarrays. Using this assay we generated DNA methylation profiles for the complete human genome at an 80 kb resolution as well as for a large set of individual CpG islands in primary and transformed cells. Our analysis reveals large chromosomal regions of differential methylation with higher abundance of methylated cytosines in gene-rich neighborhoods. Female and male primary cells show indistinguishable profiles for the autosomes but clear differences for the active and inactive x-chromosome. Unexpectedly de novo methylation of the inactive x is limited to a small subset of gene rich regions, while overall the inactive x is hypomethylated relative to its active counterpart. In cancer cells on the other hand we observe global changes in DNA methylation, which can extend over large regions and we identify gene-poor regions as the preferential target for cancer specific hypomethylation. A parallel analysis of 6000 CpG islands in primary and transformed cells provides a quantitative analysis of promoter methylation and identifies a novel set of genes inactivated by promoter hypermethylation in cancer. The results of this comprehensive analysis of genomic DNA methylation will be discussed in the light of current models of epigenetic gene regulation.

Plenary Session 03

HSP90 and epigenetic canalization in cancer
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Hsp90 is a chaperone for nearly 100 "client proteins" in the cell, most of which are involved in signaling pathways. For example, Hsp90 maintains steroid hormone receptors, such as the estrogen receptor, as inactive monomers in the cytoplasm which are capable of binding agonists. After agonist binding, the receptor-hormone complexes dissociate from Hsp90, dimerize, translocate to the nucleus, bind to the hormone response elements, activate the local chromatin, and, ultimately, activate transcription of the target genes, such as Wnt7a. More recently, Hsp90 has been shown to function as a capacitor for morphological evolution by masking both genetic and epigenetic variation. Release of the capacitor function of Hsp90, such as by environmental stress or by drugs that inhibit the ATP-binding activity of Hsp90, exposes previously hidden morphological phenotypes. The previously masked phenotypes, once revealed, can be selected in subsequent generations and increase in penetrance through both genetic and epigenetic mechanisms in a process termed "canalization" by Conrad Waddington over 70 years ago. Similarly, prenatal and perinatal exposure to diethylstilbersterol (DES), an estrogen receptor agonist, can have long-term and even trans-generational effects on predisposition to uterine developmental abnormalities and cancer, also through both genetic and epigenetic mechanisms. The unifying theme between both transgenerational epigenetic phenomena, in addition to both of them involving Hsp90, is that WNT signaling modifications are required. We propose a unifying model, which we call "WNT-mediated epigenetic reprogramming," to explain the connection between the multi-generational epigenetic capacitor function of Hsp90, and the transgenerational effects of DES on the predisposition to cancer. We also present data showing that mutations in genes encoding proteins in the WNT signaling pathway, such as Wingless/WNT and Armadillo/Beta-catenin, and in chromatin-remodeling proteins, such as the histone H3 methyl-transferase, Su(var)3-9, and the methyl-histone H3 binding protein, HP1, suppress the epigenetic canalization phenotype. We suggest that inhibitors of these enzymes might be novel anticancer agents by interfering with genetic reprogramming and canalization in cancer stem cells.

Symposia

S1 01

The mystery of conserved non-genic (CNG) sequences
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The comparison of the sequences of human chromosome 21 with that of the syntenic regions of the mouse genome revealed a large number of conserved sequences (> 100 nt in length and ? 70 % ungapped identity) that are not transcribed. We called these elements conserved non-genic (CNG) sequences. Most of these map in gene-poor regions of chromosome 21. A large majority of CNGs are also present in several mammalian species, indicating a conservation of more than 120 million years. The patterns of evolutionary conservation allow a sufficient separation of CNGs from both coding regions and non-coding RNAs. Furthermore, the evolutionary characteristics are independent of their position relative to protein-coding sequences. The overall level of conservation of CNGs is higher that exonic sequences and strongly suggests functional importance. We anticipate that mutations in CNGs may contribute to human disorders; a search for those is now in progress. The function of CNGs is largely unknown and considerable effort is now devoted to the functional analysis of these genomic elements that may account for up to1-3 % of the human genome. Some CNGs may be cis or trans regulatory elements of gene expression, others may be structural elements, and yet others may have a function totally unsuspected todate. I thank the members of the laboratory and the funding agencies for supporting our research.

S1 02

Human sequence variation for disease - The HapMap Project
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The availability of a finished reference sequence of the human genome has streamlined systematic efforts to establish a comprehensive list of common sequence variants, minor allele frequency (m.a.f) =1% and study patterns of variation at a fine-scale across the genome. The International HapMap project was launched in October of 2002 with the aim to construct a genome-wide map of common haplotypes in multiple representative ethnic groups to enable disease association studies including susceptibility to pathogens and variable response to drugs.
HapMap engaged from it's outset in SNP discovery by random whole-genome and whole chromosome shotgun sequencing. The most recent release of dbSNP (build 123) figures circa 9 M uniquely mapped SNPs. The study follows a two-phase mapping strategy in four population samples including 30 parent-child trios from CEPH (Utah residents; CEU), 30 trios from Yoruba (Ibadan, Nigeria; YRI), 45 Han Chinese (Beijing; HCB), and 45 Japanese (Tokyo; JPT). The samples have been collected with appropriate informed consent through a process of commu-

nity engagement. In phase I we are constructing maps of evenly spaced SNPs (1 per 5 kb) with m.a.f =5%. Phase II will generate data on 2.25M additional SNPs. Raw genotype data are released through the Data Coordination Center on a monthly basis (http://www.hapmap.org).
The consortium has released data on circa 1 million SNPs across the CEU panel and 0.5 million across the other two panels (YRI and CHB+JPT); phase I will be completed in February (all panels). For the first time we can observe linkage disequilibrium (LD) trends at a genome scale. For example, telomeric regions consistently display low LD whereas centromeric regions are associated with high LD. The December data-set is used to establish an analysis pipeline to assess common patterns of LD, recombination and natural selection as well as define optimal sets of tag SNPs. The analysis group has selected the 3.3M SNPs to be tested by Perlegen Inc in phase II. The set is enriched in exonic SNPs and SNPs in areas displaying high recombination rates (estimates based on population data). Phase two will be completed by next summer whereas we anticipate a possible last round to fill any remaining gaps.
Our contribution to the HapMap project includes chromosomes 1, 6, 10, 13 and 20 24% of the genome). In addition, we have initiated a systematic validation of putative functional variants. SNP analysis (dbSNP 121) in the context of protein coding genes found 48,451 non-synonymous (ns) SNPs, 1113 SNPs that introduce a STOP codon and 104 that disrupt a STOP codon. So far, we assayed 11,000 nsSNPs against the CEU panel and a further 27,468 assays are being processed. In parallel, we are resequencing all annotated human exons in 48 Caucasians.
The ultimate aim is to apply these resources to explain the genetic contribution to common diseases of major health impact. We are currently developing strategies to test large sets of markers for association in case-control study design, to confirm positive associations and assess the amount of genetic risk that is explained. We have embarked on several pilot studies mainly focusing in regions of linkage.

S1 03

Looking back: From the human karyotype to the ancestral vertebrate proto-genome
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The comparison of the human karyotype with those of our relatives reveals the human karyotype to be the more ancestral one. The chimpanzee and gorilla karyotypes differ from human mostly by multiple pericentric inversions. The molecular analysis of the inversion breakpoints in different chimpanzee species shows, that these inversions occurred in the context of the separation of the human and chimpanzee lineages and therefore may have played a causative role in the speciation process. A new model for the chromosomal speciation theory, which has been recently proposed focusses on the local suppression of recombination rates. DNA divergence data from colinear and rearranged regions between human and chimpanzee conform to this model. Comparative maps of many mammalian species which are now available allow to reconstruct the putative ancestral mammalian founder karyotype. This founder karyotype resembles the human karyotype. In 2004 the analysis of the chicken genome became available too. Once again the conservation of gene order is prevailing and can be used to reconstruct the genome of the common tetrapode ancestor of birds and mammals from 310 million years ago (MYA). In the same way the genome data from three fish species can be used to arrange a putative ancestral genome of all teleost fish. Both these ancestral genomes can be put together to get a first glimpse at the ancestral bony vertebrate founder genome from 450 MYA. This genome consists of 12 – 14 chromosomes with already huge size differences. Intrachromosomal rearrangements, such as inversion and fission are preferred in the fish lineages, whereas in the bird/mammal lineage also interchromosomal rearrangements did occur. From the two types of sex chromosomes in vertebrates the Z chromosome of the birds is more conserved. The same holds true for the whole chicken genome which despite its fissions into several macro- and microchromosomes closeley resembles the ancestral vertebrate proto-genome.

S2 01

Disorders of the human mitochondrial genome
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The true impact of mitochondrial DNA (mtDNA) mutations in human disease remains undetermined. Until relatively recently, mitochondrial disorders were considered to be obscure, exceptionally rare diseases affecting perhaps one or two per million of the population. Improvements in referral patterns and the availability of rapid, molecular diagnostic techniques have helped contribute to the expanding clinical phenotype associated with mtDNA mutations with recent epidemiological data suggesting a minimum prevalence of at least 1 in 5000, or even higher.
My talk will focus on the basic aspects of clinical mitochondrial genetics, discussing our experience in Newcastle upon Tyne of investigating, diagnosing and managing patients with primary mitochondrial disease, and the approaches we are pursuing towards developing treatments.

S2 02

Dynamics of mtDNA maintenance in health and disease
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Mitochondrial DNA in mammals is entirely dependent on nuclear encoded proteins for its maintenance. Despite early identification of some of the maintenance proteins such as mtDNA polymerase gamma (POLG), quite a few have been identified only in recent years. Good examples are the mtDNA helicase Twinkle and the transcription related factors Tfb1m and Tfb2m. The recent discovery that both the POLG and Twinkle genes are associated with various disease phenotypes which are characterized by secondary mtDNA defects has sparked renewed interest in mtDNA maintenance and its regulation.
Twinkle protein was first identified in a search for T7-helicase-like proteins in eukaryotes. The gene for Twinkle was subsequently shown to be mutated in autosomal dominant progressive external ophthalmoplegia. We have previously shown that a Twinkle-EGFP fusion protein as well as various previously characterized mtDNA maintenance proteins specifically co-localize with mtDNA in intra-mitochondrial foci designated nucleoids. MtDNA in nucleoids can be labeled with the nucleotide analogue 5-bromo-2-deoxy-uridine (BrdU) indicating that nucleoids are sites of mtDNA replication. More recently we have studied the regulation of the Twinkle protein by post-translational modification and have used various tools to study in more detail the dynamics of mtDNA nucleoids in human cells. The findings will be discussed in light of regulation of mtDNA replication and mtDNA mutation segregation and complementation.

S2 03

A new class of mitochondrial gene defects
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Mitochondria contain a separate protein synthesis machinery to produce polypeptides encoded in mitochondrial DNA and many mtDNA disease mutations affect this machinery. In humans, whilst the mitochondrial rRNAs and tRNAs are mtDNA-encoded, all proteins involved in mitochondrial translation are specified by nuclear genes. Recently, a number of pathological mutations in nuclear genes of this protein machinery have been reported defining a new class of gene defects underlying disorders of oxidative phosphorylation.

S3 01

Dyskeratosis congenita and telomeres
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Dyskeratosis congenita (DC) is an inherited bone marrow failure syndrome exhibiting considerable clinical and genetic heterogeneity. The classical form of DC is characterized by abnormal skin pigmentation, nail dystrophy and mucosal leucoplakia. A given patient may also have a variety of other somatic features. X-linked recessive, autosomal dominant and autosomal recessive forms are recognised. The gene mutated in X-linked DC (DKC1) encodes a highly conserved nucleolar protein called dyskerin. Dyskerin associates with the H/ACA class of small nucleolar RNAs in small nucleolar ribonucleoprotein particles (snoRNPs), which are important in guiding the conversion of uracil to pseudouracil during the maturation of ribosomal RNA. Dyskerin also associates with the RNA component of telomerase (TERC), which is important in the maintenance of telomeres. Mutations in TERC were recently demonstrated in patients with autosomal dominant DC and in a subset of patients with aplastic anaemia (AA) and myelodysplasia

(MDS). Additionally, some patients with the severe multi-system disorder, Hoyeraal-Hreidarsson syndrome (HH) have been found to have DKC1 mutations. These findings have demonstrated that classical DC, HH and a subset of AA are due to a defect in telomerase and cells from these patients have short telomeres. The multisystem abnormalities seen in these patients, including the increased incidence of malignancy, have highlighted the critical role of telomeres and telomerase in humans. From a clinical perspective the link between DC and AA and in turn to defective telomerase suggests that treatments directed at telomere rejuvenation might benefit DC and AA patients who do not respond to conventional therapies.
Very recently, it has been observed that "anticipation" (increasing severity and/or earlier onset of disease features with each subsequent generation) occurs in the autosomal dominant form of DC (AD DC) and that this is associated with progressive telomere shortening. Siblings that did not inherit a mutated TERC copy did not have early onset of clinical symptoms even though they inherited shorter telomeres from the affected parent. Thus, individuals with AD DC must both inherit shorter telomeres and be heterozygous with respect to TERC to show disease anticipation. This study provides the second mechanism (progressive telomere shortening thorough the generations) for disease anticipation in humans. The only other mechanism of disease anticipation relates to amplification of triplet repeats observed previously in neurodegenerative diseases.
In conclusion, recent developments in the genetics of DC have important implications for the management of patients with DC and related disorders. In addition, they have provided significant insights into the critical role of telomerase and telomeres in humans and the consequences of their dysfunction.

S3 02

Human telomere length: a prognostic marker for age-related disease and survival?
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Telomeres in most human tissues shorten with age, and there is a high degree of variation in telomere length between individuals as well as a very good correlation between telomeres in different tissues from the same individual. In recent years, telomere length in primary blood mononuclear cells (PBMC) has been associated with a wide variety of age-related diseases including atherosclerosis, myocardial infarction, stroke, immunsenescence, Alzheimer's and vascular dementia. Few retrospective and/or prospective studies so far have indicated that short telomere length does not only correlate with but actually predicts age-related mortality and morbidity. There are two interrelated causes for this:
First, short telomeres are the major trigger for cellular senescence in vitro, an irreversible growth arrest of cells accompanied by gross changes in gene expression patterns. Thus, in regenerating tissues and/or in differentiated stem cells, senescence might contribute to age-related pathology. However, the evidence that cell senescence occurs in aging human tissues is still mostly indirect. We have recently examined the signalling pathway leading to cell senescence and identified novel markers for senescence in vivo.
Second, telomeres do not shorten always by the same amount with each cell division. Rather, telomere shortening is governed by oxidative stress because of a telomere-specific deficiency in DNA strand break repair. High stress or low antioxidant defence capacity greatly accelerate telomere shortening. Telomeres are thus not only the cell's stress sentinels, but their length at any given age is a cumulative indicator of the individual's antioxidant defence, which in itself is related to the susceptibility to degenerative disease.
Contrary to e.g. genetic polymorphisms, telomeres are kinetic markers for disease and mortality risk. This has the huge potential advantage that they might be used to monitor the effect of prophylactic interventions. A major disadvantage is that PBMC telomere length can be influenced by a number of other factors as, for instance, lymphocyte subtype composition. This appears to be an issue at very advanced age: In a large population-based study of the oldest old we did not find any association between PBMC telomere length and survival or major disease.

S3 03

Role of telomere acquisition in the formation of chromosome rearrangements in human cells
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Telomeres play a vital role in protecting the ends of chromosomes and preventing chromosome fusion. We performed an extensive study of the consequences of telomere loss in a human cell model. Telomere loss results in sister chromatid fusion and prolonged breakage/fusion/bridge (B/F/B) cycles, leading to extensive DNA amplification and large deletions.We present here the consequences of the end-up of these B/F/B cycles. The large majority of telomere acquisition occurs via chromosome rearrangement. Telomere acquisition stabilizes the marker chromosome, however, two class of rearrangements are involved in telomere acquisition with dual consequences on the stability of the cell. Telomere seeding that involves Non Reciprocal Translocation and dicentrics results in a destabilisation of donor chromosomes. Chromosomes donating NRTs have lost one telomere and undergo further rearrangements or lose the donor chromosome having lost its telomere.. Final stabilisation of telomere acquisition is obtained via duplication. The duplication involves i.the subtelomeric region of the opposite arm of the marker chromosome i.i large chromosome segment of other chromosomes i.i.i formation of isochromosomes. Telomere loss is therefore an efficient mechanism for generating many of the types of chromosome rearrangements commonly associated with human cancer including allelic imbalances.The major impact of telomere loss in cancer has been proposed to occur at the time of crisis, when extensive chromosome fusion occurs when telomeres become very short due to the failure of cells to senesce However, it is now clear that cancer cells continue to have problems maintaining telomeres even after the expression of telomerase consistent with a fundamental defect affecting telomere stability in cancer. In fact, this may be the most important mechanism driving chromosome instability due to telomere loss, because crisis may selects for cells in the population that already express telomerase, and therefore cells that survive crisis will not have experienced extensive chromosome fusion during crisis.

S4 01

Disruptions of the Histone Methylase Machinery in Cancer Pathogenesis
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The Mixed Lineage Leukemia (MLL) gene codes for a histone methylase that is required for hematopoietic development and appropriate embryonic Hox gene expression. As a consequence of chromosomal translocations, MLL is mutated in a subgroup of acute leukemias that are generally associated with a poor response to current treatment regimens. MLL is considered to be an epigenetic regulator as it possesses an inherent ability to covalently mark histones to effect changes in chromatin that favor maintenance of active transcriptional states. In this capacity, MLL associates with a cohort of highly conserved accessory factors that are shared with a subfamily of histone methylases from mammals to yeast. This unique epigenetic function appears to be corrupted by MLL mutations in acute leukemias leading to disruption of the MLL macromolecular complex and inappropriately persistent Hox gene expression. Menin, a product of the MEN1 tumor suppressor gene mutated in sporadic and heritable endocrine tumors, is a component of the 1 MDa MLL complex. Abrogation of menin expression phenocopies loss of MLL, and reveals a critical role for menin in the maintenance of Hox gene expression. Oncogenic MLL fusion proteins retain an ability to interact with menin, but not with other identified complex components, and menin is an essential partner in MLL-mediated oncogenesis. These results demonstrate that a human oncoprotein is dependent on direct physical interaction with a tumor suppressor protein for oncogenic activity, and suggest a unifying model for altered epigenetic functions in the pathogenesis of endocrine and hematopoietic cancers. Novel therapeutic strategies targeting MLL-menin interactions may be particularly efficacious in acute leukemia.

S4 02

Aberrant gene methylation during cancer progression
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Aberrations of gene methylation are among the most frequent genetic abnormalities found in cancer cells. It remains an enigma whether altered methylation patterns represent a cause or a consequence of neoplastic growth. In recent years we have investigated gene methylation in early stages of cancer and precursor lesions by applying laser microdisssection. In breast cancer intraductal non-invasive precursor lesions already exhibited altered methylation patterns

when compared to normal breast epithelium. The same held true for some intraductal benign proliferations. With progression to invasiveness loss as well as gain of aberrant methylation could be observed. In liver tumours aberrant gene methylation could be found as well in benign adenoma as in carcinoma. Both tumours did not differ principally but quantitatively from each other in that in hepatocellular carcinoma the number of genes and alleles found to be hypermethylated was constantly higher. With regard to genes affected by hypermethylation no deviation was seen between benign and malignant epithelial liver tumours. In normal liver tissue some of the genes found to be constantly hypermethylated in cancer exhibited an age-dependent increase of methylation. These findings are a strict indication that gene methylation has to be studied quantitatively and in a cell type dependent manner. Applying a new quantitative assay we could show that ductal invasive can be discriminated from lobular breast cancer on the basis of different patterns of gene methylation. Furthermore, it became evident that the histological grade of some types of intraductal cancer correlated with the degree of hypermethylation. In conclusion methylation is a frequent aberration that becomes already manifest in early stages of cancer. It can be correlated to histological subtypes and grades and for this purpose it has to be investigated quantitatively. Future perspectives comprise application of array technology and analysis of hypomethylation.

S4 03

Nuclear cloning and the reversibility of cancer

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Nuclear transfer experiments allow studying the role of epigenetic modifications in cellular differentiation and transformation. Moreover, nuclear transfer provides a tool to derive autologous embryonic stem cells for a potential use in cell therapy. We have shown that nuclear transfer of mature lymphocytes produces monoclonal mice that carry a single antigen receptor in all tissues, thus demonstrating that the nucleus of a terminally differentiated cell can be reprogrammed to totipotency. Likewise, we have shown that the genomes of certain cancer cells are amenable to epigenetic reprogramming and can support partial development, indicating that the epigenetic state of at least some cancers may be reversible. To demonstrate the potential use of nuclear transfer for cell therapy, we have established a mouse model of „therapeutic cloning" by combining nuclear transfer with gene and cell therapy to treat a genetic disorder in a mouse model of disease. We will discuss the potential factors involved in epigenetic reprogramming with an emphasis on the homeobox transcription factor Oct-4 and its role in differentiation and tumorigenesis.

S5 01

Sequence-based evolutionary studies of the human sex chromosomes

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The completion of the human X chromosome project means that we have, for the first time in any organism, the completed sequences of a sex chromosome pair. This information presents an unprecedented opportunity to study the evolution of sex chromosomes and of their unique biological properties. The human sex chromosomes will become the paradigm for the study of sex chromosomes in the mammals and beyond.
Our work has been to complete and analyse the sequence of the human X chromosome. Together with colleagues in the USA and Germany, we have determined the sequence of 99.3% of the euchromatic part of the X chromosome. We have annotated only 1,098 genes on the chromosome, which is therefore among the most gene-poor in the genome. Of these genes, approximately 10% encode cancer-testis antigens, which are potential targets for tumour immunotherapy.
Comparison of the X chromosome sequence to other genome sequences provides a wealth of information about mammalian sex chromosome evolution. Alignment to the chicken genome illustrates the autosomal origins of the human X chromosome vividly. Comparison to the X chromosomes of other eutherian mammals demonstrates the remarkable stability of the X chromosome on the human lineage and the highly rearranged nature of the mouse and rat X chromosomes. And, alignment between the human X and Y chromosome sequences reveals in the greatest detail the extent of Y chromosome degeneration in non-recombining regions.
In this presentation, I will discuss our evolutionary studies of the human sex chromosomes, our observations on sequence compositional variation in different evolutionary strata of the human X chromosome, and what this might reveal about the proposed involvement of LINE1 elements in X chromosome inactivation.

S5 02

Understanding sex-specific function, mutation processes, population movements and global diversity patterns from human Y chromosome analyses

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The human Y chromosome does not recombine with the X chromosome for most of its length. The Y chromosome determines testis formation and it contains several genes and gene families necessary for male germ cell development and maintenance. Much of the chromosome consists of large, low-copy repetitive elements that contain genes expressed specifically in the testis. These paralogous repeats may be associated with Y-Y gene conversion events and they render the chromosome susceptible to interstitial deletions by non-allelic homologous recombination. Many of these deletions cause spermatogenic failure (AZFa, AZFb and AZFc deletions), some are apparently inconsequential polymorphisms (g1/g3, 12f2) and for others the precise relationship with fertility needs to be defined (gr/gr, b1/b3). I will review recent progress in this field. As well as providing direct insights into mutation processes on the Y chromosome that affect spermatogenesis, the analysis of the near-complete Y chromosome sequence has revealed many new polymorphic markers resulting in a refined Y chromosome phylogeny. The uniparental inheritance of the Y chromosome makes it ideal for the study of human origins and migration patterns. The distribution of Y chromosome lineages is mainly characterised by geographically structured drift rather than language or ethnic affiliations. The availability of these markers and the knowledge of their distribution has lead to a subtle shift in the approach used to understand the role of the Y chromosome in human pathologies. Recently a number of studies have attempted to associate Y lineages with various phenotypes including blood pressure, prostate and testicular cancer, autism, longevity and infertility/sperm counts. Some of these studies have shown promising associations and warrant the development of large-scale multinational projects.

S5 03

Early events in the onset of X-inactivation: Multiple role for non-coding RNAs.

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X chromosome inactivation is the epigenetic process which ensures dosage compensation of the X chromosome in mammals between the XX female and XY male. This chromosome wide mechanism of transcriptional silencing, is under the control of a complex master locus, the X-inactivation centre (Xic) that contains the Xist gene,the source of a large 17 kb non-coding RNA critical to the X-inactivation process. The onset of X-inactivation during early embryogenesis sees an up-regulation of Xist transcripts and the spreading of the Xist RNA along the X chromosome, followed by chromatin modifications involving both extensive histone modifications and the recruitment of polycomb group proteins to the inactivating X and the transcriptional repression of X-linked genes. Female ES cells are extensively used as models for the inactivation process as undifferentiated female ES cells have 2 X's active and X-inactivation occurs with the onset of differentiation. More recently both TS cells (Trophoblast) and XEN cells (extra-embryonic endoderm) have been used as models for imprinted X-inactivation. Integral to the initiation of X-inactivation is the counting process in which the X chromosome/autosome ratio in the cell is sensed and a choice step in which one of the two X-chromosomes in the female cell will be selected for inactivation. Both of these processes involve a region of the Xic 3' to Xist which also contains the non-coding Tsix antisense RNA which partially overlaps Xist. Recent results now also suggest an association of this antisense transcription with histone modification and chromatin remodelling around the Xist promoter prior to the onset of X-inactivation. An overview of recent progress in the field will be given.

S6 02

Insight of phenotype-genotype correlations in some CHDs

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Advances in understanding the molecular basis of congenital heart defects (CHDs), including tetralogy of Fallot (ToF), atrioventricular canal defect (AVCD), pulmonary valve stenosis (PVS) and atrial septal defect (ASD), have allowed to set forth some phenotype-genotype correlations. ToF is the most common cyanotic CHD. Distinct anatomic subtypes have been reported in del 22q11 individuals, including right aortic arch (AA), hypoplasia or discontinuity of pulmonary arteries, absent infundibular septum, absent pulmonary valve (APV), pulmonary atresia (PA), and major aorto-pulmonary collateral arteries (MAPCA). However, 22q11 FISH and TBX1 candidate gene mutation analyses in the same nonsyndromic ToF subtypes failed to detect obvious functional variations. While abnormal sidedness or branching of the AA occur with ToF in del 22q11 individuals, JAG1 gene mutations, in the setting of Alagille syndrome, can result in severe ToF, with PA and MAPCA, without left-sided AA. Low penetrant NKX2.5, GATA4, and FOG2 gene mutations have been detected in nonsyndromic ToF, with both PA and PVS. In particular, we found FOG2 mutations in individuals with "classic" anatomy or PA, hypoplastic confluent pulmonary arteries and MAPCA. AVCD is often associated with extracardiac anomalies, most commonly Down syndrome (DS) and heterotaxy. However, this heterogeneous defect can manifest with variable anatomic patterns. Complete AVCD prevails among patients with chromosome imbalances (i.e. DS, del 8p). Additional cardiac defects, in particular, left-sided obstructive lesions, occur in some mendelian syndromic disorders. Partial AVCD, with subaortic obstruction due to mitral valve anomalies, occurs in Noonan syndrome (NS), in which the distinguishing CHD is PVS. In these patients, the valvular stenosis usually consists of valve leaflet dysplasia, differently from isolated PVS, presenting with a domed form fusion of pulmonary semilunar valves. PTPN11 gene mutations have been found in about 50% of NS and 90% of LEOPARD syndrome individuals. We found a definite association between PVS and exon 8 mutations, while PTPN11 was not involved in isolated, non dysplastic PVS. Autosomal dominant PVS+ASD with normal conduction was linked to GATA4 gene mutations in a few families. We found GATA4 mutations in 40% of these patients. NKX2.5 gene mutations have been detected in individuals with ASD+other cardiac defects, in particular progressive atrioventricular block. In conclusion, understanding the genetic background underlying the different anatomic subtypes of similar CHDs provides insight of phenotype-genotype correlations, appraises the dimension of nosological splitting and lumping of CHDs, and improves their genetic counselling.

S6 03

Beyond 22q11 Deletions: Uncovering the Aetiology of DiGeorge Syndrome-like Heart Defects in Man.

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Many cases referred for deletion 22q11 screening are not found to carry a deletion, some of these have a strong phenotypic resemblance to DiGeorge or velocardiofacial syndrome (DGS/VCFS). When mouse gene targeting studies revealed Tbx1 to be haploinsufficient and necessary for the morphogenesis of structures affected in DGS/VCFS several laboratories screened their non-deletion cohorts for Tbx1 mutation. In our laboratory, over 150 cases have been screened by a mixture of direct sequencing and SSCP with no mutations found.
The multiple malformations seen in our non-deletion patients are often accompanied by a degree of cognitive impairment which suggests a chromosomal aneusomy. Therefore, we conducted comparative genome hybridization in a search for new aetiologies. Three deletions have been found in 30 patients screened so far. In one, the patient is hemizygous for the genes Islet-1, implicated in the development of the anterior heart field, and Follistatin, implicated in the development of the palate.
One congenital heart defect seen in DiGeorge syndrome, and always observed in Tbx1-/- mice, is a common arterial trunk (CAT), often diagnosed as persistent truncus arteriosus. We have analysed a family with autosomal recessive CAT and identified a homeodomain mutation F157L in the NKX2.6 transcription factor. While expression of Nkx2.6 during murine embryogenesis is strongly suggestive of a role for this gene in heart development, mice Nkx2.6-/- are normal. However, in these mice it has been shown that Nkx2.5 expression expands into regions lacking Nkx2.6 suggesting functional complementation. As transcriptional targets of NKX2.6 are unknown, we investigated functional effects of the mutation in transcriptional and protein interaction assays using NKX2.5 as a surrogate. Introduction of F157L into human NKX2.5 substantially reduced its transcription activating function, its synergism with partners at the atrial natriuretic factor (ANF) and connexin-40 (Cx40) promoters, and its specific DNA binding. We tested NKX2.5 target promoters for NKX2.6 activity. NKX2.6 was inactive at ANF but weakly activated transcription of a Cx40 promoter, whereas the F151L mutant lacked this activity. These findings indicate a previously unsuspected role for NKX2.6 in heart development which should be re-evaluated in more sophisticated model systems.

Selected presentations

SEL 01

Delineation of distinct subgroups of multiple myeloma and a model for clonal evolution based on interphase cytogenetics

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In multiple myeloma (MM), two pathogenetic pathways have been proposed, characterized by reccurent IgH-translocations versus trisomies. Due to the limitations of metaphase cytogenetics in MM, the aim of this study was to assess the existence of MM subgroups applying multiprobe interphase florescence in-situ hybridization (FISH).
Highly enriched CD138-positive bone marrow cells from 81 patients with newly diagnosed MM were analyzed by interphase FISH, using 10 region-specific probes and two probe-sets for the IgH-translocations t(11;14) and t(4;14).
Per patient, a median of 5 probes (range, 1 to 10) displayed aberrant signal numbers. Additional copies were most frequently found for chromosomes 15q22, 19q13, 9q34, 11q23, and 1q21. Common losses were observed for 13q14.3, 17p13 and 22q11. Predominance of gains or losses was quantified by a copy number score (CS) for each patient (CS equals the number of probes indicating additional signals minus the number of probes indicating losses). Two peaks (CS=+3, and CS=0) were found by plotting patient numbers over CS values, corresponding to hyperdiploid and non-hyperdiploid MM. Twenty-two of the 28 patients with one of the IgH-translocations had a CS≤0. Cluster analysis revealed four major branches: (i) gain of 9q, 15q, 19q, and/or 11q; (ii) deletion 13q and t(4;14); (iii) t(11;14); and (iv) gain of 1q. Statistical modeling of an oncogenetic tree by maximum likelihood estimation indicated as early independent events: (i) gain of 15q/9q and/or 11q; (ii) t(11;14); (iii) deletion 13q followed by t(4;14); and (iv) gain of 1q. Aberrations of 17p13, 22q11, 8p12 and 6q21 were found as subsequent events. MM with gain of 1q was characterized as a subentity with significantly higher beta-2-microglobulin and lower hemoglobin levels, indicating a poor prognosis. Based on our results, we propose a model for clonal evolution of MM.

SEL 02

DNA-repair and cell cycle checkpoint defects in Nijmegen Breakage Syndrome

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The human genetic disorder, Nijmegen Breakage Syndrome, is characterised by radiosensitivity, immunodeficiency and an increased risk for cancer. The NBS1 gene codes for a protein, nibrin, involved in the processing/repair of DNA double strand breaks and in cell cycle checkpoints. Most patients are homozygous for a founder mutation, a 5bp deletion; however, functionally relevant truncated nibrin proteins are produced. In contrast, null mutation of the homologous gene, Nbn, in mice is lethal. Mice heterozygous for an Nbn null-mutation develop tumours and die earlier than wild type mice.

We have used Cre-recombinase/LoxP technology to generate an inducible Nbn null mutation allowing the examination of DNA-repair and cell cycle-checkpoints in the complete absence of nibrin. Induction of Nbn null mutation leads to loss of the G2/M checkpoint, increased chromosome damage, radiomimetic-sensitivity and cell death. In vivo, this particularly affects the lymphatic tissues, bone marrow, thymus and spleen. In vitro, cells can be rescued from cell death by transfer of human nibrin cDNA and, more significantly, by a cDNA carrying the 5bp deletion. Thus, the expression of truncated protein is sufficient to restore at least some of nibrin's vital cellular functions. Phosphorylation of nibrin by ATM is a primary cellular response to ionising radiation. The nibrin fragment present in NBS patient cells is not phosphorylated in response to ionising radiation explaining their radiosensitivity.

Furthermore, conditional inactivation of the Nbn gene in mouse B-lymphocytes impairs immunoglobulin class switching, a process requiring the formation of DNA double strand breaks. This result implies a functional role for nibrin in non-homologous DNA-end joining during Ig class switching and probably in DNA double strand break repair generally.

SEL 03

Positional cloning of the CMT2B2 gene and its implications for CMT1A

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Charcot-Marie-Tooth disease (CMT) comprises a group of frequent, genetically and clinically heterogenous peripheral neuropathies. Two main CMT forms are distinguished: the demyelinating CMT type 1 (CMT1) and the axonal CMT type 2 (CMT2). Recently we reported linkage of the axonally pronounced CMT2B2 type to chromosome 19q13.3 (OMIM %605589). Analysis of 53 genes in the critical intervall resulted in an A335V mutation in a subunit of the mediator complex associated with RNA polymerase II. This mutation is embedded in a proline-rich motif typical for Abelson-SH3 binding sites. Wildtype and mutant peptides were incubated with Abl-SH3- and Src-family SH3 protein. While wildtype and mutant CMT2B2 strongly bind Abl-SH3, the mutation results in a drastically increased affinity for Src-family SH3 domains. This indicates a severe loss of specificity in target recognition. A mild myelin impairment in these patients guided us to investigate the CMT2B2 gene expression by qPCR in Pmp22 over- and underexpressing mice. A significant correlation of CMT2B2 expression with Pmp22 expression could be clearly shown - a high Pmp22 expression level resulted in a high CMT2B2 level and vice versa. These results were confirmed in transgenic CMT1 rats. Negative phenotypic and behavioural effects of progesterone treatment in CMT1 rats have been reported recently. We could show that progesterone-treated male CMT1 rats revealed a tremendous increase of the CMT2B2 expression correlated with increased Pmp22 expression. Our data show that the CMT2B2 A335V mutation causes an inherited peripheral neuropathy. Furthermore the tightly linked expression to Pmp22 points to a more general role in peripheral nerve pathogenesis, possibly via transcriptional (mis-) regulation of multiple nerve specific genes in a yet unknown signal transduction cascade. A common late stage feature of inherited peripheral neuropathies is the axonal damage, the CMT2B2 gene could be a crucial player in this context.

SEL 04

Rescue of photoreceptor degeneration in a genetic mouse model for X-linked juvenile retinoschisis

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Objectives: Deleterious mutations in the RS1 gene on Xp21 are associated with X-linked juvenile retinoschisis (RS), a common form of macular degeneration in males. We have generated a knock-out mouse deficient in Rs1h, the murine ortholog of RS1. The knockout mouse exhibits many characteristic features of human RS including the presence of small cyst-like structures in the inner retina, and disorganization and displacement of cells within the retinal layers. Accordingly, the Rs1h-deficient mouse serves as a valuable model system to develop possible therapeutic interventions for human RS.

Material and Methods: To explore the latter goal, we have generated a construct containing the human RS1 cDNA under the control of the mouse opsin promoter in the replication-deficient adeno-associated virus (AAV) serotype 5. The RS1-AAV construct was injected into the subretinal space of one of the eyes of 15 day old Rs1h knockout mice. The uninjected eye served as a control.

Results: The injected and uninjected eyes were evaluated at various time points by ERG and microscopic as well as immunolabeling techniques to assess the effect of gene transfer on photoresponse, protein expression and morphology of the retina. Our data show that retinoschisin is expressed and secreted from photoreceptors of the RS1-AAV injected eyes. Like normal mice, the expressed protein localizes to rod and cone photoreceptors of the outer retina and bipolar cells of the inner retina at essentially normal levels. Importantly, the injected eyes showed a significant improvement in the ERGs, retinal tissue morphology and preservation of photoreceptors.

Conclusions: Our study demonstrates that gene therapy can restore visual function in a Rs1h-deficient mouse to nearly normal levels. This visual improvement is accompanied by a corresponding recovery of retinal morphology at both the inner and outer retinal layers. Implications for clinical intervention in human RS are apparent.

Workshop Presentations

W01 Cancer cytogenetics

W01 01

Implementation of an automated scanning system for the high-throughput FA-specific interphase FISH-assay sensitively detecting MDS- and AML-associated chromosomal imbalances and further applications

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Bone marrow (BM) failure in FA patients followed by myelodysplastic syndrome or AML is frequently associated with the appearance of clonal chromosome aberrations in BM cells detectable by sensitive molecular cytogenetic techniques (e.g. FISH and CGH). We established and validated a highly sensitive interphase-FISH (I-FISH) assay for the early detection of the most common adverse clonal chromosomal imbalances in uncultivated BM and peripheral blood (PB) cells from FA patients. However, the manual counting of up to 1000 interphase cells for each individual FISH-probe and target material (e.g. BM and PB direct preparations) by a human evaluator is time consuming and restricts the number of prospective I-FISH analyses which can be performed. We know from our prospective clonality studies presented last year, that the adverse clones appear and expand very fast. To permit more frequent high-throughput I-FISH analyses, we are currently integrating an automated scanning system for unattended search and capturing of interphase nuclei in our analysis strategy. The multifunctional slide scanning system is based on a motorized fluorescence microscope equipped with a 8-bay slide scanning stage controlled by an adaptive scanning software (Metasystems). During the scanning process interphase cells and metaphase spreads are identified, pictures are saved, and I-FISH signals are counted automatically. Using this automated device and appropriate classifiers, a stable, evaluator-variation free detection and quantification of aberrant clones in BM and PB cells can be performed. First data show, that subpopulations of interphase cells from uncultivated peripheral blood mononuclear cells (e.g. granulocytes) can be evaluated selectively by their morphology without former enrichment of these cells. Furthermore, we will present other scanning-based applications for the detection and characterization of chromosome instability.

W01 02

Extensive analysis of the amplicon 11q13.5 frequently co-amplified with the MLL gene in a large collection of AML/MDS patients with MLL amplification.

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Amplification within chromosome arm 11q involving the mixed lineage leukemia gene (MLL) locus is a rare but recurrent aberration in acute myeloid leukemia and myelodysplastic syndrome (AML/MDS). In our recent study employing microarray-CGH and FISH we have shown that in addition to the core MLL amplicon, independent sequences in 11q23-24 and/or 11q13.5 were co-amplified within the same cytogenetic markers in a series of 13 AML/MDS patients with multiple copies of MLL gene. Both regions harbor a number of genes with possible oncogenic potential. In a present study we have focused on 11q13.5 amplicon represented by clone bA7H7 that has been found co-amplified in 60% of AML/MDS cases. Using semi-quantitative PCR and FISH analysis we showed in 40 AML/MDS patients that the minimal amplicon involves oncogene GRB2-associated binding protein 2 (GAB2), ubiquitin specific protease 35 (USP35), and odd Oz/ten-m homolog 4 (ODZ4) gene, but not thyroid hormone responsive (THRSP) and p21/Cdc42/Rac1-activated kinase 1 (PAK1). By this means we narrowed down the minimal amplified region of overlap bordered by clones bA7l15 and bA153F6 from previously roughly estimated 2.4 Mb down to 1.17 Mb. Results of a real-time RT-PCR based expression study of the selected genes and possible implications for leukemogenesis in the patients with 11q13.5 amplification will be also presented.

W01 03

NUP98 FISH screening in childhood and adult myeloid malignancies leads to the identification of topoisomerase 2B as a new fusion partner

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Rearrangements of the chromosomal region 11p15.5 occur in a broad range of hematologic malignancies, such as de novo and therapy related AML, CML, MDS and T ALL. They commonly result from a fusion of the *NUP98* gene with a variety of partner genes, 17 of which are currently known already. *NUP98* encodes the 98kDa nucleoporin protein NUP98, which is part of the nuclear pore complex (NPC). The respective fusion partners, on the other hand, comprise two main groups, namely HOX and non-HOX genes. We used FISH to determine the incidence and types of potential *NUP98* gene rearrangements in 59 unselected childhood AML cases that were enrolled in the Austrian AML-BFM93 clinical trial as well as in 14 adult myeloid disorders with 11p15 aberrations. This approach revealed altogether five cases with a *NUP98* gene rearrangement. Further FISH and molecular genetic analyses ascertained four cases with known fusion genes, but also suggested the presence of a new fusion partner in an adult patient with AML-M5a and a non-recurrent t(3;11)(p24;p15). The cases with previously identified gene fusions comprised one childhood AML with a t(5;11)(q35;p15) and a *NUP98/NSD1*, two adult cases with an inv(11)(p15q22) and a *NUP98/DDX10* and another one with a t(11;20)(p15;q12) and a *NUP98/TOP1* gene fusion. Moreover, we also succeeded to identify topoisomerase 2 B (*TOP2B*) as the new partner gene and, thus, also to characterize the resulting gene fusion in more detail.

W01 04

A novel DNA/RNA FISH X inactivation assay reveals a nonrandom, ploidy-dependent acquisition of the active and inactive X chromosomes in childhood hyperdiploid acute lymphoblastic leukemia (ALL) and non-Hodgkin lymphoma (NHL)

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The most common numerical chromosome aberration in childhood ALL and NHL is the gain of an extra X chromosome in both male and female patients. It was unclear whether this nondisjunction affects the active and inactive X chromosomes in a random or nonrandom fashion. The inactivation status of acquired X chromosomes is usually evaluated by methylation-specific PCR (MS PCR), which allows the simultaneous quantification of differentially methylated polymorphic DNA sequences. However, quantification with MS PCR has its limitations, especially in cases with low blast cells numbers or multiple X chromosomes. We therefore developed a simultaneous dual-color DNA/RNA FISH assay that enables the enumeration of active and inactive X chromosomes on a single cell level. FISH was performed with probes specific for the X centromere and the XIST RNA that is exclusively expressed from and covers vast parts of the inactive X in human interphase cells. Following evaluation of the assay on methanol/acetic acid-fixed cells from healthy individuals and cases with constitutional X chromosome aneusomies, we analyzed 54 hyperdiploid childhood ALL and 29 NHL cases. In contrast to all constitutional control samples, which as expected contained only one active X, the active X had been duplicated in male and females patients with three sex chromosomes. However, all female patients with four X had gained both the active and inactive X, which corroborates previously established evidence that a single nondisjunction event leads to the maldistribution of chromosomes irrespective of the ploidy range. Moreover, the ex-

clusive presence of duplicated active X chromosomes in hyperdiploid ALL concurs with the results of gene expression profiling studies, which have shown a corresponding overexpression of X-encoded genes.

W01 05

Recurrent chromosomal breakpoints in the immunoglobulin heavy chain gene locus in Reed-Sternberg cells of classical Hodgkin lymphoma

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Chromosomal breakpoints affecting immunoglobulin (IG) gene loci are the hallmark of B-cell non-Hodgkin lymphomas. In classical Hodgkin lymphoma (cHL), despite its predominant B-cell nature, the presence of chromosomal changes affecting IGH, IGK or IGL has not been systematically explored so far. Here, we have studied a large series of cHL for chromosomal breakpoints in the IGH (n=196), IGL (n=87) and IGK (n=86) loci by FISH and FICTION. By FICTION, the neoplastic Hodgkin and Reed Sternberg (HRS) cells were identified by expression of the CD30 antigen and a characteristic morphology. By FISH, only cases with large hyperploid nuclei suggestive for HRS cells were evaluated. Using these criteria 129 (66%), 45 (52%) and 50 (58%) cases were evaluable with probes flanking the IGH, IGL and IGK gene loci, respectively. Twenty-six cHL (20%) contained signal constellations indicating the presence of a breakpoint in the IGH locus. Translocations affecting the IGL or IGK loci were identified in one case (2%) and none, respectively. The partner chromosomes in four cHL with IG translocations could be identified by FISH on metaphases, and involved 2p13 (translocated to IGL), 16p13, 17q12, and 19q13. The candidate IG-translocation partners in 2p13 and 19q13 were identified as REL and BCL3/RELB. These loci encode for members of the Rel/NF-kB signalling pathway, which is constitutively activated in cHL. The candidate IG-partner genes in 16p13 and 17q12 remain to be identified. Non-Hodgkin lymphoma-associated oncogenes like CCND1, BCL2, BCL6 and MYC were ruled out as IGH-partners by FISH or FICTION in the 15 cases analyzed so far. In conclusion, we report for the first time that IG translocations are recurrent chromosomal changes in cHL. Their characterization might unravel further insights into the pathogenesis and cellular origin of this atypical B-cell lymphoma.

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W01 06

Detection of frequent chromosomal translocations and their prognostic significance in chronic lymphocytic leukemia (B-CLL)

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Objectives: Conventional metaphase cytogenetics underestimates the frequency of specific chromosome aberrations in CLL due to the low in vitro proliferative activity of CLL cells.

Material and Methods: Stimulation with CD40 ligand (CD40L) increases the frequency of metaphases (CD40L-enhanced cytogenetics; CEC) which we analyzed either by G banding or Multiplex-FISH. Blood samples were obtained from 120 CLL patients and subjected to simultaneous analysis by CEC and Interphase-FISH (I-FISH).

Results: As expected, by I-FISH, 80% of samples revealed the known aberrations. In contrast, CEC detected chromosomal aberrations in 90% of samples. Most importantly, we identified balanced and unbalanced translocations in about 35% of patients. The majority of the involved breakpoints were recurring. Breakpoint cluster regions were located within known affected regions in CLL, like 13q14, 14q32 or 18q21. In addition, we unraveled breakpoints outside of the known hotspots, e.g. on chromosomes 1, 2, 3, 6, 7 and 20. Median treatment-free survival (TFS) was significantly shorter for patients with translocations (28 mo) compared to patients without translocations (106 mo; $p<0.0001$). Overall survival was significantly decreased for patients with unbalanced translocations ($p=0.0007$). In patients with 13q or 11q deletions an additional translocation determined critically the prognosis. In a multivariate analysis including Binet stage, presence of >2 aberrations, CD38 expression, 11q- and 17p-, translocations proved to be the prognostic marker with the highest impact for a short TFS ($p<0.001$).

Conclusions: Stimulation of CLL cells by CD40L is suitable for generating enough metaphases for chromosome analysis. By CEC more chromosomal aberrations are detected compared to conventional methods and a large subset of CLL patients are identified with translocations and a poor clinical course. The chromosomal regions involved in translocations probably bear important regions for pathogenesis and progression of CLL.

W02 Clinical Genetics

W02 01

Exclusion/confirmation of Ataxia telangiectasia via cell cycle testing

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Objectives: Ataxia telangiectasia is an autosomal rezessive neurodegenerative disorder with increased radiosensitivity and cancer susceptibility. The ATM gene consists of 66 exons and a coding region of 9171 bp which renders direct sequencing costly. Colony survival assays following X-irradiation and immunoblotting for ATM protein are sensitive and specific tests but require the prior establishment of lymphoblastoid or fibroblast cell lines. The preparation of nuclear or whole cell lysates from peripheral blood needs up to $1x10^7$ cells, and protein recovery is variable and strongly dependent on the age of the blood specimen such that a recently developed immunoassay may neither be reliable nor practical with blood samples from small children shipped by mail. AT lymphocytes respond poorly to lectin mitogens which renders standard chromosome breakage analysis difficult.

Material and Methods: To circumvent these problems we use a cell cycle assay that simultaneously measures mitogen response and radiosensitivity of 72 h peripheral blood lymphocyte cultures. We have studied 327 patients with the clinical suspicion of AT, confirming the diagnosis in 82 cases. Peripheral blood mononuclear cells were X-irradiated at doses ranging from 0 to 4 Gy and incubated for 72 h in the presence of PHA. Using bivariate BrdU/Hoechst flow cytometry, the following cell cycle parameters were measured: (1) proportion of non-proliferating (G0,G1) cells, (2) proportion of first cycle G2-phase cells relative to the growth fraction.

Results: 93% of the cases could be unequivocally assigned to either the non-affected or the affected group of patients, while 7% presented with non-conclusive cell cycle results. Inclusion of AFP-measurements corrected for age further clarified nearly half of these cases.

Conclusions: Cell cycle testing in conjunction with AFP determination can thus be recommended as a rapid screening procedure in the differential diagnosis of juvenile ataxias.

W02 02

Childhood overgrowth in NF1 patients with 1.4 Mb microdeletion in 17q11.2

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Growth anomalies such as macrocephaly and short stature are well known features in patients with classical neurofibromatosis type 1 (NF1). In classical NF1 with intragenic *NF1*-mutations a mutation-specific genotype-phenotype relation does not exist. NF1 patients with a contiguous 1.4 Mb microdeletion of the entire *NF1* gene and its flanking regions, however, show a distinctive pattern of clinical features. These include mental retardation, craniofacial dysmorphism, more severe and earlier manifestations of neurofibroma and an increased risk of malignant tumors.
In a group of 21 patients with a 1.4 Mb *NF1* microdeletion - including three patients with Weaver-like appearance - we found advanced height growth and an accelerated bone age. The growth charts revealed overgrowth above the 95th centile in the preschool age.
Our observation indicates that accelerated growth in childhood is part of the phenotypic spectrum associated with the 1.4 Mb NF1 microdeletion. We postulate that the deleted chromosomal region contains a gene whose haploinsufficiency causes overgrowth.

W02 03

Systematic assessment of atypical deletions reveals genotype-phenotype correlation in 22q11.2

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Clinical variability associated with the common 22q11.2 microdeletion is well known and led to broad application of FISH diagnostics with probes for loci TUPLE1 or D22S75, although rarely reported atypical deletions associated with the same phenotypic spectrum would not be discovered by these probes. As most types of 22q11.2 deletions occurred between low copy repeats within the region (LCR22), we assumed that atypical deletions would be more common than reported. To address this question and the possibility of a deletion size related genotype-phenotype correlation, we established a set of 10 DNA probes which is capable to detect all reported and hypothetical types of deletions in between the LCR22, and analysed a total of 350 patients.
Surprisingly, investigation of 273 patients with conotruncal heart defects (ctCHD) and 14 patients with typical VCFS phenotype revealed only the common 3 Mb and nested 1.5 Mb deletions in 18.5% and 79%, respectively, while 5% of patients with mildly suggestive, atypical phenotype, showed atypical distal deletions. To exclude unspecificity of the atypical distal deletion phenotype we further analysed 250 patients with unclassified MCA/MR conditions in addition to subtelomeric FISH screening, but did not detect the atypical distal 22q11.2 deletion in any of those. This statistical significant differences demonstrate that atypical distal 22q11.2 deletions are very uncommon in patients with ctCHDs, while atypical congenital heart defects and mild dysmorphism is a recognizable feature of atypical distal deletions. Further phenotype-genotype analysis disclosed association of significant developmental delay with the distal part of the common deletion region.
Sequencing of non-deleted typical patients revealed a missense mutation within the T-box of TBX1 in one patient, thus emphasizing the association of the VCFS/DGS facial gestalt to the proximal common deletion region.

W02 04

Towards a first genotype-phenotype correlation of small supernumerary marker chromosomes (sSMC)

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Small supernumerary marker chromosomes (sSMC) are present in 0.045% of newborn. In about 30% of sSMC carriers an abnormal phenotype is observed. Clinical outcome of sSMC presence is difficult to predict as different phenotypic consequences can appear due to (i) differences in euchromatic DNA-content, (ii) uniparental disomy (UPD) of the sSMC's homologous chromosomes, and/or (iii) different degrees of mosaicism. We did own studies on >170 cases with sSMC and performed a review of the literature (i.e. presently 1628 cases with sSMC) available at http://mti-n.mti.uni-jena.de/~huwww/MOL_ZYTO/sSMC.htm. A first genotype/phenotype correlation for sSMC was deduced from that. Thus, small proximal trisomies of Xp, Xq, 1p/q, 2p, 4q, 5p, 6q, 7q, 8p/q, 12p/q, 14q, 17q, 18q, 19q, 20/20p, lead to clinical manifestations, while small partial proximal trisomies of 2q, 5q, 7p, 15q, 17p, 18p, 21q, 22q may not be associated with significant clinical symptoms. No general correlation could be found in connection with mosaicism of sSMC (47,+mar/46) and clinical symptoms. Recent own studies using new centromere and subcentromer-specific probe-sets ([sub]cenM-FISH; Starke et al., 2003, Hum Genet 114:51-67) gave evidence that this may be at least in parts due to the fact, that many patients do have different derivative-types of a sSMC – however, these could not be detected and characterized before the development of subcenM-FISH. In summary, almost 50 year after description of the first sSMC, molecular cytogenetics provides finally approaches for their comprehensive characterization. The new knowledge coming from that will lead to an improved genetic counseling of cases with de novo sSMC. Supported by the Dr. Robert Pfleger-Stiftung.

W02 05

A New XLMR Syndrome Characterized by Mental Retardation, Primary Ciliary Dyskinesia and Macrocephaly, Caused by a Novel Mutation in OFD1

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We report on a new syndrome characterized by mental retardation, primary ciliary dyskinesia (PCD) and macrocephaly in a large three-generation family with an X-linked recessive mode of inheritance. The 10-year-old index case had delayed motor development, severe mental retardation (IQ20), and macrocephaly. Recurrent respiratory infections led to the diagnosis of PCD at the age of 8 years. Eight other affected males died because of bronchopulmonary infections before the age of 5 years. All were mentally retarded, and in some hydrocephalus was suspected because of macrocephaly. In one boy, postaxial hexadactyly was described. None of the affected had situs inversus. Five obligate female carriers were clinically inconspicuous. We performed pairwise and multipoint linkage analysis using 69 SNPs as markers, and found tight linkage to DXS8019 with a maximum LOD score of 2.99. The gene defect was mapped to a 25 cM interval in Xp22.32–Xp21.3. Based on the specific phenotype of ciliary dyskinesia, the candidate interval was screened for known cilium-associated genes. No such genes were found. Therefore, we performed an in silico comparative genomic approach, comparing all genes from the candidate interval with those from Chlamydomonas reinhardii as a ciliated and those from Arabidopsis thaliana as a non-ciliated organism. Four genes have been qualified for further analyses. The causative mutation was found in OFD1, the gene known to be mutated in X-linked dominant Oral-Facial-Digital syndrome type 1 (OFD1, MIM#311200). An insertion of four nucleotides (AAGA) in exon 16, present in the affected males and all obligate carrier females, causes a frameshift that introduces a premature stop codon. Conspicuously, this mutation induces an apparently milder and different phenotype compared to those reported for OFD1. Our findings suggest that OFD1 plays also an important role in the biogenesis and/or functioning of cilia.

W02 06

Understanding computer-based decisions of syndrome diagnosis using facial traits

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Classification rates in syndrome diagnosis relying on computer based analysis of facial traits can be increased by intelligent reduction of data complexity. A data set consisting of 121 standarized photographs of patients out of 10 different syndromes was investigated (MPS III , CDL, FraX , PWS, WBS, 5p-, 22q-, Noonan S., Sotos S., SLO). Each individual was described by a roughly 4000-dimensional vector resulting from a Gabor wavelet transformation at 48 landmarks of the face. Four different classification methods of different complexity were used for multivariate data analysis (linear discriminant analysis, LDA; SVM; Jetvoting; kNN). Classification accuracy exceeds 80% of correctly diagnosed syndromes in this data set. This accuracy could be reached by choosing certain subselections of the data plus using principal component analysis. This result can be attributed to noise reduction. For LDA, classifiers were compared with clinical findings for the particular syndromes. This was done by visualisation of facial features that classifiers base their decisions on. Results show, that for most syndromes the same clinical characteristics are chosen by the computer as

compared with the clinician. Prospectively a parameterized 3D model of the face shall be used to further improve visualisation. In summary, the computer based methods presented here seem to be helpful to the clinician by offering a validated tool for including facial information into the intricate process of finding a syndrome diagnosis.

W03 Genetic Epidemiology

W03 01

Bringing into shape complex phenotypes: Methods to exploit intertwining between monogenic and complex genetic contributions for a single phenotype

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Complex traits are thought to have complex genetic contributions. Traits themselves, however, can be markedly simple, e.g., dichotomous or univariate. Many traits might be considered in a multivariate setting, e.g., analyzing hypertension jointly with metabolic disorders. In the wake of multivariate analyses like these we consider a roughly 4000 dimensional phenotype representing the face. Investigations are under way to conduct association and linkage analyses with respect to the phenotype to answer fundamental questions about the developement of the face. We here focus on the phenotype itself and present the steps needed to transform the data set into a form adequate for further analysis aiming at reduction of dimensionality and noise in the data set. We present a framework of three components and demonstrate its success on a population-based sample of 570 individuals: 1. Use of a different data set of 120 faces related to monogenic disorders to characterize variation that represents stable characteristics in human faces rather than noise. 2. Model selection procedures in conjunction with other dimensionality reduction methods like principal component analysis. 3. Heritability analysis for individual dimensions. This procedure yields a low (20 to 40) dimensional representation that captures relevant information of the data set. The parameters are the amount of variation captured, classification rates, and heritabilities. They allow further analysis in standard association or linkage settings. The knowledge gleaned from monogenic disorders substantially improved the characterization of the population-based data set - a result that we expect to hold for other phenotypes as well.

W03 02

Increased frequency of CFTR heterozygosity in infertile males

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Congenital bilateral aplasia of the vasa deferentia (CBAVD) is associated with a high frequency of cystic fibrosis transmembrane regulator (CFTR) mutations. However, there are contradictory results concerning the frequency of CFTR mutations in infertile males without CBAVD. In the present study, we examined a total of 597 infertile males for CFTR mutations prior ICSI therapy. A heterozygous CFTR mutation was observed in 34 of 597 patients (5.69%). Given that our mutation panel recognises about 82% of heterozygotes, it can be assumed that the frequency of CFTR heterozygotes in our cohort is about 6.94 %. The frequency of cystic fibrosis in the German population is 1:3300 (Bobadilla et al., Hum Mutat., 2002). Thus, a CFTR heterozygosity of 3.42 % can be estimated. This indicates that in our cohort of infertile males the frequency of CFTR heterozygosity is two-fold higher than in the general population ($p<0.0001$). The elevated frequency of CFTR heterozygosity may be caused by clinically not recognized cases of unilateral vas deferens aplasia. However, the frequency of the 5T allels known to be associated with vas deferens aplasia was in the normal range.

W03 03

Cardiovascular morbidity and mortality in dialysis patients: a 10 year follow-up

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Objectives: Lipoprotein(a) [Lp(a)] is a genetically determined risk factor for atherosclerosis. About 30-70% of the Lp(a) plasma concentrations are determined by the apo(a) size polymorphism. This polymorphism is caused by a kringle-IV repeat polymorphism in the LPA gene with more than 30 isoforms. These isoforms can be grouped in low (LMW) and high molecular weight (HMW) apo(a) isoforms. Patients with kidney disease have a 10- to 20-fold increased risk for cardiovascular complications which can not be explained by traditional atherosclerosis risk factors. Lipoprotein(a) is significantly elevated in these patients. Previous studies, however, demonstrated that the apo(a) size polymorphism is a better predictor for cardiovascular disease in dialysis patients than Lp(a) concentrations.

Material and Methods: We are following a group of more than 600 dialysis patients recruited between 1991 and 1996 in five Austrian dialysis centers. By the end of 2004 we will have finished the follow-up of on average 10 years in these patients.

Results: Preliminary results show that about 65% of the patients have already died. A Cox hazard regression model reveals that the following variables contributed significantly to total mortality (see Table):

Variable (increment) HR, hazard ratio (95% CI) P Age (year) 1.066 (1.057-1.075) <0.001 Diabetes mellitus (0=no, 1=yes) 1.79 (1.45-2.22) <0.001 Apo(a) phenoytpe (1=HMW, 2=LMW) 1.46 (1.17-1.89) 0.001 Current smoking et study entry 1.40 (1.09-1.80) 0.008 **Conclusions:** From the pathogenetic standpoint the LMW apo(a) phenotype is mainly associated with atherosclerotic complication. Therefore, we conclude that the association of the apo(a) phenotype with atherosclerosis in dialysis patients is so strong that we can even observe an association with total mortality to which cardiovascular events might contribute 50%. The ongoing validation of all fatal and non-fatal cardiovascular events will be of major importance for further analysis of the data.

W03 04

The rare ERBB2 variant Ile654Val is associated with an increased familial breast cancer risk

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Overexpression of the proto-oncogene ERBB2 (HER2/NEU) has been observed in 20-30 % of breast cancers involving poor prognosis. Genetic alterations within ERBB2 have been shown to induce carcinogenesis and metastasis. We tested eight annotated single nucleotide polymorphisms for occurrence in familial breast cancer samples. The confirmed variants Ile654Val, Ile655Val and Ala1170Pro were analysed in subsequent epidemiological studies on familial breast cancer risk. While Ala1170Pro resides within a C-terminally located regulatory domain, both adjacent polymorphisms Ile654Val and Ile655Val are part of the transmembrane domain. The case-controls study analysing a cohort of 348 German familial breast cancer cases and 960 corresponding controls showed no association of both Ile655Val and Ala1170Pro with familial breast cancer risk. Differences of haplotypes between cases and controls could not be detected either. The ERBB2 variant Ile654Val, however, revealed an increased risk for carriers

of the heterozygous Val654 allele (OR = 2.56, 95 % C.I. = 1.08-6.08, p = 0.028). The rare Val654 is linked to the more frequent Val655, resulting in two consecutive valine instead of two isoleucine residues within the transmembrane domain. Computational analyses suggest that the Val654-Val655 allele provokes receptor dimerisation and activation thus stimulating kinase activity and cell transformation. These findings, for the first time, reveal ERBB2 Val654 as an oncogenic variant which might, in addition, influence clinical outcome and predict worse prognosis.

W03 05

Common Gene Variants in Myocardial Potassium Channel Genes Act Additively to Modify the QT-Interval in the general population. Results from the KORA Study

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Altered myocardial repolarization is one of the important substrates of ventricular tachycardia and fibrillation. The influence of rare gene variants on repolarization is evident in the familial long QT-syndrome. To investigate the influence of common gene variants in candidate genes we performed a linkage-disequilibrium based SNP fine mapping and association study to the corrected QT-interval. In a two-step investigation we analyzed 174 SNP-markers from the KCNQ1, KCNH2, KCNE1 and KCNE2 genes in 689 individuals from the population-based KORA study and 13 SNP-markers with results suggestive of association in a confirmatory sample of 3271 individuals from the same survey. We detected association to a gene variant in intron 1 of the KCNQ1 gene (+1.72 ms/allele, p<0.00005) and observed weaker association to a gene variant upstream of the KCNE1 gene (+1.38 ms/allele, p=0.0063). In addition we detected association to two gene variants in the KCNH2 gene, the previously described K897T variant (-1.79 ms/allele, p=0.0003) and a gene variant that tagged a different haplotype in the same haplotype block (+1.84 ms/allele, p=0.0001). The analysis of combined effects by an allelic score of the three most significantly associated SNP markers was able to explain a +14.3 ms difference in corrected QT-interval length between extreme score groups (p<0.00005). These results confirm previous heritability studies indicating that repolarization is a complex trait with a significant heritable component and demonstrate that high-resolution SNP-mapping in large population samples can detect association to quantitative trait loci even if locus specific heritabilities are small.

W03 06

Genomwide Linkage Scan of Idiopathic Generalised Epilepsy reveals Oligogenic Susceptibility Loci on 5q31, 13q32, 16q22 and 19q13

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Idiopathic generalised epilepsies (IGE) affect about 0.3% of the general population and account for 30% of all epilepsies. The aetiology of common IGE syndromes, such as juvenile myoclonic epilepsy (JME) and idiopathic absence epilepsies (IAE), is genetically determined, but the complex pattern of inheritance suggests an oligogenic predisposition. The aim of the present collaborative European genome scan was to map IGE loci and to dissect its oligogenic aetiology. Our linkage study included 96 European families ascertained through a proband with either IAE or JME, and one or more siblings affected by an IGE trait (IGE or generalised spike wave EEG discharges). In total, 561 microsatellite polymorphisms with an average intermarker spacing of 7 cM were genotyped in 377 family members. Non-parametric multipoint linkage analyses, using the GENEHUNTER program, revealed suggestive evidence (P < 0.01) for novel IGE loci in the chromosomal regions 5q31 (ZNPL = 2.86, P = 0.0022) and 13q32 (ZNPL = 3.63, P = 0.0002). To dissect seizure type-specific susceptibility loci, two distinct family subgroups were selected by the presence of either myoclonic seizures (JME-families; n = 41) or absence seizures (IAE-families; n = 55). In the JME families, maximum evidence for linkage was found in the chromosomal regions 16q22 (ZNPL = 2.87, P = 0.0021) and 19q13 (ZNPL = 2.56, P = 0.0052). In the IAE families, linkage hints were obtained on chromosome 11q13 (ZNPL = 3.08, P = 0.0011) and 13q32 (ZNPL = 3.40, P = 0.0004). Our present linkage results indicate an IGE locus in the region 13q32 that confers susceptibility to a broad IGE spectrum, whereas three additional susceptibility loci on 11q12, 16q22 and 19q13 seem to specify various seizure types in an age-related manner. These genomic regions contain high-ranking candidate genes (e.g. GABRG2, GABRA1, HTR4) for mutation analyses.

W04 Functional Genomics, New Technologies

W04 01

Miniaturised Real-Time PCR Using Universal Reporters

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Real-time quantitative PCR is a sensitive and accurate method for gene expression studies. While detection systems with small molecules binding to double-stranded DNA like Sybr Green I offer the advantage of low reagent cost, they do not allow multiplexing. In contrast the sequence specific Taqman based detection system enables multiplexed expression analysis but requires expensive unique probes or modified primers for each target. Therefore we developed the cr-real-time PCR assay which uses tailed locus-specific amplification primers for the initial PCR cycles. In the following the PCR-Product is amplified with one common and one locus specific primer and monitored by a universal Taqman probe hybridising to the common tail. This cost reducing method has shown its high robustness and accuracy in single gene as well as multiplexed setups and is easily applicable for various loci. As a proof of principle we applied the cr-real-time PCR to study previously published, differentially expressed candidate genes for congenital heart defects. Moreover, in order to utilise this new assay in high throughput applications as Nanowell Technology we scaled the reaction volume down to 1 ul. Even in the miniaturised format the cr-real-time PCR demonstrated high accuracy, sensitivity and robustness and is therefore a valuable tool for various gene expression studies.

W04 02

Genome-wide identification and functional characterization of novel factors regulating cellular sterol metabolism

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Here we describe the establishment of a combined expression profiling and microscope-based functional screening approach that allowed us to systematically identify new candidate genes which are regulated by sterols and themselves are involved in regulating cellular sterol metabolism. Genome wide expression analysis was determined by using the Human Transcriptome Microarray containing 51.145 cDNA clones of the Unigene Set RZPD3. A total number of 465 gene targets showed statistically significant changes in expression in HeLa cells at different time points in response to sterol depletion. A subset of 51 genes met the stringent combinatorial criteria of being regulated at all time points investigated, 31 of which have not yet been identified as involved in cellular sterol metabolism. Control experiments with primary human fibroblasts from healthy individuals as well as individuals affected by autosomal-dominant familial hypercholesterolemia confirmed the specificity and significance of our results. A putative function of the newly identified candidate genes is now being further characterized by using cDNA-/RNAi-based human live cell arrays and high-content screening microscopy. Our study contributes an important step toward a more comprehensive understanding of the molecular basis of cellular sterol regulation, with our methodology being suitable for addressing a wide range of biological and medical questions.

W04 03

Application of High Resolution genome wide DNA Array CGH analysis within a diagnostic setting

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The power of array-based DNA comparative genomic hybridisation (array CGH) analysis to identify submicroscopic genetic imbalances has already been proven and demonstrated in various research settings by our group and others. Genome wide DNA array CGH analysis has also been implemented for diagnostic purposes in our department in order to enable physicians to make use of this powerful tool. Patients with mental retardation and multiple congenital anomalies with a normal karyotype (including normal subtelomeric regions) are included in this diagnostic process. The array we use for these patients consists of 32,000 BAC clones, evenly spaced over the human genome with an average resolution of 100 kb. Each patient sample is analysed twice with label swap (Cy3 / Cy5) in patient versus pooled control hybridisations. If analytical data are indicative of DNA copy number aberrations, which do not match with known polymorphisms, then DNA samples from the respective parents are collected and subjected to array CGH analysis. If necessary, apparently non-polymorphic imbalances are subsequently validated either by Fluorescence In Situ Hybridisation analysis or Multiplex Ligation-dependent Probe Amplification analysis of the patient sample and the accompanying parental samples.
The strategy to use array CGH analysis in a diagnostic setting and the resulting data from the first set of at least 80 patients in combination with more than 20 parental couples will be presented here. With our approach we were able to identify disease-associated losses and gains, including complex microduplications, and new polymorphic imbalances, that otherwise would have remained unrevealed.

W04 04

Functional analyses of the AZFa genes USP9Y and DBY during human spermatogenesis reveal expression at different phases by translation control

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We explore the male germ line function of the human Ubiquitin specific protease 9Y (USP9Y) and DEAD BOX Y RNA helicase (DBY) genes located in the AZFa locus of the human Y chromosome (Yq11.21). Deletion of both Y genes is known as a major cause for occurrence of a severe testicular pathology, the Sertoli-cell-only (SCO) syndrome (Kamp et al. 2001; Mol. Hum. Reprod. 10:987-994). USP9Y and DBY have a structural homologue on the short arm of the X chromosome (Xp11.4): USP9X and DBX. Their high sequence similarity (> 95%) throughout their coding regions suggests that X and Y copies should have a similar function. Surprisingly, however, USP9X and DBX is not able to compensate for the loss of the USP9Y and DBY germ line function in men with an AZFa deletion. One explanation might be that the Y copy has a different role in spermatogenesis not provided by the X copy. We therefore studied the function of the USP9Y/USP9X and DBY/DBX genes in the human male germ line, separately. Their transcriptional profiles were distinguished by gene copy-specific RT-PCR, their translational profiles by antisera which mark specifically only the USP9Y- or USP9X, respectively only the DBY- or DBX-protein. Our results strongly suggest a translational control for DBY transcripts; the protein is synthesised only in the germ line, while DBX protein was expressed in all tissues analysed (Ditton et al. 2004: Hum. Mol. Genet. 13:2333-2341). In testis tissue sections, DBY and USP9X proteins were found predominantly in spermatogonia, whereas DBX and USP9Y proteins were expressed after meiosis in spermatids. We conclude that although USP9Y/USP9X and DBY/DBX proteins are structurally very similar, they have diverged functionally by translation control to fulfil different roles in the RNA/protein metabolism of human spermatogenesis, and that deletion of DBY cause the SCO syndrome observed in men with AZFa deletions whereas USP9Y deletions only cause a post-meiotic male germ line defect.

W04 05

From fishes, birds and mammals to the bony vertebrate proto-karyotype - a comparative study

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The recent advantages of whole genome sequencing of certain species provides the possibility to try whole genome comparisons. From these comparisons one gets ideas about karyotype evolution. In our current work we compared ~3300 genes spread along the human genome with their orthologous genes in chicken (Gallus gallus), and the three fish species pufferfish (Tetraodon nigrovirides), medaka (Oryzias latipes) and zebrafish (Danio rerio). This dataset allows to perform potential karyotype reconstructions of last common ancestors. In a first step, we have reconstructed the karyotype of the progenitor species of the bird and mammalian lineages which divided 310 million years ago (MYA). This karyotype consists of 16 chromosomes. In almost the same manner it was possible to reconstruct from the three fish species an ancestral teleostean karyotype consisting of 12-14 chromosomes. This karyotype represents the situation before the whole genome duplication unique for teleostean fishes. Finally, reconstruction of a putative ancestral proto-karyotype of all bony vertebrates was tried. This karyotype represents the status 450 MYA and consists of 12 chromosomes of different size. There are some chromosomes, such as HSA 4 and 10, which have been completely conserved, whereas e.g. HSA 3 and 6 represent a collection of genes from different proto-chromosomes. It is also evident from our data, that the bird Z chromosomal arrangement is in fact more ancestral than the mammalian X, which is the fusion product of segments from at least 3 different proto-chromosomes. In fishes the karyotypes are made up by a relatively stable modal number of chromosomes, whereas land vertebrates differ in chromosome number. This is due to the common view that in fishes more intrachromosomal rearrangements (inversions) and in land vertebrates more interchromosomal rearrangements (translocations) took place.

W04 06

Clustered organization and asynchronous replication of imprinted gene orthologues are conserved on macrochromosomes in the chicken genome

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Genomic imprinting is the epigenetic marking of a gene that results in preferential expression of one specific parental allele. An intriguing characteristic of nearly all imprinted genes is that they occur in clusters forming large imprinted domains. In contrast to the “average” genome, imprinted chromosome regions replicate asynchronously during the cell cycle. It is likely that clustered organization and asynchronous replication relate to the use of shared regulatory elements across multiple genes. These cytological hallmarks of imprinted genes might have evolved before the emergence of genomic imprinting as a prerequisite for imprinted gene expression. To analyze the evolutionary conservation of imprinting clusters, mouse cDNA sequences of 68 imprinted genes were subjected to an Ensembl BLASTview search against the chicken genome sequence. 42 chicken orthologues of mouse imprinted genes were identified and in silico mapped to chicken (GGA) chromosomes, 34 (81%) to a macrochromosome and 8 (19%) to a microchromosome. Gene content and gene order of the major mouse imprinted clusters were highly conserved, mapping to clusters on macrochromosomes GGA2, GGA3, and GGA5. Interphase FISH dot assays on nuclei from exponentially growing chicken fibroblasts were used to compare the replication timing of orthologues of imprinted genes and non-imprinted control genes. Preliminary evidence suggests that asynchronous replication is also detectable for chicken orthologues of imprinted genes, but limited to orthologues residing on macrochromosomes. In the light of recent estimates suggesting that microchromosomes contain at least as twice as many genes as macrochromosomes our results strongly indicate a preferential localization of chicken orthologues of mammalian imprinted genes on macrochromosomes. Thus, one could speculate that macrochromosomes provide a better microenvironment for the establishment of asynchronous replication and imprinted gene expression later in evolution.

W05 Cancer Genetics

W05 01

Involvement of the transforming growth factor-beta signaling pathway in rhabdomyosarcomagenesis of Patched mutant mice

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The putative tumor suppressor gene PATCHED1 (PTCH1) is a member of the sonic hedgehog (SHH) signaling pathway and causatively associated with several human sporadic and familial cancers, including those of the skin, muscle and brain. Inactivation of one Ptch1 allele in mice results in the development of rhabdomyosarcoma (RMS), a malignant tumor of skeletal muscle origin. To identify genes involved in the pathogenesis of RMS, we have monitored the expression of 588 genes in RMS and normal skeletal muscle of heterozygous Ptch1neo67/+ mice using cDNA array technology. RMS displayed increased transcript levels of several genes such as transforming growth factor-beta1 (Tgfb1), inhibin alpha, Sloan-Kettering viral oncogene homolog, integrin alpha 1, insulin-like growth factor 2, insulin-like growth factor binding protein 3, as well as numerous genes coding for structural components of myogenic cells like atrial fetal myosin alkali light chain, non-muscle myosin alkali light chain, and vimentin. Genes with a decreased expression in RMS comprised cyclin G, transcription factor S-II as well as several protein kinases. Differential expression of these genes was verified by Northern blot analysis.
Furthermore, we wanted to ascertain whether Tgfb1 might be transcriptionally regulated by a constitutively activated Shh signaling pathway. Tgfb1 expression was moderately increased in C2C12 myoblasts in the presence of murine Shh-N peptide. This effect was totally blocked by simultaneous administration of cyclopamine, a specific inhibitor of the Shh pathway. However, using reporter assay we show that the P2 promoter of the murine Tgfb1 gene is not responsive to Gli1, a known down-stream transcription factor of Shh signaling. Moreover, TGFB1 was strongly expressed in human RMS cell lines independent of the GLI1 expression status. Thus, our results suggest that aberrant expression of members of the Tgf-beta superfamily may be involved in RMS development independent of Shh signaling.

W05 02

Characterization of CATS which interacts with the leukemogenic fusion protein CALM/AF10

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The CATS gene was first identified in a yeast two hybrid screen as CALM interacting protein expressed in thymus and spleen. The CATS interaction region of CALM is contained in the leukemogenic fusion protein CALM/AF10, which is found in acute myeloid leukemia, malignant lymphoma and acute lymphoblastic leukemia.
We performed CoIP experiment to confirm CALM-CATS interaction found in yeast system. Using antibodies against CATS we could show a high expression of the protein in different human leukemia, lymphoma and solid tumor cell lines, as well as in normal proliferating cell lines (HEK293 and WI38) but not in normal non-proliferating T-cell lines (TYRF8 and JB4).
Transient transfection studies showed that CATS is localized mainly to the nucleus in nodular structures. Coexpression of CFP-CATS with YFP tagged nucleolar proteins showed that CATS is found predominantly at the nucleoli. Coexpression of CFP-CATS with YFP-CALM or YFP-CALM/AF10 was able to markedly increase the nuclear localization of both CALM and the CALM/AF10 fusion protein. This effect of CATS is stronger on the YFP-CALM/AF10 fusion protein than on the CALM protein. When fused to a GAL4 DNA binding domain, CATS acts as a strong repressor of transcription in reporter gene assays.
Whole mount in situ hybridization on mouse embryos showed a ubiquitous expression of CATS in early embryonic stages and a distinct expression pattern in the developing limbs in later stage embryos. Our results indicate that the subcellular localization of CALM and CALM/AF10 could depend in part on the presence of CATS with greater portion of CALM or CALM/AF10 being present in the nucleus in cells with high CATS expression (e.g. lymphoid cells). High expression of CATS in proliferating cells and in tumor cells together with its nucleolar localization suggest that CATS is involved in controlling cell proliferation. CALM-CATS interaction might thus play an important role in CALM/AF10 mediated leukemogenesis.

W05 03

Immunohistochemical tissue localization and confirmation of cancer biomarkers detected by ProteinChip technology (SELDI): Calgranulin A, B and calgizzarin in head and neck cancers

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Head and neck cancer are frequent malignancies with a complex and up to now unclear molecular etiology. Therefore we investigated tissue localization and distribution of three cancer biomarkers identified in a former study by ProteinChip technology (SELDI) after laser microdissection.
Tissue probes from head and neck cancers (HNC) (n = 5) and normal pharyngeal tissue (n = 5) were analysed by immunohistochemistry (IHC) using antibodies against calgranulin A (S100A8), calgranulain B (S100A9) or an anti-calgizzarin (S100A11), respectively. A Jenchrom pxbl-kit was used to visualize the location of the antibodies in the tissue.
Calgranulin A and calgranulin B showed identical strong immune reactivity in the normal epithelium, except the basal and parabasal cells. In epithelial tumor tissue, no expression could be detected for either protein. The normal epithelial connective tissue was completly negative, whereas in the stroma of HNCs fibrocytes and macrophages showed a positive reaction, and additionally depositions of calgranulin A and B on collagenic fibers could be observed. Calgizzarin was expressed in all normal epithelial cells and stromal cells (fibrocytes). In HNCs calgizzarin was expressed in epithelial tumor cells, in constituents of the connective tissue (fibrocytes, fibroblasts, macrophages, leukocytes), in the epithelium of the glandular ducts and in endothelial cells.
The results show that: (1) proteins identified by SELDI after preceded microdissection can be found and localized successfully in the starting tissue probes by IHC. (2) Reanalysis of IHC-positive and –negative tissue areas by microdissection and proteomic profiling confirms the identities of differentially expressed peaks in SELDI analysis. (3) IHC demonstrates the heterogenous distribution of the analysed proteins in the tissue probes underlining the indispensability of tissue microdissection prior to all analyses. This work was supported by the IZKF Jena and the BMBF

W05 04

Mutation Spectrum, Frequency and Phenotype in German Patients with MUTYH-Associated Polyposis (MAP)

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Background: MUTYH-associated polyposis (MAP) is a recently described precancerous adenomatous polyposis syndrome of the colorectum (MIM 608456) that is caused by germline mutations in the base excision repair gene MUTYH (MYH). In contrast to familial adenomatous polyposis (FAP), the most important differential diagnosis, MAP is inherited in an autosomal recessive way.
Methods: We performed a systematic search for MUTYH mutations by sequencing the complete coding region of the gene in 96 unrelated patients with multiple colorectal adenomas (63 attenuated FAP, 14 typical FAP, 16 unknown phenotype), in whom no mutation in the APC gene had been detected by conventional mutation analysis (PTT, DHPLC, MLPA).
Results: Biallelic germline mutations in MUTYH were identified in 12 of 96 patients (12.5%). In 7 patients biallelic mutations for the hot-spot missense changes Y165C and/or G382D were identified, in one proband a stop mutation (Q377X) in homozygous state was found, 4 more patients were either homozygous or compound-heterozygous for yet unclear variants (P143L; P281L; R168H; c.1395_1397delGGA). In 9 of the 12 patients with biallelic mutations an attenuated phenotype is described, in 3 patients the phenotype was unknown. In 8 patients the family history was compatible with autosomal recessive inher-

itance, in 2 cases vertical segregation was suspected. Monoallelic mutations were found in 7 probands (7.3%).
Conclusions: MAP should be considered in genetic counselling and follow-up since biallelic mutations of the MUTYH gene are the underlying genetic basis in a substantial fraction of patients with multiple colorectal adenomas. Mutation analysis in the MUTYH gene should be implemented in routine mutation detection protocols, possibly restricted to patients with a more attenuated adenomatous polyposis and to segregation pattern consistent with autosomal recessive inheritance.

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W05 05

Genetic alterations in desmoplastic medulloblastomas: Evidence for monoclonal tumor origin and identification of novel amplified and overexpressed proto-oncogenes
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Desmoplastic medulloblastomas (dMBs) are histologically characterized by two distinct tumor components, the so-called pale islands and the desmoplastic areas. Previous molecular studies have shown that dMBs frequently carry PTCH mutations. However, little is known about other genetic and chromosomal aberrations associated with these tumors. We investigated total tumor DNA of 23 sporadic dMBs using comparative genomic hybridization (CGH). Chromosomal imbalances were identified in 17 tumors (74%). The number of aberrations detected per tumor varied from 1 to 12, with an average of 4.61 ± 0.73 (mean ± SEM). Recurrent chromosomal gains were detected on chromosomes 3, 9 (6/23); 2, 20 (5/23); 6, 7, 17, 22 (4/23 each) and 1 (3/23). Recurrent losses were found on chromosomes X (8/23); Y (6/13 male patients); 9, 12 (4/23 each) as well as 10, 13 and 17 (3/23 each). Amplifications were detected in 4 tumors and mapped to 1p22, 5p15, 9p, 12p13, 13q33-q34 and 17q22-q24. To address the question of clonality of the two components in dMBs, we performed CGH analysis on microdissected pale islands and desmoplastic areas. In 5/6 informative tumors both histological components shared common chromosomal imbalances, indicating an origin from a single progenitor cell. We additionally characterized the amplicons detected on 5p15, 9p and 17q22-q24 in 2 dMBs using matrix-CGH on genomic arrays of 6.000 large insert clones. Subsequent molecular analyses of amplified candidate genes identified by matrix-CGH confirmed amplification of several genes on 17q23 in three dMBs and the JMJD2C gene on 9p24 in one dMB, respectively. Expression analysis suggested RPS6KB1 as the most important target on 17q23, which was found to be markedly overexpressed in 10/11 medulloblastomas investigated. Taken together, our study provides strong genetic evidence for a monoclonal origin of dMBs and implicates RPS6KB1 and JMJD2C as novel proto-oncogenes that are aberrantly activated in these tumors.

W05 06

Leupaxin plays a role in adhesion and invasion of prostate carcinoma cells
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We analyzed the expression levels of more than 400 cancer-related genes using the cDNA array technique in a set of capsule-invasive prostate tumor, central tumor and matched normal prostate tissue. The overexpression of the leupaxin gene in capsule-invasive tumor cells was confirmed using quantitative real-time RT-PCR. Leupaxin is a cytoskeleton adaptor protein belonging to the paxillin- protein family of LIM-domain containing proteins and here we demonstrate that leupaxin is expressed in several prostate carcinoma (PCa) cell lines. Furthermore, down-regulation of leupaxin expression using small interfering double-stranded RNA (siRNA) oligonucleotides in different PCa cell lines resulted in morphological changes and detachment of androgen-dependent, non-invasive LNCaP cells. More than 50% of the LNCaP cells underwent apoptosis 5 days after transfection with siRNA against leupaxin, whereas control transfected LNCaP cells, leupaxin-siRNA transfected PC-3 and DU 145 cells did not show apoptotic effects. By using the Matrigel invasion assay we could demonstrate that down-regulation of leupaxin expression in invasive PC-3 and DU 145 prostate carcinoma cells leads to a 70% reduction of PCa cell invasiveness in vitro. Immunohistochemical staining of PCa cells using a leupaxin-specific antibody showed that leupaxin is localized in focal adhesion sites of PCa cells. In addition, the expression of leupaxin was investigated on tissue sections from 64 different human prostate carcinomas. In 21% of prostate carcinomas analyzed expression of leupaxin in prostate carcinoma cells was detected. Taken together, our results indicate that leupaxin could serve as a putative candidate gene involved in invasive properties of prostate carcinoma cells.

W06 Cytogenetics

W06 01

The pericentromeric region of the human Y chromosome: comparative FISH analysis of a segmental duplication cluster in human and higher primates
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We have identified an euchromatic island within the pericentromeric repeats on the long arm of the human Y chromosome which is not part of the male specific sequence published by Skaletsky and colleagues (2003). This 450 kb region is covered by four overlapping BAC/PAC clones and is embedded within satellite 3 sequences. FISH analyses of the clones revealed that these sequences are not restricted to the Y chromosome. Signals were detected on 15 different chromosomes and 18 different loci. All four clones hybridized most prevalently to pericentromeric and subtelomeric regions. Furthermore, sequence homologies to two ancestral pericentromeric regions could be detected. Striking signals were also found on all acrocentric short arms. Thus, this 450 kb interval on human Yq represents a segmental duplication cluster that is widely distributed over the human genome.
To elucidate the evolutionary fate of this duplicon cluster we hybridised all four clones to metaphase spreads of chimpanzee, bonobo or pygmy chimpanzee, gorilla, orangutan and gibbon as well as the macaque as an "out-group" species. In all great apes a human like distribution of the sequences was found especially on their autosomes, while the hybridisation patterns on the different Y chromosomes point to the well known species-specific Y chromosomal rearrangements in human and great apes. In contrast, signals were found mainly on the rDNA-containing marker chromosome pair in macaque and only on a few autosomal pairs in gibbon. No signals could be detected on their Y chromosomes.
Investigating this euchromatic island encompassed by satellite sequences has illuminated its complex structure and the dynamic history of sequences located in this region.

W06 02

Re-evaluation of probability estimates for the occurrence of unfavourable pregnancy outcomes in families of Robertsonian der(13;14) carriers
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Robertsonian chromosomal translocations (RobCT) families are asking for the occurrence probability rate for unfavorable pregnancies outcomes and infertility. Data of 473 pregnancies from 165 families of 90 der(13;14) smaller and larger pedigrees were collected from the contributing centers and from the literature to re-evaluate probability rates of occurrence for un-

balanced progeny at birth as well as at prenatal diagnosis of second trimester pregnancy and for other types of unfavorable pregnancy outcomes (unkaryotyped miscarriages and stillbirths/early newborn death).To improve the cytogenetic characterization of der(13;14) the distinction among dicentric and monocentric translocations was introduced. Additionally we performed UPD studies in a selected group of children/fetuses with abnormal phenotypes and balanced der(13;14). Probabilities rates for occurrence of the unfavourable pregnancy outcomes were performed according to the method of Stengel-Rutkowski et al (1988) with ascertainment correction according to Stene (1970). It was found, that probability rate for a child's birth with translocation trisomy 13 for total group of pregnancies after ascertainment correction is 0/365, i.e.<0.1 %. In addition rate for unbalanced foetuses at second trimester prenatal diagnosis is estimated as 2/24 i.e. 8.3±5.6%, for a stillbirth as 8/301 i.e. 2.7 0.9% and for miscarriages as 52/365 i.e. 14.2±1.8%. Slightly higher rates for maternal carriers have been obtained at prenatal diagnosis and for miscarriages in comparison to paternal carriers. Interestingly, the probability rate for miscarriages were similar for the couples with dicentric translocations carriers comparing to monocentric ones and to the group of carriers with unclassified centromeric region, namely 16.4±3.5% (18/110) 14.1±4.3% (9/64)and 13.0±2.4% respectively. No case with the UPD 14 syndrome was identified. It supports, that parental UPD responsible for clinical effect in abnormal progeny of der(13;14) are extremely rare findings. In addition we found nine cases of der(13;14) with coexistent trisomy 21 among 473 pregnancies.
(Project BMBF POL 03/02)

W06 03

Genome-wide array-CGH screening of 11 patients with mental retardation of unknown etiology – Detection of a case with cryptic interstitial deletion 1q24

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Microdeletions and microduplications of the subtelomeres and certain interstitial chromosome regions are a major cause of mental retardation (MR). With the advent of genome-wide array-based comparative genomic hybridization (array CGH / matrix CGH), high-resolution screening for such microdeletions and –duplications in mentally retarded patients is possible by hybridizing differentially labelled patient and reference DNAs to arrays consisting of thousands of genomic clones.
We analyzed 11 well characterized patients with MR of unknown etiology (n=3 with mild MR, n=8 with moderate to severe MR) with a genome-wide array of approximately 2,400 BAC clones. Five patients additionally displayed dysmorphisms as well as malformations, 1 had malformations only, 4 had dysmorphisms only and 1 had isolated MR.
We detected one de novo interstitial deletion, one familial polymorphism (a paternally inherited copy number gain of the beta-defensin cluster in 8p23.1) and one deletion-polymorphism or polymorphism-related artefact (identical in three patients). All findings were verified with independent methods (metaphase FISH, semi-quantitative FISH, MAPH) on patient material and, where appropriate, on parental material. Four patients were re-analyzed with an array of 6.000 large insert clones and no copy number changes were detected.
The de novo interstitial deletion 1q24 was detected in a 6 year old girl with a severely delayed language development, several dysmorphic signs, flat occiput, hyperextensibility of joints, muscular hypotonia, seizures and reduced sense of pain. The size of the deletion was determined to be approximately 4 megabases by matrix CGH with an array of 7.000 clones and with a panel of FISH clones. Its de novo occurrence was demonstrated by corresponding metaphase FISH experiments in both parents.
Our study shows that matrix CGH is a powerful tool for the investigation of cryptic chromosomal changes associated with mental disability and dysmorphism.

W06 04

L1 elements facilitate X-inactivation spreading onto trisomic chromosome 15q in an unbalanced translocation t(X;15)(q22.3;q11.2) stimulated by low-copy repeats.

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Few cases of *de novo* unbalanced X;autosome translocations associated with a normal phenotype have been described. We report a now 3-year-old dizygotic female twin with prenatally ascertained nuchal translucency and growth retardation, in whom prenatal chromosome studies revealed a *de novo* unbalanced translocation 46,X,der(X)t(X;15)(q22;q11.2) with nearly complete trisomy 15 and a normal karyotype in her male twin. Karyotyping after birth confirmed the initial diagnosis. Replication timing studies showed that the der(X) chromosome was late-replicating with variable spreading of inactivation onto the translocated 15q segment. The der(X) was determined to be of paternal origin by both CGG-repeat analysis of *FMR1* and an *AR* methylation assay. Methylation analysis at the *SNRPN* locus and analysis of microsatellites on 15q revealed paternal isodisomy with double dosage for all markers and the unmethylated *SNRPN* gene. The Xq breakpoint was mapped to within two overlapping BAC clones RP11-575K24 and RP13-483F6 and the 15q breakpoint within overlapping clones RP11-509A17 and RP11-382A4 that are all significantly enriched for LINE1 elements (36.6%, 43.0%, 26.6%, 22.0%, respectively). It has been proposed that L1 elements may serve as DNA signals for X-inactivation propagation along the normal X. We hypothesize that in X;autosome translocations, efficient spreading of X-inactivation may be mediated only from specific regions of the X chromosome. In addition, *in silico* analysis of breakpoint regions revealed the presence of highly identical low-copy repeats at both breakpoints, suggesting that they may be responsible for the formation of the translocation.

W06 05

Analysis of the classical morphological mitotic transitions in cells of patients with premature chromosome condensation syndrome

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Mitosis is a fundamental and tightly regulated process ensuring the proper segregation of replicated chromatids to the daughter cells. Prerequisite is the chromosome condensation, the active and complex folding of interphase chromatin to metaphase chromosomes. Recently, we described the first congenital disorder in man which is associated with a chromosome condensation defect (OMIM 606858). Patients with the PCC syndrome (premature chromosome condensation), an autosomal recessive disorder, show primary microcephaly and mental retardation. Chromosome condensation is remarkably affected in all patients cells. In dividing tissues an increased proportion of prophase-like cells can be detected by conventional-cytogenetic techniques. These cells exhibit premature chromosome condensation in G2- and delayed decondensation in the G1-phases of the cell cycle. Classically, observation of distinct changes in the cell morphology, apart from the condensation state of the chromatin, are used to subdivide different stages of mitosis: assembly or breakdown of the nuclear envelope, number and distribution of the centrosomes and formation of the mitotic spindle, number of nucleoli and their disintegration. These changes are cell cycle-dependent. Our analyses of these classical mitotic transitions with conventional staining methods (Giemsa- and silverstaining), FISH and immunocytological techniques did not reveal any further misregulations or disturbances of chromosome segregation in the cells with the chromosome condensation defect. Regarding these results we suppose that only the chromosome cycle is affected, while the remaining morphological changes of mitosis do not show detectable misregulations – obviously they are not influenced by the condensation defect. Thus, the regulation of chromosome condensation in the patient cells seems to be autonomous from the other major morphological changes during mitotic cell division.

W06 06

Sequential application of interphase-FISH and CGH to single cells

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A comprehensive genomic analysis of single cells is needed for numerous scenarios in tumor genetics, clinical diagnostics and forensic application. PCR protocols were developed which allow an unbiased amplification of the whole genome of a single cell for subsequent analyses by comparative genomic hybridization (CGH). However, verification of single cell CGH results has been impossible as the procedure naturally involves the destruction of the respective cell. Here we show that the genome of individual cells can be analyzed by two different single cell techniques applied sequentially to the same cell. In a first step, interphase FISH is applied. After evaluation of the interphase-FISH signals, cells of interest can be selected for a further analysis. Single cells are collected by laser microdissection, the DNA is amplified by linker-adaptor PCR and subjected to CGH-analysis. This strategy offers new opportunities for a sophisticated selection of cells based on interphase FISH signals. Furthermore, the sequential application of two different single cell approaches to the same single cell represents the only option to control and verify the single cell CGH results. We demonstrate the feasibility of this approach with a series of experiments including cells from pre- and postnatal diagnostics, e.g. cells with trisomies 13, 18, or 21, respectively, leukemia and tumor cells and tissue sections.

W07 Genotype and Phenotype

W07 01

A mouse model for the study of craniofacial defects seen in patients with campomelic dysplasia

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Campomelic dysplasia (CD) is a rare skeletal disorder characterised by bowing of the lower limbs, severe respiratory distress and XY sex reversal. CD patients also present with craniofacial abnormalities including a large dolichocephalic skull and a dysmorphic face with low-set ears, flat nasal bridge, hypertelorism, a long philtrum, micrognathia and midline posterior cleft palate. CD is caused by heterozygous mutations in and around the SOX9 gene which encodes a transcription factor essential for chondrocyte and testis differentiation. In addition to these and other tissues, Sox9 is also expressed in cranial neural crest cells (CNCCs), embryonic multipotent cells which delaminate from the neural tube, migrate dorsoventrally, and contribute to a variety of craniofacial tissues including the sensory and parasympathetic ganglia, cartilage, bone, muscles and connective tissue. It is known that Sox9 is necessary for CNCC development in frog and chicken. In mice, Sox9 has so far been shown to be necessary for the CNCC-derived chondrogenic lineages.We have used the Cre/loxP recombination system to homozygously inactivate Sox9 during early CNCC development. In contrast to previous studies, in our mutant embryos, CNCC differentiation is affected already at a very early stage, so that not only the CNCC-derived chondrogenic but also other lineages are affected. Thus, our mutants have a phenotype already at E9.5 before chondrocyte differentiation, revealing severely hypoplastic branchial arches. At E15.5, mutant embryos show complete exencephaly and deformities in the development of all craniofacial structures. Analysis of early CNCC molecular markers (Sox10, Ap2, Dlx5, Alx3, Alx4) in E8.5 and E9.5 mutant embryos revealed a drastic reduction in the number of CNCCs. These results indicate an essential role for Sox9 in early CNCC development, providing a molecular basis for the understanding of the various craniofacial abnormalities seen in CD patients.

W07 02

Trimethylaminuria, an important cause of body malodour

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Trimethylaminuria (TMA-uria) is an autosomal recessive disorder caused by a deficiency of flavin-containing monooxygenase isoform 3 (FMO3), a hepatic enzyme required for N-oxidation and detoxification of many endogenous and exogenous compounds including biogenic amines and common drugs. Individuals with severe FMO3 deficiency display a constant fish-like body malodour (fish odour syndrome) due to a reduced capacity to oxidize dietary-derived, malodourous trimethylamine (TMA) to its non-volatile, non-odourous N-oxide (TMAO). So far, 29 mutations or amino acid variants in the FMO3 gene have been identified in a limited number of patients. We previously showed that a common FMO3 variant [E158K,E308G], homozygous in 4 % of the German population, is associated with reduced FMO3 function and mild trimethylaminuria. We now report the result of extensive molecular studies, using DGGE and sequencing, in individuals with constant or intermittent malodour from 38 independent families. We found a total of 18 different mutations, 13 of which are novel. Malodour in two of the investigated patients was related to carnitine treatment, a well-known but previously unexplained side effect. Mild genetic variants were identified in combination with severe mutations in patients with mild trimethylaminuria as well as patients with malodour related to carnitine treatment. Mutations in the FMO3 gene thus cause a broad spectrum of phenotypes ranging from severe fish odour syndrome to mild enzyme deficiency that is common in the general population but only intermittently associated with malodour. Considering additional functions of this enzyme, mild FMO3 deficiency may play a role as susceptibility factor in pharmacological and other pathophysiological conditions.

W07 03

Infantile-lethal non-lysosomal heart glycogenosis caused by a recurrent activating mutation in the γ2 subunit of AMP-activated protein kinase (PRKAG2), not by phosphorylase kinase deficiency

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Massive, infantile-lethal, non-lysosomal glycogenosis of the heart is a very rare form of glycogen storage disease. It has been attributed to a subtype of phosphorylase kinase deficiency, but the underlying genes and mutations have not been identified. Analyzing four unrelated patients, we found no mutations in the eight genes encoding phosphorylase kinase subunits, and in the two genes encoding the muscle and brain isoforms of glycogen phosphorylase. We then analysed the PRKAG2 gene, encoding the γ2 subunit of AMP-activated protein kinase and identified in three patients identical, recurrent, heterozygous R531Q missense mutations. AMP-activated protein kinase is a key regulator of energy balance. Other PRKAG2 missense mutations were previously identified in familial hypertrophic cardiomyopathy with Wolff-Parkinson-White syndrome, a mild cardiac glycogenosis of autosomal-dominant inheritance with juvenile or adult clinical onset, characteristic disturbances of excitation conduction, and enhanced risk of sudden cardiac death in middle life. Biochemical characterization of the recombinant R531Q-mutant protein shows >100-fold reduction of binding affinities for the regulatory ligands AMP and ATP but an enhanced basal activity and increased phosphorylation of the γ 2 subunit. These perturbations of molecular function are much more severe than those observed with any other PRKAG2 mutations analysed to date, in accordance with the particularly severe clinical phenotype of the R531Q mutation.In conclusion, recurrent, heterozygous R531Q missense mutations in PRKAG2 give rise to a massive, infantile-lethal non-lysosomal cardiac glycogenosis which can hence be seen as a clinically distinct, particularly severe variant of hypertrophic cardiomyopathy with Wolff-Parkinson-White syndrome. The existence of a heart-specific primary phosphorylase kinase deficiency is questionable. The

R531Q mutations enhance the basal activity of AMP-activated protein kinase.

W07 04

Shox2 acts upstream of Bmp4 in regulation of sinus venosus and venous valve development

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The striking expression pattern of the homeodomain transcription factor SHOX2 in the heart of human and mouse embryos suggests its involvement in the early stages of heart development. We have created a null allele of the Shox2 gene by targeted mutation and here report on fatal heart malformations in Shox2 homozygous mutant embryos. Shox2-/- mouse embryos exhibit retarded development of the sinus venosus region, lack the sinoatrial valves, and develop atrial dilation and thoracic oedema. They die from heart failure between embryonic day 11 and 13. In situ hybridization revealed a dramatic down regulation of Bmp4 expression in the myocardial wall of the sinus venosus and in the proepicardial organ. Interestingly, Zebrafish embryos injected with Shox2 specific antisense morpholino oligonucleotides exhibit cardiac conduction failure leading to bradycardia and eventually to cardiac arrest. These observations demonstrate an essential function of Shox2 upstream of Bmp4 in the development of the inflow tract region and suggest an involvement in the formation of the cardiac conduction system.

W07 05

THE NEUROLOGICAL PHENOTYPE OF HUMAN LAMININ β2 DEFICIENCY - EVIDENCE OF GENOTYPE-PHENOTYPE CORRELATIONS IN PIERSON SYNDROME

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Pierson syndrome (PS; OMIM 609049) is a new autosomal-recessive oculorenal syndrome caused by LAMB2 mutations leading to laminin β2 deficiency. Previously reported patients died in the newborn period or early infancy due to renal failure. Aside from its renal and ocular expression, laminin β2 is thought to play an important role at the neuromuscular synapse and is present in certain regions of the brain. This raises concerns about a neurological phenotype of PS which may be obscured by early lethality but become evident in long-term survivors. Herein we first report on PS patients surviving beyond infancy.
Patient 1 is the product of a German couple originating from an isolate in Romania. She had congenital nephrotic syndrome and developed end stage renal failure in the newborn period requiring chronic dialysis. She died at age 1.5 years after kidney transplantation. By that age she was found to have severe muscular hypotonia, did not achieve sitting and speech abilities, and was apparently blind. The patient was homozygous for a truncating LAMB2 mutation, 5259insA. Immunohistochemistry for laminin β2 on kidney tissue was negative.
Patient 2 was born to unrelated French parents. She had typical ocular features of PS, early-onset nephrosis (age 2 months), and required peritoneal dialysis from age 3. She is now 3.5 years old, has a normal psychomotor development and moderate vision impairment. She was found to be compound-heterozygous for a truncating LAMB2 mutation, 1477delT, and a splice site mutation IVS22+2T>G which may retain some residual protein function.
Objectives: Our observations suggest that complete laminin β2 deficiency is associated with a severe neurological phenotype. In the presence of specific (milder) LAMB2 mutations, however, PS might be associated with milder neurological features and even lack a neurological phenotype. These issues are important for the counselling of affected families and therapeutic decisions.

W07 06

The mutational spectrum of NSDHL in CHILD syndrome: Functional implications

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CHILD syndrome (Congenital Hemidysplasia with Ichthyosiform Nevus and Limb Defects, MIM 308050), is an X-linked dominant, male-lethal trait characterized by an inflammatory nevus that usually shows striking lateralization with strict midline demarcation as well as ipsilateral hypoplasia of the body. Recently, we were able to demonstrate that this trait is caused by mutations in the gene NSDHL (NAD(P)H steroid dehydrogenase-like protein) encoding a 3ß-hydroxysteroid dehydrogenase functioning in the cholesterol biosynthetic pathway. NSDHL maps to Xq28.
We report the results of mutational analysis in more than 30 familial and sporadic cases. The phenotype appears to be caused by loss of function because it can be associated with nonsense- and missense mutations as well as with deletions eliminating several exons or the complete gene. Amino acids of NSDHL located outside the predicted functional domains (co-factor binding site, catalytically active site, transmembrane helix) which are highly conserved in evolution may pinpoint positions of potential functional importance. We generated by mutagenesis human NSDHL transgenes reflecting missense-mutations observed in CHILD patients or having altered other potentially functionally important sites. GFP-NSDHL fusion protein constructs with wild type or mutated NSDHL were transiently expressed in different cell lines. By comparing the localization of GFP-NSDHL with the sites taken by different cellular compartments (identified by immunohistochemistry) we demonstrate that the wild type protein primarily localises to the surface of lipid storage droplets (LDs) and to the ER. In contrast, most of the mutant NSDHL variants show disturbed subcellular distribution. Complementation analysis by transfer of mutated human NSDHL into the erg26ts yeast strain, which is mutated in the orthologous gene, ERG26, suggests functional differences between mutants, which are not reflected in the human phenotype.

W08 Neurogenetics

W08 01

The possible role of Ca2+ in the pathophysiology of BSCL2 resulting in autosomal dominant Silver syndrome

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Silver syndrome is a complicated hereditary spastic paraplegia associated with amyotrophy of small hand muscles. Recently two heterozygous missense mutations in the Berardinelli-Seip congenital lipodystrophy gene (BSCL2), causing Silver syndrome, were found. In this study we investigated the function of the protein seipin. Colocalisation studies with calreticulin showed that seipin and seipin-mutants are located in the ER and ER-membrane respectively. Therefore it is tempting to speculate that these proteins are involved in the control of the Ca2+ concentration within the ER. Furthermore involvement of Ca2+ in neurodegenerative diseases is well established. We determined intracellular [Ca2+] in human umbilical vein endothelial cells transfected with wild type and mutant seipin-EGFP constructs. Subsequently we quantified the Ca2+ concentration in the cytoplasm of single cells with the Fura-2 technique in time laps experiments. Changes of intracellular [Ca2+] were measured by a variation in the fluorescence intensity of Fura-2. Differences in Ca2+ concentration were observed between cells transiently transfected with wild-type and mutations N88S and S90L. Upon stimulation with an IP3 generating agonist (histamine 100 M) initial cytosolic [Ca2+] elevation was significantly reduced in cells expressing N88S mutant compared to control (WT mutant or not transfected cells). In addition the cytosolic [Ca2+] during stimulation with histamine in the absence of extracellular [Ca2+] was not transient in contrast to control cells. Due to the observed decelerated [Ca2+] release out of endoplasmic reticulum further investigations of calcium pumps will be carried out.

W08 02

JARID1C, a novel gene involved in X-linked mental retardation, is frequently mutated

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More than 30% of mutations in families with non-syndromic X-linked mental retardation (NS-XLMR) seem to cluster on proximal Xp and in the pericentric region. In a systematic screen of brain-expressed genes from this region in 354 families with XLMR, we have identified 12 different mutations in JARID1C, including one frameshift and three nonsense mutations introducing premature stop codons as well as eight missense mutations changing evolutionarily conserved amino acids. In two of these families, expression studies revealed an almost complete absence of the mutated JARID1C transcript, suggesting that the phenotype in these families results from a loss of JARID1C function. JARID1C belongs to the highly conserved ARID protein family. It contains several DNA binding motifs linking it to transcriptional regulation and chromatin remodeling, a process, which is defective in various other forms of mental retardation. The mutation frequency of 3.4% in our patient cohort suggests that mutations in JARID1C are a relatively common cause of XLMR, and that this gene might play an important role in human brain function. Further studies will include the characterization of mice deficient in Jarid1c and expression profiling of the respective brain tissues. These investigations should shed more light on the pathogenesis of mental retardation.

W08 03

Identification of potential gene modifiers in SMA discordant families

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Spinal muscular atrophy (SMA) is an autosomal recessive disorder which affects the alpha motor neurons of the spinal cord. SMA is caused by deletions and/or mutations in the survival motor neuron gene 1 (SMN1).The SMN gene is duplicated on chromosome 5q13 and the second copy (SMN2) differs in only 5 bp. Most of the SMN2 transcripts undergo alternative splicing of exon 7, which encode in a truncated and unstable SMN protein. SMN protein interacts with multiple proteins with functions in snRNPs biogenesis, pre-mRNA splicing, gene repression and presumably neural transport. In rare cases, sibs with identical SMN1 mutations and identical SMN2 copies can show variable phenotypes from unaffected to affected indicating the existence of SMA modifying factor(s). Recently, we showed that the modifier is influencing the SMN protein level. The expression profiling of EBV-transformed cell lines belonging to one discordant family (2 sibs unaffected and 2 sibs affected), 2 type I and 2 type III SMA patients revealed up-regulation of 6 genes differentially expressed above 2-fold threshold. All the candidate genes were found to be expressed in brain, spinal cord and muscle, tissues which are involved in the development and the progression of the disease. After statistical evaluation and further validation on RT-PCR in all discordant families, two promising transcripts were identified. These showed in 4 additional discordant families the same significant difference between affected and unaffected siblings. So far, no mutation within the coding region and no association with a certain SNP were found. The analysis of the promoter region is in progress. However, an in vivo protein interaction with SMN was shown for one candidate so far. These interesting findings open new prospects to better understand the mechanism of pathology and the regulation of the SMN protein, as well as to develop additional therapies for SMA.

W08 04

Morphological and functional changes in motoneurons from an animal model for Spinal Muscular Atrophy (SMA)

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The most common form of childhood spinal muscular atrophy (SMA) is caused by a defect in the survival motoneuron 1 (SMN1) gene. We have previously shown that mice which lack Smn protein, either as Smn heterozygous (Smn+/-) or Smn-/- mice with two transgenic copies of the SMN2 gene (Smn-/-;SMN2), develop postnatal motoneuron degeneration. Cell loss and severity of the motoneuron degeneration depend on Smn protein levels. However, it is still unknown why specifically motoneurons are so vulnerable to Smn loss.

We have observed that cultivated embryonic spinal motoneurons from Smn-deficient mice exhibit normal survival in cell culture, but show reduced axon growth. Reduced axon growth of Smn-deficient motoneurons is associated with reduced beta-actin protein and beta-actin mRNA accumulation in the distal part of the axons. This correlates with reduced beta-actin mRNA levels in axon terminals of Smn-/-;SMN2 motoneurons. We have also found that the 3'UTR of the beta-actin mRNA specifically interacts with a complex of Smn and hnRNP-R, suggesting that Smn plays a role in the axonal translocation of beta-actin mRNA.

In order to investigate the consequences of disturbed beta-actin distribution along the axon and the mechanisms leading to reduced axon length, we have analyzed the excitability of Smn-deficient motoneurons using Calcium imaging techniques. Spontaneous excitability was tested by spike frequency of intracellular Ca2+ transients, caused by opening of voltage-gated Ca2+ channels. Our experiments show that Smn-deficient motoneurons, in comparison to wildtype controls, exhibit reduced frequency of spikes. The reduced frequency of Ca2+ transients indicates that motoneurons in SMA mouse models and patients are functionally impaired by a reduced capacity to depolarize and thus to release neurotransmitters at the motor endplate. This might significantly contribute to the disease phenotype, and muscle weakness might occur before motoneurons start to degenerate.

W08 05

Periphilin maps to the *PARK8* region and is a new interactor of Synphilin-1, a protein involved in Parkinson's disease

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Parkinson's disease (PD) is neuropathologically characterized by the loss of dopaminergic neurons and the presence of intracytoplasmic inclusions (Lewy bodies, LB). Alpha-synuclein, a presynaptic protein, and synphilin-1, a synuclein-interacting protein, were found to be major components of the LB. We recently identified a mutation in the synphilin-1 gene in two PD patients. In order to further elucidate the role of synphilin-1 in the pathogenesis of PD, we searched for novel interacting proteins of synphilin-1 and isolated the highly insoluble protein periphilin by yeast-two hybrid screening. Immunohistochemical studies identified periphilin as a component of LB in brains of PD patients. Interestingly, the periphilin gene maps to the PARK8 region. A mutation search identified a missense mutation in two PD patients of one pedigree. However, immunocytochemistry data provide no indication of co-localization of the nuclear periphilin with synphilin-1. The mutation does neither affect the cellular distribution nor the susceptibility to cellular stressors such as the proteasome inhibitor MG132 or H2O2. However, HEK cells stably expressing mutant periphilin appeared to be more susceptible to the nitric oxide donor S-nitroso-N-acetylpenicillamin (SNAP). Since periphilin accumulates under proteasomal inhibition with MG132, it seems likely that this protein is degraded by the proteasome, fitting periphilin in the ubiquitin-proteasome degradation pathway. In summary, our results link periphilin with synphilin-1 and open up new vistas for periphilin as a novel potential pathogenesis factor in PD.

W08 06

A novel mouse model for Spinocerebellar Ataxia Type 3 containing 148 polyglutamine repeats

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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. SCA3 therefore belongs to the group of the so called polyglutamine diseases. In order to study the course of the disease we generated transgenic mouse models for this disorder. We used the prion protein (PrP) promotor to control the expression of the full length Ataxin-3 gene containing 70 or 148 CAG repeats, respectively. As controls transgenic mice expressing ataxin-3 with 15 CAG repeats were used. Western blot analyses confirmed the expression of the transgenic Ataxin-3 proteins in the brain. The mice carrying 148 polyglutamine repeats were severely affected by a remarkable neurological phenotype with aberrant behaviour, conspicuous footprint pattern as well as poor rotarod performance. 148 polyglutamine repeats in the Ataxin-3 protein lead to early death at about 15 weeks of age. Neuropathological examination by immunohistochemical stainings revealed Ubiquitin- and Ataxin-3-positive intranuclear inclusion bodies in a multitude of neurons. The transgene containing 70 polyglutamine repeats give rise to a comparable but significant milder phenotype resulting in death at about 30 weeks of age. The phenotypical observations as well as the neuropathological results correspond to the mouse model with 148 CAG repeats. On the other hand mice carrying the Ataxin-3 transgene with a normal repeat length (15 CAG repeats) were phenotypically normal with no neuropathological findings. We believe that our mouse models will be a very helpful tool to study the progression of pathology in SCA3. In addition the different repeat numbers allow choosing from a more severe (148 repeats) or milder (77 repeats) phenotype.

W09 Molecular Genetics

W09 01

Mutations in RDH12, encoding a photoreceptor cell retinol dehydrogenase, in autosomal recessive retinal dystrophy

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We recently identified *RDH12* (14q23.3-q24.1) as a novel disease gene finding 5 most likely pathogenic changes (T49M, R62X, Q189X, 806delCCCTG, Y226C) in homozygous or compound heterozygous form in patients with childhood-onset severe retinal dystrophy (Janecke et al. Nature Genet. 36, 850, 2004). Retinol dehydrogenase-12 (RDH12) is a photoreceptor enzyme proposed to function in the visual cycle to convert all-trans retinal (released from bleached visual pigments) to vitamin A that is used by the RPE to reform the chromophore 11-cis retinal. The purpose of the current study is to determine the mutation spectrum and prevalence of RDH12 mutations in autosomal recessive retinal dystrophy (arRD). We performed *RDH12* mutation screening in 990 patients with arRD. Patient missense mutations were introduced into RDH12.pcDNA3.1 constructs that were used to transfect COS-7 cells, and the enzyme activity of the recombinant proteins was assayed using HPLC analysis. We have now identified 17 additional RDH12 mutations in patients with arRD: M1?, A47T, N34fsX62, T55M, R65X, L99I, G127X, [c.429C>G;c.430C>G;c.432delG], G145E, H151D, T155I, Y195X, A206D, R239W, L274P, C285Y, and R295X. Each of the missense variants tested exhibited decreased or aberrant activity relative to wild-type when assayed for their ability to catalyze the interconversion all-trans retinol and all-trans retinal. In all patients with RDH12 mutations, the disease affected both rods and cones with onset of symptoms in early childhood (2-4 y) and progression to legal blindness in early adulthood (18-25 y). Our studies suggest that RDH12 mutations account for about 5% of cases of severe, childhood-onset arRD, and for 1.5% of arRD cases in total. Our studies identify a cohort of patients whose disease may be amenable to therapies effective for visual cycle defects currently under development by the vision research community.

W09 02

A two step imprinting defect involving CTCF binding sites results in Beckwith-Wiedemann syndrome and Wilms'tumor

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Genetic diseases can be caused by incorrect genomic imprinting, a mechanism that normally leads to epigenetic silencing of one parental allele. A developmental disorder associated with imprinting defects in the chromosomal region 11p15.5 is Beckwith Wiedemann syndrome (BWS), a congenital overgrowth condition, with about 5% of children developing embryonal tumors, most frequently Wilms'tumor (WT). The disease causing epigenetic alterations described to date cluster in two regions, that are coordinated by imprinting-centers (ICs), termed KvDMR1 (or IC2) and IC1. The latter is positioned between H19 and IGF2 and harbors differentially methylated target sites for the insulator protein CTCF. Based on an extraordinary familial case of BWS with WT we describe a microdeletion in IC1, associated with IGF2-loss of imprinting and upregulation of IGF2 mRNA. A second genetic lesion - a duplication of maternal 11p15.5 - was found to be restricted to clinically affected family members. The BWS-WT phenotype is associated with/characterized by a further increase of IGF2 levels resulting in critical activation of a receptor signaling cascade (PI3K-AKT-mTOR-4EBP1/S6K). Activation of this cascade seems to be a tumor predisposition marker of BWS with various predictive and even therapeutic implications.

W09 03

An inversion involving the Sonic Hedgehog locus results in the mouse Short Digits (Dsh) phenotype through dysregulation of Shh expression

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Short digits (Dsh) is a radiation induced semidominant mouse mutant. Dsh/Dsh mice exhibit multiple internal and skeletal defects strongly resembling the Shh-/- mouse. In contrast to Shh +/- mice, Dsh/+ mutants are characterized by a limb reduction phenotype with fusion of proximal and middle phalanges in all digits, reminiscent of human brachydactyly type A1. We mapped Dsh to mouse chromosome 5 in a region harboring the Shh gene. We were able to demonstrate an inversion comprising 11.7 Mb and identified both breakpoints. The distal breakpoint resides 13.298 kb upstream of Shh and separates the gene from several conserved noncoding elements, that may represent cis-regulatory enhancers. Using extensive in situ hybridization analysis and quantitative RT-PCR we were able to show an almost complete downregulation of Shh expression during developmental stages E9.5 to E12.5 in Dsh/Dsh mice, leading to the Shh -/- like phenotype. However, in Dsh/+ mice Shh is upregulated at stages E13.5 and E14.5 and ectopically expressed in the phalanges, at a time point when Shh has normally been turned off in the limb. As a consequence of local Shh upregulation and misexpression, hedgehog target genes, as Gli1-3, Patched and Pthlh are overexpressed, and Ihh and Gdf5 are downregulated. This results in the disruption of digit growth and joint formation explaining the brachydactyly phenotype. The Dsh mutant represents a so far undescribed example for the temporal and spatial misregulation of gene expression due to a chromosomal rearrangement. The developmental pathology associated with this misexpression extends our understanding of normal and abnormal limb development.

W09 04

Microarray analysis combined with microdissection of fetal human neocortex identifies candidate genes for brain development and function

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Development of the human neocortex depends on spatially and temporally correct expression of numerous genes. Disturbances of this highly coordinated process are an important cause of mental retardation and many other brain disorders. It is plausible that the same set of genes also contributes to cognitive variation. Identification of genes involved in human brain development and characterization of their expression patterns may provide a better understanding of cognitive processes. To this end we have developed a cDNA chip with approximately 600 genes that are known to influence some aspect of cognition in humans, mice and/or Drosophila, along with 100 control house keeping genes. This customized gene chip was used to quantify the mRNA expression levels in fetal (weeks 15-25 of gestation) brain samples from frontal cortex (prospective area A10). Approximately 300 genes on the chip displayed detectable expression levels. A subset was expressed differentially at different time points of gestation. Approximately 50 genes showed at least twofold expression differences between normal and trisomy 21 brain samples of the same gestational weeks and/or changes in their temporal expression patterns. Some of these genes were reported previously to be expressed differentially in trisomy 21 brains. However, most identified genes, including genes of the MAPK signaling and the Alzheimer disease pathways, have not been associated with trisomy 21 in the literature. Microarray results were validated with reverse Northern blots and real time PCR. Because the topography of gene expression during early human cortical development is largely unknown, we have microdissected the six neuronal cell layers from normal and trisomy 21 neocortex. The extracted and amplified RNA samples will be hybridized on our microarray chip. Collectively, our results will provide new insights into the genetics of human brain development (corticogenesis) and cognitive processes

W09 05

Nijmegen Breakage Syndrome: Proteomic analysis of Nbn null-mutant mice.

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Nijmegen breakage syndrome (NBS, MIM 251260) is a rare autosomal recessive genetic disease belonging to a group of disorders termed chromosome instability syndromes. Patients affected by NBS have a range of symptoms including microcephaly, growth retardation, radiosensitivity, immunodeficiency and an increased cancer risk, particularly for B-cell lymphoma. Over 90% of all NBS patients are homozygous for a 5 bp deletion (657D5) in exon 6 of the NBS1 gene.
To circumvent the embryonic lethality of null mutation of the murine NBS1 homologue, Nbn, we have generated mice with an inducible null mutation. LoxP-sites were introduced into the murine genome either side of Nbn exon 6. Expression of cre-recombinase leads to exon 6 deletion: a lethal mutation. Mice were crossed with transgenic mice expressing Cre recombinase under the control of the interferon responsive promoter, Mx1, allowing induction of Cre-mediated exon 6-deletion by injection of polyI:polyC. A semi quantitative PCR on DNA extracted from various organs revealed the highest deletion efficiency in the liver of treated mice (ca. 90 %) suggesting that this organ is most suitable for identification of proteins that are affected by the absence of nibrin.
In order to identify such proteins, we performed 2 dimensional gel electrophoresis on liver proteins isolated from mice at various times after ionising irradiation. Already in unirradiated livers, 11 proteins were aberrantly expressed in the absence of nibrin. The number of proteins with altered expression increased dramatically to 123 24h after irradiation. The proteins were isolated and identified by mass spectrometry, they can be classified into several ontologic categories such as cell cycle regulation, translation/transcription and protein folding. These data demonstrate that the mutagenised cell is strongly affected by the absence of nibrin reflecting its central role in the cellular response to DNA damage.

W09 06

The impact of ovarian stimulation on imprinted gene methylation in preimplantation stage mouse embryos

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Several lines of evidence suggest a relationship between assisted reproductive technology (ART) and genomic imprinting disorders. Recent studies reported an increased incidence of Angelman syndrome and Beckwith-Wiedemann syndrome associated with methylation imprinting defects in children conceived through in vitro fertilization (IVF) and/or intracytoplasmatic sperm injection (ICSI). Several animal studies showed that embryo culture conditions during ART affect the expression and methylation of imprinted genes. Ovarian stimulation, i.e. superovulation with gonadotrophins, which is used to increase the number of oocytes that can be retrieved for ART is known to impair implantation and fetal development in mice. These data prompted us to analyze the effects of ovarian stimulation on methylation of the imprinted genes <H19>, <Snrpn>, <Igf2r>, <Lit1>, <Mest> and <Peg3> in preimplantation mouse embryos. Stimulated (by i.p. injection with 7.5 IU pregnant mare's serum gonadotrophin followed 44–48 h later by an injection of 7.5 IU human chorionic gonadotrophin) and non-stimulated C57BL/6J females were mated with C57BL/6J or <Mus musculus castaneus> males. Morula stage embryos were recovered from oviducts on gestational day 2.5. DNA was isolated from single embryos or pools of 5-12 embryos and subjected to bisulphite modification, PCR amplification, subcloning and sequencing. Preliminary evidence suggests that morula stage embryos from stimulated females exhibit a loss of DNA methylation at the imprinting control regions of the <Snprn> and <H19> genes. These preliminary findings support the hypothesis that developmental abnormalities associated with ovarian stimulation are due to impaired imprinted gene methylation.

W10 Genetics of Mendelian Traits

W10 01

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is caused by a mutation in the calcium-binding protein calsequestrin 2

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Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a heart muscle disease that causes arrhythmias, heart failure, and sudden cardiac death. A specific form of ARVC (named ARVD2) is associated with catecholamine-induced polymorphic ventricular tachycardia (CPVT) and caused by dominant mutations in the cardiac ryanodine receptor 2 gene (RYR2).
We now clinically characterized a family with an ARVD2 similar phenotype. The 26 year-old index patient presented with recurrent syncopes, structural changes of the right ventricular wall due to fibro-fatty replacement of the myocardium, and CPVT. A sister of the index patient, showing the same phenotype, suddenly died at the age of 15. Mutation screening of the cardiac calcium-binding protein calsequestrin 2 (CASQ2) gene, which was recently identified to cause CPVT without any structural abnormality, revealed a heterozygous missense mutation in exon 5 of the CASQ2 gene. The mutation, that was not present in 322 control chromosomes, is located in the highly conserved core domain II of the protein. Three-dimensional modeling of the mutant protein suggested that the hydrophobicity of the acidic core of the protein might be changed, which could lead to a calcium disturbance in cardiac myocytes. Functional analysis of the mutation is currently performed to understand the pathophysiological mechanism of this specific phenotype.
Our study shows for the first time that mutations in CASQ2 cause the ARVC/CPVT phenotype, most likely by a calcium disturbance of the sarcoplasmic reticulum.

W10 02

Extended heterogeneity in hemophagocytic lymphohistiocytosis: UNC13D and PRF1 mutations in childhood patients

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Familial hemophagocytic lymphohistiocytosis (FHL) is an autosomal recessive disease affecting young children. It presents as a severe hyperinflammatory syndrome with activated macrophages and T lymphocytes. Mutations in the perforin 1 gene (*PRF1*) were reported in FHL-2 in 15–50% of all cases. Defective granule exocytosis caused by mutations in *UNC13D* has recently been described as the defect underlying FHL-3. Both types are phenotypically indistinguishable. We have analyzed a large cohort of 61 patients with hemophagocytic lymphohistiocytosis from different geographic origins by linkage analysis and direct sequencing. We identified mutations in 28 samples investigated, 18 in *PRF1* (30%) and 10 in *UNC13D* (16%). Besides several known mutations, novel deletions, missense and nonsense mutations were detected in both genes throughout the coding regions. In 24 patients from Turkey, 13 had mutations in *PRF1* (54%) and 4 had mutations in *UNC13D* (17%). The mutation Trp374X, found in 11/13 patients with *PRF1* mutation from Turkey, was the only mutation observed repeatedly in patients from a common geographic origin. Surprisingly, only 7/28 patients from Germany showed mutations in either *PRF1* (3; 11%) or *UNC13D* (4; 14%). Furthermore, 4 out of 9 patients from other countries showed mutations in one of these genes. We detected only one mutation in *UNC13D* repeatedly in 10 heterozygous patients so far. Moreover, a genome-wide scan for a novel locus for FHL is under way using homozygosity mapping in a large consanguineous family from Turkey. Our results indicate that FHL-2 and FHL-3 account for more than 70% of FHL cases from Turkish origin, however, mutations were identified in only 25% of cases from German descent. Our data demonstrate extensive genetic heterogeneity in FHL, differences in epidemiology depending on ethnicity, and the importance of further, yet unknown loci for the etiology of FHL.

W10 03

The Role of Common Single-Nucleotide Polymorphisms on Exon 9 and Exon 12 Skipping in Nonmutated *CFTR* Alleles

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Classic cystic fibrosis (CF) is caused by two loss-of-function mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, whereas patients with nonclassic CF have at least one copy of a mutant gene that retains partial function of the CFTR protein. In addition, there are several other phenotypes associated with *CFTR* gene mutations, such as idiopathic chronic pancreatitis. In *CFTR*-associated disorders and in nonclassic CF, often only one *CFTR* mutation or no *CFTR* mutations can be detected. In this study, we screened 23 patients with *CFTR*-associated disorders for *CFTR* mutations by complete gene testing and quantitative transcript analysis. Mutations were found in 10 patients. In cells from respiratory epithelium, we detected aberrant splicing of *CFTR* mRNA in all investigated individuals. We observed a highly significant association between the presence of coding single-nucleotide polymorphisms (coding SNPs, or cSNPs) and increased skipping of exon 9 and 12. This association was found both in patients and in normal individuals carrying the same cSNPs. The cSNPs c.1540A/G, c.2694T/G, and c.4521G/A may have affected pre-mRNA splicing by changing regulatory sequence motifs of exonic splice enhancers, leading to lower amounts of normal transcripts. The analysis of *CFTR* exons indicated that less frequent and weak exonic splicing enhancer (ESE) motifs make exon 12 vulnerable to skipping. The number of splice variants in individuals with cSNPs was similar to previously reported values for the T5 allele, suggesting that cSNPs may enhance susceptibility to *CFTR* related diseases. In addition, cSNPs may be responsible for variation in the phenotypic expression of *CFTR* mutations. Quantitative approaches rather than conventional genomic analysis are required to interpret the role of cSNPs.

W10 04

Mutation in the Transcriptional Co-activator Eya4 Causes Dilated Cardiomyopathy and Sensorineural Hearing Loss

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Background: The pathophysiology of heart failure is complex and not fully understood. Dilated cardiomyopathy (DCM) is an important form of heart failure and appears familial in 25-30% of cases. Mutations in several cytoskeletal proteins have already been identified.
Results: We identified a four generation pedigree with a novel syndrome characterized by autosomal-dominant co-inheritance of DCM and sensoneural hearing loss (DCM/SNHL). Hearing loss was evident by late adolescence, whereas congestive heart failure appeared later. A linkage study demonstrated that polymorphic loci on chromosome 6q23 to 24 were co-inherited with the disease (maximum LOD=4.88 at D6S2411). The minimal disease interval measures 2.0Mb and is defined between loci D6S975 and a CA-repeat on BAC RP1-32B1. The locus contains 8 known genes. Epicardin, SGK and EYA4 could be detected in cDNA libraries from both heart and cochlea. Screening of all three genes identified a mutation in EYA4, a transcriptional co-activator that has recently been described in autosomal-dominant hearing loss DFNA10. A deletion of 4846 bp affecting exons 9 and 10 leads to a frameshift with a premature stop codon at aminoacid 193 in DCM/SNHL. To elucidate the role of Eya4 in heart function, antisense morpholino oligo-injected zebrafish embryos were studied; attenuated Eya4 transcript levels produced morphologic and hemodynamic features of heart failure. To understand why previously described mutant Eya4 alleles cause hearing loss without heart disease, biochemical interactions of mutant Eya4 peptides were examined. Hearing loss-associated Eya4 peptides, but not the foreshortened 193 amino acid peptide encoded by the DCM/SNHL Eya4 allele, bound wildtype Eya4 and associated with Six proteins.
Conclusions: These data define unrecognized and critical roles for Eya4-Six mediated transcriptional regulation for normal heart function and thereby provide novel insights into the pathophysiological pathways of heart failure.

W10 05

Very high prevalence of hereditary prosopagnosia - a first report

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Prosopagnosia (syn. face blindness) is defined as the inability to associate a face with a person, while faces as such and facial expressions are recognized. Acquired prosopagnosia is a rare condition after a right hemispheric stroke or brain injury. Until recently, the congenital form of prosopagnosia, was generally considered to be even less common. Until 2001 only 11 cases in seven single case studies and one family with three affected members have been published. In our recent examinations of more than 180 prosopagnosics and more than 30 families we could show that this cognitive disorder is hereditary and compatible with simple autosomal dominant inheritance. We therefore introduced the term "hereditary prosopagnosia" (Kennerknecht et al. 2002, Grüter et al. 2005).
Here, we present evidence that hereditary prosopagnosia is a very common cognitive disorder. Probands were recruited by questionnaire based screening in local secondary schools and among medical students of our university. 28 out of 571 probands, who were highly suspicious of a possible visual recognition impairment were interviewed in detail for symptoms of prosopagnosia, i.e. (1) recognition of a familiar face or of faces outside the normal context is significantly reduced or nearly impossible; (2) not possible to say whether a face is familiar or not results in false positive and negative decisions; (3) decision time is prolonged; (4) no recognition after only a short contact; (5) development of adaptive strategies (Grüter et al. 2005). A diagnosis of hereditary prosopagnosia was established in 13 individuals. This gives a prevalence of 2.6 % at least in this population. This frequency is

among the highest known for a monogenic disorder.

W10 06

Whole gene deletions of SALL4 as well as deletions of single exons are a common cause of Okihiro and acro-renal-ocular syndromes and confirm haploinsufficiency as the pathogenetic mechanism

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Okihiro (Duane Radial-Ray) syndrome is an autosomal dominantly inherited malformation syndrome characterized by radial malformations of upper limbs in combination with Duane anomaly. Previously, mutations in the putative zinc finger transcription factor gene SALL4 were reported to cause Okihiro and acro-renal-ocular syndrome. However, in a substantial proportion of patients with such phenotypes no SALL4 mutation was found. Segregation analysis of five intragenic SNPs was informative in four out of seven families and suggested a heterozygous SALL4 deletion of at least exon 2. To confirm these findings and establish the deletion size, quantitative real time PCR was applied. In this way, we identified three families with deletions of all four exons as well as two families with deletions of exons 1-3. In one family uninformative for the five intragenic SNPs, a heterozygous deletion of exon 4 was detected, and in a further family, a heterozygous exon 1 deletion of 8.9 kb in size was determined with one breakpoint residing within an Alu-element. In another family, the deletion size was 59.4 kb with both breakpoints within Alu-elements, suggesting that Alu-mediated recombination is responsible for at least some of the deletions. In a further patient who had developmental delay and some other features not associated with Okihiro syndrome, a heterozygous deletion of approximately 2 - 3.5 Mb was detected by real time PCR. In addition to the SALL4 locus the deletion was found to harbor at least 15 genes flanking SALL4. These results show that, in contrast to the likely dominant-negative action of SALL1 mutations causing Townes-Brocks syndrome, Okihiro and acro-renal-ocular syndromes are clearly resulting from SALL4 haploinsufficiency. Furthermore, we will discuss the influence of haploinsufficiency of flanking genes on the phenotype in the patient with a larger deletion.

W11 Prenatal Diagnosis

W11 01

Stem cells in human amniotic fluid

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Currently it is the hope of both patients and investigators that human progenitor cells and stem cells can be widely used to replace dysfunctional cells within tissue. It is speculated that such cells may prove to have the potential to treat or cure a myriad of diseases. A major aim in this respect is the identification of new sources for pluripotent stem cells. Oct-4 is a marker for pluripotent human stem cells so far known to be expressed in embryonal carcinoma cells, embryonic stem cells and embryonic germ cells. We provided evidence that human amniotic fluid may represent a new source for the isolation of human Oct-4-positive stem cells without raising the ethical concerns associated with human embryonic research. In addition, we used proteomic approaches to provide new insights into the spectrum of cells contained in human amniotic fluid. Recently, we also described that human amniotic fluid contains cells that express markers for neuronal stem and progenitor cells, which harbour the potential to differentiate into neurogenic cells. Using constructs harboring GFP or a neomycin resistance gene under the control of the Oct-4 promoter or the Rex-1 promoter we a currently isolating Oct-4/Rex-1-positive populations of human amniotic fluid cells. These cells will be treated with differentiation media to analyse their potency to differentiate into neurogenic, chondrogenic, osteogenic, myogenic and adipogenic lineages.

W11 02

Attitudes of parents towards their child with Down syndrome before and after the introduction of prenatal diagnosis: Comparison 1970 vs. 2003

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In 1970, a questionnaire study concerning the social and emotional situation of mothers of children with Down syndrome was carried through in Bavaria, at a time when the chromosomal cause of Down syndrome had been discovered but prenatal diagnosis was not yet feasible. In the meantime, prenatal diagnosis of Down syndrome has been firmly established in medicine and society, and nowadays parents frequently complain about being held socially responsible for not having "avoided" their child.

To assess the psychosocial impact of the availability of prenatal diagnosis on parents of genetically handicapped children, the 1970 study was repeated over thirty years later, using the same questionnaire plus questions concerning attitudes towards prenatal diagnosis (participants 1970 = 282 mothers; 2003 = 410 mothers).
The results reflect the mixed blessings of medical and societal progress for families affected from genetically diagnosable conditions:
While mothers' feelings of guilt for having a disabled child stayed on a low level, today mothers have a stronger feeling of being segregated in society. On the other hand, they more often experience support and respect through others today, in particular through self-support groups; moreover, tendencies of withdrawal from social life have decreased.
Overall, the emotional situation of mothers of children with Down syndrome has considerably improved, despite adverse societal effects of prenatal diagnosis being misunderstood as a 'tool' to guarantee the birth of healthy children. Consequently, the vast majority of the mothers support the general availability of prenatal diagnosis, although their wish to use it for themselves is lower than in the general population.

W11 03

Knowledge about the risks for congenital malformations and Down syndrome in general population.

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Knowledge about risks for congenital malformations (CM) and Down syndrome (DS) is poor in the general population. In order to assess the knowledge about the etiology and risks for CM and DS, 3000 questionnaires comprising 60 questions were distributed, of which 66,8% were returned for analysis (mean age: 30.6±16,3y, m:f: 32:68%). Incidence of CM: 46,4% agreed with the given categories: 1-2% or 3-5%, while the majority presumed higher risks. The risk was overestimated by younger participants (< 20 years (OR 1,7) as well as by women (OR 0,5). There was a significant correlation between the number of correct answers and the level of education. DS: 81,6 % knew that DS is a chromosomal disorder. Adolescents < 20 years and women gave significantly more correct answers. 10,5% believe that perinatal asphyxia is a cause of DS, while 33,1% indicated 'I don't know'. Women, subjects with a medical background and those with a higher education were more often correct. Those with a history of miscarriages and with personal experience regarding malformations and those at an reproductive age were more likely to give correct answers. 10% of participants thought that alcohol consumption causes DS, and at least 36% were not sure about a relationship between drinking and DS The key question 'What is the risk for a 40year-old woman to bear a child with DS?' indicates a dramatic overestimation with answers according to the given categories: <1%:3,8%; about 1%:9,9%; 2-5%:23%; 6-10%:17,7%; 11-15%:12,0%; 16-20%:11,0%; 21-30%:8,8%; 31-40%:8,5%; >41%:5,3%. Thus 86,9% overestimated this risk. Participants < 20 years and women significantly overestimated this risk as well as those with a lower educational level. A dramatic overestimation of the risk for DS leads

to an increasing demand for prenatal diagnosis. Our data show that information about genetic risks should be given in a much broader context, possibly starting at school which has been recognised in the Netherlands since many years.

W11 04

Polar body diagnosis for monogenic disorders by one step-multiplex fluorescence PCR

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Under the German Embryo protection law preimplantation genetic testing for monogenic disorders can only be performed as polar body diagnosis and thus is restricted to female mutation carriers. In order to select against pronuclear stages with the respective mutation, the diagnosis in Germany has to be performed within 24 hours after oocyte biopsy. Here we report about the experimental design and the results of three different assays to analyze both a particular mutation and two closely linked informative markers in first and second polar bodies of oocytes from female carriers of the characteristic repeat expansion in the Huntingtin gene, the ΔF508 mutation in the CFTR gene and a missense mutation in the Norrie disease (ND) gene, respectively. The CAG repeat in the Huntingtin gene as well as the 3bp deletion were directly detected by separation of fluorescently labeled PCR products on an ABI310. Minisequencing was used to detect the disease causing missense mutation in the ND gene.

Transfer of 14 embryos in 9 ICSI cycles at our center so far resulted in 4 biochemical pregnancies, two of them with early abortion. After transfer of 2 embryos in the fourth cycle in the family with the ND mutation carrier prenatal diagnosis confirmed that the male fetus with normal 46,XY karyotype had inherited the ND wildtype allele from his mother. In August 2004 a healthy boy was delivered at term as the first child born after polar body diagnosis for a monogenic disorder in Germany. In the family with the CFTR mutation carrier transfer of 2 embryos in the first ICSI cycle resulted in an ongoing clinical singleton pregnancy.

The experimental setup of single cell assays for polar body diagnosis requires the implementation of a wide variety of safety measures, which has to be complemented by a close interdisciplinary care for the couple during the entire process of pre-treatment counseling, assisted reproduction with polar body diagnosis and the subsequent pregnancy.

W11 05

Prenatal exclusion/confirmation of Fanconi Anemia

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Objectives: There are three main indications for prenatal exclusion/confirmation of Fanconi anemia (FA): (1) FA-negative family history, but suspicious ultrasound findings such as radial ray aplasia, (2) FA-positive family history, but lack of information about the affected gene and/or mutation. (3) FA-positive family history and knowledge of the affected gene and/or mutation permitting either indirect or direct molecular testing.
Methods: Molecular testing is the method of choice, but, in our experience, applicable in less than 50% of requests for prenatal diagnosis. The first two indication groups require functional testing of prenatal cell cultures which our lab assays for typical cell cycle changes (G2-phase accumulations) without and with Mitomycin C-treatments using single and dual parameter (BrdU-Hoechst) flowcytometry.
Results: Single parameter flow-cytometry correctly identified 2 positive and 9 negative cases on the basis of MMC-sensitivity of cultivated amniotic fluid (AF) cells. Likewise, 8 negative cases and 2 positive cases were correctly predicted using bivariate flowcytometry of 72 h umbilical cord (UC) blood cultures. In contrast, bivariate flowcytometry applied to AF cells grown in the presence of bromodesoxyuridine (BrdU) yielded false positive and false negative results.
Conclusion: Single parameter flowcytometry of AF-cell cultures and bivariate flowcytometry of UC-cell cultures have the potential to correctly predict the affected status in cases at risk for FA, whereas bivariate flow cytometry proved unreliable when applied to BrdU-substituted AF-cell cultures. Cases with a low a priori risk (e.g. sonographic finding of radial ray abnormalities and negative family history) would benefit most from cell cycle testing as a prenatal screening procedure.

W12 Complex Disease

W12 01

Systematic linkage disequilibrium analysis of SLC12A8 at PSORS5 confirms a role in susceptibility to psoriasis vulgaris

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The gene for the solute carrier family 12 member A8 has recently been proposed as a candidate gene for psoriasis susceptibility (PSORS5) on chromosome 3q based on association of five intronic SNPs in a Swedish psoriasis cohort. To investigate whether this susceptibility locus is also relevant for German patients suffering from psoriasis vulgaris we analysed two samples of psoriasis patients: the previously recruited group of 210 parent-offspring trios as well as new samples of 375 single patients with psoriasis vulgaris and of 376 controls for a case-control study. Based on our systematic investigation of the linkage disequilibrium (LD) haplotype structure of SLC12A8 we assayed a total of 35 tag SNPs, which we grouped into nine LD-blocks of 3-20 kb size each. In the case-control samples we detected significant association of six different SNPs for psoriasis vulgaris at single SNP level, while three LD-based haplotypes showed association. Association was strongest for SNP 34 (2 = 11.224, p = 0.0008) and haplotype E-2 (2 = 11.788, p = 0.00059) and was independent of the presence of an HLA-associated PSORS1 risk allele. Through extended haplotype analysis we could show, that at least two independent association signals exist in SLC12A8, indicating the existence of allelic heterogeneity. None of the SNPs showed association in the parent-offspring trios, apart from a weak association of SNP 68 (TDT statistics p = 0.048), probably due to lack of power in that group. We conclude that SLC12A8 is a susceptibility locus for psoriasis vulgaris. In order to establish the exact nature of this association, efforts to identify the disease-causing variants are ongoing.

W12 02

Supportive evidence for a relationship between genetic variants at the brain-derived neurotrophic factor (BDNF) locus and depressive symptoms in affective disorder and schizophrenia

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Objectives: There is evidence from clinical, pharmacological, animal, and genetic studies that the *brain derived neurotrophic factor (BDNF)* is involved in the pathogenesis of neuropsychiatric disorders and in the therapeutic action of at least some effective drugs. The aim of this study was to investigate the potential influence of genetic variation at the *BDNF* locus on the development of major depressive disorder (MDD), bipolar affective disorder (BPAD), and schizophrenia.
Material and Methods: We genotyped three polymorphisms at *BDNF* (rs6265 leading to Val66Met, rs988748, and a $(GT)_n$ repeat) in DSM-IV diagnosed patients with major depression (MDD), schizophrenia, bipolar affective disorder (BPAD), and in healthy controls. There were 465 MDD patients, 533 schizophrenia patients, 281 BPAD patients, and 1,097 controls of German origin (Bonn sample). A second sample (312 MDD patients, 444 controls) was recruited in Bavaria (Munich sample). Single-marker and haplotype analysis for association with the phenotype was performed.
Results: Three-marker haplotype rs988748-$(GT)_n$ -rs6265 produced significant associations with all phenotypes under investigation

(p=0.00006 for Bonn MDD, p=0.0092 for Munich MDD, p=0.0057 for BPAD, p=0.016 for schizophrenia). To explore the effect of BDNF variants on the development of depressive symptoms, we stratified the schizophrenia sample according to the presence or absence of comorbid mood or depressive disorder. The p-value for schizophrenia with comorbid depression became much smaller (p=0.0011) in comparison with the complete sample.
Conclusions: In conclusion, we find supportive evidence for a relationship between genetic variants at the *BDNF* locus and depressive symptoms in affective disorder and schizophrenia.

W12 03

A common haplotype in the 5 region of the SCN5A gene is associated with ventricular conduction impairment
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Background: The SCN5A gene encodes the α-subunit of the cardiac voltage dependent sodium channel. Coding region mutations cause Brugada Syndrome and other familial conduction disturbances. Recent studies have suggested SCN5A promoter mutations may also contribute to arrythmias.
Aim: We investigated the influence of common SCN5A promoter and gene variants on ECG parameters in a central European general population sample. Methods: We genotyped 702 individuals from the population based KORA S2000 survey for 55 SNP markers. Haplotypes were inferred by the Haploview software package.
Results: We identified a block of high linkage disequilibrium extending from 10 kb upstream of noncoding exon 1 to 10 kb into intron 1. Within the block the third most frequent haplotype (hap3, AF= 16.8%) was significantly associated with the width of the QRS complex (p=0.0075; QRS 93.4 ms in wt/wt (n=474), 96.3 ms in wt/hap3 (n=201) and 100.8 ms in hap3/hap3 (n=18)). The association was confirmed in the entire sample of the KORA S2000 survey (p=0.0021). Is was stronger in older individuals and in those with preexisting cardiac and cardiovascular disease. These data support the concept that variability in channel expression by polymorphisms in the regulatory region of the gene influences cardiac conduction even in unselected individuals from the general population.

W12 04

Sarcoidosis is associated with a truncating splice site mutation in the BTNL2 gene
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Objectives: Sarcoidosis is a polygenic immune disorder with predominant manifestation in the lung and the lymph system. A previous genome wide linkage analysis identified the extended MHC region on chromosome 6p as a susceptibility region.
Material and Methods: A systematic three-stage single nucleotide polymorphism (SNP) scan of 16.4Mb on chromosome 6p21 was performed in up to 947 independent cases of familial and sporadic sarcoidosis.
Results: This strategy identified a 15kb disease-associated segment of the butyrophilin-like 2 (BTNL2) gene. The major disease variant (provisional designation "BTNL2_SAR", p TDT = 3*10-6, p case/control = 1.1*10-8, replication p TDT = 0.0018; p case/control = 1.8*10-6) represents an independent risk factor from DRB1.
Conclusions: BTNL2 is a member of the immunoglobulin superfamily and has been implicated as a co-stimulatory molecule involved in T-cell activation on the basis of its homology to B7-1. The G to A transition at "BTNL2_SAR" leads to an alteration of the splice site anatomy and unmasks a splice site 4 base pairs upstream from the wild type donor site. Thus, transcripts of the risk allele include a premature stop in the spliced mRNA. The resulting protein lacks the C-terminal IgC domain and transmembrane helix, thereby disrupting the membrane localization of the protein as shown by GFP and V5-tag fusion protein experiments.

W12 05

Genetic association of psoriatic arthritis but also of psoriasis vulgaris with haplotypes at PTPN22 and evidence for epistasis with the HLA-C associated risk factor at PSORS1
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We investigated whether variant R620W in PTPN22, which was previously described to be associated to rheumatoid arthritis, systemic lupus erythematodes and diabetes mellitus type I, is also associated with psoriatic arthritis. Three independent cohorts of Caucasians patients with either psoriatic arthritis, psoriasis vulgaris without joint manifestation or rheumatoid arthritis were analysed for this variant as well as for linkage disequilibrim-based haplotypes. All three patient cohorts showed association to same PTPN22 haplotype. In contrast, polymorphism R620W was only associated in the rheumatoid arthritis group (OR=1.606, p=0.003). The same frequent haplotype but without variant R620W conferred risk to psoriasis vulgaris (OR=1.378, p=0.011) as well as psoriatic arthritis (OR=1.331, p=0.024) suggesting the existence of a different, yet unknown variant within this haplotype and presumably within the PTPN22 gene. Regression analyses between the PTPN22 risk haplotype in both psoriasis cohorts with the major risk allele for psoriasis at HLA-C (PSORS1) revealed evidence for epistasis in both the psoriasis vulgaris (p=1.27 E-09) and the psoriatic arthritis cohorts (p=3.78 E-05). While the PTPN22 risk haplotype has no measurable effect on its own, it enhances the risk conferred by PSORS1 alone, indicating that PTPN22 is a subsidiary partner that may interact with the HLA-C associated risk factor in a common pathway.

W12 06

Molecular Genetic Dissection of Photosensitivity and its Relationship to Idiopathic Generalised Epilepsy
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Photosensitivity or photoparoxysmal response (PPR) is a common and highly heritable electroencephalographic trait characterised by an abnormal visual sensitivity of the brain in reaction to intermittent photic stimulation (IPS). The evoked cortical response ranges from occipital-spikes only to generalised spike-and-wave discharges (PPR type I–IV). Family and twin studies provide unequivocal evidence that PPR is genetically determined. The familial clustering of the different PPR types suggests that they share a common genetic predisposition. PPR occurs frequently associated with idiopathic generalised epilepsies (IGEs). Using a standard IPS procedure, PPR is found in 13-18% of idiopathic absence epilepsies, and in 30-35% of patients with juvenile myoclonic epilepsy. The present genomewide linkage scan was designed to map susceptibility loci for PPR and to explore its genetic relationship with IGE. The study sample included 60 families with at least two siblings displaying PPR. To dissect PPR-specific and IGE-related susceptibility loci, two distinct family subgroups were defined, comprising 19 families with predominantly pure PPR and photosensitive seizures (PPR-families), and 25 families, in which PPR was strongly associated with IGE (PPR/IGE-families). MOD-score analyses provided significant evidence for linkage to the region 6p21.2 in the PPR-families (pointwise empirical P = 0.00004), and suggestive evidence for linkage to the region 13q31.3 in the PPR/IGE families (P = 0.00015), both with a best-fitting recessive mode of inheritance. Our study reveals two PPR-related susceptibility loci, depending on the familial background of IGE. The locus on 6p21.2 seems to predispose to PPR itself, whereas the locus on 13q31.3 also confers susceptibility to IGE.

Poster

P01 Cancer cytogenetics

P001

Deciphering the pathogenetic importance of t(8;14)(q24;q32) and variants in lymphomagenesis by comprehensive morphologic, genetic and expression analyses within the Deutsche Krebshilfe Verbundprojekt „Molekulare Mechanismen bei Malignen Lymphomen“

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The Burkitt translocation t(8;14)(q24;q32) has been the first recurrent chromosomal change identified in a human lymphoma entity. Meanwhile, the t(8;14) and its variants are supposed to be the hallmark of Burkitt's lymphoma (BL). Nevertheless, chromosomal translocations juxtaposing the MYC gene next to an IG- or non-IG-locus are recurrent in lymphomas other than BL, too. The Deutsche Krebshilfe Verbundprojekt „Molekulare Mechanismen bei Malignen Lymphomen“ offers for the first time the possibility of a comprehensive interdisciplinary characterization of these MYC-positive high-grade lymphomas. Conventional morphologic, molecular genetic and cytogenetic approaches along with novel genome wide high-resolution techniques including matrix-CGH and gene expression profiling are being applied. Currently, gene expression profiling using the Affymetrix U133A GeneChip has been performed in 166 high grade B-cell lymphomas, which have been morphologically classified by a panel of seven expert reference pathologists. Of these, 155 lymphomas have already been analyzed by interphase-FISH using probes for the detection of chromosomal aberrations affecting the IGH, MYC, BCL2, BCL6, MALT1 and REL loci. Moreover, genome-wide matrix-CGH has been performed in 106 of these cases. The BCL6 and IGHV mutation status has been determined in 114 and 63 lymphomas, respectively. The presence of a breakpoint affecting the MYC locus separates the study population into two groups of nearly identical size. Supervised bioinformatic analyses identified gene expression patterns associated with these groups. The group of MYC-positive lymphomas was heterogeneous with regard to morphology, chromosomal aberrations and gene expression patterns. Ongoing analyses suggest the existence of at least two subgroups of MYC-positive high-grade B-cell lymphomas associated with particular gene expression profiles and recurrent genomic imbalances.

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P002

Detection of telomeric repeats using the single cell gel electrophoresis-FISH technique in DNA damaged by cytostatics

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Objectives: The involvement of telomeric repeats in DNA damage induced by cytostatics was determined.
Material and Methods: Directly labeled telomere-specific PNA probes were applied in Comet-FISH to detect DNA fragmentation. The effect of bleomycin (BLM) and mitomycin C (MMC) was measured in normal human peripheral leukocytes, and in three transformed cell lines (HT1080, CCRF-CEM and CHO).
Results: Involvement of telomere repeats in DNA fragmentation by BLM was highly specifically detected by the assay. With respect to total DNA damage CHO cells reacted most sensitively just at low doses, in human leukocytes the highest dose-related effect was found. A rather linear dose response characterized the HT1080 cells while the lowest effect was seen in CCRF-CEM cells. MMC at lower doses increased the percentage of migrating DNA dose-dependently, at higher doses its effect decreased in all cell lines.
Obvious differences characterized the examined cell types with respect to the "head/tail" distribution of telomeric signals after BLM exposure. A large number of signal spots of various sizes were found in CHO cells, very small signals could be detected in the "comets" of both neoplastic cell lines. Dose dependence of telomeres in the"tail" was most pronounced in CCRF-CEM and normal leukocytes,less in HT1080. The steepest dose-related increase of telomeric signals in the tail was found in CHO cells. The ratio between the telomeric signals in the tail and the migrated DNA varied distinctly between the examined cell types. In normal human lymphocytes the breakage frequency for telomere associated DNA was proportional to that of the total DNA, pointing to a random induction of the DNA breaks by BLM and MMC.
Conclusions: The finding that Comet-FISH can detect mutagenic effects on specific DNA sequences may be of high practical meaning if, for instance, amplified DNA sequences will be addressed by those examinations in future.

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P003

Detection of cryptic chromosomal aberrations in acute lymphoblastic leukaemia (ALL)

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Bone marrow samples of 16 (according to routine banding cytogenetics) karyotypically normal patients with ALL were studied applying mMCB (Weise et al., 2003, Cytogenet Genome Res 103:34-39) and subCTM-FISH (i.e. 24 chromosome-specific probe sets consisting of subcentromeric, subtelomeric and whole chromosome painting probes). Using the mMCB approach, it was possible to identify in six cases cryptic chromosomal aberrations like del(4)(q31), der(4)t(4;18), del(5)(q21)/del(5)(q11.2)/r(5q), ins (5;7)(q21;p14p22)/t(3;7)(p21;q11.23) and t(7;17)(p22;q22). Application of the subCTM probe set confirmed and characterized the mentioned chromosomal aberrations in more detail. Furthermore, additional chromosomal aberrations including a cryptic del(7)(q11.23), del(9)(p24), del(11)(q25), del(11)(p15.5), del(12)(p13.33) in 3 cases, del(12)(p12), del(13)(q34), t/del(17)(q25) and del(18)(p)/dup(18)(q11) were recognized. The most frequent abnormalities were subtelomeric deletions of the short arm of chromosome 11 (2 cases) and chromosome 12 (4 cases). As allelic losses of chromosome 12p is associated with childhood ALL and several solid neoplasias this suggests the presence of a tumour suppressor locus there. This pilot-study demonstrate an unexpected high rate of cryptic chromosomal aberrations of 75 % of ALL-cases.

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P004

Chromosome-torsions detected in cytogenetic preparations of bone-marrow – preparation induced artifacts or something leukemia-specific?

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Multitude multicolor banding (mMCB) studies were performed on, according to routine cytogenetics, karyotypically normal cases with AML. In one of the studied cases with an AML-M6 in 4/18 of the analyzed metaphase plates (mpp) an unusual condition of one chromosome #1, each, was noted. Instead of an alignment of the chromatides as expected, the chromatides of the p- and the q-arm of one #1 were located side by side. This condition was called a 'torsion', as it can be explained by a rotation of 180° within the centromere. To study this phenomenon in more detail two-color-FISH studies using probes for 1p and 1q were applied to in summary eight cases with AML-M6. For the initial case the torsion of #1 was detected in 10/70 mpp (14%), in the other seven cases torsions of #1 were detected in 2.4 to 6%. For the same cases torsions of #9 and #16 were detectable in 2 to 10% and 4 to 10%, respectively. In one case each of AML-M2, AML out of MDS and AML-M5 torsions of #1, #9 and #16 were also detected in comparable rates. In contrary, they were (almost) absent in bone marrow samples derived from one case each with leukopenia, unclear autoimmune disease and thrombocytemia and completely undetectable in samples of peripheral blood lymphocytes (6 cases), fibroblasts and amniocytes (3 cases, each) and in 1 chorion-sample. Such torsions have been observed previously during leukemia banding cytogenetic diagnostics (pers. com.: Dr. E. Gebhart, Erlangen), however, to the

best of our knowledge have never been analyzed in detail for their biological significance. It still has to be tested in further studies if this phenomenon of torsions is something associated with (acute myelogenous) leukemia only, or if torsions are tissue-specific preparation induced artifacts. Studies on bone marrow samples derived from patients with rheumatoid arthritis are planned to be done.
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P005

A novel t(14;19)(q32;q12) juxtaposes the CCNE1 candidate oncogene to the IGH locus in a leukemic B-cell lymphoma

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Translocations juxtaposing oncogenes next to the immunoglobulin heavy chain (IGH) locus in chromosomal region 14q32 are the hallmark of various B-cell malignancies. These lead to deregulation of the oncogenes by IGH enhancers resulting in cell proliferation and transformation. Here we have characterized a novel translocation, t(14;19)(q32;q12), in a case of leukemic B-cell lymphoma. The immunophenotype of the tumor revealed expression of CD19, CD20, CD38, IgKappa, and partially CD5, but lacked CD23, which is the typical profile for mantle cell lymphoma (MCL). The IGVH locus showed a 5% hypermutation rate and used the VH4-34 segment. In addition to the t(14;19), the tumor cells show a complex karyotype including a t(8;14)(q24;q32). By fluorescence in situ hybridization (FISH) the two IGH alleles were shown to be translocated, one to MYC in 8q24, and the second to 19q12. Using BAC-clones located in 19q12, the breakpoint region was narrowed down to 100kb. Among the genes located near the breakpoint we identified the CCNE1 locus, that encodes for Cyclin E1. Cyclin E1 enhances transition from G1 to S- phase of the cell cycle and subsequently promotes cell proliferation. Genomic amplifications of CCNE1 are recurrent in solid tumors, and overexpression of Cyclin E1 leads to chromosomal instability. These facts qualify CCNE1 as a good candidate oncogene to play a role in the present t(14;19). Besides, the lymphoma reported here shares common features with a MCL, but lacks the characteristic t(11;14)(q13;q32), leading to deregulation of Cyclin D1. Thus, it is tempting to speculate that overexpression of Cyclin E1 replaces that of Cyclin D1 in this MCL-like lymphoma. Western Blot analyses are underway to determine whether the Cyclin E1 protein is overexpressed in the tumor material.
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P006

Characterization of chromosomal aberrations in glioblastoma multiforme

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Gliomas are variable central nervous system neoplasms derived from glial cells, including astrocytomas, ependymomas, oligodendrogliomas and glioblastoma multiforme. Among these tumors Glioblastoma multiforme shows the highest grade of malignancy. It occurs with an incidence of 7:100.000. In spite of chemotherapy and radiotherapy the prognosis is poor. For enhancement of the treatment the classification of gliomas should be improved based on genetic findings. Earlier studies described an association of gliomas to specific chromosomal regions or single genes.
Therefore we carried out cytogenetic and molecular cytogenetic investigations in cultured lymphocytes as well as in cultured tumor cell from 15 glioblastoma patients. We performed routine cytogenetic analysis from both cultures. For identification of cryptic translocations we completed the analysis with Spectral Karyotyping (SKYÔ, ASI Ltd., Israel) in tumor cell lines.
The most common numerous chromosome abnormalities were the loss of one sex chromosome - X, - Y, and autosomal imbalances + 7, - 7, - 11 and - 8. Furthermore this study confirmed various structural aberrations like deletions and translocations in single cells. With this methods we could not identify clonal structural anomalies, especially translocations or deletions of specific chromosomal regions like 10p or 10q. Further studies are needed to determine quantitative aberrations in consequence of duplications or interstitial deletions to identify gene loci which play a potential role in tumor progression.

P007

Identification of genetic subgroups and progression markers of oligodendrogliomas by meta-analysis of comparative genomic hybridization (CGH) results

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In order to characterize the molecular basis of oligodendroglioma (ODG) formation and to identify the genetic events at the transition from low to high grade ODG many non-anaplastic and anaplastic ODGs have been investigated by CGH. The loss of 1p and 19q has been identified as the most characteristic pattern of genomic imbalances in these tissues. However, the existence of genetically distinct subgroups and progression markers of ODGs is still a matter of debate.
Analysing the detailed CGH results of the 89 ODGs published between 1992 and 2004, in a meta-analysis we could confirm the existence of three disjunct subgroups of ODGs, characterized by the existence of the aberration pattern -1p/-19q (n=58, 65%, group A), +7/-10 (n=6, 7%, group B) or the absence of either of the two pattern (n=25, 28%, group C). The distinct aberration pattern of group C (-1p31, -4q, -11p15, -18q, -22q, +17p, +17q) could indicate an alternative pathway of ODG formation. Another previously proposed subgroup, -9p (n=19, 21%), showed a considerable overlap with both the group A (overlap n=15) and group B (overlap n=3). Many genomic imbalances have been claimed to be associated with a progression of the histopathological grade of malignancy. However, analysing our database, we found that only the individual events -10, -15q and +7 were significantly correlated with a higher grade of malignancy in the total group of ODG. Within the group A only the additional loss of 15q was significantly associated with tumor progression. No statistically significant progression markers were found in group B or C.
Our data clearly demonstate the urgend demand for the establishment and statistical analysis of CGH databases for the reliable prediction of molecular pathways of ODG development and identification of progression markers.

P008

Identification of chromosomal imbalances in a large series of human oligodendrogliomas and oligoastrocytomas: Loss on 9p and 14q are associated with tumor progression

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Genomic losses on the short arm of chromosome 1 and the long arm of chromosome 19 are well established as frequent alterations in oligodendrogliomas. To identify other chromosomal regions of importance particularly for tumor progression, we studied 70 oligodendrogliomas and oligoastrocytomas using chromosomal comparative genomic hybridization (CGH). Of these tumors, 21 were classified as WHO grade II (16 oligodendrogliomas and 5 oligoastrocytomas) and 49 as WHO grade III (25 anaplastic oligodendrogliomas and 24 anaplastic oligoastrocytomas).
Most frequent genomic gains were identified on 7q (24 %), 19p (19 %) and 7p (17 %). Genomic losses were most frequently found on 19q (64 %), 1p (59 %), combined loss 1p/19q (59 %), 9p (26 %), 4q (21 %), 10q (19 %), 18q (17 %), 4p, 13q, 14q (16 % each). Amplifications were found in 8 tumors and mapped to 1q21, 1q31-q32, 3p24-p26, 4q12-q13, 5q14, 6p12-p21.1, 7p12, 8q23-q24, 10p12-p13, 12p12-p13, 12p, 21q11.2-q21.

Statistical analyses showed that gain on 7p is associated with WHO grade II tumors (p= 0.03) whereas loss on 14q is significantly more common in WHO grade III tumors (p= 0.03). When comparing only oligodendroglial tumors of different grades, loss on 9p is associated with WHO grade III tumors (p=0.03). Loss on 12q is preferentially found in mixed oligoastrocytomas versus oligodendrogliomas (p=0.02).
The ongoing correlation of longterm patient survival with chromosomal imbalances aims at the identification of genomic alterations with predictive value for disease outcome.

P009

Molecular cytogenetic investigation of t(11;14)(q13;q32)-positive B-cell NHL cell lines increases the suspicion of tumor-associated genes on chromosome 1

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Introduction: Translocation t(11;14)(q13;q32) has been considered to be the initial step in tumor development in a subset of B-cell non-Hodgkin lymphomas (NHL). The juxtaposition of CCND1/BCL1/PRRAD-1 on 11q13 to the IgH enhancer on 14q32 leads to the overexpression of cyclin D1 and loss of cell cycle control. Whereas the IgH/CCND1 rearrangement has been detectable in nearly all mantle cell lymphoma (MCL) cases, translocation t(11;14)(q13;q32) occurs in only 15-20% of multiple myelomas (MM). Experimental data have shown that secondary genetic changes are required for the malignant transformation in MCL. As secondary events, alterations of chromosome 1 have been observed with a high incidence but relevant genes remain unknown. The aim of this study was to better characterize aberrations of chromosome 1 in t(11;14)(q13;q32)-positive B-cell NHL cell lines.
Methods: Eleven t(11;14)(q13;q32)-positive B-cell NHL cell lines, including 6 MCL and 4 MM cell lines were analyzed using spectral karyotyping (SKY), R-banding, fluorescence in situ hybridization (FISH), and multicolor banding (MCB) analysis of chromosome 1.
Results: Our data show that chromosome regions 1p31 and 1p21 are the most frequently altered and deletion-prone loci in MCL cell lines. In contrast, in MM cell lines breakpoints most often involved the heterochromatic regions 1p11-12, 1q12, and 1q21.
Conclusions: These data show that different mechanisms inducing the chromosomal instability act in t(11;14)(q13;q32)-positive MCL and MM cell lines. These data are in accordance to alterations found by Au et al. (2002) and Nilsson et al. (2003) and we are confident that the detailed cytogenetic analysis of established cell lines is a useful tool for the identification and characterization of genes like PDZK1 that might be involved in the pathogenesis of B-cell NHL.
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P010

Genomic profiling of pleomorphic xanthoastrocytomas by chromosomal-CGH and matrix-CGH

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Pleomorphic xanthoastrocytomas (PXAs) are rare astrocytic neoplasms corresponding histologically to WHO grade II. They usually show circumscribed growth and favourable prognosis despite exhibiting a high degree of cellular pleomorphism. PXAs mainly affect children and young adults.
Here we present genomic profiling experiments of 50 PXAs. Chromosomal-CGH revealed a distinct pattern of chromosomal imbalances. The hallmark alteration detected in 50% of PXAs was loss on chromosome 9. Less common recurrent losses were on chromosome 17 (10%); 8, 18 and 22 (4% each) as well as a loss of the Y chromosome in 7.7% of tumors from male patients. Recurrent gains were identified on chromosome X (16%); 7, 9q and 20 (8% each) as well as 4, 5 and 19 (4% each). Amplifications were found in 2 tumors and mapped to 2p23-p25, 4p15, 12q13, 12q21, 21q21 and 21q22. To achieve a higher resolution, 7 PXAs were analyzed with a whole genome microarray of 6.000 large insert clones resulting in a resolution of at least 1Mb. In each of these cases, the results obtained by chromosomal-CGH could also be detected by the matrix-CGH experiments. In 3 of the 7 PXAs, additional aberrations were found by matrix-CGH. Imbalances detected by matrix-CGH were verified by interphase-FISH on tumor tissue sections. In particular, breakpoints were confirmed in one case with partial deletions on 9p and 18p. In conclusion, our study provides a comprehensive overview of DNA copy number changes in PXAs at a high genomic resolution and indicates that chromosome 9 carries one or more not yet identified tumor suppressor gene(s) with relevance to the molecular pathogenesis of these tumors.

P011

Recurring Pattern of Genomic Imbalances in Retinoblastoma

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Retinoblastoma (Rb) is the most common primary intra-ocular tumor in childhood. It has been shown that genetic abnormalities in addition to biallelic RB1 are necessary for tumor progression in Rb. We studied genomic imbalances of tumors of 103 patients with non hereditary (nhRb) and of 65 patients with hereditary (hRb) Rb using comparative genomic hybridization (CGH). Ages at operation of nhRb patients were 2-97.7 months, and of hRb cases 1.2-67.7 months, and included 96 male and 72 female patients. To investigate genetic pathways of tumorprogression, the genomic imbalances of 106 abnormal cases were analysed with respect to the time and the order of the appearance, as well as to the nonrandom joint occurrence of recurring changes using the cytogenetic data analysis system CyDAS (http://www.cydas.org). The copy number changes were summarized on chromosome arm level. Gains at 6p (68.9%) and 1q (44.3%) were most frequent. Both aberrations were observed in 58% and 6.5% of 31 case with a single genomic imbalance, respectively. Loss at 16q was observed in 31.1% of the abnormal cases and was never seen as a single aberration. This indicates that 6p gains represent primary aberrations whereas +1q and -16q occur later in tumor evolution. Among different combinations of copy number changes (CNC), +6p, +1q and -16q were most frequently observed (18.9%) and may, therefore, represent a common pattern of genomic changes in Rb. The nonrandom joint occurrence of +1q and -16q (24 cases, 22.6%), and of +2p and +17q (5 cases; 4.7%) was demonstrated ($p<0,05$). Whereas a der(16)t(1;16)(q11:q11) resulting in +1q and –16q has been described in Rb, the cause of a joint gain of 2p and 17q remains unclear. Overall, we observed a recurring pattern of CNCs which may point to the localization of genes which may have a synergistic effect on the tumor progression in Rb. Funded by the DFG, Ri-1123/1-1

P012

Assessment of the reliability of conventional chromosome analysis based of R- and G-banding and M-FISH for the detection of chromosomal changes in high grade B-cell lymphomas

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The importance of the cytogenetic analysis for the diagnosis and prognosis of malignant lymphomas is irrefutable, as highlighted in the novel WHO classification for hematological neoplasias. R- or G-banding chromosome analyses are conventionally used, but other more advanced techniques like multiplex fluorescence in situ hybridization (M-FISH) are gaining importance. Here, we aimed to determine the power of M-FISH as compared to conventional cytogenetics to detect chromosomal changes in high grade B-cell lymphomas. To that purpose, 17 diffuse large B-cell lymphomas (DLBCL) were systematically studied by M-FISH, R- and G-banding (group A), 10 DLBCLs by M-FISH and G-banding (group B), and 2 DLBCLs and 2 Burkitt lymphomas (BL) by M-FISH and R-banding (group C). All cases were selected due to the presence of an aberrant clone. Both R- and G-banding chromosome analysis proved to be limited to fully resolve complex changes, which were described as „add" (additional material of unknown origin) or marker chromosomes. By M-FISH, new findings (mostly identification of unresolved changes) were detected in 11 (64%), 8 (80%), and 1 (25%) case in groups A, B, and C, respectively. In group A, M-FISH and R-banding showed identical chromosomal changes in 5 (30%) cases, which were not detected by G-banding. R- and G-banding showed discrepant results in 16 (94%) cases. M-FISH was in 22 (71%) cases unable to precisely define breakpoints or small deletions, and the help of conventional cytogenetics was required. Although a more detailed technical comparison is underway, our preliminary results show that conventional chromosome analysis and M-FISH are complementary for the cytogenetic characterization of high grade B-cell lymphomas. The application of both approaches leads to a more complete cytogenetic description, but the high cost of M-FISH has to be considered.

Supported by the Deutsche Krebshilfe (Verbundprojekt "Molekulare Mechanismen bei malignen Lymphomen", 70-3173-Tr3)

P013

INTERPHASE CYTOGENETICS IN 174 PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Objectives: Chromosome aberrations in CLL have proven to be important predictors of disease progression and survival time (Döhner et al., 2000). In contrast to classical cytogenetics, fluorescence in situ hybridization (FISH) on interphase nuclei allows the detection of genomic anomalies in up to 80% of patients with B-cell chronic lymphocytic leukemia (CLL).

Results: In our study peripheral blood mononuclear cells of 174 patients with CLL were tested at time of diagnosis, about 25 cases were additionaly monitored for up to 3 years. FISH-analyses were done for deletions in chromosome bands 11q22~23 (ATM locus), 13q14 (D13S272 probe) and 17p13 (p53 gene), for trisomy of chromosome 12 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 27% of the aberrant cases. Consistent with data from other studies, the most frequent anomaly was a deletion in 13q14 followed by trisomy of 12q13 and the loss of one 11q22~23 signal.

Furthermore it could be demonstrated that mRNA expression of ZAP-70 and AID (activation-induced cytidine deaminase) is significantly associated with cytogenetic aberrations indicating good or poor prognosis, respectively.

P014

Radiosensitivity detected by the Micronucleus Test is not generally increased in Sporadic Prostate Cancer Patients

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The micronucleus test (MNT) has shown increased micronuclei (MN) frequencies in BRCA associated and sporadic breast cancer patients, Ataxia teleangiectasia and Nijmegen Breakage Syndrome patients, demonstrating a common cellular phenotype of increased radiosensitivity. Some genes causative of these diseases have recently been associated also with prostate cancer. In order to investigate if prostate cancer exhibits the cellular phenotype of increased radiosensitivity, we performed MNT analysis on 22 sporadic prostate cancer patients and 43 male controls. We determined the baseline MN frequency, in order to see in-vivo chromosomal damage without radiation, and induced (after irradiation with 2 Gy) frequency of MN, both in binucleated cells (BNC) obtained from cultured peripheral blood lymphocytes. An automated image analysis system was used to score the MN employing two different classifiers (Classifier A and B) for detection of BNC. The mean baseline frequencies were 48/43 (A/B) MN/1000 BNC for the controls and 42/50 (A/B) for prostate cancer patients. The induced MN frequencies amounted to 107/111 (A/B) MN/1000 BNC for controls and 111/114 (A/B) MN/1000 BNC for prostate cancer patients. The obtained MN frequencies did not result in a statistically significant difference between unselected cases and controls. However, restricting the analysis to young patients (50-60 years, N=7) and age matched controls (N=7) revealed marginally significant higher MN frequencies in patients. We conclude that increased radiosensitivity is not a property of prostate cancer patients in general.

P015

Recurrent tumor arising in a pleomorphic adenoma characterized by karyotypic rearrangements besides the t(3;8)(p13;q12)

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Pleomorphic adenomas are the most frequent tumors originating from the major and minor salivary glands. Extensive cytogenetic studies of these tumors have shown that besides cases with an apparently normal karyotype two different groups of cytogenetic abnormalities exist. The first subgroup is characterized by structural aberrations involving chromosomal band 12q13-15 and the second subgroup by alterations of the chromosomal region 8q12. In pleomorphic adenomas with 12q13-15 aberrations, fusion genes involving the HMGA2 have been described, whereas tumors with 8q12 translocations are affected by PLAG1. Here, we describe the results of cytogenetic investigations of a recurrent tumor (12th recurrence) with the karyotype 47,XX,der(3)t(3;8)(p13;q12),-4,+5,der(5)t(3;5)(p13;p14),der(6)t(4;6)(q12;q13),+7,inv(7)(p12q31.2),del(8)(q12) arising in a pleomorphic adenoma. Though the tumor did not display histologically clear signs of malignancy, its high tendency to recur and its locally aggressive growth may be associated with the additional karyotypic abnormalities besides the complex translocation involving 8q12 and 3p13.

P02 Clinical Genetics

P016

Clinical and molecular analyses in patients with Cornelia de Lange syndrome

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Cornelia de Lange syndrome (CdLS) is a clinically heterogeneous disorder characterized by distinct facial dysmorphism, short stature, microcephaly, limb reduction defects, a variety of internal abnormalities, and mental retardation. The distinctive facial features include synophrys, long eyelashes, depressed nasal bridge, an upturned nasal tip with anteverted nares, and a thin upper lip.

In many patients, diagnosis is easily made on the basis of the characteristic facial gestalt cooccurring with limb reduction defects. However, mild cases are challenging. To explore the range of phenotypic expression of CdLS we evaluated clinical data from 51 patients. One of the most interesting findings of this analysis is the diagnostic value of the metacarpo-phalangeal pattern profile (MCPP): patients with CdLS have a distinct pattern characterized by very short first metacarpal, brachymesophalangy V and less severe shortening of terminal phalanges. Especially in patients with mild facial signs of CdLS, this distinct MCPP pattern can be of high diagnostic value.

Recently, patients with CdLS were found to be heterozygous for mutations in NIPBL. This gene codes for a protein that is a homolog of the Drosophila melanogaster Nipped-B that facilitates enhancer-promoter communication of remote enhancers and plays a role in developmental regulation. It is also homologous to a family of chromosomal adherins which have roles in sister chromatid cohesion, chromosome condensation, and DNA repair. In order to confirm

the diagnosis of CdLS on the molecular level we started mutation analysis in 14 patients with variable clinical expression of CdLS. We currently perform sequence analysis of all exons of the NIPBL gene and will establish quantitative assays for detection of gross alterations. Identification of mutations in patients with variable phenotypic expression will help to determine the relations between genotype and phenotype which would be of importance for genetic counseling.

P017

EFNB1 gene mutations in familial and sporadic Craniofrontonasal syndrome (CFNS)

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Craniofrontonasal syndrome (CFNS [OMIM 304110]) is an X-linked disorder characterized by a more severe manifestation in heterozygous females than in hemizygous males. Heterozyous females have frontonasal dysplasia and extracranial manifestations including skeletal abnormalities and body asymmetry. Manifestation in hemizygous males includes hypertelorism and occasionally cleft lip and palate. Recently, we identified a deletion and two missense mutations in the EFNB1 gene (OMIM 300035) in three families with CFNS (Wieland et al. 2004, AJHG 74, 1209-1215). We now report on mutation analysis in 9 families and 29 sporadic patients with CFNS. DNA sequencing revealed mutations in 33 (87%) cases including 26 distinct novel mutations. A recurrent mutation, R66X, was detected in one family and 4 sporadic patients. The majority of mutations (26/33) were located in exons 2 and 3 of the EFNB1 gene encoding the extracellular ephrin domain. The mutation spectrum includes frameshift, nonsense, missense and splice site mutations with a predominance of frameshift and stop mutations resulting in premature termination codons. Of particular interest are frameshift mutations located in the last 25 codons of EFNB1 encoding the carboxyterminal end of ephrinB1. These mutations disrupt the intracellular binding sites for Grb4 and PDZ-effector proteins involved in reverse ephrinB1 signaling. Comparing the mutations and phenotypes no phenotype/genotype correlation could be deduced. We conclude that the major cause for familial and sporadic CFNS are loss of function mutations in the EFNB1 gene. We proposed cellular interference as a mechanism for the sex-dependent manifestation of CFNS. Accordingly, the severity and variable expression of the disease may be explained in the context of cellular interference by the stochastic event of X inactivation and the following degree of mosaicsm in the affected tissues.

P018

Valproic acid treatment of spinal muscular atrophy patients as a chance to increase SMN2 gene expression and improve motor abilities

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Spinal muscular atrophy (SMA) is an autosomal recessively inherited motoneuron disease representing one of the most common genetic disorders leading to death in childhood. The disease determining survival motor neuron gene (SMN) is present in a telomeric and a centromeric version on chromosome 5q13. While SMA is caused by homozygous deletion or mutation of the telomeric copy (SMN1), each patient retains at least one almost identical centromeric copy (SMN2). However, SMN2 fails to compensate for the loss of SMN1 due to a silent mutation leading to exon 7 skipping in the majority of SMN2 transcripts encoding a protein that is not functionable.

Using fibroblasts from SMA patients, our lab demonstrated that valproic acid (VPA), a well-known drug successfully used in epilepsy treatment, increases full-length (FL) SMN2 mRNA/protein levels via stimulating transcription of SMN2 and promoting exon 7 inclusion. In a first clinical trial enrolling SMA carriers, we showed that under therapeutic serum levels of VPA 6/10 carriers revealed increased FL SMN mRNA levels.

Meanwhile, we collected blood from SMA type I-III patients treated with VPA in individual attempts of healing. From each of the patients, 2 blood samples have been taken before VPA treatment to detect baseline levels of FL and truncated SMN2 transcripts. After reaching serum levels of 70-100 mg VPA/l (common in epilepsy therapy), another 3 blood samples have been collected within a time period of several weeks. Levels of FL and truncated SMN2 mRNA are analyzed by real time quantitative PCR and compared to baseline values before VPA treatment. Data will be presented to evaluate the suitability of that approach for monitoring the potential success of a VPA therapy in SMA patients. Moreover, a comparison of FL and truncated SMN mRNA baseline levels in freshly isolated blood cells from patients, carriers and controls will be shown to further characterize the value of these parameters for clinical study monitoring.

P019

Molecular support for MEHMO (mental retardation, epileptic seizures, hypogonadism and –genitalism, microcephaly, obesity) as a distinct nosologic entity

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MEHMO syndrome (OMIM #300148) is a rare X-linked recessive disorder characterized by mental retardation, epileptic seizures, hypogonadism and –genitalism, microcephaly, and obesity. Patients usually die within the first two years of life. The disease gene has been assigned to Xp21.1-p22.13 by linkage analysis [Steinmüller et al., 1998].

During the last few years mutations were found in the gene ARX, located within the MEHMO critical interval, in a wide spectrum of X-linked mental retardation syndromes, including ISSX (infantile spasm syndrome, X-linked), Partington syndrome (dystonia, ataxia, mental retardation, seizures), West syndrome (infantile spasms, epileptic seizures, arrest of psychomotor development), and in non-syndromic intellectual disability with myoclonic epilepsy and generalized spasticity, with or without hyptonia and macro- or microcephaly. Furthermore, ARX mutations were described in X-linked lissencephaly with abnormal genitalia (XLAG).

Given the location of ARX in Xp22.13, its function, the wide spectrum of phenotypic variation in mutation carriers, and the pronounced overlap of signs and symptoms with those observed in MEHMO, ARX mutations might also underlie MEHMO syndrome. Sequencing of all exons, intron-exon boundaries and of the promoter region of ARX in a MEHMO patient did not reveal a mutation in any portion of the gene. These findings provide strong molecular evidence for MEHMO being a nosologic entity independent of the wide spectrum of mental retardation syndromes caused by ARX within the MEHMO critical interval.

Literature: Steinmüller R, Steinberger D, Müller U. Eur J Hum Genet 1998; 6:201-206

P020

A FISH study of supernumerary marker chromosomes (SMCs) identifies a novel type of bisatellited SMC(22) predicting chromosome 22q duplication syndrome, not CES, and six intervals on chromosome 22q that are relevant for diagnostics

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Supernumerary marker chromosomes (SMCs) are frequently found at pre- and postnatal cytogenetic diagnosis and require identification by FISH. An overproportionally large subset of SMCs is derived from the human chromosome 22 and confers tri- or tetrasomy for the cat eye chromosomal region (CECR, the first 2 Mb of 22q) and/or other segments of 22q. Using FISH and 15 different DNA probes, we studied 9 unrelated patients with a SMC(22) that contained the CECR. Five patients showed the small (type I) cat eye syndrome (CES) chromosome and each one had the larger (type II) CES chromosome, a novel type of bisatellited SMC(22), small ring extrachromosome 22, or der(22)t(11;22) extrachromosome. By size and morphology, the novel bisatellited SMC(22) resembled the typical (type I and II) CES chromosomes, but it predicted the chromosome 22q duplication syndrome, not CES. This SMC included a marker from band 22q12.3 and conferred only one extra copy each of the 22 centromere, CECR, and common 22q11 deletion area. There has been no previous report of a bisatellited SMC(22) predicting the chromosome 22q duplication syndrome. Accounting for the cytogenetic resemblance to CES chromosomes but different makeup and prognosis we propose naming this an atypical (type III) CES chromosome. In this study, six distinct intervals on 22q were found to be relevant for FISH diagnostics. We propose to identify

SMCs(22) using DNA probes corresponding to these intervals.

P021

Narrowing the candidate region of Albright Hereditary Osteodystrophy-like syndrome by quantitative real-time PCR in seven patients with a deletion of chromosome 2q

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There have been a number of reports concerning patients who present with an Albright Hereditary Osteodystrophy (AHO)-like syndrome concomitant with a deletion of chromosome 2q, an entity that is also called brachydactyly-mental retardation-syndrome. The AHO-like syndrome has certain features, like short stature, mental retardation, short hands and feet due to an abnormal shortening of one or more metacarpals or –tarsals, especially the IVth and Vth. We analysed 7 patients with a deletion of various sizes of chromosome 2q with a real time-PCR based aproach. The smallest deletion found in a patient with AHO-like syndrom has a size of 2.5 Mb, together with a duplication of chromosome 10qter, another patient suffering from mild mental retardation and autism but not affected by AHO-like syndrome has a deletion of 1.5 Mb and additionally a duplication of chromosome 18pter. Our data indicate, that the „AHO-like syndrome critical region" thus has a size of about 1.0 Mb and contains several genes, for e.g. glypican-1, that was already suggested as a candidate gene in other studies. To elucidate the role of glypican-1 in the AHO-like syndrome, a knockout approach in mice is planned.

P022

Genomic variants of TNFα and TNFβ and their impact on TNFα gene and protein expression in vivo

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TNFα and β are important cytokines in the complex signalling pathway involved in the development of CAD. Methods: Influence of 2 polymorphisms (PM) of TNFα(c.-238G>A) and TNFβ (T60N) on the in vivo TNFα-expression in the context of CAD(176 age matched patients with angiographically confirmed diagnosis: 88 patients with severe CAD and 88 patients without any coronary symptoms as controls). Results: The c.-238G>A-PM is influencing TNFα mRNA-expression significantly in the whole patient group (GG: 6.2+5.9ag/cell; AG: 8.8+6.2ag/cell; p=0.019). This influence is mainly due to variation in the CAD-patients (p=0.013). A significant effect of the T60N PM on TNFα gene expression in the whole patient group (AA: 4.3+4; AC: 7.7+6.7; CC: 5.7+5.4ag/cell; p=0.007). However, this regulation was more pronounced in the control group (p=0.011) than in the CAD-group (p=0.079). A haplotype-analysis revealed that the c.-238G>A PM is a stronger predictor for an elevated TNFα mRNA-expression. The heterozygous variant AC-AG (T60N-c.-238G>A) causes the highest expression (9+7.6ag/cell) followed by the variant CC-AG (8.4+2.8ag/cell). All other combinations showed significant lower expression rates (AC-GG: 7.5+6.6; CC-GG: 5.5+5.5; AA-GG: 4.3+4ag/cell; p=0.002). On translational level only the T60N-PM, could be referred as a predictor of TNFα protein-expression in the group of CAD-patients but not in the whole patient group: A-allele carriers showed a significantly increased TNFαprotein-expression (p=0.045). Conclusions: genomic variants in both TNFα and TNFβ gene are proven to affect in vivo TNF-α gene-expression. Especially in CAD, an effect of the TNFα-PM c.-238G>A and the TNFβ-PM T60N on gene and protein expression, respectively, could be proven.

P023

Novel and Recurrent Mutations in Patients with Androgen Insensitivity Syndromes

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Objectives: Androgen insensitivity syndrome caused by mutations within the androgen receptor gene represents a variety of phenotypes from females with 46,XY-karyotype over individuals with ambiguous genitalia to infertile males. The majority of mutations consists of individual mutations, while only few mutational hot spots are known. In comparison to missense mutations only few nonsense mutations, deletions, duplications, or insertions have been reported.
Material and Methods: We studied 18 patients with androgen insensitivity syndromes by sequencing. 14 of the investigated patients were affected by complete androgen insensitivity syndrome (CAIS) and 4 suffered from partial androgen insensitivity syndrome (PAIS).
Results: We detected 6 so far unreported mutations as well as 9 recurrent mutations (three recurrent mutations were detected twice) in exons 2-8 of the androgen receptor gene. Three of the novel mutations cause a frameshift with subsequent premature termination and were found in patients with CAIS. These frameshifts were induced by single nucleotide deletion or insertion or in one case by a 4 bp-duplication, respectively. Another premature stop codon found in a CAIS-patient results from an already reported nucleotide substitution in exon 5. All other mutations caused single base substitutions spread through exons 2-8 and were associated with CAIS or PAIS.
Conclusions: We report a broad spectrum of different mutations within the AR gene leading to various manifestations of AIS. Apart from truncating mutations, a reliable genotype/phenotype correlation can not be established. Therefore, modifying factors must be effective.

P024

THREE UNRELATED PATIENTS WITH NICOLAIDES-BARAITSER- SYNDROME

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We report on three unrelated boys with concordant medical histories and consistent phenotypic and clinical findings - similar facial dysmorphic signs, sparse scalp hair, short stature, severe mental retardation and an idiopathic epilepsy resistant to pharmacotherapy.
All three boys were born to healthy non-consanguineous parents. Measurements at birth were in the lower range, especially for length and weight. Motor development was retarded (walking at 2 to 3 years). All three had no speech development. At the age of about 2 years, they developed grand mal seizures which are difficult to treat. Clinical examination at the age of 16 (patient 1), 13 (patient 2), and 18 years (patient 3) revealed short statures (about – 4 SD), dystrophy, and small head circumferences (about – 2 SD). There was severe mental retardation and no speech. Concordant facial dysmorphic signs in all three were a coarse triangular face with flat philtrum, prominent lower lip, large mouth and low set ears. Beside this, sparse scalp hair with normal texture was another characteristic finding. Hands were short, with enlarged, non inflammatory interphalangeal joints. X-rays of the hands showed a retarded bone age and brachydactyly. Patient 3 additionally developed a severe thoracolumbar scoliosis.
In all three boys, chromosome analysis showed normal male karyotypes. Subtelomeric screening, metabolic screening, thyroid hormone levels, brain CT/MRI scans as well as ophthalmologic and audiologic investigation were normal where performed.
Based on the characteristic dysmorphic facial features, sparse hair, epilepsy, mental retardation and short stature we diagnosed Nicolaides-Baraitser syndrome. After the initial delineation of this syndrome by Nicolaides and Baraitser in 1993, only four more patients were published in the literature up to now. We review the literature of Nicolaides-Baraitser syndrome and propose the important clinical features necessary for diagnosis.

P025

Screening of NSD1 mutations by DGGE and MLPA in Sotos patients.

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Sotos-syndrome is an autosomal dominant overgrowth syndrom with distinctive craniofacial features, advanced bone age and various degree of mental retardation.
It was shown that haploinsufficiency of the NSD1 gene is the major cause of Sotos syndrome. Interestingly, in the caucasian population

point mutations of the NSD1 gene are found in most patients, whereas submicroscopic deletions have been found in the majority of Japanese Sotos patients.
The NSD1 gene consist of 23 exons. Point mutations are scattered through almost all exons. Therefore we established a DGGE (denaturing gradient gel electrophoresis) screening protocol for the detection of mutations in all exons of the NSD1 gene. Additionally, we have utilized MLPA (multiplex ligation-dependent probe amplification) to identify submicroscopic deletions of NSD1 exons.
Till now there have been 12 patients with suspected Sotos-syndrome genotyped in our laboratory. We were able to detect 10 different sequence alterations in our ongoing study demonstrating that DGGE is a reliable technique.
In a 12 year old boy with classical Sotos symptoms a novel nonsense mutation (Q925X) was detected.
No deletions were found in our patients. Our first data confirm the observation that haploinsufficiency in caucasian patients is mostly the result of nonsense mutations rather then the result of deletions. Furthermore, we could demonstrate that DGGE-analysis is a reliable and easy to perform technique for the detection of mutations in the NSD1-Gen.

P026

Bloom Syndrome - The Extended Phenotype
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Bloom syndrome (MIM 210900) is a unique human hereditary disorder with a distinctive clinical and cellular phenotype, first described 50 years ago. Its main manifestations are (i) pre- and postnatal growth retardation, (ii) characteristic facial features consisting of dolichocephaly and a narrow face, (iii) light-sensitive facial telangiectasia in most patients, (iv) extensive signs of genomic instability as revealed by a 10-fold increase of spontaneous sister chromatid exchanges, chromosomal breaks and exchanges between homologous chromosomes, evident at metaphase, and an increased rate of somatic mutations. The underlying molecular defect is a wide range of mutations of different types in the BLM gene encoding a DNA helicase with homology to RecQ in E. coli at human chromosome 15q26.1. Individuals with Bloom syndrome are at increased risk to develop different forms of cancer at a greatly advanced age (about 1 in 4). We have followed the natural history of this disorder for 35 years in 14 patients in Germany. We will document that, as adults, individuals with Bloom syndrome change their characteristic phenotype. Their facial features become less destinct than in young patients. In addition, most develop learning difficulties and exhibit a reduced attention span. Furthermore, in an ongoing investigation with J. German and M. Sanz, New York, we find an increased risk for diabetes mellitus of both type 1 and type 2 (in 27 of 117 patients, 23%). The main clinical, cellular, and molecular aspects in the light of new findings will be reviewed. In addition, the inconspicuous phenotype of a patient will be described who was diagnosed prenatally and subsequently followed after birth.
Bloom syndrome has been recognized in different parts of the world. One particular mutation, a 6 base pair deletion/7 bp insertion at nucleotide position 2281 occurs in Ashkenazi Jewish populations as a result of founder effect.

P027

Xq28 submicroscopic duplication causes increased expression of the *MECP2* gene in a boy with severe mental retardation and features of Rett syndrome
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Rett syndrome has been recognized as one of the major causes of mental retardation in girls. Over the last years, numerous point mutations and deletions affecting the *MECP2* gene have been identified in girls with this neurodevelopmental disorder. In contrast, only few boys with missense mutations of *MECP2* have been described, most of these with a severe neonatal encephalopathy. We describe an 8 year old boy with severe mental retardation, autistic features, and other signs of Rett syndrome. Retardation of psychomotor development became apparent within the first year of life. Stereotypic hand movements were noted around the age of 4 years. At the age of 6 years, the patient had learned to walk a few steps assistedly and could hold toys, but the parents then observed a gradual loss of purposeful hand use. At the same time, the boy developed complex seizures.
Molecular genetic diagnostics revealed no mutations within exons 1 to 4 of *MECP2*, but a complete duplication of *MECP2* due to a submicroscopic duplication of approximately 430 kb in Xq28. The size of the duplication was analyzed by quantitative PCR. No other genes than *MECP2* known to be involved in mental retardation or essential for neural development were found completely duplicated or disrupted. The proximal border of the duplicated segment was located to a region at least 8.4 kb 5' from the first exon of *L1CAM*. The distal border lies within the *FLNA* gene, but leaving the functional gene copy intact. Quantitative RT-PCR analyses show that the duplication leads to elevated *MECP2* transcript levels in lymphoblastoid cells. The mother of the patient carries the same duplication but is asymptomatic, which may be attributed to extremely skewed X-inactivation. We are currently analyzing another male patient with *MECP2* duplication in order to compare the breakpoints.

P028

Ring chromosome 4 with complex karyotypes in two patients
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Ring chromosomes are usually the result of two breakages at the p- and q- arm of a chromosome and a subsequent fusion of both ends. They often show a mitotic instability which results in a mosaicism of different karyotypes. Ring chromosome 4 has been described in several patients showing variable clinical features depending on the amount of lost material of chromosome 4. There have been some reports on patients with ring chromosome 4, normal intelligence, some dysmorphic facial features but severe growth retardation. These patients have no visible loss of genetic material. Some other reports on ring chromosome 4 with remarkable loss of chromosome 4p and 4q material exist. These patients show profound psychomotoric delay, malformations, and dysmorphic features corresponding to the amount of the lost chromosomal fragment and usually including the critical WHS (Wolf- Hirschhorn syndrome) - region.
We focus on two patients with predominantly ring (4) karyotypes but also many metaphases with complex aberrant karyotypes showing fragmentations of the ring chromosome 4. The analysis was done with conventional Giemsa banding and multicolour banding according to Liehr et al. 2002. Using the subtelomeric localized probes of chromosome 4p and 4q it was shown that the derivative chromosome 4 lost the terminal regions of the 4p and 4q arms. Both patients are deeply retarded with microcephaly, muscular hypotonia, and craniofacial dysmorphic features. No internal malformations were found, only an anal stenosis was present in one patient.
We will discuss the patients phenotypes and their chromosomal findings in regard to previously published cases.
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P029

Genetic information in the workplace - Results of a survey of German occupational health physicians
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Objectives: The German government currently is discussing draft legislation to regulate genetic testing in the workplace. Mainly by prohibiting DNA - and chromosomal analysis, genetic discrimination in this field should be prevented. However, the actual usage of personal genetic information in or before employment has not been investigated for Germany so far. With our study we are aiming to elucidate the relevance of the different methods of genetic testing in the workplace.
Material and Methods: A postal questionnaire was sent to the 3300 members of the professional organization of German occupational health physicians (Verband Deutscher Betriebs- und Werksärzte e.V., VDBW). It contained twelve questions on the usage of methods, which provide personal genetic information, in the companies, they are in charge of. The questionnaire was designed to be returned anonymously.
Results: Completed questionnaires were obtained from 304 physicians, 78,9% (240) of whom were in charge of large companies (more than 500 employees). The majority of physicians stated, that neither for occupational health safety (94,7%) nor in pre-employment medical examinations (96,7%) genetic laboratory testing is performed in their companies. Family medical histories are part of 86% of pre-employment medical examinations though, which most of the companies surveyed (90%)routinely ask for.

Conclusions: Our results indicate that (1) in accordance with results from other European countries genetic laboratory testing still is of minor importance for pre-employment medical examinations as well as for occupational health safety. Family medical histories, which also can provide significant information (of sometimes predictive value) on the genetic status of a person, seem (2) to be performed routinely especially in the pre-employment situation. Legislative action on this issue should deal with the usage of personal genetic information in general - irrespective of its origin - to prevent genetic discrimination.

P030

A new patient with Shprintzen Omphalocele Syndrome?

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We report on a boy with short stature, facial dysmorphism, laryngeal abnormality, renal agenesis on the left and a large umbilical hernia. Dysmorphic features include brachycephaly, round face, frontal bossing, hypertelorism, epicanthal folds, broad nasal bridge, downturned mouth, thin lips, microgenia and malformed deeply set ears. The voice is abnormal. The boy has a weak cry and high pitched voice. Bronchoscopy showed a laryngeal abnormality. Motor and mental development are retarded.

The boy is the second child of nonconsanguineous parents. Family history is unremarkable. He was born in the 40th gestational week with a length of 44 cm (< 3rd centile), a birth weight of 2700g (< 3rd centile), and a head circumference of 34 cm (15th centile). Length at age 10 months was 67,5 cm (< 3rd centile), and head circumference 46 cm (50th centile).

Cytogenetic analysis was performed on peripheral blood lymphocytes. The analysis of G-banded chromosomes revealed a normal male karyotype 46,XY. Fluorescence in situ hybridization analysis excluded a microdeletion of the short arm of chromosome 5 (5p15.2) and a microdeletion of the short arm of chromosome 4 (4p16.3). Shprintzen and Goldberg [Birth Defects Orig Artic Ser. 1979; 15(5B): 347-53] described a malformation syndrome which includes dysmorphic facies, omphalocele, laryngeal and pharyngeal hypoplasia, scoliosis and learning disabilities. Our patient resembles the clinical features of this syndrome. This condition was only published once. Therefore we want to contribute this patient as a possibly second observation of this rare entity.

P031

High intrafamilial variability of BOR syndrome and a newly recognized EYA1 Mutation.

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Branchio-Oto-Renal (BOR) syndrome is an autosomal dominantly inherited disorder, characterized by the association of branchial fistulae and cysts, ear and kidney malformations and hearing impairment. Exceeding clinical variability is known, even within a family. Mutations in the EYA1 gene are detected in up to 40% of persons with BOR syndrome.

We present the variable expression of clinical features in a family with BOR syndrome: Our female index patient was born with bilateral branchial fistulae. Progressive hearing loss developed in her infancy. Ultrasound examination showed kidneys of normal size, shape and structure on both sides. Two of her pregnancies were complicated by oligohydramnion. The children were stillborn after 18 and 24 weeks of gestation. Postmortem examination revealed bilateral renal agenesis in both. 'Potter facies' but no further malformations could be detected.

We assumed BOR syndrome as the cause of the malformations in this family. Genomic sequencing of the EYA1 gene showed an up to now undescribed splice site mutation of exon 10: c.1041+2 T>G. The mutation was detected in DNA from the mother and subsequently in a stored DNA probe from one fetus. This mutation disrupts the splice-donor site of intron 10.

Our observations confirm previous studies of BOR syndrome families that could show a highly variable expressivity ranging from mild to lethal phenotypes. Phenotype cannot be predicted from mutation analyses results, not even in a single family. BOR syndrome should be reminded in differential diagnosis of hearing loss and of renal anomalies. In many cases DNA analysis of the EYA1 gene detects mutations, thus confirming BOR syndrome.

P032

High intrafamilial variability in a family with Opitz GBBB syndrome

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We report on two brothers with Opitz GBBB syndrome, their unaffected mother and their mildly affected maternal grandfather.

The propositus is the first child of a healthy, non consanguineous German couple. The pregnancy was complicated by polyhydramnios beginning at 32nd week of pregnancy. The boy was born at 42nd week of pregnancy with normal birth measurements (weight 3959 g, length 56 cm, OFC 34,5 cm). Hypertelorism, laryngo-tracheomalacia and penoscrotal hypospadias with bifid scrotum were noted and led to the diagnosis of Opitz GBBB syndrome.

His brother was born at 39th week of pregnancy after an uneventful pregnancy. Birth measurements were normal (weight 3140 g, length 51 cm, OFC 34 cm). In addition to hypertelorism and hypospadias with bifid scrotum he had a complex heart defect with ASD, several VSDs, coarctation of the aorta and persistence of the left vena cava superior as well as anal atresia. Surgical corrections of the heart defect and the anal atresia were successful. Both patients show normal mental and motor development.

The mother of the boys is healthy and has no hypertelorism or other midline defects. Her father however has hypertelorism and hypospadias, but no further problems are reported.

Chromosomal analysis in the propositus was normal, as were FISH 22q11.2 investigations.

Mutation analysis in MID1 gene, presuming an X-linked recessive mode of inheritance, showed no mutation, but RNA studies will be performed. This family demonstrates the high intrafamilial variability of the phenotype in Opitz GBBB syndrome and might help to identify both, X-chromosomal sequences that, in addition to the known parts of MID1 are involved in the development of the ventral midline and second of factors that modify the development of an Opitz GBBB phenotype.

P033

Dispermic chimerism in a female blood-donor

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Here, we report on chimerism in a phenotypically normal individual. The 19 year old healthy woman is mother of a now 3 year old child. Blood group typing revealed three alleles (AB0*A^2, AB0*0^1 and AB0*B). Following molecular HLA-genotyping using peripheral blood samples and skin fibroblasts confirmed these results. Also three HLA-alleles for HLA-A and HLA-DRB1 loci could be demonstrated. In all these cases the third allele was of paternal origin. For further evaluation microsatellite markers were examined. Therefore, DNA from the probands lymphocytes and skin fibroblasts together with DNA of the parents was amplified with primers for ten markers from nine different chromosomes. A third allele of paternal origin was present for two markers while a third maternal allele never was observed. Y chromosomal sequences could not be detected. No difference could be seen between DNA isolated from lymphocytes and skin fibroblasts. Blood group typing, HLA-typing and microsatellite analysis together, demonstrate that the third allele being present in the proband is exclusively of paternal origin. Therefore dispermic chimerism in the proband is suggested. The most likely explanation is fertilization of one oocyte and the corresponding polar body after meiosis II with two different sperms.

P034

Atypical tuberous sclerosis with NF1-like multiple hyperintense (T1) lesions in MRI caused by TSC2 missense mutation

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Tuberous sclerosis is an underdiagnosed multisystem genetic disorder because of its variable expressivity. The classical triad of clinical features is mental retardation, epilepsy and skin lesions as angiofibromas, hypomelanotic macules and Shagreen patches. Nevertheless, these features are not always present.

Therefore we present a 9-year old boy who was referred with the temptative diagnosis of Neurofibromatosis due to multiple hyperintense (T1) lesions in MRI and who turned out to suffer from an atypical form of tuberous sclerosis.
The boy had developmental delay, especially regarding speech development. He attends normal school but had to repeat the first school year because of difficulties in calculating.
Clinical examination revealed most notably auburn, but also partly scarred looking skin lesions, neither typical for Neurofibromatosis nor for tuberous sclerosis. Skin biopsy revealed fibromatous naevus like changes. He showed an intermitted anankasting winking which was suspected to be a kind of epilepsy but EEG studies revealed normal results.
While sequence analysis in the NF1 gene showed normal results, sequencing of all coding exons of both TSC genes revealed a 3230C/T (Thr1071Ile) mutation in exon 27. This missense mutation leads to a substitution of Threonine with Isoleucine and is not described in literature but concerns a conserved amino acid.
Both parents of the boy were initially unconspicuos and did not have any difficulties in school or signs of epilepsy. However, in the father clinical investigation showed little areas with scarred looking skin lesions as well as areas of net-like hypopigmentation on both thighs which were only visible by wood-light examination. Molecular genetic testing confirmed the same mutation in the boy's father.

P035

Defining the heterochromatin localization and repression domains of SALL1

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SALL1 has been identified as one of four human homologues of the Drosophila region-specific homeotic gene spalt (sal), encoding a zinc finger protein of characteristic structure. Mutations of SALL1 on chromosome 16q12.1 were demonstrated to cause Townes-Brocks syndrome (TBS, OMIM# 107480). We have reported previously that SALL1 acts as a strong transcriptional repressor in mammalian cells when fused to a heterologous DNA-binding domain. Here we report that SALL1 contains two repression domains, one located at the extreme N-terminus of the protein and the other in the central region. We further identified a domain which mediates the heterochromatic localization of the protein. For this purpose, we transiently transfected NIH-3T3 cells with the SALL1 deletion constructs used for mapping the repression domains. Indirect immunofluorescence staining with a primary antibody directed against the heterologous GAL4-DNA-binding domain revealed that a fusion protein covering amino acids 618 to 690 of SALL1 (and any other SALL1 fragment containing this region) localizes to chromocenters in interphase nuclei. The full-length SALL1 protein is known to display a similar punctate distribution pattern at these DAPI-bright sites of clustered pericentromeric heterochromatin in murine fibroblasts. The data presented shed light on the possible connection between heterochromatin localization and repression.

P036

Oesophageal atresia, hypoplasia of zygomatic complex and mandibula, microcephaly, microtia, and congenital heart defect - new syndrome in two affected sibs and a mildly affected mother?

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Treacher Collins syndrome (TCS) was suspected in two siblings with hypoplasia of the zygomatic complex, hypoplasia of mandibula, microtia, microcephaly, and oesophageal atresia, but screening of the TCOF1 gene revealed no pathogenic mutation. The facial phenotype is different from TCS and the association with oesophageal atresia and congenital heart defect is uncommon. Therefore a new syndrome is considered.
The 12-year-old girl was the first child of apparently healthy parents. Pregnancy was complicated by polyhydramnios due to oesophageal atresia and birth occurred at term with normal measurements. She developed postnatal microcephaly, had hypoplastic zygomatic bones, cup-shaped ears and conductive hearing loss, a mild global developmental delay, slightly reduced mouth opening, and an atrial septal defect.
After birth of two healthy sisters, a more severely affected brother was born. Pregnancy was again complicated by polyhydramnios due to oesophageal atresia. Birth occurred after premature rupture of membranes at 34 weeks of gestation with normal measurement apart from OCF. He presents with microcephaly with prenatal onset, postnatal dystrophy and short stature, cup-shaped ears associated with conductive hearing loss, severe developmental delay, and a reduced mouth opening (10 mm). Due to upper airway obstruction he required a tracheostoma at the age of eight months. In addition, he has a median cleft palate and a ventricular septal defect. A 3D MRI scan of the head showed bilateral aplasia of the zygomatic arches.
The mother presented with a scar on her right cheek. A radiograph confirmed the clinical suspicion of aplasia of the zygomatic arch on this side of her face. In addition, she has a reduced mouth opening. No further anomalies or dysmorphic signs were present.
We suggest that this previously unreported combination of clinical findings is a new hitherto undescribed syndrome with autosomal dominant inheritance and reduced penetrance.

P037

Dissecting the pathogenesis of dilated cardiomyopathy due to a heterozygous Arg 9 Cys mutation in phospholamban

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We recently identified a heterozygous Arg 9 Cys mutation in phospholamban (PLN^{R9C}) in a family with autosomal dominant inheritance of dilated cardiomyopathy (DCM) (Schmitt et al., 2003). Phospholamban (PLN) is a cardiac-specific transmembrane protein of the sarcoplasmic reticulum (SR) that inhibits the Ca^{2+}-ATPase SERCA2a.
Our investigations using transgenic mice (Tg-PLN^{R9C}) and human cell lines revealed that PLN^{R9C} blocked protein kinase A (PKA)-mediated phosphorylation of both PLN^{R9C} and wild type PLN resulting in constitutive inhibition of SERCA2a and reduced SR Ca^{2+} uptake rates. To better understand the dominant acting mechanism of the heterozygous mutation we ablated wild type PLN from TgPLN^{R9C}-mice (Tg-$PLN^{R9C,PLN-/-}$). Surprisingly, these mice lived for longer than 1 year, while TgPLN^{R9C}-mice died of heart failure at the age of 21±6 weeks. Tg$PLN^{R9C,PLN-/-}$—myocytes demonstrated rescued kinetics of SR Ca^{2+} uptake and myocyte relaxation. The findings were explained by in vitro experiments showing that PLN^{R9C} exhibited only about 25% of wild type PLN function.
We further wanted to analyze the mechanism of PKA inhibition due to PLN^{R9C}. Different aminoacid substitutions were introduced in position 9 of PLN and expressed in HEK 293 cells. Intriguingly, the phosphorylation patterns of PLN^{R9A} and PLN^{R9S} turned out to be indistinguishable from wild type PLN. Only the PLN^{R9C} mutant demonstrated a clear reduction of ^{32}P incorporation.
We conclude that (i) specific changes in myocyte Ca^{2+} cycling can lead to DCM, (ii) heterozygous PLN^{R9C} can exhibit its detrimental effects only in the presence of wild type PLN and (iii) malphosphorylation of PLN^{R9C} is not caused by the loss of an arginine residue or a change in charge, but by the gain of a cysteine residue.

P038

De novo interstitial deletion of 4q[46,XY,del(4)(q25q31.1)] in a 35 year old patient with multiple congenital anomalies but without Rieger anomaly.

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Interstitial deletions of the long arm of chromosome 4 involving the region of 4q25-q31.1 are rare. Previous reported cases mostly involve either the region 4q25-q27 often associated with Rieger syndrome, or the region 4q31-qter which has been suggested to lead to a characteristic syndrome of facial dysmorphism, cardiac defects and developmental delay. Here we report clinical and chromosomal findings in a 35 year old patient with a de novo interstitial deletion del(4)(q25q31.1). The clinical findings include mental retardation, membranous aortic stenosis

and subaortal ventricular septal defect, craniofacial and skeletal anomalies including micrognathia and dental crowding and mild iris hypoplasia but no Rieger anomaly. Using peripheral lymphocytes the deletion was cytogeneticaly chrarterized by G-banding and further fluorescent in situ hybridisation (FISH) has been performed to narrow the breakpoints of the deletion of the abberant chromosome 4. Alltogether 21 Bac-clones were ordered from the RZPD (Deutsches Ressourcenzentrum für Genomforschung) characterized by molecular means and hybridized to chromosomal metaphases using standard FISH protocols. 7 of these isolated Bac clones could be mapped to the region of the proximal breakpoint, 9 Bacs did neither show a signal on the derivative chromosome 4 nor on any other chromosome besides the normal chromosome 4 and were considered to bind within the deleted region. 3 Bac clones could be located distal to the telomeric breakpoint, whereas 2 clones did not show specific signals. In an effort to characterize the deletion breakpoints the proximal one could be defined between Bac clones RP11-265M21 and RP11-326N15 and the distal breakpoint between RP11-435P6 and RP11-1062K20. The phenotypic expression noted in patients with specific 4q25-q33 deletions seems to be highly variable. This case could therefore contribute to delineate phenotypes and specific malformations compared to other cases reported in the literature.

P039

Genetic counseling from the clients' perspective

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Objectives: Given the rapidly growing human genetic knowledge and diagnostic options today genetic counseling must respond dynamically to these developments. We wanted to see what genetic counseling clients expect from this service, how their expectations are met, how 'successful' they perceive the counseling and what they like and dislike about it.

Methods: In a prospective exploratory study 56 clients of 4 middle European genetic counseling centers were asked to answer anonymous questionnaires before and after the counseling session. Those included scale ratings and multiple choice and open questions.

Results: The most frequently stated expectations are 'certainty' and 'a good result', followed by 'information'. 30% said they did not have any specific expectations. Realistic expectations like information, psychologic support and practical help were adequately met. Also the other outcomes measured quantitatively (gain of knowledge, gain of control, perception of counsellors and satisfaction) were evaluated quite positively with an overall grade point average of 1,7 (1 being 'very good' on a 5-point scale). However, answers to the open questions revealed a number of things clients disliked about the counseling.

Conclusions: 1. Unrealistic or missing expectations in so many clients show deficits in the preparatory stage of the counseling process. 2. The quantitative measurement of currently used quality criteria of genetic counseling like gain of knowledge or satisfaction is not sufficient to evaluate the 'success' of genetic counseling from the clients' perspective. Unless it is supplenmented by open questions it may fail to show the 'real' picture and thus to serve as a valuable means of feedback and quality control.

P040

A new locus for brachydactyly type A2 maps to chromosome 20p

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Brachydactyly type A2, an autosomal dominant hand malformation, is characterized by short and laterally deviated second and fifth fingers. Also, the first and second toes can be affected in a similar way. Heterozygous missense mutations in the gene coding for bone morphogenetic protein receptor 1b (BMPR1B) were shown to cause brachydactyly type A2 in some cases by acting in a dominant negative manner. Recently, we performed a linkage analysis in a large Brazilian pedigree presenting with brachydactyly type A2 using the SNP-GeneChip Human Mapping 10K Array/Assay Kit from Affymetrix. Additional polymorphic microsatellite markers were used for the fine mapping to narrow the defined region. Using this approach, we mapped a new locus for brachydactyly A2 to a 17 cM region on chromosome 20p. As a candidate gene located within this new locus, we first sequenced the coding region of BMP2, known as an important ligand of the BMPRs that mediates essential functions in chondrocyte differentiation and bone formation, but no mutation was found. Several other candidate genes located within the disease locus will now be sequenced.

P041

Clinically atypical Brachydactyly A2 with a missense mutation in BMPR1B

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Isolated brachydactylies (BD) are phenotypically and genetically heterogeneous and have been classified into at least 5 different groups A-E (Bell 1951). Subgroups BDA1-A3 are considered as autosomal dominant traits. Linkage analysis in 2 unrelated German families mapped the BDA2 locus to 4q21-q25. Mutation analysis of the positional candidate gene BMPR1B identified the first 2 BDA2 causing missense mutations, considered to act in a dominant negative manner (Lehmann K et al. 2003). We describe a 34-year-old woman with isolated brachydactyly of fingers II and III. In contrast to the affected members of the two BDA2 families, fingers III on both hands instead of fingers II are severely affected in our patient. She also has preferential involvement of the right foot with deviation of the first toe, cutaneous syndactyly between 2nd and 3rd toes as well as shortening of toes III to V instead of I and II. X rays of both hands and feet at an age of 10 yrs. documented brachydactyly of fingers II and III. Both parents and her sister appear to be not affected by BDA2. Mutation analysis identified a transition 1457G>A resulting in a missense mutation R486Q in exon 10 of BMPR1 by which arginine is being replaced by glutamin. This is the 2nd mutation affecting the identical amino acid position 486 within the intracellular NANDOR domain of BMPR1B. The other mutation described once before at position 486 is due to a 1456C>T transition resulting in an arginine to tryptophan replacement. It appears that in our patient the arginine-to-glutamin substitution is related with a slightly different manifestation compared with the arginine-to-tryptophan substitution. Functional analysis of the 2 missense mutations within the highly conserved NANDOR box may help to elucidate the mechanism of BMPR1B function.

P042

Two different chromosomal aberrations, of 17q11.2 and 7qter, in a child with neurofibromatosis von Recklinghausen, developmental delay, facial dysmorphy, and mild overgrowth

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Most cases of Neurofibromatosis type I result from a point mutation and some represent chromosomal microdeletions at NF1. Patient was born from the 2nd pregnancy to young, healthy, non-consanguineous but endogamic parents (no contact to mutagenes, teratogenes). There are no relatives with a similar phenotype. The pregnancy, delivery and newborn period were normal. At the age of 1 month a large cafe-au-lait spot was noted, later numerous pigmented spots were apparent. At the age of 1 year she showed a coarse face and was not yet able to walk and speak. At the age of 3 years (first seen by E.S.) she was eutrophic, eutonic, and tall (97th percentile) with mild thoracal scoliosis. The face was elongated with relative microcephaly (25th percentile), prominent supraorbital arches, convergent strabism, long nose, crowded teeth, and retromandibula. She had several large cafe-au-lait spots, numerous pigmented lesions, and axillary freckling. She also had mild developmental delay, a hoarse voice, and poor and dyslalic speech. A presumptive diagnosis of NF1 was made, but the molecular analysis of the NF1 gene was normal. Because of the retardation, facial dysmorphy, and mild overgrowth, FISH was performed and revealed a deletion of 17q11.2 between BACs 271K11 and 640N20, which

yielded diminished signals: ish del(17)(q11.2q11.2)(tp53+,RH65670/RH71187+dim,RH71187-,NF1/RH106225-,RH78003/ RH26307-,RH55147/RH26307+dim). During the introduction of the FISH subtelomere screen at the cytogenetic laboratory in Prague, random samples were chosen for training and one indicated a distal chromosome 7q trisomy, karyotype ish der(?14)t(7;?14)(q36;?p12)(G31340+). The sample was traced back to this child with 17q11.2 microdeletion, and further studies for confirmation are pending. The finding of two seemingly unrelated chromosomal micromutations raises mainly two issues: the contribution of the individual mutations to the phenotype and the underlying cytogenetic mechanism.

P043

Progeroid syndrome in a 26 years old male patient

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Progeroid syndromes are a heterogeneous group of disorders including Hutchinson-Gilford-Progeria syndrome and other lamin A/C related disorders, some chromosome instability syndromes, Werner syndrome, Wiedemann-Rautenstrauch syndrome, some types of Ehlers-Danlos syndrome, Geroderma osteodysplasticum and further very rare entities.
We report on a 26 years old male patient affected by growth retardation (< 3 centile), microcephaly, alopecia, hyperpigmentation, multiple lentigines, muscle atrophy, osteolysis and multiple contractures. Mental development is normal.
Chromosome analysis revealed a 46,XY, inv(2)(p11.2q13) karyotype. This inversion is a common rearrangement so far not associated with a disease phenotype. There was no elevated rate of spontaneous or induced chromosome breakage or SCE. DNA analysis of the coding sequence of LMNA revealed no mutation.

P044

Atypical 22q11.2 deletions in two patients with palmoplantar hyperkeratosis and unusual clinical features

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The occurrence of frequent deletions and other chromosomal rearrangements at 22q11.2 makes it one of the most extensively studied regions in the human genome. The 22q11.2 deletions are estimated to occur at a frequency of 1/3000-4000 live births. Deletions are found in 90% of patients presenting with DiGeorge/Velocardiofacial syndrome. The presence of chromosome specific low copy repeats LCR (LCRs) predispose the region to genomic rearrangement. Eight copies of such LCR's have been identified in 22q (named A-H). Of the microdeletions associated with DGS/VCFS, deletions flanked by LCR's A and D are the most common (85%). The less frequent rearrangements are those flanked by LCRs A and B (8%), by A and C (2%) and some atypical deletions. We examined two male patients with features of DGS. They both presented with significant hyperkeratosis on their hands and feet as well as some unusual features. Cytogenetic and FISH analysis using the TUPLE 1 probe revealed a de novo deletion of 22q11.2 in both patients. To further assess the size of the deletion in these patients several cosmid and BAC probes mapping to the 22q11 region were used. FISH mapping in the first patient showed that probes flanking LCR B extending to LCR D were deleted. The deletion breakpoints were placed between LCR A and B on the centromeric side and between LCR D and E on the telomeric side suggesting a large deletion that extends distally beyond LCR-D, the distal BP of the common large 3 Mb deletion. In the second patient the deletion involved LCR-A and extended beyond LCR-C where probes flanking LCR-D were not deleted suggesting that this patient falls into a group of atypical deletions. According to our knowledge palmoplantar hyperkeratosis has not been reported so far in association with 22q11 deletions. Further cases are required to evaluate how frequent this feature is being found with atypical 22q11 deletions.

P045

A novel HRPT2 mutation segregating in a multigeneration family with hyperparathyroidism-jaw tumor syndrome (HPR-JT)

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Hyperparathyroidism-jaw tumor syndrome (HPR-JT) (OMIM *145001, an autosomal dominant disorder, is caused by mutations in the gene HRPT2 (Carpten et al., 2002) mapped to the chromosomal region 1q25-q32. HRPT2 is suggested to function as a tumor-suppressor gene supported by the identification of loss of heterozygosity (LOH) in some tumors and inactivating somatic mutations in parathyroid adenomas with cystic features. Individuals with HPR-JT demonstrate recurring pancreatitis, renal lesions, ossifying fibromas of the mandible and maxilla as well as hyperparathyroidism. The risk for development of tumors such as parathyroid carcinomas is greatly increased in individuals with HPR-JT. We report on a multigeneration family with the hyperparathyroidism-jaw tumor syndrome (HPR-JT) segregating with a novel point mutation within HRPT2. Sequencing of genomic DNA prepared from peripheral blood of our index proband identified a heterozygous deletion 76delA in exon 1. The deletion results in a frameshift mutation leading to a premature termination signal at amino acid position 36 (V36X). HRPT2 encodes a ubiquitously expressed protein called parafibromin consisting of 531 amino acids; its functional role remains to be elucidated. By sequencing we also detected an RFLP (Sau3AI) closely linked with the 76delA mutation. Analysing DNA from thirteen additional family members, some of them affected by tumors, revealed 4 additional mutation carriers. Individuals without the mutation do not show any tumor so far. Thus heterozygosity for the 76delA mutation correlates with the clinical phenotype in our family.

P046

Analysis of Intrachromosomal Aberrations Using High Resolution Multicolour Banding - Advantages and Limitations

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The multicolour banding (MCB) technique uses region specific partial chromosome paints (RPCPs) which are generated by microdissection.Theses probes that partially overlap with the adjacent regions are tagged with different fluorochromes thus resulting in a colour banded chromosome with a greater number of pseudocolour bands than the number of libraries used. In order to illustrate the advantages and limitations of MCB, we reinvestigated ten clinical cases with different aberrations previously identified by conventional banding techniques;these included deletions (three cases), duplications (four cases), inversions (two case), and complex rearrangement (one case).
MCB redefined breakpoints of G-banded chromosomes in five cases, in two it merely confirmed previous cytogenetic results.
In two other cases in which conventional cytogenetics and fluorescence in situ hybridisation (FISH) failed to reveal the precise karyotype, MCB was able to resolve the structural aberrations in detail. One of these cases was an inserted duplication in the p arm of chromosome 4. Another was a complex rearranged X-chromosome showing a terminal deletion of Xp and an inverted duplication of the terminal Xq region translocated to the tip of Xp, thus resulting in a partial monosomy Xp as well as a partial trisomy Xq.
In one case MCB gave no hint of an intrachromosomal aberration although G-banding of the chromosomes suggested an inversion and also a small deletion in the p arm of chromosome 17. The results of MCB were successfully confirmed with other molecular cytogenetic techniques.
MCB allows high resolution analysis of the fine structure of chromosomes at various band levels. The highly reproducible MCB pattern can be used to characterise abnormalities that remain unresolvable by G-banding analysis. We can therefore say that high resolution multicolour banding is a helpful additional method in clinical genetics which is especially useful for the analysis of intrachromosomal aberration.

P047

Third Case of Carnevale Syndrome

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We report on a 14-year-old boy who was referred to us because of an unclear dysmorphism syndrome with mild mental retardation. Y is the child of healthy, consanguineous parents. He has one healthy brother. Y showed the following dysmorphisms: bilateral ptosis, hypertelorism, downslanting palpebral fissures, prominent nose, left half of face is more prominent than the right one, head slightly inclined to the right, high arched palate, clinodactyly. Y is shortsighted. Besides, there is a profound hearing impairment at the left ear due to recurrent infections. At the age of one year a pelvis kidney was detected by ultrasound. At the age of two weeks an omphalocele and at the age of two months bilateral inguinal hernias were operated.
Pregnancy and birth were uneventful. Milestones of development were achieved at the following ages: crawling at 7-8 months, free walking at 1 ½ years, speaking at 3 years. Y is mildly mentally retarded and attends a school for children with hearing impairment.
Conventional cytogenetic analysis revealed a numerically and structurally normal male karyotype of 46,XY. Screening for metabolic disorders was without pathological findings.
Y's facial dysmorphisms such as bilateral ptosis, hypertelorism, downslanting palpebral fissures and a head slightly inclined to the right are in accordance with the facial dysmorphisms reported by Carnevale et al. (1989) as part of a new syndrome. The predominant symptoms of this new syndrome, found in two brothers of consanguineous parents, are bilateral ptosis, strabismus, diastasis recti, hip dysplasia, cryptochidism and mild mental retardation. We will discuss the clinical features of our patient and the two brothers described by Carnevale et al.

P048

Late diagnosis of Marshall-Smith Syndrome (MSS)

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Marshall-Smith Syndrome is a rare condition characterised by facial dysmorphism, failure to thrive and advanced bone age. The syndrome was first described in 1971 by Marshall et al. and until now about 30 patients were published. Most patients die in early infancy because of respiratory failure. Our female patient was born in 1995 as the second child of healthy, non consanguineous parents. After an uneventful pregnancy caesarean section was performed because of premature labour. The newborn experienced repiratory distress and needed intubation. Recurrend apneas and infections as well as a abnormal epiglottis required tracheotomy. Failure to thrive make a PEG necessary. Later on she developed seizures. Examination at the age of 6 years showed turricephaly and a coarse face with high forehead, shallow orbits with ocular proptosis, small, upturned nose with anteverted nostrils, large mouth with full lips, glossoptosis and micrognathia. Psychomotor developmental was severely delayed. She couldn't walk alone, and was unable to speak, but she understood simple orders. Despite her remarkable phenotype, which suggests a storage disease, it was quite difficult to make a diagnosis. Unfortunately, there were no x-rays available from the neonatal period. However, the combination of the mildly accelerated bone age at 6 years and the facial dysmorphism may well suggest the diagnosis of Marshall Smith syndrome. The recently published 7 year old patient of Diab et al (2003) shows an surprising facial similarity. In this report we wish to emphasise the phenotypic aspects of older patients with MMS.

P049

Duplication of 17p11.2 in two patients identified by cytogenetic and molecular genetic analyses - is the duplication of the Smith-Magenis region underdiagnosed?

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The molecular mechanism of a number of contiguous gene syndromes can be explained only recently. Most of these recurrent chromosomal rearrangements are caused by nonallelic homologous recombination within region-specific low-copy repeats (LCRs). The proximal short arm of chromosome 17 is rich of such LCRs and thus disposed to deletions and duplications. Genomic disorders mapped within this region include Smith-Magenis syndrome, dup(17)(p11.2p11.2) syndrome, Charcot-Marie-Tooth disease type 1A (CMT1A) and hereditary neuropathy with liability to pressure palsies (HNPP).
Whereas Smith-Magenis syndrome (SMS) results from a ~4Mb deletion of 17p11.2, the duplication of the same region leads to dup(17)(p11.2p11.2) syndrome. In both, the deletion and the reciprocal duplication, these repeats act as substrates for recombination in approximately 80-90 % of the patients. Although the duplication is predicted to occur at the same frequency as the SMS deletion (1:20.000), only a few patients with dup(17)(p11.2p11.2) have been described to date. Because the clinical findings of this syndrome are less severe and more inconsistent than those associated with deficiency of the same genomic region, the dup(17)(p11.2p11.2) syndrome seems to be underdiagnosed.
Here, we report two further cases with a duplication of 17p11.2 initially identified by chromosome analysis and FISH. The duplication could be confirmed subsequently by using MLPA. The comparison of the clinical features of our patients with the cases previously described demostrates the variable phenotypical expression and the difficulties in clinical diagnosis of this syndrome.

P050

A novel nonsense mutation in LMNA leads to nonsense-mediated decay and causes early atrial fibrillation, stroke, AV block, dilated cardiomyopathy and limb girdle musculat dystrophy through haploinsufficiency

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Mutations in the lamin A/C gene (LMNA) lead to a variety of phenotypes including Emery-Dreifuss-muscular dystrophy (EDMD2), limb-girdle muscular dystrophy (LGMD1B), partial lipodystrophy type 2 (FPLD2), and mandibuloacral dysplasia type A with partial lipodystrophy (MADA). In addition, familial dilated cardiomyopathy associated with conduction system disease (CMD1A) and atrial fibrillation has been related with mutations in LMNA. A clear genotype-phenotype correlation for LMNA mutations has not been established. More than 90 % of LMNA mutations represent missense mutations. It has been proposed that the disease causing mechanism would primarily be a dominant negative effect of mutated "poison polypeptides" that disrupt the three dimensional structure of the filaments.
Here we report a four generation pedigree of a german family with varying degrees of early atrial fibrillation, subsequent stroke, AV-Block, cardiomyopathy and limb girdle muscular dystrophy. Sequence analysis of coding exons of LMNA identified a C to T transition in exon 4 at nucleotide position 700 (C700T) that co-segregates with the phenotype in an autosomal-dominant pattern. The mutation was not found in 180 control DNAs. The C to T transition introduces a stop codon at amino acid position 234 (Q234X) of lamins A/C, presumably leading to a protein that is truncated in coil 1b of the alpha-helical rod domain.
However, RT-PCR of lymphocyte RNA from an affected individual failed to detect the mutant allele but not the transcript from the unaffected allele, indicating that the mutated transcript is subject to nonsense-mediated decay. Hence the DCM in this family would be caused by lamin A/C haploinsufficiency and not by a dominant-negative effect of "poison peptides".

P051

Molecular cytogenetic characterisation of a terminal 2q37 microdeletion

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We report a 18 month old boy with deep set ears, congenital abnormalities of the brain, and minor facial anomalies. The sister of the patient is autistic. The patient's chromosomes were examined by routine cytogenetics and fluorescence in situ hybridisation analyses, using commercial whole chromosome painting and telomer-specific probes as well as mapped BAC clones from the genomic region of 2q37. Molecular-cytogenetic analysis defined a deleted region of about 300 kb at the telomer of the long arm of chromosome 2. Only 2 known genes were found to map within this region. GAL3ST2 (Galactose-3-O-sulfotransferase 2), coding for a ubiquitous protein which takes part in the biosynthesis of sulfated glycoproteins and NEU4 (sialidase 4), coding for a glycohydrolytic enzymes which remove sialic acid residues from glycoproteins and glycolipids. Further transcripts close or within the deleted region will be investigated for their contribution to the phenotypic anomalies in our patient.

P052

Partial deletions of chromosomes 9 and 22 in a child with distinct morphological phenotype

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We report on a child with a de novo combined deletion of del(9)(pter->p22) and del(22)(pter->q11.2). The girl presented clinical signs consistent with DiGeorge syndrome and was referred for molecular cytogenetic diagnosis at the age of 8 days.
Cytogenetic and molecular cytogenetic analyses identified a numerical and structural aberrant karyotype with loss of one normal chromosome 22 and translocation of the distal part of chromosome 22 to the short arm of chromosome 9. Hybridization with the probe TUPLE1 specific for the DiGeorge critical region showed only one signal on the normal chromosome 22. FISH analyses with probes assigned to q11.2 and q13 revealed the translocation of part of the long arm of chromosome 22 on the derivative chromosome 9. Hybridization with chromosome 9 and chromosome 22 specific painting probes confirmed the unbalanced karyotype. Based on these studies the karyotype is 45,XX,der(9)t(9;22)(p22;q11.2),-22. Chromosome analyses of lymphocytes from the parents revealed normal karyotypes.
The girl is the second child of a 34-year-old woman and a 36-year-old man. After an uneventful pregnancy the girl was delivered spontaneously at term, being small for gestational age. The child presented with a conotruncal heart defect (truncus arteriosus communis type II), cleft palate and thymus aplasia, a condition consistent with DiGeorge Syndrome. Most of the dysmorphic features seen in our patient like hypertelorism, small eyes, retrognathia, and abnormal auricles are reported in both, 9p- and 22q11.2 deletion syndrome. Some distinct anomalies were present as short, broad distal phalanges with square- shaped nails and flat nasal root which are likely to be attributed to chromosome 9p- syndrome, whereas trigonocephaly, a characteristic symptom of 9p- syndrome was not observed.

P053

Genetic analysis of familial DIDMOAD

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We report on a German family affected by the rare autosomal-recessive DIDMOAD syndrome. DIDMOAD, a neurodegenerative disorder is characterized by diabetes insipidus, early onset diabetes mellitus, progressive optic atrophy and deafness.
The family history shows two affected children of healthy parents. The 30 years old daughter suffers on insulin dependent diabetes mellitus, a severe vision impairment and a profound bilateral sensorineural hearing loss of the higher frequencies. Her 20 years old brother shows a severe bilateral high frequency hearing loss. Audiological evaluation of the parents resulted in normal findings.
The gene responsible for DIDMOAD was identified on 4p16.1 (WFS1). It encodes a putative 890 amino acid transmembrane glycoprotein localized in the membrane of the endoplasmic reticulum (ER).
The mutational screening of the family revealed in the identification of a c.579-594ins16 and a c.1266 C>T mutation resulting in a frameshift V142fsX251 and a nonsense mutation Q366X. Both mutations lead to a termination before or after the 1st transmembrane domain leading to a non functional protein product. Because of the combination of two severe mutations in the children of this family, we will present further data of the patients phenotype.

P054

Molecular analysis of a translocation t (17;20) (q25;q13) in a patient with Silver-Russell syndrome

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In 1992 Ramirez-Dueñas et al. reported an autosomal translocation t(17;20)(q25;q13) in a patient with Silver-Russell syndrome (SRS), a heterogenous disorder characterised by pre- and postnatal growth retardation, lateral asymmetry and other dysmorphologies.
Here we report the molecular analysis of both breakpoint regions based on specific PAC and BAC clone contigs. Several clones could be identified giving signals on chromosomes 17, der17 and der20 in FISH analysis using proband metaphase chromosomes so establishing a refined clone contig for the 17q25 region. According to the mutation analysis of the candidate region for hereditary neuralgic amyotrophy (HNA) we could localise the gene SEC14L1 nearby the region of interest by PCR approach. STS content mapping and sequencing of the clone insert ends resulted in locating the breakpoint within 81,5 kb in the 5'- region of SEC14L1.
Corresponding FISH analysis resulted in the identification of a PAC clone RP1-232n11 spanning the breakpoint on chromosome 20 including parts of the Receptor Protein Tyrosine Phosphatase Rho gene (PTPRT) considered to be another SRS candidate gene. In order to establish a precise physical map of the region of interest we located one XhoI fragment of RP1-232n11 distal to the 20q13 breakpoint. Several BamHI fragments of the same PAC clone are now being subcloned and used as FISH probes, thus leading to a high resolution map of the 20q breakpoint region and giving more insight into the genetic background of SRS.

P055

First report on a child with partial monosomy 4q33->qter and partial trisomy 20p13->pter.

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We report on a female infant with mild dysmorphisms, congenital heart defect, and mild developmental delay through partial monosomy 4q33->qter combined with partial trisomy 20p13->pter. Prenatally, tetralogy of Fallot was diagnosed by ultrasonographic investigations, the parents refused prenatal cytogenetic analysis.
The female infant was born to a 28-year-old mother and a 32-year-old father, both healthy and nonconsanguineous, at 36 weeks of gestation by cesarean section after premature rupture of the amnion, birth weight 2120 g. Artificial respiration was necessary because of respiratory insufficiency.
Clinical findings of the index patient: craniofacial anomalies with telecanthus and Pierre-Robin sequence, tetralogy of Fallot, pes calcaneovalgus with irregular toes; at the age of six months mild statomotoric retardation, body weight and length above 10th centile.
The unbalanced translocation of our patient was due to a balanced paternal rearrangement as shown by GTG banding technique and FISH.

P056

Spondylocostal Dysostosis with anal atresia and urogenital anomalies (Casamassima-Morton-Nance-Syndrome) in a newborn

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The Spondylocostal dysostoses (SCD) are a heterogenous group of disorders characterised by multiple vertebral segmentation defects and rib anomalies. DLL3 is the most commonly mutated gene in autosomal recessive SCD1. Even though additional malformations can be found in SCD1, the association with urogenital malformation is rare and has been described as a distinct entity by Casamassima et al. in 1981: "Spondylocostal Dysostosis with anal and urogenital anomalies (OMIM 271520)".
We report on a female newborn (36 weeks of gestation) who died shortly after birth because of respiratory insufficiency. The parents are from the same Turkish village, consanguinity was denied. The pregnancy was the sixt in this partnership: there are three healthy daughters, two pregnancies resulted in an abortion. In the 22. week of gestation of this pregnancy a maternal

glucosuria occurred. Mild gestational diabetes mellitus was not treated.
Radiographic examination of the affected newborn revealed multiple vertebral segmentation defects in the thoracical and upper lumbar spine (hemivertebrae, wedge shaped vertebrae), agenesis of sacral vertebrae and fusion of ribs. Additionally bilateral club foot deformation and dolichocephaly were found.
Multicystic renal dysplasia and anhydramnia were detected prenatally by ultrasound. Postmortal examination showed additionaly severe urogenital malformation: no definable ureteres, streak-shaped urinary bladder, uterus bicornus with rudimentary cornu left and right, cervical and vaginal atresia, anal atresia, recto-cloacal-fistula.
Chromosomal analysis of the newborn and the parents were normal. A mutation in DLL3 was excluded by direct sequencing. On the basis of distinct pattern of malformations the diagnosis of "Spondylocostal Dysostosis with anal atresia and urogenital anomalies" is suggested in this presented case.

P057

Analysis of skeletal phenotype and exclusion of mutation in BMP7 gene in two cases of short rib polydactyly syndrome type Majewski

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The short-rib-polydactyly syndromes (SRPSs) are a heterogenous group of skeletal dysplasias characterized by short-limb dysplasia, narrow hypoplastic thorax with short ribs, polydactyly and associated visceral abnormalities, mainly polycystic kidneys. To date a responsible mutation in a defined gene could be identified only for the Ellis-van-Creveld syndrome (EVC). This EVC gene was excluded as causative for other SRPS, as demonstrated by linkage analysis. In order to further elucidate the genetic background of SRPSs we compared phenotypic features of affected individuals with developmental defects of knockout models in mice. Previous studies analyzed the histological changes in endochondral ossification in SRPS, mentioning mainly a disorganization and irregularity of the growth plate in long bones.
Our studie showed that changes in endochondral ossification represent a partly arrested orthotopic ossification at the stage of the hypertrophic chondroblast and heterotopic perichondral ossification with accessory ossicles in the lower leg of one case. Impaired renal development is indicated by polycystic kidney disease with dilated collecting tubules and glomerulocystic changes in the other case. Excessive polysyndactyly and median cleft palate was apparent in both cases. Due to apparent phenotypic analogies to the knockout mouse model for BMP-7 we excluded impaired expression or mutation of this morphogen as a causative factor for SRPS in the two clinical cases by immunohistochemical methods and analysis of genomic sequence.

P058

Clinical and Mutational Spectrum of Mowat-Wilson Syndrome

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Mowat-Wilson syndrome is a recently delineated mental retardation syndrome usually associated with multiple malformations and a recognizable facial phenotype caused by defects of the transcriptional repressor ZFHX1B. To address the question of clinical and mutational variability, we analysed a large number of patients with suspected Mowat-Wilson syndrome (MWS). Without prior knowledge of their mutational status, 70 patients were classified into "typical MWS", "ambiguous" and "atypical" groups according to their facial phenotype. Using FISH, qPCR and sequencing, ZFHX1B deletions, splice site or truncating mutations were detected in all 28 patients classified as typical MWS. No ZFHX1B defect was apparent in the remaining 15 cases with ambiguous facial features or in the 27 atypical patients.
Genotype-phenotype analysis confirmed that ZFHX1B deletions and stop mutations result in a recognizable facial dysmorphism with associated severe mental retardation and variable malformations such as Hirschsprung disease and congenital heart defects. Our findings indicate that structural eye anomalies such as microphthalmia should be considered as part of the MWS spectrum. We also show that agenesis of the corpus callosum and urogenital anomalies (especially hypospadias) are significant positive predictors of a ZFHX1B defect. Based on our observation of affected siblings and the number of MWS cases previously reported, we suggest a recurrence risk of around 1%. The lack of missense mutations in MWS and MWS-like patients suggests there may be other, as yet unrecognised phenotypes, associated with missense mutations of this transcription factor.

P059

Extremely high load of internal tumors determined by whole body MRI scanning in a patient with neurofibromatosis type 1 and a non-LCR-mediated 2-Mb deletion in 17q11.2

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Large deletions in 17q11.2 occur in 5% of patients with NF1. The two major types of NF1 deletions encompass 1.4- and 1.2-Mb, respectively, and have breakpoints in the NF1 LCRs or in the JJAZ gene. Deletions larger than 1.4-Mb are rare and only few cases have been reported so far. Here, we describe an NF1 patient with an "atypical" NF1 deletion of 2-Mb. In contrast to the common 1.4-Mb deletions, which preferentially occur on the maternal allele, the deletion described here affects the paternally inherited chromosome. Precise breakpoint mapping revealed that the proximal deletion breakpoint occurred in an L1-element located 1.3-Mb proximal to the NF1 gene. The distal deletion boundary is located in a single copy segment between an AT-rich segment and an AluSx-element in intron 15 of the JJAZ1 gene. Structural analyses imply that non-B DNA conformations at the breakpoints destabilized the duplex DNA and caused double-strand breaks. Although the breakpoints of this 2-Mb deletion are not recurrent, it is conspicuous that one breakpoint is located in the JJAZ1 gene. Paralogous recombination between the JJAZ1 gene and its pseudogene is causing the recurrent 1.2-Mb deletions. We conclude that the genomic architecture of the NF1 gene region, influenced by paralogous sequences like the JJAZ1 gene and its pseudogene also stimulates the occurrence of non-recurrent deletions mediated by non-homologous end joining. In addition to mental retardation, the patient with the 2-Mb deletion suffers from an extremely high burden of subdermal neurofibromas. Magnetic resonance imaging scanning of the whole body further revealed numerous internal plexiform neurofibromas and spinal tumors suggesting that deletions larger than the common 1.4-Mb deletions lead to extremely severe phenotypes. We further demonstrate the value of whole-body magnetic resonance imaging in determining total tumor load which is an important aspect in genotype/phenotype correlations regarding large NF1 deletions.

P060

FREQUENCY OF A TGT INSERTION AND ADDITIONAL NUCLEOTIDE POLYMORPHISMS IN A FAR UPSTREAM ENHANCER OF THE CYP3A4 GENE IN CAUCASIANS

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Objectives: Cytochrome P4503A4 enzymes are involved in the metabolism of more than 50% of all drugs. Genetic polymorphisms lead to a difference in metabolic capacity and, therefore, might contribute to therapeutic failure. The variability of the CYP3A4 activity has been described as a five-fold interindividual variability in the oral clearance of midazolam. A TGT insertion in the far upstream enhancer of the CYP3A4 gene has been described (-11,129_-1,128insTGT). In vitro, the TGT insertion results in a 36% reduction of the enhancer activity in French lung cancer patients. We tested the DNA of a group of 122 Caucasians for this and additional polymorphisms.
Material and Methods: DNA has been isolated from whole blood or mouth swabs of healthy volunteers. Four allele-specific PCRs were developed which allowed for specific amplification of the wild-type or the TGT-ins allele. Furthermore, 900bps flanking the polymorphic site were sequenced in order to characterize the genetic variability of this region.
Results: Allele-specific PCR allows fast, reliable and cost efficient detection of polymorphic sites. Seven DNA samples out of 122 have been test-

ed positive for the mutant allel (5.7%). The allele frequency of the INS-polymorphism was estimated at 2.85% (95% confidence interval: 1.4-5.8%) which is in good accordance with a 3.1% frequency found in the large group of French lung cancer patients. So far, the enhancer region surrounding the polymorphic site of thirty DNA-samples has been tested for additional polymorphisms. No further nucleotide polymorphisms were found.
Conclusions: The presence of a functional TGT-insertion polymorphism in the far-upstream enhancer of CYP3A4 was confirmed in Caucasian healthy subjects predominantly of German descent. The functional significance of the polymorphism in vivo remains to be determined.

P061

Townes-Brocks syndrome due to a novel SALL1 mutation and trisomy 8 mosaicism in one patient
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Townes-Brocks syndrome (TBS) is an autosomal-dominant malformation syndrome caused by SALL1 mutations. The three major criteria for TBS are malformations of the thumbs, dysplastic ears and imperforate anus. A strong inter- and intrafamilial variability in expression is known. Here we report a non-consanguineous English family. The index patient is a 14 years old girl presenting with a macrocephaly, distinctive facial features, normal ears, bilateral triphalangeal thumbs, arachnodactyly, bilateral pes cavus, very long and clawed toes and learning difficulties. She has had an anorectal malformation and ureteric stenosis. Karyotyping revealed a trisomy 8 mosaicism. As triphalangeal thumbs do not fit into the picture of trisomy 8 mosaicism, TBS was suspected, too. Mutation analysis showed the novel SALL1 mutation c.2779C>T (Q927X). Four other family members show some clinical features of TBS but not the typical phenotype. The mother carries the mutation but has no signs of TBS but a scoliosis. In three other family members TBS can suspected due to anorectal malformation, hearing loss and learning difficulties, respectively. The mutation Q927X is the most 3' positioned nonsense mutation found in SALL1 up to now. It is localized in exon 2 and creates a preterminal stop codon 5' to the region encoding the third double zinc finger motif. So far all known TBS-causing mutations are truncating, so this mutation is most likely responsible for the TBS features in the index patient. Since more zinc finger domains will be present in the truncated Q927X protein as compared to hotspot mutations, it might have a preserved residual function. This could explain the mild phenotypes or reduced penetrance in the other family members. In that respect, one could assume that the mosaic trisomy 8 has aggravated the effect of the SALL1 mutation. Some features of the index patient are not reported for either SALL1 mutations or trisomy 8 mosaicism and may result from a combination of both.

P062

Extended clinical spectrum of patients with Cohen syndrome caused by novel mutations in COH1
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Cohen syndrome is an uncommon autosomal recessive disorder whose diagnosis is based on a variable clinical picture of psychomotor retardation, microcephaly, typical facial dysmorphism, progressive pigmentary retinopathy, early onset of severe myopia, and intermittent neutropenia. Recently, mutations of the gene COH1 were shown to be responsible for the Cohen syndrome phenotype in different populations. In order to investigate the clinical and mutational spectrum, the present study included the analysis of 12 patients with Cohen syndrome from 7 families, originating from a wide geographical distribution. All patients were compound heterozygous for different mutations in COH1 including 9 novel mutations. The families investigated comprised a German family with 5 male patients, in which an X-linked mode of inheritance of an unknown mental retardation syndrome was initially assumed based on the pedigree. However, the facial gestalt of these brothers affected with pigmentary retinopathy was consistent with Cohen syndrome, which was subsequently confirmed by mutational analysis. In our series of patients with Cohen syndrome, the degree of mental retardation was remarkably variable. Although all patients had visual abnormalities, myopia, which is usually of early onset, was absent in a 9-year-old patient. Typical craniofacial features were evident in all patients, however, facial dysmorphism was very subtle in a 17-year-old patient who presented with bulbous nasal tip, everted lips, and a normal appearing philtrum. Other findings of the patients studied here indicate high variability of growth parameters such as head circumference and height. The present study confirms that absence of microcephaly and neutropenia does not rule out the diagnosis. A comparison of clinical signs among our patients with COH1 mutations facilitated the determination of improved clinical criteria, upon which the diagnosis of Cohen syndrome can be established.

P063

The concept of German skeletal dysplasia network SKELNET - an interactive telemedical research and diagnostic approach to rare diseases
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Skeletal dysplasias represent a large, very heterogenous group of rare genetic diseases with patients located all across Germany. Due to the rare appearance of these conditions, only a few experts in the field exist, lacking a forum for the exchange of scientific knowledge or the consultation of colleagues on controversial cases. In consequence, patients suffer from low efficiency in diagnosis, treatment and after-care. The current situation often results in extremely prolonged intervals between upcoming symptoms and correct diagnosis, and probably in a high number of unknown and insufficiently treated cases.
By collecting patient related data in an internet-based database and establishing an infrastructure for data exchange and discussion, we are implementing a network of actively participating experts. This will contribute to better scientific knowledge on the nature of skeletal dysplasias. As the physicians, who contribute their knowledge to the X-ray focused PACS-like RDE-system, are often not acquainted with the others' field of practice, one of our major efforts will be to join the different areas of clinical and research information, making data accessible and transparent to the specialists. Thus we target the development of new strategies resulting in high level patient care, where physicians have access to cutting-edge research results and researchers use current patient data and medical expertise. The database is currently in development and located in Mainz, Germany, at http://www.skelnet.de, providing access to the SKELNET members located at various centres in Germany. Patient's data privacy policies are as strictly applied as recommended by BMBF and TMF for telemedical networks and data exchange (e.g. regarding pseudonymization, data separation, encryption within a public key environment, secure DICOM/SSL transport mechanisms through firewalls for the immediate collection of X-rays).

P064

A severe congenital muscular nemaline myopathy associated with a de novo missense mutation in the skeletal alpha-actin gene (ACTA 1)
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Nemaline myopathy constitutes a clinically and genetically heterogeneous group of congenital

myopathy. The disease is characterized by a variable degree of generalized muscle weakness and presence of nemaline bodies in muscle fibers. The majority of mutations associated with nemaline myopathy have been identified in the gene encoding nebulin (NEB) and skeletal muscle alpha-actin (ACTA1). Only in rare cases mutations of tropomyosin 2 and 3 (TMP2, TMP3) and troponin T1 have been detected. ACTA1 gene mutations are most often associated with a more severe form of nemaline myopathy. The majority of cases are sporadic. Segregation in some families indicate both an autosomal dominant and an autosomal recessive mode of inheritance. Our female patient was born at week 37 after an uncomplicated pregnancy. She is the second child of non-consanguineous healthy parents. After birth the clinical features were characterized by severe hypotonia, generalized muscle weakness, lack of spontaneous movement, feeding difficulties and respiratory insufficiency. Both myotonia congenita and spinal muscular atrophy had been excluded following molecular analysis. Muscle biopsy showed intranuclear nemaline (rod) bodies. Mutation analysis was performed by direct sequencing of the ACTA1 gene mapped to chromosome 1q42.1. A CGC<TGC (Arg372Cys) heterozygous missense mutation in exon 7 was disclosed. Molecular analysis of both parents using DNA from peripheral blood did not reveal the same mutation. This missense mutation has not been seen in > 600 sequenced chromosomes of controls. Though the mutation R372C has not been described previously, it is consistent with being the cause for the severe form of nemaline myopathy in the patient. Our patient is now 1 year and 14 months old and requires assisted respiration in a domiciliary service.

P065

Four novel mutations in hereditary motor and sensory neuropathies

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Hereditary motor and sensory neuropathies (HMSN; CMT) are the most common inherited peripheral neuropathies in humans. In most of the cases a duplication of chromosome 17p11.2-12 (containing the peripheral myelin protein-22 gene; PMP22) is associated with the disease. In other cases of HMSN mutations in ca. 30 genes (e.g. the PMP22, MPZ and GJB1 or Connexin 32), causing a variety of HMSN phenotypes have been described.
Here we report on four novel mutations found in HMSN cases after excluding the dup17p11.2-12. Using the denaturing high pressure liquid chromatography method and sequencing, we found novel mutations in the PMP22, MPZ and Cx32 genes. These mutations were confirmed by cosegregation analysis and excluded in healthy controls.

P066

Pregnancy and delivery in women with myotonic dystrophy type 2/proximal myotonic myopathy

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The myotonic dystrophies (DM) subdivided into DM1 and DM2/PROMM share common pathogenic mechanisms and result in abnormal RNA splicing. DM1 women face a considerable risk in pregnancy which can partly be attributed to congenitally affected fetuses, but is also related to uterine and placental dysfunction. The question, whether reproductive risks are also increased in DM2, has so far not been systematically addressed.
We recruited 33 women with genetically confirmed DM2 for a retrospective evaluation of their obstetric history. The participants completed different questionnaires and gave their consent to review the medical reports.
The 33 patients had a median age at onset of 35 years, most of them being unaware of their disease when becoming pregnant. First symptoms were proximal pareses, myalgia, myotonia and cataracts. Seven women (30%) had an onset <30 years and mostly had symptoms prior to at least one pregnancy (early onset = EO group). A total of 80 pregnancies were recorded, 10 (13%) resulted in 1st trimester miscarriages. Five 2nd trimester fetuses, including a twin pregnancy, were lost to 3 women, exclusively from the EO group. Of the 66 children delivered (62 singletons, 4 twins), 8 (12%) were born preterm (<36 weeks), 6 of whom were born in the EO group. Further 3 children were small for gestational age (2 in the EO group). Altogether, 11 out of 30 gestations >12 weeks (37%) in the EO group were complicated by fetal loss, prematurity or intrauterine growth restriction (IUGR). Most women coped well with their pregnancies and tasks as a mother, however, 9 patients (27%) noted first signs of DM2 associated with gestation.
To conclude, there was no significantly increased risk for obstetric complications or adverse neonatal outcome in the overall group. In contrast to most DM1 study groups, DM2 presents later in life and rarely affects family planning. Although based on a few cases only, our data suggest a higher fetal risk to women with early onset DM2.

P067

Mutations in the glucokinase gene (*GCK*) in gestational diabetic women

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Maturity-Onset Diabetes of the Young (MODY) is a genetically and clinically heterogeneous form of non- insulin dependent diabetes mellitus, characterized by early onset, usually before 25 years of age and primary defect in insulin secretion. Glucokinase-related MODY 2, autosomal dominant disorder, is detected in children with mild hyperglycemia and in women with gestational diabetes and positive family history. Glucokinase mutations resulted in reduction of the beta cells sensitivity to glucose and in disturbance of glycogen synthesis in the liver. The aim of this study was to estimate the prevalance of MODY 2 in Polish gestational diabetic patients. Selected gestational diabetic women fulfilled the following criteria: age<35 years, BMI< 25, a small increment (<3 mmol/l) during 2-h oral glucose tolerant test and a family history of type II or gestational diabetes in a first or second-degree relatives. The analysis involved the coding regions of the 12 exons and the intron-exons boundaries of the *GCK* gene, which were amplified by PCR and electrophoresed on glycerol-containing and glycerol- free nondenaturing polyacrylamide gel at room temperature or 4°C. Abnormally migrated PCR products were sequenced directly using a dideoxynucleotide- cycle sequencing method. In 9 (~8%) of the 119 patients three novel *GCK* mutations: Y215Y (exon 6), E312Q (exon 8), GfsinsG (exon 10) have been detected. In addition three previously unreported intronic variants: IVS2-12C>T, IVS3-8G>A, IVS7-13A>G have been identified. The obtained results indicated on relatively low prevalance of MODY 2 in Polish gestational diabetic patients.

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P03 Genetic Epidemiology

P068

Die Analyse des Oberflächen-EKGs in Familien einer Isolatpopulation offenbart hohe Heritabilitäten

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Die Signaturen des Oberflächen-EKGs (QT-, QRS-Intervall etc.) widerspiegeln den Ablauf der kardialen Elektrophysiologie in physiologischen und pathologischen Zuständen. Die Bedeutung einzelner Signaturen für die nichtinvasive Risikostratifizierung ist, nach anfänglichen Überbewertungen, in den letzten Jahren genauer verstanden und definiert worden, z.B. der prädiktive Aussagegehalt der QRS-Verbreiterung für Tachycardien und Mortalität in der MUSTT-Studie (Zimetbaum et al., 2004). Viele dieser Signaturen weisen neben bekannten exogenen Einflußfaktoren hohe Heritabilitäten auf. Um zugrundeliegende Gene zu identizfizieren und Genvarianten auf ihre potentielle Bedeutung als genetische Risikomarker zu testen, sind Familienstudien von zentraler Bedeutung. Wir haben in einer familienbasierten Studie mit 500 Personen die Heritabilitäten vieler dieser Signaturen, darunter auch innovativer Signaturen aus dem signalverstärkten EKG mit Hilfe der Varianzkomonenten-

analyse bestimmt. Die Familen entstammen einer isolierten Population. Die Standardabweichungen waren im Vergleich zu nichtisolierten Populationen nicht signifikant vermindert (QT:±27.9ms in der isolierten vs. ±28.1ms in einer nichtisolierten Population; QRS:±13.8ms vs. ±12.5ms), die Heritabilitäten für einige der analysierten Signaturen jedoch signifikant(QT: 47±12%,p=0.00004, QRS: 21±11%, p=0.0159). Mit Hilfe von Kopplungsuntersuchungen lassen sich die zugrundeliegenden häufigen Genvarianten chromosomal lokalisieren und in einem nächsten Schritt auf ihren Effekt in der Normalbevölkerung und auf ihre Eignung für eine molekulargenetische Risikostratifizierung von arrhythmogen prädisponierten kardiologischen Patienten untersuchen.

P04 Functional Genomics, New Technologies

P069

CGHPRO - A software tool for array CGH data analysis

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Array based comparative genomic hybridisation (array CGH) is a high resolution screening tool for the genome wide detection of chromosomal imbalances. The exponential growth of array CGH data necessitates the availability of a user-friendly data analysis software.
We have developed an easy-to-use and versatile tool for the normalization, visualization, breakpoint detection and comparative analysis of array CGH data. CGHPRO is a stand-alone JAVA application that guides the user through the whole process of data analysis. Several image analysis data formats are covered by the import option, but users can also customize their own data formats. Graphical representation tools assist in the selection of the appropriate normalization method. Intensity ratios of each clone can be plotted in a size-dependent manner along the chromosome ideograms. The interactive graphical interface offers the chance to explore the characteristics of each clone, such as the involvement of the clones sequence in segmental duplications. Circular Binary Segmentation and unsupervised Hidden Markov Model algorithms facilitate objective detection of chromosomal breakpoints. The storage of all essential data in a back-end database allows the simultaneously comparative analysis of different cases. The various display options facilitate also the definition of shortest regions of overlap and simplify the identification of “weird” clones. By providing all of its features offline, CGHPRO is particularly suitable for clinical application where protection of sensitive patient data is an issue. Array CGH in conjunction with CGHPRO will become an important tool in clinical genetics.
We will give a demonstration of the software by analysing a male-to-female co-hybridisation onto a 14000 BAC array.

P070

easyLINKAGE - A Windows based program for rapid and automated single- and multipoint linkage analyses

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Most of linkage analyses software was traditionally developed for UNIX environments restricting calculations to a limited number of experienced users. The time-consuming generation of input files as well as missing/restricted graphical outputs were further limits of those programs. Nowadays, scientists prefer to perform their own analyses right after generating the genotypes. However, a tool for performing linkage analyses under the same operating environment where scientists get their daily PC work done was not available so far.
We have developed the program easyLINKAGE that combines an automated setup and performance of linkage/simulation analyses under a graphical user interface for Windows 2000/XP. The program supports FastLink, SuperLink, SPLink, GeneHunter-/Plus, Allegro, and SLink. easyLINKAGE provides genome-wide as well as chromosomal postscript plots of LOD scores, NPL scores, P values, and other parameters. The program generates input files for pedigree/haplotype drawing software such as HaploPainter. We also implemented the program PedCheck, which does an automated Mendel check prior performing linkage analyses.
To run the program, the user must provide marker files containing subject IDs and genotypes, a pedigree structure information file, and a marker database with chromosomal positions. Genotypes will be automatically assembled to the pedigree file, therefore allowing rapid and fully automated linkage analyses of whole genome scans in a few minutes. Markers will be analyzed according to their positions on either autosomes or the X chromosome. All options for the covered linkage programs can be set interactively.
easyLINKAGE boosts the use of most known linkage programs under a Windows environment and enables to perform analyses for a wide audience. All windows binaries including instructions and recompiled runtimes for the linkage programs can be found at http://www.uni-wuerzburg.de/nephrologie/molecular_genetics/download.htm.

P071

NGFN 2 Platform ‘Models’ Subproject 1-3: Chemical mutagenesis of mouse ES cells

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The sequencing of the genomes of man and mouse has lead to the identification of about 30.000 genes coding for proteins. The function of most of these genes has remained elusive. Mutations are the key for an understanding of gene function, when the phenotype that is produced by a mutation is analysed in comparison to the wildtype situation. Many genes have not been targeted in collections of mutants that arose spontaneously (termed the ‘phenotype gap’ by Steve Brown). Therefore, mutations have to be created artificially, either by random mutagenesis, or be gene targeting. Chemical mutagenesis of mouse embryonic stem (ES) cells combines the random approach with gene targeting. The exposure of ES cells to a mutagen (e.g. ethylnitrosourea; ENU), randomly introduces mutations in the genome of ES cells. During NGFN1, we have established libraries of chemically mutagenised ES cells. Currently, libraries of 40,000 clones in pooles (MPI) and 10,000 single clones (GSF) are available. In parallel, we have developed and optimised screening strategies to retrieve mutant clones harbouring specific gene defects from our libraries. Screening of ES cell libraries is carried out using either cDNA or genomic DNA as template. These two complementing screening strategies allow the identification of different types of mutations, i.e. missense, nonsense, as well as splice site mutations, the phenotypic effects of which will vary depending on which domain(s) of the protein are affected. The clones that contain interesting mutations within the gene under study can be used to generate a mouse mutant. Libraries of chemically mutagenised ES cells are therefore valuable tools for the analysis of genes, and in particular so in a biomedical context.

P072

Analysis of a conserved long-range sox9 notochord enhancer in zebrafish

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Heterozygous de novo mutations in and around the human SOX9 gene on 17q cause campomelic dysplasia (CD; MIM 114290), an autosomal dominant skeletal malformation syndrome with XY sex reversal. About 90% of the classical CD cases have mutations in the open reading frame, while the minority of patients exhibit chromosome rearrangements in the vicinity of SOX9. The large 5’ intergenic region of SOX9 contains several sequence elements highly conserved between human, mouse, and Fugu (Bagheri-Fam et al. 2001). In CD translocation and inversion cases, some or all of these elements are separated from SOX9. A 516 bp fragment containing the 130 bp conserved element E1, located 28 kb 5’to human SOX9, directs expression of a reporter gene to the notochord in mice (Bagheri-Fam and Scherer, unpublished). To define the sequences and transcription factors responsible for this E1-directed expression more precisely, we have started to use zebrafish as a model system. Because of a large-scale duplication event in ray fin fish phylogeny, zebrafish has two sox9 homologues, termed sox9a and sox9b, both of which contain the conserved element E1. We assayed the regulatory potential of the 130 bp core sequence of the sox9a E1 element, located 7.8 kb 5’ to the gene, in transient transgenic zebrafish embryos, using a gfp reporter gene driven by the sonic hedgehog (shh) promoter. We

observed specific expression in the notochord and, in contrast to mouse, also in the floor plate in 24 h-old embryos. E1 contains an evolutionarily conserved binding site for the winged-helix transcription factor FoxA2 (Hnf3b). Mutations in and around this binding site caused a simultaneous loss of notochord and floor plate expression, indicating that sox9a might be a direct target of this transcription factor. To test this hypothesis, binding assays of FoxA2 to the E1 element will be performed, as well as knock-down of FoxA2 by co-injection of morpholinos together with the E1-shh-gfp construct.

P073

Localization of novel binding partners via retroviral targeting

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Endostatin is an inhibitor of angiogenesis and tumour growth mutated in Knobloch syndrome. The fact that a high affinity endostatin cell membrane receptor has thus far not been identified may be due to very low expression on endothelial cells. In order to circumvent the problem of low receptor expression, we have started to develop a sensitive retroviral targeting approach. It has been demonstrated that bridge proteins comprised of avian leukosis virus (ALV) receptors fused to epidermal growth factor (EGF) can be used to selectively target retroviral vectors with ALV envelope proteins to cells expressing EGF receptors. In analogy to these experiments, we have generated a set of receptor ligand bridge proteins containing the extracellular domain of the TVA receptor for subgroup A avian leukosis virus (ALV-A) fused to various endostatins via a proline-rich linker. Flow cytometric analyses are used to assay for specific binding of TVA-Endostatins to endothelial cells. Binding is detected with the fusion protein SUA-rIgG which contains the ALV-A surface protein (SUA) of the viral glycoprotein and is recognized by a secondary antibody (anti-rabbit FITC). To determine whether bound TVA-Endostatins can also mediate ALV-A entry, cells are infected with ALV-A encoding the enhanced green fluorescent protein. Approximately 72 hours after viral challenge, the cells are either visualized under an inverted fluorescence microscope or removed from plates and identified by flow cytometry. Once a cell line expressing an endostatin receptor has been identified, the viral targeting strategy can be used to expression clone a novel receptor.

P074

Mutations in the Gene encoding Neutrophil Elastase cause Alteration of Intracellular Trafficking and Proteolytic Function of NE Protein in Congenital Neutropenia

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Severe congenital (SCN) and cyclic neutropenia (CyN) are characterized by early-stage maturation arrest of myelopoiesis and often are found associated with different heterozygous mutations in the gene encoding neutrophil elastase (ELA2). Neutrophil elastase (NE) is a serin protease stored mainly in azurophilic granules. However, the consequences of ELA2 mutations are not clear. Recent studies suggest a correlation with incorrect intracellular transport leading to mis-localization of the mutant protein. In the present study, we have generated an ELA2-inducible expression system based on the monoblast-like cell line U937. This system allows controlled expression of the transgene to study functional correlations with specific mutations of ELA2. Applying this system, we have studied intracellular processing and proteolytic activities of ten different mutations of ELA2 that are typically detected in patients suffering from SCN or CyN. We detected two clusters within the neutrophil elastase protein, in which mutations caused alteration of protein glycosylation, associated with prevention of membrane localization of the mutant protein. Instead, the mutant protein was incorrectly directed to the nuclear region. Consequently, NE protein also was not secreted in these mutants as it is the case for wildtype NE protein. Additionally, proteolytic activities of NE protein against different substrates were measured. NE activities were suppressed in most but not all mutations tested. In summary, our data suggest that intracellular processing and localization is altered due to specific mutations of ELA2, which may be crucial for the molecular mechanisms resulting in the neutropenic phenotype.

P075

Extensive amplification of exonic sequences of a fetal brain-specific ROBO2 isoform during hominoid evolution

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Human homologs of the Drosophila roundabout (Robo) gene function as transmembrane proteins that have been implicated in the guidance and migration of axons, myoblasts, and leukocytes and also may have a role as tumor suppressors. The human ROBO2 gene lies 400-500 kb proximal to an evolutionary breakpoint that distinguishes human chromosome 3p12.3 and orangutan chromosome 2. In the course of positional breakpoint cloning we have identified two new exons in the 5' region of ROBO2. By RT-PCR the newly discovered isoform of ROBO2 was expressed abundantly in the developing fetal human brain, whereas the known isoform was expressed in multiple human tissues, most highly in fetal and adult brain. The two different ROBO2 isoforms have been conserved between humans and mice, indicating their functional significance. Sequences syntenic to the evolutionarily rearranged chromosome region appear to be specific for the 3p12.3-syntenic region in humans and chimpanzees, but were duplicatively transposed to numerous pericentromeric and subtelomeric sites in the orangutan and siamang genomes. The amplified DNA segments contain the first two exons of ROBO2, which may confer a fetal brain-specific expression pattern, whereas the remaining exons were not amplified. Interestingly, the amplicon size is smaller but the copy number is much higher in siamang gibbon than in orangutan. The amplified sequences which are paralogous to ROBO2 exons account for several percent of siamang genomic DNA. Northern blotting, cDNA library screening, and RACE experiments are underway to identify new transcripts containing exons 1 and/or 2 of ROBO2 in the orangutan and siamang genomes. We propose that regions that have been involved in evolutionary chromosome rearrangements have served as acceptor and/or donor sites of duplicated genic DNA segments for the formation of new transcripts.

P076

Gene-targeted mouse models Timp3(S156C), Timp3(S156M), Timp3(-/-) to investigate the role of TIMP3 in angiogenesis

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Objectives: Sorsby fundus dystrophy (SFD) is a progressive disease of the macula caused by mutations in the tissue inhibitor of metalloproteinases-3 (TIMP3). Choroidal neovascularization is a hallmark closely resembling the exudative form of age-related macular degeneration (AMD), a prevalent blinding condition of multifactorial etiology. To study the mechanism by which TIMP3 mutations lead to SFD-typical lesions, we have generated three Timp3-targeted mouse models including two knock-ins (Ser156Cys; Ser156Met) and one knock-out (del exon 3). Here, we are interested in the role of TIMP3 as a potent inhibitor of angiogenesis.
Material and Methods: A number of in vitro and in vivo assays are used including the tube formation, the mouse aortic ring and the chick chorioallantoic membrane assays. Protein analysis is done by Western blotting and immunoprecipitation.
Results: In vitro and in vivo assays allow us to quantitate a vascular response subject to (i) a complete lack of Timp3 (knock-out), (ii) the SFD-related mutation Ser156Cys and (iii) the missense mutation Ser156Cys which so far has unclear consequences on the human phenotype. Further, we are addressing the ramifications of Timp3 mutations Ser156Cys and Ser156Met on binding of the vascular endothelial growth factor (VEGF) to its receptor, VEGFR2 and the downstream effects on angiogenesis. In particular, we are investigating VEGFR2 autophosphorylation as well as downstream signalling molecules of VEGFR2. Additionally, we are evaluating the AKT/NO pathway critical for VEGF-induced endothelial cell migration, proliferation and tube formation.
Conclusions: The analysis of TIMP3 mutant protein in tissue explants and cell lines derived from gene-targeted mice allows an unbiased assessment free of artefacts often encountered with overexpressed protein. Understanding the angiogenic properties of TIMP3 may contribute to develop novel therapies in retinal diseases with sight-threatening complications such as choroidal neovascularization

P077

Analysis of subunit assembly in bestrophin, the protein mutated in Best vitelliform macular dystrophy

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Objectives: The VMD2 gene is mutated in Best vitelliform macular dystrophy (BMD), an early-onset autosomal dominant disorder characterized by deposits of lipofuscin-like material in the subretinal space. Functionally, VMD2 has been suggested to encode a putative Ca2+-dependent chloride channel, termed bestrophin. The number of subunits forming the Cl-channel are estimated at 4 or 5. With the first N-terminal half of bestrophin there is a high sequence identity to the three family members VMD2L1, VMD2L2 and VMD2L3 suggesting similar functional properties for this recently identified protein family. Little is known about the molecular mechanism underlying the more than 90 distinct mutations associated with BMD. With this study, we aim to identify the structural properties of bestrophin required for oligomerization and functional channel formation in wild type and mutated protein.
Material and Methods: C-terminal truncated wild-type and mutant VMD2 constructs including tagged epitope sequences were cloned and cotransfected with wt VMD2 in EBNA cells. Similarly, constructs containing tagged proteins of the bestrophin family members were generated. Solubilized protein was co-immunoprecipitated with a polyclonal VMD2 antibody.
Results: The oligomerization complexes obtained by co-immunoprecipitation were analyzed for their ability to bind the respective mutant and wild type subunits. The bestrophin-like family members were analyzed similarly. Distinction of the protein subfragments was achieved by Western blotting using the different tag epitopes. We show that the full-length but also C-terminally truncated bestrophin co-precipitates with itself but also with its family members.
Conclusions: Our results demonstrate that the N-terminus of bestrophin may be critical for subunit binding. Channel formation may also include family members. Subsequent analyses of mutant bestrophin species will address a possible influence of the mutations on functional subunit assembly.

P078

Transgenic studies on physiological function of testicular insulin

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Transgenic studies in our group revealed that the Leydig insulin-like hormone (Insl3) produced in pancreatic β-cells is able to restore the cryptorchidism phenotype in Insl3- deficient mice. These results demonstrate regulated secretory mechanisms of processed proInsl3 to mature Insl3 by proteolytic cleavage of C-peptide in β-cells of the pancreas. In this report, we investigated the efficiency of testicular Leydig cells to secrete mature insulin and the functional consequences of overexpression of human insulin in Leydig cells. To address that, we have generated three transgenic mouse lines expressing the human insulin gene under the control of the Insl3 promoter (I3I2). Northern blot analysis showed that more than ten times insulin mRNA is present in testis than in pancreas of transgenic mice. Immunhistochemical analysis revealed that the translation of the human insulin is restricted to Leydig cells. To determine the efficiency of the testicular human insulin to rescue diabetes development in Pax4-deficient mice resulting from the developmental impairment of β-cells, we introduced the I3I2-transgenic allele in the genome of Pax4-/- mice. Analyses of I3I2: Pax4 double transgenic mice revealed that testicular human insulin failed to compensate the deficiency of pancreatic insulin. We have then determined the level of the human proinsulin in testis and pancreas and the level of human C-peptide in serum of transgenic mice. The level of secreted human C-peptide in serum was found to be significantly lower than that of murine C-peptide in serum of wild-type mice. These results suggest that Leydig cells are not able to process the proinsulin efficiently and/or to regulate insulin secretion. The analysis of underlying factors is in progress.

P079

Identification of a ß-intercalated cell-specific promoter

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AE4 is an anion-exchanger located in ß-intercalated cells of renal collecting ducts, which are specialized for the secretion of HCO3- into the urine. It was suggested that AE4 serves as apical anion-exchanger. We report cloning and transcription analysis of the mouse homologue of AE4. Northern analysis showed expression of Ae4 exclusively in kidney. With a 5'-RACE approach 4 additional transcription start points were identified and subsequently verified by Northern analysis. Constructs with genomic sequences located immediately 5' of the individual transcriptional start sites displayed transcription activation properties in a cell-based reporter gene-assay. A ß-galactosidase (lacZ) cassette driven by the promoter of full-length Ae4 cDNA was introduced into the mouse genome by pronucleus injection. In transgenic mice, lacZ expression was confined to the kidney. Histochemical analysis showed expression of the reporter gene exclusively in ß-intercalated cells indicating that the promoter construct reproduces endogenous Ae4 expression. To our knowledge this is the first promoter identified with specificity for ß-intercalated cells, thus providing a tool for expression of transgenes specifically in ß-intercalated cells.

P080

The forkhead transcription factor Foxi1 regulates AE4 expression

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AE4 belongs to the gene family of sodium-independent anion-exchangers, which transport intracellular chloride out of the cell in exchange of extracellular bicarbonate, thereby functioning as acid-loaders. The expression of AE4 is restricted to ß-intercalated cells in the kidney. The knockout mouse of the forkhead transcription factor Foxi1 shows a disturbed differentiation of intercalated cells with the absence of several intercalated cell markers, including AE4. We addressed the question whether AE4 is a direct target gene of Foxi1. Compared to a control plasmid, co-transfection of Foxi1 cDNA and an Ae4 promoter construct resulted in a more than 100-fold activation in a cell-based reporter–gene assay. Truncation of the Ae4 promoter at the 5'-end revealed that a fragment of about 450 bp upstream the transcription start point is sufficient to mediate activation by Foxi1. Sequence analysis identified a total of 9 potential binding sites for Foxi1 in this region in both sense and antisense orientation. Only one element was bound by recombinant Foxi1 protein in bandshift assays. Mutation of this binding site abolished both binding in bandshift assays and transcriptional activation by co-transfection of Foxi1 in the reporter–gene assay. We thus identified Ae4 as a direct target gene of Foxi1.

P081

Microarray-based comparative genomic hybridization with microdissection derived DNA-probes

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Comparative genomic hybridization (CGH) is a well established technique in molecular cytogenetics for the identification of chromosomal copy number changes. This approach is used mainly in the field of tumor cytogenetics but also to study imbalances in pre- and postnatal genomes. In array CGH the metaphase chromosomes are replaced by immobilized DNA fragments (e.g., BAC-, PAC-, YAC-clones, oligonucleotids, cosmid, or cDNA probes) leading to a high genomic resolution. Gains and losses of larger genomic regions do have clinical relevance as shown by conventional CGH. To create a routinely applicable CGH-array for a fast detection of chromosomal imbalancies (e.g., in tumor samples) we used microdissection derived chromosome fragments as probes covering the whole human genome. So far 120 DNA-probes are spotted on Epoxy-slides (Nexterion slide E, SCHOTT Nexterion) after the amplification by DOP-PCR. In first experiments we could demonstrate loss of chromosome 7 in a tumor sample and the imbalances in chromosome X between male and female genomic DNA. Current work focus on optimization of signal to background ratio, data calculation and extension of the probe number on the array. The sensitivity of this rou-

tinely applicable CGH-array is being investigated.

P082

Sensitivity and specificity of a MAPH assay for the detection of subtelomeric copy number changes - Retrospective and prospective studies of patients with mental retardation of unknown aetiology

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Subtelomeric chromosome rearrangements are an important cause of mental retardation. Since the most common detection method, fluorescence in situ hybridisation / FISH, is expensive and time consuming, alternative testing methods have been pursued. Here, we tested a multiplex amplifiable probe hybridisation (MAPH) assay as a high throughput technique for the detection of copy number changes of all euchromatic chromosome ends.

The probe set consisted of 42 probes for all relevant subtelomeres except 16q. The sensitivity and probe reliability were determined in a retrospective analysis of 59 negative and 7 positive controls (4 deletions, 1 duplication-polymorphism, 2 unbalanced translocations) previously ascertained by FISH as well as prospectively on 10 samples. Each sample was tested in duplicate and the standard deviation (SD) of "normal" probes (normalised ratios between 0.8 and 1.2) within each patient was calculated. In order to combine reliable detection with low numbers of false positive results, we simultaneously applied two thresholds (SD times two and three, respectively - "2SD","3SD") of different stringency for calling a potential aberration.

5 probes were excluded from the analysis because of an SD >0.15 in more than 30% of the experiments (1p, 5p, 22q), undefined peaks (20q) or undetected copy number differences between males and females (Xp).

No false negative results were observed using both 2SD and 3SD, demonstrating the high sensitivity of the method. The false positive rates were 1.01% (2SD) and 0.05% (3SD). The number of retests (e. g. because a single probe's SD was >0.15 in one of the duplicate assays) was 0.32% / 0.05%, respectively. Thus, only 0.5 subtelomeric regions per sample (2SD) or 0.04 regions per sample (3SD) had to be verified by FISH.

Our results demonstrate that MAPH is a highly sensitive method suitable for high throughput screening of subtelomeric copy numbers and can be useful in reducing the FISH workload by at least 98

P05 Cancer Genetics

P083

Discovery and identification of a low abundant tumor derived serum markers in colorectal cancer by ProteinChip technology (SELDI)

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Although colorectal cancer is one of the best characterized tumors with regard to the multistep genetic progression it remains one of the most frequent and deadly neoplasms in the western countries. This is mainly due to the fact that up to clinically relevant serum markers could not be established at all in an early routine diagnostic.

In our study, we comparatively analysed microdissected normal and tumorous colonic epithelium by ProteinChip technology to detect and localize proteins specific for the tumor directly in the tissue which would certainly be of a very low concentration in serum. By this approach we found and identified one protein which was more highly expressed in the tumor than in normal epithelium and one protein expressed more highly in normal colonic epithelium, which could also be confirmed by immunohistochemistry (IHC). Detection of these peptides in the corresponding serum samples was subsequently performed with ELISA resulting in a high sensitivity and specificity for diagnosis. The direct analysis of microdissected tissue for the discovery of tumor specific markers followed by the specific detection of those markers by antibody based methods proved to be a successful strategy in this study. So we can conclude that these promising markers would not have been found in serum without the information gained through the analysis of microdissected tissue by ProteinChip technology.

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P084

Differential sensitivity to doxorubicin-induced cardiotoxicity in two inbred mouse strains

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Anthracyclines are well established as highly efficacious antineoplastic agents for various haematopoietic and solid tumours. Heart failure following anthracycline therapy is a major clinical problem in cancer treatment. Several lines of evidence indicate that the individual genetic make-up may be a major determinant of the individual sensitivity to anthracyclines-induced cardiotoxicity. However, nothing is known about the identity of the underlying genes and variants thereof. We investigated doxorubicin-induced cardiotoxicity in inbred mouse strains by means of echocardiography. We confirmed C57Bl/6 mice as doxorubicine-sensitive whereas Balb/c mice turned out to be essentially insensitive. Gene expression analyses in the hearts of doxorubicin-treated mice revealed differentially expressed genes. Among them was Carp (cardiac adriamycin-responsive protein), which had been previously implicated in doxorubicin-induced heart failure. Doxorubicin accumulation was higher in hearts of C57BL/6 mice in comparison to Balb/c mice, which may have contributed to cardiotoxicity observed in this strain. F1 animals from a C57Bl/6xBalb/c cross were insensitive to doxorubicin, indicating that the sensitivity of C57Bl/6 mice is caused by a recessive locus (or loci). This data provide a basis for the identification by means of QTL of the locus/loci underlying doxorubicin sensitivity, currently in progress. The identification of these loci may allow in the long term for development of less toxic treatments with anthracyclines.

P085

Evaluation einer unklassifizierten Variante des BRCA1-Genes in einer Hochrisikofamilie für Mamma- und Ovarialkarzinom durch LOH- und Segregationsanalyse

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Thema: In einer Hochrisikofamilie für Mamma- und Ovarialkarzinom identifizierten wir im BRCA1-Gen die unklassifizierte Variante 5628 T>C, W1837R. Zur Differenzierung zwischen einem Polymorphismus und einer pathogenen Mutation führten wir LOH- und Segregationsanalysen durch.

Methoden: Zur Mutationssuche in den Genen BRCA1 und BRCA2 erfolgte ein Vorscreening mittels DHPLC-Methode mit anschließender direkter Sequenzierung der auffälligen PCR-Fragmente. Blutproben wurden von der Indexpatientin, ihrer ebenfalls erkrankten Tochter und einer nicht erkrankten, erstgradigen Verwandten mit fortgeschrittenem Lebensalter zur Durchführung einer Segregationsanalyse abgenommen. Es standen zusätzlich Paraffinblöcke von Tumorgewebe zweier Mammakarzinome und eines Ovarialkarzinoms zur Verfügung. Im Anschluß an die DNA-Präparation wurde die LOH-Analyse mittels Fragmentanalye durchgeführt.

Ergebnisse: Die Segregationsanalyse zeigte das Voliegen der unklassifizierten Variante bei beiden der an Mamma- und/oder Ovarialkarzinom erkrankten Frauen, während bei der gesunden, 76-jährigen Verwandten die Genveränderung nicht nachweisbar war. Das Ergebnis der LOH-

Analyse demonstrierte in zwei der Tumore einen Heterozyotieverlust im Bereich des Aminosäurenaustausches. Der Befund des zweiten Mammakarzinoms der Tochter der Indexpatientin war mit Normalgewebe vergleichbar.
Diskussion: Studien über die Häufigkeit der Genvariante in der Allgemeinbevölkerung und biochemische Tests weisen bereits auf eine pathogene Rolle der untersuchten unklassifizierten Variante W1837R hin. Der beschriebene Aminosäureaustausch erfolgt in einem funktionell bedeutsamen Bereich am C-terminalen Ende des BRCA1-Proteins. Unsere Ergebnisse unterstützen den Verdacht auf das Vorliegen einer prädisponierenden Mutation. Der fehlende Heterozygotieverlust des zweiten Mammakarzinoms könnte auf eine durch LOH nicht erfaßbare Tumorgenese, z.B. Methylierung zurückgeführt werden.

P086

Downregulation of the DICE1 gene by CpG hypermethylation in the promoter region
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The critical region of loss of heterozygosity on human chromosome 13q14 harbors the tumor suppressor gene DICE1 (DDX26). 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. Promoter activity was identified within three overlapping fragments of the 800 bp sequence upstream of the DICE1 gene using a β-galactosidase reporter assay. This DICE1 promoter binds proteins from HeLa nuclear extract enriched for transcriptional factors as could be shown by retardation in a gel shift assay. A 13 bp deletion polymorphism detected in a variant DICE1 promoter exhibited a decreased activity compared with the undeleted promoter. This 13 bp deletion was detected in healthy control samples and patients with prostate cancer in similar frequencies. A reduced DICE1 expression was observed in prostate cancer cell lines DU145 and LNCaP. This downregulation of DICE1 is associated with hypermethylation of the promoter region. Treatment of both prostate cancer cell lines with 5-azacytidine leads to upregulation of DICE1 expression. From these results we conclude that transcriptional repression of DICE1 is caused by promoter hypermethylation of the DICE1 promoter region in prostate cancer cells. These results emphasize the significance of DICE1 inactivation in the pathogenesis of prostate cancer.

P087

The CALM/AF10 Fusion: Molecular Analysis of the Fusion Transcripts in 16 Cases of AML and ALL; Gene Expression Profiling Reveals HOX Gene Deregulation
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The t(10;11)(p13;q14) is a recurring translocation associated with the CALM/AF10 fusion gene which is found in undifferentiated leukemia, acute myeloid leukemia, acute lymphoblastic leukemia and malignant lymphoma with poor prognosis. The CALM/AF10 fusion protein was reported to be the most common fusion protein in T-ALL with TCR gd rearrangement. We have analyzed samples from 12 patients with different types of leukemia and a t(10;11) translocation suggesting a CALM/AF10-rearrangement. The samples were analyzed for the presence of the CALM/AF10 and AF10/CALM fusion mRNA by RT-PCR and sequence analysis. All these patients were found positive for the CALM/AF10 fusion. In addition, we analyzed a series of twenty-nine patients with T-ALL with gd rearrangement. Among these patients, four were positive for CALM/AF10 transcripts, indicating a high incidence of CALM/AF10 fusions in this group of leukemia. We found three different breakpoints in CALM at nucleotide 1926, 2091 and a new exon, with 106 bases inserted after nt 2064 of CALM. In AF10 four breakpoints were identified: at nucleotide position 424, 589, 883 and 979. In seven patients it was also possible to amplify the reciprocal AF10/CALM fusion transcript. There was no correlation between disease phenotype and breakpoint location. Ten samples were analyzed using oligonucleotide microarrays representing 33,000 different genes (U133 set, Affymetrix). Analysis of microarray gene expression signatures revealed high expression levels of the polycomb group gene BMI1, the homeobox gene MEIS1 and the HOXA cluster genes HOXA4, HOXA5, HOXA7, HOXA9, and HOXA10. The overexpression of HOX genes seen in these CALM/AF10 positive leukemias is reminiscent of the pattern seen in leukemias with rearrangements of the MLL gene, normal karyotypes and complex aberrant karyotypes suggesting a common effector pathway (i.e. HOX gene deregulation) for these diverse leukemias.

P088

Process of genetic diagnostics at cancer patients - analysis of medical records
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Purpose: Genetic factors are likely to be the primary determinants of the cancer seen in around 5-10% of all cancer patients. For some of these cases the possibility exists to accomplish molecular genetic diagnostic (mutation analysis). The detection of a mutation at a diseased person (diagnostic testing) is on the one hand precondition for offering special surveillance for this person and on the other hand pre-condition for genetic diagnostic in relatives (predictive testing). Therefore purpose of investigation was to describe the implementation of genetic diagnostics in the clinical care of cancer patients.
Methods: In order to represent the real workflows in the genetic care of cancer patients, a non reactive method of measurement was used – a retrospective analysis of medical records. In doing so existing medical records from cancer patients that frequented a human geneticist between 2002 and 2004 in two settings were analysed. So the total sample (n=96) consist out of two sub samples: records from an office of a human geneticist (n=57) and from a human genetic department at the university (n=39). Diagnostic testing sub sample (n=61) contained different cancer sites at the patients: mainly colorectal cancers (FAP and HNPCC) and breast cancer. 35 non-affected relatives represent the predictive testing sub sample.
Results: Data for waiting times, referral requests to the geneticist and length of singular sessions (first counselling, blood withdrawal and communication of diagnostic findings) are available. Further data of the involvement of psychosocial professionals were presented.
Conclusion: First of all findings show that most of the patients carry out the whole process of genetic diagnostics (only a minor part of dropouts). Reviewing the shown results it is to emphasize that this study had an explorative character. Further investigation is required e.g. in order to derive appropriate modes of cooperation between primary care physicians and human geneticists.

P089

Molecular-cytogenetic analysis of two cases of leiomyosarcoma
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Leiomyosarcoma is a malignant tumour composed of cells showing distinct smooth muscle features. Five to 10% of all soft tissue tumours are leiomyosarcomas. The most common locations are the gastrointestinal tract, retroperitoneum and uterus. Children with leiomyosarcoma are very rare.
Cytogenetic studies of this entity are rare. So far, only about 100 patients with leiomyosarcomas have been analysed by conventional cytogenetic methods. Most karyotypes are complex without consistent aberrations. We performed detailed karyotypic analysis with comparative genomic hybridisation (CGH) of two patients with leiomyosarcoma. Case 1 was a 61 years old woman with a leiomyosarcoma of the vena cava, grade III. Case 2 was a 6 years old girl with a histological characterized epithelioid leiomyosarcoma of the jaw, grade III. She had three relapses localised in the jaw and the tongue and died three years after primary diagnosis. In case 1 we detected a very complex aberrant karyotype with losses of the long arm of chromosome 2 and 13, gains of chromosome 1 and the short arm of chromosome 17 as well as amplification of the whole chromosome X, which are commonly reported in leiomyosarcomas. Beside these alterations case 1 revealed losses of the whole chromosome 9, the long arms of chromosomes 16 and 18 and the short arm of chromosome 20 as well as gains of the long arm of chromosome 11 and the short arm of chromosome 18, which represent novel observations in leiomyosarcomas. Although, case 2 had a very fulminant course of disease, we could not detect any cytogenetic aberration.

Our results of case 1 confirm that karyotypes of adult leiomyosarcomas are very complex and indicate (case 2) that the pathogenesis between children and adults may be different. Additionally, this is the first report of a girl with a leiomyosarcoma of the jaw.

P090

The Codon 242 G/A polymorphism in the CCND1 gene is not associated with age of disease onset in mantle cell lymphoma with IGH/CCND1 fusion

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The CCND1 gene encodes Cyclin D1, a major regulator of the cell cycle transition from G1 to S phase. A single nucleotide polymorphism (G to A) at codon 242 of CCND1, the boundary of exon 4 and intron 4, results in a splice variant of Cyclin D1 with loss of exon 5 in the A allele. Since exon 5 is involved in rapid turnover, the variant cyclin D1 protein corresponding to the A allele may have a longer half-life. Previous studies have demonstrated that the age of onset of both hereditary nonpolyposis colorectal cancer and hepatoblastoma is associated with this polymorphism. The present study aimed to find out whether this CCND1 polymorphism influences the development and progression of mantle cell lymphoma (MCL) in a similar way. This subtype of lymphoma is characterized by overexpression of Cyclin D1 resulting from a t(11;14)(q13;q32), whereby the CCND1 gene is juxtaposed to the IGH locus. We analyzed tumor DNA of 98 patients with mantle cell lymphoma. The age of diagnosis varied between 30 and 81 years. The codon 242 polymorphism was detected by PCR-based restriction fragment length polymorphism (RFLP) analyses. The allele frequencies were 47% for the A-allele and 53% for the G-allele. 22 cases showed the genotype AA, 27 cases the genotype GG and 49 samples were heterozygous. Subsequent statistical analysis failed to detect any association of one of the alleles or genotypes of the described CCND1 polymorphism with the age of onset of MCL. In summary, there was no evidence for the CCND1 SNP analyzed in the present study to influence the age of onset of disease in mantle cell lymphoma.

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P091

The Four and a Half LIM domain protein FHL2 interats with CALM

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The balanced t(10;11)(p13;q14) translocation results in the CALM/AF10 fusion gene. This translocation is found in acute myeloid leukemia (AML), T-cell acute lymphoblastic leukemia (ALL) and malignant lymphoma. CALM (Clathrin Assembly Lymphoid Myeloid Leukemia Protein) is a clathrin assembly protein which plays role in clathrin mediated endocytosis and trans Golgi network trafficking. CALM is a member of the growing family of endocytic proteins which are found altered in tumors. AF10 is a putative transcription factor that is probably involved in processes related to chromatin organization and appears to have polycomb group gene like properties. To learn more about the function of CALM, a yeast two hybrid assay was performed. Seven putative protein interaction partners of CALM were detected: FLNA (filamin A), FABP4 (fatty acid binding protein), PCBP1 (poly (rC) binding protein 1), DPP7 (dipeptidyl-peptidase 7), CALM, CATS (CALM interacting protein expressed in Thymus and spleen) and FHL2 (Four and a half LIM domain protein 2). The interactions were confirmed by co-transformation assay in yeast. For both CALM-CALM and CALM-FHL2 interactions the mapping of interaction domains using CALM deletion constructs is in process. FHL2 was shown to interact with PLZF (promyelocytic leukemia zinc finger protein) and the proto-oncogene SKII that has a role in the Wnt signalling pathway. FHL2 transmits Rho signals from the cell membrane to the nucleus, and is a coactivator for the CREM/CREB transcription factors (Fimia et al, 1999). Vecchi et al .(2001) demonstrated that inhibition of CREM-mediated nuclear export leads to the accumulation of CALM in the nucleus and that CALM has transcriptional activator properties, suggesting participation of CALM in nuclear events, possibly including regulation of transcription via FHL2 interaction. We are currently performing co-localization studies of CALM and FHL2 and are testing whether FHL2 is a transcriptional activator for CALM.

P092

LOH within the genomic region of the pseudoautosomal protein phosphatase gene PPP2R3B in breast cancer tissue

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Breast cancer is the most common malignancy to affect women. Roughly 5 % of all cases are familial forms due to mutations in genes such as BRCA1 and BRCA2, while up to 95 % of cases are thought to be sporadic. Cytogenetic investigations and microsatellite loss of heterozygositiy (LOH) analysis have revealed that mamma carcinoma cells exhibit deletions in the terminal part of Xp. We chose a MALDI-TOF-MS approach to analyze the genomic region of PPP2R3B located in the terminal band Xp22.3. Due to its potential function as a regulator of initiation of replication and its localization in the terminal part of the X-chromosome within the pseudoautosomal region it is a potential tumor suppressor gene. PPP2R3B has been characterized as a variable regulatory subunit of the protein phosphatase 2A (PP2A). PP2A is a trimeric enzyme consisting of a catalytic, a constant regulatory and a variable regulatory subunit. PP2A has long been known as a tumor promoter, and some of its subunit genes have been discussed as potential tumor suppressor genes. SNPs are very frequent in the genomic region of PPP2R3B. The identification of 13 SNPs within a genomic region of about 25 kbp therefore offered a set of polymorphic markers for analysis of LOH. DNA analysis of 29 patients revealed that three patients had lost one allele. Two patients exhibited LOH at least in the region spanning exon 11 to exon 12, the third one has a deletion extending at least from exon 2 to the 3'flanking region of the PPP2R3B gene. Sequencing of exons did not result in the identification of an aberration in the remaining allele as it would be expected for a classical tumor suppressor gene. But recently, it was shown that haploinsufficiency of tumor suppressor genes may also lead to carcinogenesis.

P093

Frequent aberrant methylation of tumor-related genes in thyroid cancer

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Methylation profiling of tumor-related gene promoter regions in thyroid cancer has not been reported. We assessed the methylation of 17 cancer-related genes by MSP in nine thyroid cancer cell lines. 12 of 17 genes (RASSF1A, p16INK4A, TSHR, MGMT, DAPK, ERa, ERß, RARß, PTEN, CD26, SLC5A8 and UCHL1) showed frequent hypermethylation (25-100%) in the cancer cell lines and were furthermore analyzed in 38 primary thyroid carcinomas (6 medullary MTC, 10 follicular FTC, 13 papillary PTC, 8 undifferentiated UTC and 1 poorly differentiated thyroid carcinoma PDTC), 12 struma nodosa colloides, 10 follicular adenoma and 14 normal thyroid tissue probes. UTC showed in several genes (p16INK4A, TSHR, MGMT, DAPK and UCHL1) a significant higher methylation rate compared to MTC's, FTC's, PTC's, struma nodosa colloides and normal thyroid tissue (UTC vs. MTC, UTC vs. FTC etc., $p<0,05$). Moreover, in 5 of 8 UTC's (62,5%) we found a simultaneous promoter methylation among a great number of analyzed candidate genes (5 to 9 genes), whereas only 15,3% (2/13) of PTC's, 8,3% (1/12) of struma nodosa colloides, 10% (1/10) of follicular adenomas and none of MTC's, FTC's and normal thyroid showed such a high number of concurrent methylated genes (UTC vs. PTC, UTC vs. struma etc., $p<0,03$). In our study the partial methylated cases were additional semiquantified in low methylated (<50%) and strong methylated (=50%) probes. The primary thyroid carcinoma had the highest rate of strong till complete methylated probes (44/106, 41,5%) among all analyzed genes. Compared to it normal thyroid tissues showed in only 12% (3/25) of methylated cases a strong pattern. For RASSF1A the older tumor patients (>50 years) had significant higher frequence of methylation than younger one (83,3% vs. 50%, $p=0,029$). There was no correlation of methylation rate in analyzed genes to gender, tumor stage, local nodes and distant metastasis of investigated patients. This work was supported by the BMBF grant (NBL3-FKZ 01ZZ0104).

P094

Identification of a novel germline mutation of the MEN1 gene in a family with MEN1

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Introduction: Multiple endocrine neoplasia type 1 (MEN1) is an autosomal-dominant inherited disease with over 95% penetrance. It is characterized by tumors of parathyroid glands, endocrine pancreas and anterior pituitary gland.
Patient and family: The index case is a 36-year-old patient from Turkey, suffered from bone pain, nausea and emesis. An endocrine examination confirmed a primary hyperparathyroidism (pHPT).
His medical record showed that a transsphenoidal adenomectomy was performed four years ago (chromophobic adenoma, PRL immunopositive). Two years ago, multiple neuroendocrine tumors of the pancreas were dissected and confirmed as carcinoids. He also had a medical history of bleeding gastric ulcers and elevated gastrin levels.
The patient has two brothers and two sons, who had shown no symptoms of MEN1 until then. Genetic testing for the novel mutation was done on these family members subsequent to the results from the patient.
Methods: Genomic DNA was extracted from peripheral blood leukocytes, followed by PCR amplification of the coding exons (2-10 for the patient, and 3 for the family members) of the MEN1 gene, including corresponding exon-intron boundaries. PCR products were sequenced directly.
Results: Sequence analysis of the MEN1 gene shows a novel heterozygous germline mutation in exon 3, codon 199 of the patient and his 4-year-old son. This mutation, a 1bp deletion (705delC), results in a truncated and inactive menin protein due to a premature termination codon at position 223.
Conclusion: Genetic counseling and careful genetic testing of the MEN1 gene should be done in these families of sporadic MEN1 cases, to confirm the diagnosis and define presymptomatic gene carriers. Periodic screening should be carried out for endocrine tumor manifestation, as we do with the son of the patient, for whom pHPT was diagnosed in the meantime.

P095

Specific detection of Flt3 point mutations by highly sensitive realtime PCR in acute myeloid leukemia

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Among activating Flt3 mutations internal tandem duplications of Flt3 (Flt3-ITD) are detected in about 25% of patients with acute myeloid leukemia (AML). In contrast, mutations within the tyrosine kinase domain of Flt3 (Flt3-TKD mutations) are less frequent (approximately 7%) while there are only few data on frequency of recently demonstrated activating Flt3 point mutation at codon 592 (Flt3-V592A mutation).
We evaluated a new approach for rapid screening of Flt3-TKD and Flt3-V592A mutations using the FRET (fluorescence resonance energy transfer) principle in 122 patients. Based on individual Flt3-TKD mutations we designed patient specific primers to perform a highly sensitive polymerase chain reaction (PCR) assay for rapid detection minimal residual disease (MRD).Furthermore, a model system using MonoMac-6 cells carrying the Flt3-V592A mutation was used to establish a mutation specific real- time-PCR approach also for this molecular aberration. We identified nine patients (8%) with Flt3-TKD mutations (5 pts. D835Y, 3 pts. D835H, 1 pt. Del836), while none of the 122 patients we investigated had a Flt3-V592A mutation. Screening for Flt3-TKD mutations with fluorescent probes is equivalent with conventional screening using standard PCR followed by EcoRV restriction. Furthermore, we present a real-time PCR protocol that can be used for MRD analyses based on individual Flt3-TKD mutations. Examples for MRD analyses are presented for all three subtypes of Flt3-TKD mutations that were identified in this study. In summary, we demonstrate new methodological approaches for rapid screening of Flt3 point mutations as well as the detection of MRD based on patient specific Flt3-TKD mutations.

P096

Different molecular pathways in the development of periocular sebaceous gland carcinomas

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Periocular sebaceous gland carcinomas (SGC) are rare malignant skin tumors occurring sporadically or as a phenotypic feature of the Muir-Torre syndrome (OMIM #158320). The fragile histidine triad gene (FHIT) encompassing the most active common human chromosomal fragile region, FRA3B, was proposed as a tumor supressor gene for many human cancers. Fhit deficient knockout mice develop Muir-Torre-like symptoms including SGC. In the current study, periocular sebaceous gland carcinoma obtained from three patients (male, * 1961; male *1960; male *1944) were analyzed for FHIT, hMSH2, hMLH1 and hMSH6 expression by immunochemistry (IHC). Polymerase chain reaction (PCR)-based analysis of the markers BAT25, BAT26, BAT40, D2S123, D5S346 and D17S250 was performed for microsatellite instabilty (MSI). FHIT expression was detectable in one SGC with high-grade microsatellite instability (MSI-H) accompanied by loss of MSH2 immunostaining. The two other tumors, which were negative for FHIT as revealed by IHC, demonstrated microsatellite stability (MSS). To explore the mechanism responsible for loss of FHIT, we studied loss of heterozygosity (LOH) at 3p14.2 by utilization of a panel of intragenic FHIT specific CA-markers, genomic multiplex PCRs for combinations of FHIT encoding exons and by methylation specific analyses of the FHIT promoter including MSP and COBRA. Our data demonstrated complete methylation of the FHIT transcription regulatory region in one case. In the other SGC loss of FHIT protein appeared to be due to intragenic deletions affecting coding exons. Hence, either inactivation of the FHIT gene associated with MSS or inactivation of the mismatch-repair system resulting in microsatellite instability may contribute to the development of periocular sebaceous gland carcinomas.

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P097

Genetik and epigenetic alterations of tumor-related genes in soft tissue sarcoma

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A main mechanism of carcinogenesis is tumor suppressor gene inactivation caused by aberrant methylation of promoter CpG islands. In this study, the methylation status of RASSF1A, p16, MLH1, MSH2 and ERalpha was investigated in 84 primary soft tissue sarcomas (STSs), including 22 liposarcomas, 18 malignant fibrous histiocytomas (MFHs), 18 leiomyosarcomas, 6 rhabdomyosarcomas, 6 neurogenic sarcomas and several other sarcoma entities. RASSF1A hypermethylation was detected in 17 of 84 (20%) STSs; however, methylation was more frequent in leiomyosarcomas (39%) compared to MFHs (6%; $p < 0.015$) and liposarcomas (18%). The p16 CpG island was methylated in 22 out of 82 (27%) cases. In 7 out of 81 (9%) STS samples, the promoter of MLH1 was methylated and in liposarcoma the methylation frequency was higher (14%). For MSH2, no hypermethylation was detected. Methylation of ERalpha was detected in 48 of 63 (76%) STSs, but also in 4 of 8 (50%) normal tissue samples. Furthermore, we analyzed mutational activation of K-ras and BRAF. In 4 out of 84 (5%) of STSs, a substitution at codon 599 of BRAF was found; however, no alteration of K-ras was detected. The samples have been analysed for the occurence of the RASSF1A polymorphism 133 (GCT>TCT). 8 out of 69 soft tissue samples showed this base change. Interestingly this substitution was detected more frequently in liposarcomas (4 out of 15; 26,7 %) compared to leiomyosarcomas (1 out of 16; 6,3 %). In an univariate Cox proportional-hazards regression model, we found that the risk of a tumor-related death for STS patients with methylated RASSF1A was significantly increased (RR = 2.9; $p = 0.037$). In summary, our data indicate that inactivation of RASSF1A is a common event in STS, especially in leiomyosarcoma. Thus, the methylation status of cancer-related genes was distinct in different STS and methylation of RASSF1A promoter can serve as prognostic marker in STSs.

This work was supported by BMBF, Land Sachsen-Anhalt and DFG.

P098

Communicating BRCA1 and BRCA2 test results: Data from a telephone interview with 332 women tested within the German Consortium on Hereditary Breast and Ovarian Cancer

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Objectives: In order to promote safe and effective testing for BRCA1 and BRCA2 mutations in clinical practice the German Consortium on Hereditary Breast and Ovarian Cancer (HBOC) has been established. To improve practice, evaluations based upon the views of patients who have undergone testing concerning the impact of the genetic diagnosis and the quality of the services they receive and require are undertaken.
Material and Methods: 332 women from HBOC families who had obtained their test results at least 6 months earlier were interviewed by telephone. The interview includes open and standardised questions.
Results: Almost all women (91%) had consulted at least one family member before undergoing testing. 10% reported conflicts with at least one family member about whether or not the test should be taken. The communication process about the test was characterized by selective and preferential information of female family members both before testing and after obtaining the test result. Women with breast cancer showed a greater tendency to inform their children than women without breast cancer. 10% were asked by at least one family member not to tell results. Mutation carriers reported more conflicts with other family members in communicating test results than non-carriers (22% vs. 3%, $p<0.05$).
Conclusions: Genetic counselling needs to raise awareness that both sexes may inherit the mutation and may benefit from information. Strategies need to be developed and evaluated regarding how to facilitate the dissemination of information within families without potentially 'overstraining' the 'messenger patient' at least when a mutation has been found.

P099

Molecular characterization of gross rearrangements in the BRCA1 and BRCA2 genes in a large cohort of hereditary breast and ovarian cancer cases of German origin

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Objectives: Disease-associated point mutations and small insertions/deletions in the BRCA1 and BRCA2 genes are found in up to 25 % of hereditary breast and ovarian cancer (HBOC) families in Germany. Thus far, the proportion of large genomic rearrangements in both BRCA1 and BRCA2 have not been determined in larger sample sizes.
Material and Methods: The multiplex ligation dependent probe amplification (MLPA) technique was used to investigate 226 index patients. Prior to this analysis, HBOC families recruited within the setting of the Deutsche Krebshilfe Familial Breast and Ovarian Cancer Centres at Wuerzburg and Muenster, were tested negative for small nucleotide alterations in BRCA1 and BRCA2.
Results: Six genomic rearrangements were identified in BRCA1 while no large alterations were found in BRCA2. The 6 BRCA1 mutations include two novel rearrangements with a deletion of exon 5 and a deletion comprising exons 5 to 7, as well as four previously described gross alterations including a deletion encompassing exons 1A, 1B and 2, two cases of duplications of exon 13 and a deletion of exon 17. We have defined the respective breakpoint regions on the sequence level. In all cases, crossing over events must have occurred between direct repeat sequences flanking the deleted regions. These direct repeat sequences range from 9-bp to 188-bp of perfect base pair matches (del exon 1 -2, del exon 5) while the breakpoint regions of the exon 5 to 7 deletion, the exon 13 duplication and the exon 17 deletion are flanked by Alu sequences.
Conclusions: The frequency of large genomic rearrangements in BRCA1 accounts for up to 3% (6/226) of germ-line predisposition mutations in our HBOC cohort of German ancestry. BRCA2 seems significantly less affected by gross deletions/insertions. We therefore recommend to include screening for germ-line rearrangements in the BRCA1 gene on a routine basis in high risk HBOC families.

P100

Aberrant splicing in MLH1 and MSH2 genes due to single base substitutions in exonic sequences

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Germline mutations in DNA mismatch repair genes, mainly MLH1 and MSH2, are the cause of hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome). While mutations leading to truncated proteins are considered to be pathogenic per se, the evaluation of single base substitutions predicted to result in missense or silent mutations or intronic variants outside the highly conserved splicing region remains difficult. There is growing evidence that some single base substitutions may have detrimental effects due to influence on splicing.
We evaluated the effect of several variants of unknown relevance by examination of mRNA. As our results show, the substitution MLH1,c.2103G>C in the last position of exon 18 does not result in a missense mutation as theoretically predicted (p.Gln701His), in fact it reduces splice efficiency and leads to a loss of exon 18. For the substitution MLH1,c.1731G>A (p.Ser577) at the last position of exon 15, which is predicted to be a silent mutation, our results demonstrate a complete skipping of exon 15. Thus, these two variants are pathogenic and allow a clear statement about cancer risks in the setting of predictive genetic testing for family members. In contrast, the substitution MSH2,c.1275A>G (p.Glu425) at the penultimate position of exon 7 results in activation of a cryptic splice site and only partial deletion of the last 16 codons of exon 7. Therefore, this variant cannot be classified as definitely pathogenic.
By computer programs consequences of single base substitutions on splicing can be predicted by theoretical calculation. For the variant MLH1,c.1731G>A (p.Ser577) only a slight influence on splicing was predicted which is not according to our result of mRNA-analysis. In conclusion RNA studies should be performed for each variant of unknown relevance prior to other functional tests at protein level in order to evaluate the pathogenicity of a mutation.
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P101

Characterization of splice site mutations in two patients with Hereditary Non-Polyposis Colorectal Cancer (HNPCC)

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HNPCC represents the most common entity of autosomal-dominant inherited colon cancer diseases. Mutations in the mismatch repair genes *MLH1*, *MSH2*, *MSH6* and *PMS2* lead to tumour development. These genes are responsible for the recognition and excision of mismatched bases and mutations in these genes give rise to a defect mismatch repair system (MMR) which is recognized by changes in the length of microsatellite loci (microsatellite instability, MSI). This MMR defect leads to an accumulation of mutations in several genes resulting in tumour development. We have analyzed the effect of 5'splice site mutations in the splice donor site in two HNPCC-patients. Sequencing revealed the following sequence changes: a) in patient 1: *MSH2* c.1276+1G>A at the splice donor site of exon 7 and b) in patient 2: *MLH1* c.677+3A>G at the splice donor site of exon 8. In order to characterize these two sequence changes the following experiments were performed: a) for patient 1: total RNA prepared from lymphocytes was reverse transcribed into cDNA and a subsequent RT-PCR was performed using specific primers of the adjacent exons. We could show that due to the mutation +1G>A splicing does not occur at this site, but another cryptic splice site in exon 7 was activated leading to skipping of 16 codons of exon 7 (in frame deletion); b) for patient 2: in this case we have designed minigene constructs, which were analysed in transient transfection assays using the cell line Hela T4+. After transfection, the RNA was isolated and analyzed using RT-PCR. We could show that due to the sequence change +3A>G, splicing does not occur at this site, leading to skipping of exon 8. Two further ±1 splice site mutations were also analyzed with RT-PCR of lymphocyte RNA or in transient transfection assays. These mutations resulted also in exon skipping. Seven missense mutations located in or close to putative ESE elements studied, had no influence on splicing.

P102

Association of a CAV-1 Haplotype to Familial Aggressive Prostate Cancer

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Objective: Multiple lines of evidence have implicated the CAV-1 gene in prostate cancer progression. CAV-1 is located within the prostate cancer aggressiveness locus at 7q31-33, and was identified as being overexpressed in prostate tumors.

Mutation screening was performed as well as a case-control study to examine if polymorphisms in CAV-1 are associated with prostate cancer aggressiveness in a German population.

Methods: We sequenced the CAV-1 promoter region and its open reading frame in prostate cancer families with linkage to chromosome 7q31-33. Additionally, 105 unrelated familial prostate cancer probands, 190 sporadic cases and 191 controls were genotyped at four intronic single nucleotide polymorphisms. Resulting haplotypes were tested for association using age at diagnosis, tumor grade, TNM stage, and follow up information to stratify for aggressive disease.

Results: No mutation was found in the CAV-1 coding region or in the promoter. One of the eleven observed haplotypes showed an increased frequency in cases with high tumor stage (p = 0.03).

Conclusion: This is the first report providing evidence for CAV-1 being involved in predisposition to aggressive prostate cancer. The association of a potential risk haplotype agrees well with a role of CAV-1 in tumor progression but needs further confirmation.

P103

HNPCC based on MSH6 mutations is characterized by later age of disease onset and lower incidence of colorectal cancer compared to MLH1 or MSH2 mutations: The German HNPCC-Consortium

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Germline mutations in the mismatch repair (MMR) gene MSH6 predispose to hereditary nonpolyposis colorectal cancer (HNPCC), but detailed characterisation of the involvement and phenotypic manifestations of MSH6 has been hampered by the limited number of identified mutations.

Patients were pre-selected by microsatellite instability, loss of protein expression and/or exclusion of MLH1 or MSH2 mutations, and subjected to MSH6 analysis. Clinical and molecular data of MSH6 mutation families were compared to data from families with MLH1 and MSH2 mutations.

We identified 24 different MSH6 germline mutations in 27 families. These families represented 3.8% of the total of families (n=706), and 14.7% of all families with MMR gene mutations (n=183). The median age of onset of colorectal cancer (54 years) in putative MSH6 mutation carriers was 10 years higher than in MLH1 and MSH2 mutation carriers (44 years). Colorectal cancer was statistically less frequent in MSH6 families compared to MLH1 and MSH2 families. In contrast, the frequency of non-HNPCC-associated tumors was increased.

Later age of disease onset and lower incidence of colorectal cancer may contribute to a lower proportion of identified MSH6 mutations in families suspected of HNPCC. However, in about half of these families at least one patient developed colorectal or endometrial cancer in the fourth decade of life. Therefore, a surveillance program as stringent as that for families with MLH1 or MSH2 mutations is recommended.

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P104

Matrix-CGH enables the identification of genomic imbalances in childhood MDS with monosomy 7

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Objectives: MDS is believed to originate from a hematopoietic stem stell, but it is not known which myeloid progenitor is targeted by genetic events leading to neoplastic transformation. The most common cytogenetic abnormality in MDS is monosomy 7 or del(7q). This is also true for pediatric MDS developing in the context of different inherited predispositions such as severe congenital neutropenia. The aim of this study in the context of the European working group on MDS in childhood (EWOG-MDS) is to clarify if monosomy 7 is the only chromosomal aberration or whether additional subtle chromosomal gains or losses are present.

Methods: Genomic profiling by means of matrix-/array-CGH, a molecular tool that allows the genome-wide screening of chromosomal imbalances was applied to ten cases of primary MDS. DNA isolated from granulocytes and from mononuclear cells (MNC) was compared to normal blood cells. A genome-wide resolution of 1 Mb and an even higher resolution of up to 100kb for recurrently aberrant regions in cancer as well as for regions containing known tumor suppressor genes and oncogenes was reached by the selection of 6251 individual BAC/PAC clones.

Results: Three different groups were detected: a first with the monosomy 7 solely, a second with few additional alterations like -5, -17p, -6q, +21, +22, +1p which were also detected by cytogenetics and a third group displaying several genomic abnormalities in addition to monosomy 7. Interestingly, the highly genomic instability observed in the third group was more extensive in MNC compared to granulocytes.

Conclusions: Matrix-CGH is a helpful tool to unmask the subtle underlying genetic alterations and to identify genes located in these regions leading to the development of MDS. The different genomic profiles may reflect the clinical heterogeneity of childhood MDS and have to be correlated with clinical data.

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P105

EGFR mutations in head and neck cancer

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Objectives: Somatic mutations in the tyrosine kinase domain of the epidermal growth factor receptor (EGFR) gene are associated with sensitivity to gefitinib (Iressa®) and present in at least 10% of non-small cell lung carcinoma (NSCLC) [LynchTL. et al., N Engl J Med 350, 2004; Paez JG. et al., Science 304:1497-1500, 2004]. Inhibition of activated tyrosine kinases with targeted small molecule drugs has emerged as an effective approach to cancer therapy. It was also observed in about 10% of head and neck squamous cell carcinoma (HNSCC) that gefitinib can lead to a rapid and often dramatic clinical response [Cohen EE. et al., J Clin Oncol. 21:1980-7, 2004, Shintani S., et al., Oral Oncol. 40:43-51, 2004]. Furthermore, EGFR is overexpressed in about 90% of all oral cancers [Grandis JR. Cancer Res 53, 1993].

Material and Methods: We searched for mutations in exons 19 and 21 of EGFR gene in the DNA of tumor samples from 79 patients with HNSCC. Among them, 6 patients are treated with gefitinib in an ongoing study. Furthermore, one patient with squamous cell carcinoma of the skin and known response to gefitinib was included. The DNA was extracted from paraffin embedded tumor tissues. PCR and sequencing was performed with primers already described in [LynchTL. et al., N Engl J Med 350, 2004].

Results: None of the already published mutations described to be associated with a response to gefitinib had been detected in this cohort of patients. In intron 19 two polymorphisms IVS19+69GA with 0.06 and IVS19+96AG with 0.2 allele frequency were found. In one patient a 1 bp deletion in intron 18 (IVS18-24delG) was observed. In exon 21 R836R occurred with 0.2 allele frequency.

Conclusions: In contrast to non-small cell lung carcinoma activating EGFR-mutations were not found in head and neck squamous cell carcino-

ma. The relevance of the intron 18 mutation has to be determined. Other molecular mechanisms than EGFR-mutations may influence the response to gefitinib in HNSCC.

P106

Protein profiling of Imatinib sensitive and resistant leukemic cell lines with ProteinChip technology (SELDI)

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The development of resistance to the tyrosine kinase inhibitor Imatinib (STI571, Glivec) is a widely spread problem during the therapy of chronic myeloid leukemia (CML). In many cases the resistance is caused by a mutation of the ATP binding site of BCR-ABL but also an overexpression of BCR-ABL protein can be relevant. However, there is a group of patients, where none of the just named mechanisms seems to be responsible for resistance. An experimental system for research of the background of such resistances is the cell line KCL22 R1, which is initially drug-resistant, and its sensitive counterpart KCL22 S. The protein profiles of these two cell lines were compared by ProteinChip technology (SELDI). Protein lysates were fractionated by pH-gradient and eluats were bound to three different chemical surfaces (IMAC30 Copper, CM10, H50). Fractionation allows the description of almost all proteins of the cell lines. In first experiments approximately 16 differentially expressed proteins were observed. Especially one protein with a molecular weight of 19 kDa was very prominent. This protein was only found in the fractions two and three of the resistant cell line and is potentially linked to the drug-resistance of KCL22 R1. Protein identification by trypsine digestion is currently in progress to get closer to function of the 19 kDa protein in the mechanism of drug-resistance to Imatinib.

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P107

Mutation screen and association study of the EZH2 gene in PCa patients

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Several linkage studies have provided evidence for a prostate cancer susceptibility gene on chromosome 7q. EZH2 (enhancer of zeste homolog 2) is a potential candidate gene for the development of aggressive prostate cancer because its location at 7q35 and its increased expression in metastatic prostate cancers. We genotyped eleven polymorphisms, spanning the entire EZH2 gene inclusively the promoter region, in genomic samples of 96 unrelated familial prostate cancer patients, 192 sporadic prostate cancer probands and 96 unaffected controls. We examined the polymorphisms individually for association with prostate cancer taking into account the TNMG classification (tumor staging, tumor grading) and the follow up of the patients without detecting an association. In addition, we analyzed the resulting haplotypes for association with prostate cancer with or without stratification according to clinical characteristics of prostate cancer. Using the program FAMHAP9 statistical significance of the polymorphisms and haplotypes was evaluated. Whereas no overall differences between cases and controls could be detected, two haplotypes showed an unequal distribution: One has a higher frequency in controls (11.5% versus 6.4%, p=0.042) and the other one that is mostly defined by complementary alleles is more frequent in cases with a favourable disease characteristic (e.g. GI/II or NED) (7.3% versus 3.2%, p=0.037). EZH2 is a polycomb group transcriptional repressor. Since upregulation of wildtype EZH2 promotes aggressive prostate cancer a beneficial effect could be expected from any genomic variant. We may have identified haplotypes which mark such alleles that reduce the transcriptional activity and therefore affect the development of the disease. This hypothesis can be tested by functional assays.

P108

Characterization of secondary alterations in mantle cell lymphomas using matrix-/ array-CGH

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Overexpression of Cyclin D1 due to the chromosomal translocation t(11;14)(q13;q32) alone is not sufficient to induce the development of mantle cell lymphoma (MCL). Secondary genetic alterations seem to be necessary for the malignant transformation and may determine the clinical course of the disease. In order to define chromosomal imbalances more precisely, we analyzed six t(11;14)-positive lymphoma cell lines (Granta-519, NCEB1, JeKo-1, Rec-1, SP-53 and HBL-2) by means of matrix-/array-based comparative genomic hybridization, a new molecular tool that allows the genome-wide screening of chromosomal imbalances. A genome-wide resolution of 1 Mb and an even higher resolution of up to 100kb for recurrently aberrant regions of MCL as well as for regions containing known tumor suppressor genes and oncogenes was reached by the selection of 6251 individual BAC/PAC clones. Based on the normalized fluorescence ratios computed as log2 values, we were able to detect amplifications as well as single gains and losses. The most frequent alterations were losses in 9p21.3, 2p11.2, 22q11.22, 1p21.1-p31.2, 13q14, 11q22, 6q21, 6q27, 17p13 and gains in 7p14.1, 7q11.2, 2q37.1, 8q24, 12q13 and 18q21. These results are in agreement with the recently published data from deLeeuw et al. (HMG 2004 Sep 1;13):1827-37). Up to now, losses of 2p11.22 (all cell lines except for JeKo-1) and 22q11.22 (all cell lines except for Rec-1) have not been known as recurrent aberrations of MCL. Selected chromosomal losses (p16, p53) and the amplification of BCL2 in Granta-519 were confirmed by means of fluorescence in situ hybridization using commercially available probes (Abbott Diagnostics). Matrix-/array CGH analyses enabled us to further delineate important regions of gain and loss like 13q14. In conclusion, the cell lines are excellent model systems which will facilitate the identification and characterization of novel genes which play important roles in the pathobiology of MCL.

P109

Haplotype-based analysis of BRCA1 and BRCA2 in German high risk breast and/or ovarian cancer families

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The 'Berlin Center for Hereditary Breast and Ovarian Cancer' as one of twelve centers of the 'German Consortium for Hereditary Breast and Ovarian Cancer' (GCHBOC) investigated about 220 German high risk breast and/or ovarian cancer families for mutations in the breast cancer susceptibility genes BRCA1 and BRCA2 from 1997-2004. By direct sequencing of all exons and exon flanking regions of the introns of both genes, 22 single nucleotide polymorphisms (SNPs) spanning 80.78 kb of the BRCA1 gene and 25 SNPs spanning 83.01kb of the BRCA2 gene were detected among our German breast and/or ovarian cancer cases. We performed a haplotype-based study of BRCA1 and BRCA2 based on SNPs that were genotyped in 149 investigated index patients. Out of four different BRCA1 haplotypes with a frequency of > 5%, two common BRCA1 haplotypes accounted for more than 67% of all chromosomes in our collective. In contrast, analysis of BRCA2 haplotypes revealed a higher haplotype diversity where the most abundant haplotype reached a frequency of 18%. We further estimated the pattern and extent of linkage disequilibrium (LD) between the BRCA1/2-SNPs. We also utilized various methods based on pairwise and multilocus LD measures to define block structures at each genomic region. While most methods agreed on two blocks of elevated LD that spanned most of BRCA1, BRCA2 showed only low levels of LD in general and only a single block with strong LD. Our results on LD architecture and haplotype-tagging SNP definition of BRCA1 and BRCA2 will be presented and discussed with respect to their relevance in BRCA1/2 diagnostic.

P110

Gene expression analysis of clear cell and chromophile renal cell carcinomas

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The underlying molecular mechanisms of renal cell carcinoma (RCC) are poorly understood and more reliable diagnostic markers are needed. Hence, alternative strategies for biomarker discovery with appropriate validation technologies for the different subtypes must be pursued. To elucidate the genesis and progression of RCC we used high parallel chip-based gene expression profiling comparing normal and tumour tissues of clear-cell renal cell carcinoma and chromophile renal cell carcinoma. We analysed corresponding non-tumorous and tumour tissue samples from 10 patients with clear cell RCC and 12 corresponding non-tumorous and tumour tissue samples derived from patients with chromophile RCC. We isolated RNA from histologically well-characterized tissue sections and performed reverse transcription, labelling, and a linear RNA amplification. Samples were hybridized on microarrays containing 642 human cDNAs. We found several differentially expressed genes for both tumour types, including genes previously found as markers for renal cell carcinomas by other groups. Furthermore, we found over-expressed genes in the tumour samples, which may provide a way to discriminate between normal and tumour tissue. Those genes were selected for further evaluation by real-time RT-PCR. Increased mRNA expression of these genes in tumour cells could be correlated to their expression at the protein level by immunohistochemistry (IHC), where at least one gene showed very strong expression in all tumour samples examined but no expression in adjacent normal kidney tissue. Although the results are pointing towards possible marker genes for diagnostic purposes, more samples are needed to validate the study.

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P111

AKAP12/Gravin is down regulated in breast cancer

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We identified AKAP12 (A-kinase anchoring protein) as a candidate tumor suppressor gene in breast cancer. AKAP12, also known as gravin, has been mapped on chromosome 6q24-25.2, a hot spot region of loss of heterozygosity (LOH) in breast cancer. AKAP12 is a scaffold protein involved in signalling pathways. Significant down regulation of AKAP12 expression in breast tumors was shown by cancer profiling and chip array experiments. In different breast cancer cell lines AKAP12 is also expressed at low levels as demonstrated by RT-PCR. Northern blot analysis and 5'/3'-RACE were used to identify distinct transcripts of AKAP12. To evaluate whether down regulation of AKAP12 expression is due to aberrant methylation in these transcripts we performed methylation-specific PCR and 5'-Aza-dC treatment. Based on the expression data we investigated the effect of AKAP12 on breast cancer cell growth by transient transfection into a breast cancer cell line. Data as to the gene expression, structure of transcripts and ability of the gene to retard tumor cell growth will be presented with respect to their relevance in tumorigenesis of breast cancer.

P112

CpG island methylation and expression of tumor-associated genes in lung carcinoma

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In this study, we investigated the methylation of promoters of tumor-related genes, which were downregulated in micro-array analyses of 89 lung cancer patients by bisulfite methylation assays. In 15 lung cancer cell lines, frequent CpG island methylation was detected for SOX18 (73%), CD105 (71%), SEMA2 (55%), SLIT2 (100%), TIMP3 (29%), TIMP4 (64%), DLC1 (18%), p16 (66%), RASSF1A (71%) and EGF-like domain 7 (56%), but methylation was rarely observed for SLIT3 (18%), DLC1 (18%), Purα (10%). In primary lung tumor, methylation of SOX18 (100%), CD105 (69%), SEMA2 (93%), SLIT2 (100%), TIMP3 (13%), TIMP4 (94%), DLC1 (61%), p16 (41%), RASSF1A (44%) and EGF-like domain 7 (100%) was detected. Methylation of several promoters (SOX18, SLIT2, EGF-like domain 7, CD105, SEMA2 and TIMP4) was frequently found in normal lung tissue of cancer patients. In summary, frequent methylation of several cancer-related genes was observed in lung carcinoma.

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P113

Detection of translocations and amplifications of the *MLT*/*MALT1* gene in Non-Hodgkin's Lymphoma using *MLT*/*MALT1* specific FISH probes

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The t(11;18)(q21;q21) and the t(14;18)(q32;q21) involving *MLT* are the main structural abnormalities in extranodal marginal zone lymphoma (MZL). In addition to translocations, amplification of the *MLT* gene has been proposed as pathogenetic mechanism in NHL. Amplifications in the 18q21 region frequently involve the *BCL2* gene, however, a recent study described amplification of *MLT* without *BCL2* involvement, suggesting that *MLT* and *BCL2* are independent targets of amplification in NHL. In order to screen for translocations and amplifications of *MLT*, we have analyzed 276 NHL with FISH assays using probes flanking *MLT* (PACs 117B5 and 59N7 in 207 cases; MALT BAP Assay, Abbott Vysis, in 69 cases). These assays were applied to 91 MALT lymphomas, 19 splenic MZL, 17 nodal MZL, 17 follicular lymphomas (FL), 8 mantle cell lymphoma (MCL), 15 CLL, 3 PLL, 23 Burkitt's lymphomas (BL), 78 DLBCL, 5 T-cell NHL, and 10 cell lines. In 20 MALT lymphomas a translocation involving *MLT* was detected. FISH analyses with *API2* and *IGH* specific probes revealed the t(11;18) in 13 cases and the t(14;18) in 7 cases. Amplification of *MLT* was observed in 4 DLBCL, 1 BL, the MZL cell line SSK41, and the BL cell line NAMALWA. Further FISH analyses showed a concomitant amplification of *BCL2* in 2 DLBCL; in the remaining cases (2 DLBCL, 1 BL, SSK41 and NAMALWA) amplification of *MLT* without *BCL2* involvement was found. Aneuploidy of the 18q21 region was found in 43 cases from various NHL subtypes. In addition, a heterozygous deletion of *MLT* was detected in 2 MALT lymphomas and 6 DLBCL.
We conclude, that *MLT* associated translocations occur exclusively in MALT lymphomas and represent either the t(11;18) or the t(14;18); true amplifications of *MLT* occur in some aggressive NHL and some transformed cell lines; and aneuploidy of 18q21 is seen in different subtypes of NHL.

P114

Multicolor fluorescence in situ hybridization (M-FISH) identifies novel chromosomal abnormalities in t(8;14)-positive high grade Non-Hodgkin's Lymphomas

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The chromosomal translocation t(8;14)(q24;q32) and its variants t(2;8)(p12;q24) and t(8;22)(q24;q11) involve the *CMYC* gene on 8q24 and are consistently present in Burkitt's lymphomas (BL) and acute lymphoblastic leukemia (ALL), subtype L3. Secondary chromosomal abnormalities occur in about 70% of the cases, but their spectrum, incidence and prognostic relevance are not well known. In order to better characterize primary and secondary chromosomal abnormalities and to screen for new aberrations, we applied multicolor fluorescence in situ hybridization (M-FISH) to 8 BL cell lines and 11 patients with t(8;14)-positive high grade lymphoma. M-FISH allows the identification of each individual chromosome by its fluorescence pattern and hereby greatly enhances the resolution of conventional cytogenetic analysis, like the accuracy of characterization of marker chromosomes or complex chromosomal rearrangements.
All cases revealed the t(8;14) or its variants. In two cases, one additional abnormality was detected, whereas the remaining 17 cases revealed a complex karyotype. In all of the latter cases, M-FISH could specify the results obtained by conventional cytogenetic analysis or add new data. Interestingly, 4 cases revealed cytogenetically unrecognized three-way-translocations involving 8q24/*CMYC*, 14q32 and unknown partner genes on 4q12 and 18q21 (3 cases), respectively. In addition, a novel chromosomal rearrangement involving the immunoglobulin heavy chain locus in 14q32 was detected in one case: der(14)t(X;14)(p11;q32).
The most frequent secondary abnormalities were duplications (5 cases, 26%) and structural abnormalities (4 cases, 21%) of 1q, trisomy 7 (8 cases, 42%), as well as deletions and structural aberrations of 17p (4 cases, 21%) and 6q (3 cases, 16%).
Our study underlines the importance of M-FISH in the detection of novel chromosomal abnormalities and cryptic translocations, which are the basis for further molecular analyses.

P115

Frequent loss of differential methylation at the imprinted ARHI locus in uveal melanoma

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Uveal melanoma is the most common primary ocular tumor of the eye. Approximately 50% of the patients die of metastases, which mainly arise from primary tumors that show loss of an entire chromosome 3 (monosomy 3). Loss of the entire short arm of chromosome 1 is another frequent alteration only observed in tumors with monosomy 3. This indicates a potential role of genes located in this region in tumor progression. The imprinted putative tumor suppressor gene ARHI (Ras homolog member I), which frequently shows loss of imprinting (LOI) in breast and ovarian cancer cells, is located within this region. We compared the ARHI mRNA levels in uveal melanomas and cultured melanocytes and found that the ARHI expression is strongly reduced in all primary tumors. We next analyzed the ARHI methylation pattern in DNA from 41 primary uveal melanomas. Among 32 uveal melanomas with retention of heterozygosity of 1p we found loss of differential methylation in 50% of tumors, which was irrespective of the chromosome 3 status. In five of nine tumors showing loss of heterozygosity of 1p the remaining allele was methylated and in four tumors ARHI was unmethylated. As we could not detect any aberrant methylation pattern of the imprinted SNRPN promoter/exon 1 region on chromosome 15, it is unlikely that LOI is due to a general impairment of the imprinting/methylation process. Based on the observation of hypomethylated as well as hypermethylated ARHI alleles in primary tumors we conclude that LOI of ARHI is a random rather than specific alteration in uveal melanomas. Furthermore, a specific role of ARHI in metastatic progression of disease is unlikely as tumors with disomy 3 and monosomy 3 are similarly affected by LOI. We also determined the ARHI methylation status in six neuroblastomas and thirteen retinoblastomas, which are early childhood tumors, and could not find altered methylation in any of these tumors. This suggests that LOI of ARHI might be related to the age of the patients.

P116

The translocations t(6;18;11)(q24;q21;21) and t(11;14;18)(q21;q32;q21) represent two novel variant translocations of the t(11;18)(q21;q21) associated with extranodal MALT lymphomas

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The translocation t(11;18)(q21;q21) represents the most frequent structural abnormality in MALT lymphomas. The t(11;18) leads to a fusion of the *API2* gene on 11q21 and the *MLT* gene on 18q21 resulting in activation of NF-κB. Recently, we reported the first variant translocation of the t(11;18), a t(11;12;18)(q21;q13;q21) in a MALT lymphoma. The cloning of the t(11;12;18) showed that the pathological relevant event was, similar to the standard t(11;18), the fusion of *API2* to *MLT*.
We herein describe two new variant translocations of the t(11;18), the t(6;18;11)(q24;q21;q21) and the t(11;14;18)(q21;q32;q21), occurring in MALT lymphomas of the stomach and the lung, respectively. Conventional cytogenetic analysis revealed: I.46,XY,t(6;18;11)(q24;q21;q21)[7], II.46,idem,del(1)(q43)[10], III.46,XY[3] in the first case; and 46,XY,t(11;14;18)(q21;q32;q21)[23] in the second case. In both cases, fluorescence in situ hybridization (FISH) with *API2* (PAC166G16) and *MLT* (PAC59N7) specific probes showed *API2-MLT* fusion encoded on the der(11) as in the standard t(11;18). Split hybridization signals of *API2* were located on the der(6) and the der(14), indicating the presence of a three-way-translocation. Further FISH analyses demonstrated a colocalization of genomic sequences derived immediately upstream of *MLT* (PAC117B5) and sequences of the variable region of the *IGH* locus on 14q32 (YAC Y6) in the t(11;14;18). For the t(6;18;11), painting probes for chromosomes 6, 11, and 18 were applied to confirm the three-way-translocation.
We conclude that, analogously to the standard t(11;18) and the t(11;12;18), the new variant translocations t(6;18;11) and t(11;14;18) lead to an *API2-MLT* fusion and that due to an additional translocation event the expression of the reciprocal *MLT-API2* is excluded. Further molecular analyses to determine the translocation partners on chromosome 6 and 14 are in progress.

P117

The translocation t(8;14) distinguishes two groups of aggressive lymphoma with different IGVH and BCL6 mutational status

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During lymphomagenesis, oncogenes can be activated through chromosomal aberrations, like MYC through the t(8;14)(q24;q32), or somatic hypermutation (SHM), as is the case for BCL6 in 75% of diffuse large B-cell lymphomas (DLBCL). Moreover, somatically mutated IGVH genes are a hallmark of germinal center (GC) B-cells and allow to trace the developmental stage at which neoplastic transformation occurred. The SHM status of IGVH differs between the histologic lymphoma entities.
We investigated 116 B-cell lymphomas for their IGVH SHM status and aberrant BCL6 hypermutation within the Deutsche Krebshilfe Verbundprojekt „Molekulare Mechanismen bei Malignen Lymphomen". 43 B-cell lymphomas possessed a t(8;14) by FISH analysis, 73 were t(8;14) negative.
Interestingly, t(8;14)-positive B-cell lymphomas carried VH genes with a considerably lower mutation frequency (range 0,44-11%, median 4,44%) than t(8;14)-negative B-cell lymphomas (range 0-25%, median 12,23%). The IGHV mutation status was independent of the histologic diagnosis. In contrast to published results demonstrating an unbiased usage of VH genes in DLBCL we observed a strong bias in VH gene usage in both groups with and without t(8;14) towards overrepresentation of the VH4 (50% in t(8;14)+, 40% in t(8;14)-) and VH3 genes (26% in t(8;14)+, 49% in t(8;14)-). Furthermore, the incidence of SHM of BCL6 was significantly different: in the t(8;14) positive group only 11/43 (25%) were mutated (median mutation frequency 0,13%) compared to 31/73 (42%) of the cases lacking a t(8;14) (median mutation frequency 0,22%).
Our results reveal that within aggressive lymphomas the t(8;14) identifies groups with differ-

ent VH and BCL6 mutation status independent from the histologic subtype. Ongoing studies investigate whether hypermutation patterns can also identify different subgroups within the t(8;14) positive lymphomas.

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P118

Amplification of the *MLT/MALT1* gene in a hsr(18) occurring in a case of diffuse large B-cell lymphoma of the tonsil evolving from an extranodal MALT lymphoma

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The t(11;18)(q21;q21) and the t(14;18)(q32;q21) involving the *MLT* gene are the main structural abnormalities in extranodal MALT lymphomas. In addition, amplification of *MLT* was proposed as pathogenetic mechanism in non-Hodgkin's lymphomas (NHL). Amplifications in 18q21 frequently involve the *BCL2* gene, which lies about 5Mb telomeric to *MLT*. However, a recent study described amplification of *MLT* without *BCL2* coamplification, suggesting that *MLT* and *BCL2* are independent targets of amplification in NHL. We herein report the first case of a MALT lymphoma in transformation to diffuse large B-cell lymphoma with a *MLT* amplification. In August 1999, this 60-year-old male presented with tonsil enlargement without other organomegalies and normal levels of lactate dehydrogenase. Bone marrow aspiration revealed an infiltration of around 20% leading to stage IV disease. The patient achieved complete remission after 8 courses of intensive chemotherapy. The MALT lymphoma of the tonsil was cytogenetically characterized by a complex karyotype including a homogeneously staining region in chromosome 18p (hsr(18)(p11)). The karyotype was described as follows: 47,XY,add(1)(p35-36),add(2)(p2?1),add(4)(q25-26),del(6)(q21q25)x2,-8,-12,del(13)(q2?1),hsr(18)(p11),+3mar[20], II.46,XY[2]. To determine the origin of the genomic material of the region described as hsr(18), fluorescence in situ hybridization (FISH) with PAC clones (117B5 and 59N7) flanking *MLT* was performed. FISH analysis revealed a 4-fold amplification of *MLT* in the region previously identified as hsr(18). Further FISH experiments showed a concomitant overrepresentation of *BCL2*.

We conclude that given the importance of *MLT* in the pathogenesis of MALT lymphomas and the absence of a translocation involving *MLT* in this case, the described *MLT* amplification likely represents the pathogenetic relevant abnormality in this case.

P119

The p53 codon 72 variation is associated with the age of onset of hereditary non-polyposis colorectal cancer (HNPCC)

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Background: The polymorphic variants at codon 72 of the *p53* gene were shown to be functionally distinct *in vitro*, whereby the arginine (arg) variant induces apoptosis more efficiently than the proline (pro) variant. Due to the evidence that the DNA mismatch repair system and *p53* interact to maintain genomic integrity, we hypothesized that the codon 72 variation may influence the age of onset of disease in HNPCC patients.

Methods: 538 patients were tested for *p53* codon 72 variants. These included 167 unrelated patients with pathogenic germline mutations in *MSH2* or *MLH1* and colorectal carcinoma as first tumour, 126 patients with sporadic microsatellite stable colorectal cancers and 245 healthy controls.

Results: The median age of onset was 41, 36 and 32 years in *MSH2* or *MLH1* mutation carriers with arg/arg, arg/pro and pro/pro genotypes, respectively. The log-rank test revealed significant differences in the age of onset between arg/arg and pro/pro individuals (P=0.0002) and arg/pro versus arg/arg and pro/pro individuals (P=0.0026 and P=0.0217, respectively). A Cox regression model indicated an additive mode of inheritance. No significant differences in age of onset were observed among different genotype carriers with microsatellite stable tumours.

Conclusions: Our results suggest that *p53* codon 72 genotypes are associated with the age of onset of colorectal carcinoma in a mismatch repair deficient background in a dose-dependent manner. These findings may be relevant for preventive strategies in HNPCC.

P120

Do histological stains affect the analysis of microdissected tissue by ProteinChip technology

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ProteinChip array technology (SELDI; surface enhanced laser desorption/ionisation - mass spectrometry) allows to analyse protein extracts from small amounts of cells (i.e. areas of microdissected tissue) using affinity chromatographic surfaces. In connection with laser based microdissection (LMPC; laser microdissection and pressure catapulting) it therefore presents a powerful tool for proteomic analysis of tumors.

To find out whether histological stains influence the quality of SELDI analysis we microdissected defined areas (about 5000 cells) of mouse liver sections unstained or stained with different dyes (cresyl violet, nuclear fast red, HE). All dyes were additionally combined with different fixation protocols.

Microdissected tissue areas were transferred in a lysis buffer and proteins were applied to a strong anion exchanger (SAX2) ProteinChip arrays with appropriate binding buffer. Spectra were read out in a defined analysis mode on a PBSII instrument (Ciphergen).

On this specific affinity chromatographic surface the HE-stained cells resulted in a poor spectra. Nuclear fast red showed a better spectra. Only the cresyl violet spectra showed a comparable rich spectra as is was detectable for the unstained cells.

This result is especially important for the microdissection of tumor tissue which has to be done presently on unstained sections, because of the interference of HE. Further on it is a prerequisite for an automated software aided laser microdissection (Definiens) which is highly dependent on a histologically stained section.

P121

Alteration of β-Catenin in Wilms Tumors

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Wilms tumors can be divided in two groups based on the presence or absence of WT1 mutations. It has been described that Wilms tumors have a high percentage of ß-Catenin mutations. We have analysed 106 tumors for ß-Catenin mutations and found 23 mutations (22%). The mutation occurred in Ser45 in 16/23 cases, two were in Thr41 and five in His36. Two of these amino acids are targets for phosphorylation of the ß-Catenin protein (Thr41 and Ser45) and His36 is next to Ser37, another phosphorylation site in the protein. Previously the WT1 gene was analysed in 97 of these tumors and 17 mutations (16%) were identified, demonstrating that the percentage of mutations in ß-Catenin is higher than in WT1. The analysed tumors belong to different histologic subtypes and were from patients either treated preoperatively with chemotherapy or not. If the different histologic

subtypes are viewed separately, we found that 64% of the stromal type tumors and 26% of the triphasic tumors had ß-Catenin mutations. Both mutations were simultaneously present in 15, i.e. 88% of the tumors with WT1 mutations also had a ß-Catenin mutation. This extends and confirms a previous observation of a significant correlation of WT1 and ß-Catenin mutations, suggesting that the presence of both mutations is important for the development of a specific subtype of Wilms tumors. ß-catenin mRNA expression was studied using semiquantitative RT-PCR analysis and only a low amount of mRNA was detected in the tumors with mutations. In contrast tumors without WT1 and ß-Catenin mutations of the blastemal type had a higher expression of ß-Catenin mRNA. The immunohistochemical (IHC) analysis of several Wilms tumors of the stromal type, revealed a weak to moderate staining and some staining in the nucleus was found in limited areas of the tumor. Activation of the wnt signalling pathway by mutations in ß-Catenin does not result in a nuclear localisation of this protein in all cells.

P122

Fluorescence in situ hybridization reveals new translocations involving the *ETV6* gene in myeloid malignancies

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Rearrangements affecting band 12p13 are recurring aberrations in hematological malignancies. The main target of these rearrangements is the *ETV6* gene, which has been involved in more than 41 translocations.

In order to screen for new *ETV6* rearrangements, we analyzed 32 cases with structural abnormalities of 12p, using FISH. Conventional cytogenetic analysis of the cases, referred to our center from 1990 to 2003, identified del(12)(p) in 19 cases, add(12)(p) in 5 cases, and translocations in 8 cases.

FISH was performed using the cosmid clones 179A6, 50F4, 163E7, and 148B6 covering *ETV6*. By FISH, heterozygous deletions of *ETV6* were detected in 16 cases. These results were in accordance with our cytogenetic findings in 13 cases; 3 cases were described as add(12)(p12) and t(X;12)(q28;p11), respectively. Of the 8 cytogenetically shown del(12)(p), 3 were not confirmed by FISH. 7 translocations of *ETV6* were shown by FISH, including the cytogenetically described t(3;12)(q26;p13), t(4;12)(q12;p13), and t(5;12)(q33;p13). In addition, 4 cryptic translocations involving *ETV6* were seen: the t(12;17)(p13;p12-13) and 3 novel translocations with unknown partner genes in 12q24, 2?q33, and 17q25. Summarizing cytogenetic and FISH results, the revised partial karyotypes were determined as follows: inv(12)(p13q24), previously del(12p)(13); t(2;12)(?q33;p13) previously del(12)(p12); and der(3)t(3;12)(p21;p13),der(12)del(12)(p13)t(3;12)(p21;p13),der(17)t(12;17)(p13;q25), previously t(3;12)(p21;p13). The translocations occurred in patients with acute myeloid leukemia and the breakpoints within *ETV6* were located as follows: 12q24 in intron 1, 2?q33 distal to exon 5, and 17q25 distal to exon 2. The molecular cloning of these translocations is in progress.

Our study demonstrates the high frequency of cryptic translocations involving *ETV6* on 12p13 and underlines the importance of FISH to detect and characterize these translocations.

P123

Poor response to preoperative chemotherapy in Wilms' tumors with WT1 mutations and rhabdomyomatous differentiation

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To evaluate the response to preoperative chemotherapy and the clinical outcome in patients with WT1 mutations and abundant rhabdomyomatous differentiation we have retrospectively reviewed 60 patients of the German SIOP 9/GPOH study. 16/60 patients (five bilateral and 11 unilateral tumors) had WT1 mutations.

Tumors with and without WT1 mutations differed significantly in histopathological and clinical features. Tumors with WT1 mutations showed a massive rhabdomyomatous differentiation in the viable part of the tumor compared to tumors without WT1 mutations (mean 63% vs. 8.6%; $p<0.01$). Response to chemotherapy was poor in 90% of tumors with WT1 mutations compared to 40% of tumors without mutation ($p=0.018$) and they showed only mild histopathological regressive changes (mean 20% vs. 52%; $p<0.01$). None of the patients with WT1 mutations presented with distant metastases compared to 11/45 (24,4%) of children without WT1 mutations ($p=0.012$), whereas most of the bilateral tumors (5/6) were found in children with WT1 mutations ($p<0.01$). The 5-year relapse-free survival for patients with WT1 mutations was 91.7% (11/12) as opposed to 88.1% (37/42) in patients without WT1 mutations. None of the 16 patients with WT1 mutations died due to progressive disease.

This study demonstrates that WT1 mutations and a rhabdomyomatous differentiation are highly valuable prognostic markers in the identification of tumours which respond poorly to preoperative chemotherapy but having a favourable outcome at least in low stages. This data will help to refine risk adapted and individualised therapeutic strategies and to identify patients who may benefit from reduced preoperative chemotherapy.

P124

Fusion of the H4/D10S170 to the PDGFßR gene in a patient with chronic myelomonocytic leukemia (CMML) and resposivness to Imatinib

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Cytogenetic analysis of a patient with CMML showed a translocation t(5;10)(q33;q22).

The PDGFßR gene is located at chromosome 5q33 and is disrupted in some cases of hematologic malignancies producing different fusion transcripts. To prove whether the PDGFßR gene is affected by the translocation a FISH analysis was performed and indeed the PDGFßR gene was disrupted. A candidate gene as partner for the fusion transcript is the H4/D10S170 gene because of its location on chromosome 10q22. The disruption of the H4/D10S170 gene was described for two cases of myeloproliferative disorders.

To test whether these two genes were involved a RT PCR with specific primers for the possible fusion transcript was performed. A PCR product of the expected size was observed. Sequencing of this product showed the presence of the fusion transcript with the identical breakpoints described previously in another case. It is of interest that the described breakpoints in the PDGFßR gene occured at the same site in intron 9 resulting in a transcript starting with exon 10 of the PDGFßR gene.

Because of the involvement of the PDGFßR gene the patient was treated with Imatinib, a new drug which interacts specifically with the function of the activated kinase. Hematologic remission of the patient was observed within one week.

P125

Molecular and cytogenetic characteristics of the Imatinib-resistant cell line KCL22-r and its sensitive counterpart KCL22-s

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Tyrosine kinase inhibitor Imatinib (STI571, Glivec) is an effective drug in therapy of chronic myeloid leukemia (CML). However, some patients present an intrinsic resistance to Imatinib, especially in advanced disease stage without an overexpression or mutation of the BCR-ABL kinase. Blast crisis in CML is often accompanied by karyotype changes like trisomy 8, +Ph, isochromosome 17q and trisomy 19. To investigate whether KCL22-r and KCL22-s show divergent chromosomal aberrations, both cell lines were analyzed by 24-colour- and interphase-FISH. KCL22-r shows the same aberrations as KCL22-s plus a gain of the derivative chromosome 8 and loss of chromosome 13. Additionally, KCL22-r exhibit a translocation t(6;13). Interphase-FISH detected two cytogenetically divergent sub clones in KCL22-s. Whether these aberrations contribute to the Imatinib-resistance in KCL22-r remain obscure. This data will be compared with the protein profile determined by protein chip technology (SELDI), to see whether the differences in the protein profile can be

linked to the chromosomal changes as the genetic basis for the resistance to Imatinib.

P126

Improving for-client letters in routine genetic counseling

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Genetic counseling should help clients to arrive at valid decisions concerning health-related problems with a genetic component. However, maintaining continuity and coherence of the counseling process becomes difficult, when this process gets more and more cognitively complex, fragmented and scattered over time. Therefore, supporting the dialogical phase by means of communication media might be increasingly important.
For-client letters are supposed to serve as a medium, facilitating clients' deliberations beyond the temporal limitations and other constraints of the actual counseling session. In a former study we had investigated effectiveness, scope, and ethical significance of enhancing the flow of communication by such letters. An established format (standard letter) had been enriched systematically (enriched letter), based on a taperecording of the counseling session. Standard and enriched letters were randomly and doubleblindedly given to clients and compared. Altogether we had counseled 142 families, 70 of them because of suspected hereditary breast/ovarian cancer (HBOC). The results had proven that clients, who received enriched letters, had a significantly better and temporarily sustained knowledge and comprehension of relevant facts.
However, prerequisite for employing "enriched" letters in routine genetic counseling is a simple and not time consuming procedure. Consequently, we simplified the process for writing enriched letters and tested its usefulness in counseling clients with suspected HBOC: An established enriched letter for HBOC was modified according to notes made by the counselor during the counseling session, avoiding any tape recording. Analysis of now 30 families revealed that clients receiving the "simplified enriched format" had a significantly better knowledge and comprehension of relevant facts. Therefore it seems feasible to write communicatively enriched for-client letters also in routine genetic counseling.

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P127

Investigation of alterations in fanconi anemia genes FANCE,FANCL and FANCB in human breast cancer.

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Breast cancer occurs at a relatively high frequency of 10% in the female population of the western world. Only a small fraction of it manifests itself clustered in families mainly due to germline mutations in the BRCA1 and BRCA2 genes.The rest 95% of the breast cancers occur spontaneously as somatic mutations of probably polygenic origin and the underlying gene defects and mechanisms are not clear. The likely candidates are defects in processes of cell cycle, DNA repair,and apoptosis including especially the genes whose products interact or interfere with DNA repair functions of BRCA1 and BRCA2 proteins. Recently a novel Fanconi anemia/BRCA pathway has been elucidated which shows at least 8 of the 11 known FA proteins (A,B,C,D1,D2,E,F,G,I,J,L) interacting with BRCA proteins in a common signalling pathway involved in DNA repair. In order to evaluate the contribution of the alterations in the FA genes we started screening some of these genes at genomic and expression level in our collective of sporadic breast tumors. In this report we present the results of mutation screening in FANCE,FANCL and FANCB gene(ongoing).
In a subcollective of 40 breast cancers and 5 breast cancer cell lines without mutations in BRCA genes all exons and intron exon boundaries of FANCE,FANCL and the newly discovered FANCB gene are investigated by PCR amplification,SSCP and direct sequencing. Uptil now only one mutation in exon 5 of FANCE gene has been found.The work on FANCB gene is still in progress. This would argue that mutations in FANCE and FANCL genes are unlikely to be involved as frequent cause of defects in Fanconi/BRCA pathway.

P128

Clinical manifestation of non-hereditary retinoblastoma is influenced by a parent-of-origin effect in a subset of patients

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Mutation analysis is part of routine management in patients with isolated unilateral retinoblastoma (RB). To date, we have identified both oncogenic RB1 gene mutations in tumors from 230 of 263 of patients (87%). In 34 patients (13%), a mutation was also detected in constitutional DNA (peripheral blood), including 14 with obvious mutational mosaicism. To identify any differences in phenotypic expression between patients with and without a constitutional mutation we analyzed all relevant data including clinical data, histopathology, and genetic findings. Data were extracted from source systems (patient oriented databases), transformed, and loaded into a data mart using business intelligence software (COGNOS). Datasets complete with all relevant data on phenotypic expression were available from 237 of 263 patients. Among other findings we found that the distribution of age at diagnosis in patients with a constitutional mutation was not significantly distinct from that of patients without a constitutional mutation. This contrasts the findings in many hereditary cancers, where age at diagnosis is earlier compared to that of sporadic disease. To identify factors associated with early diagnosis we compared distributions grouped by certain attributes (drilling down). Overall, multifocal RBs were diagnosed earlier than unifocal RBs. However, in several patients with multifocal RB both oncogenic mutations were not detected in blood DNA. The distributions of age at diagnosis of tumors with different RB1 mutations were not distinct. Analysis of age at diagnosis of tumors with LOH showed that several patients with non-hereditary RB and retention of the paternal allele were diagnosed early. This caused an extra peak early in the distribution that was absent in non-hereditary patients with retention of maternal alleles. Our findings suggest that in a subset of patients with non-hereditary RB age at diagnosis of is influenced by a parent-of-origin effect.

P129

Exhaustive mutation analysis of RNASEL in a hereditary prostate cancer family with high linkage to 1q25

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Linkage analysis identified RNASEL at 1q25 as a strong candidate for the hereditary prostate cancer 1 (HPC1). The gene encodes an endoribonuclease which is a member of the interferon-regulated 2-5A system. In vitro experiments suggested that the RNASEL gene may function as a tumor suppressor gene. In our genome wide linkage study for prostate cancer, the family with the highest individual evidence (ZLR = 2.86, p= 0.002) to the locus of the RNASEL gene, included four affected brothers and an unaffected one. In the course of a mutation screening, exon sequencing did not identify nonsense mutation in this pedigree. However, the common polymorphism R462Q was found heterozygous in the affected brothers, while the unaffected one had two Q alleles. Any mutation leading to prostate cancer would be expected to alter either (1) the peptide sequence, (2) splice pattern or (3) the expression level of the gene. Since no deleterious protein variant had been observed by sequencing the coding region, we searched for aberrant splicing. PCR products from cDNA did not reveal any length variation in three affected brothers, thus transcripts were spliced correctly. In order to test if both RNASEL alleles are expressed we determined their presence in cDNA compared to genomic DNA by SNaP Shot analysis for the R462Q variant. The result confirmed, that in every proband both alleles were present at equal amounts. The absence of RNASEL mutation in this significantly linked family may ask for further candidate genes in 1q25.

P130

Deleterious germline mutations of the MSR1 gene in prostate cancer families from Germany

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The short arm of chromosom 8 was indicated by linkage analyses to harbour a gene pedisposing to hereditary prostate cancer. Germline mutations were found subsequently, in the gene encoding the Macrophage Scavenger Receptor

(MSR1) at 8p22. These variants include one nonsense (R293X) and several missense mutations within the family collection that initially provided evidence for linkage. To date however, the role of MSR1, as of other suggested hereditary prostate cancer genes, has remained unclear. This is due to a pronounced heterogeneity, which has been complicating the confirmation of linked loci and candidate genes therein, on the basis of an independent familiy collection. We have gained evidence of linkage to 8p22 previously in a genome wide scan in prostate cancer families from Germany. These families have been used in the present study in order to (1) clarify, if MSR1 is the relevant gene at 8p, and (2) what would be its impact for prostate cancer susceptibility in the German population. The candidate gene approach included 139 prostate cancer families with at least two affected relatives. Out of each family the index case was selected for sequencing of the 11 exons of the MSR1 open reading frame. In the ongoing screenig we recovered the known R293X variantion in two of our familial prostate cancer cases. Moreover, two further deleterious germline mutations were identified each in a single family: a nonsense mutation S84X, and a splice site mutation IVS5-1. Rare missense mutations include the variants H235Y, P286S, P392R, A398G and K430R. To our knowledge, our study is the first to provide further deleterious variants of MSR1 apart from the initially reported R293X. Genotyping of additional nonfamilial (n = 380) cases and healthy controls (n = 210) is in process, and will help to evaluate the impact of the identified variants in the development of prostate cancer.

P131

Genexpressions-Profile zur Prognose-Bestimmung beim Mammakarzinom

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Nach einer Umfrage ist die Möglichkeit einer individualisierten adjuvanten systemischen Therapie die wichtigste Herausforderung bei der Behandlung von Brustkrebs. Dabei sind die bisherigen klassischen Kriterien nur unzureichend in der Lage, Patientinnen zu identifizieren, die ohne adjuvante Therapie Metastasen entwickeln würden und daher eine adjuvante Therapie benötigen. Um solche Patientinnen zu identifizieren, ist eine genaue Bestimmung des Metastase-Risikos notwendig. Die dazu durchgeführte Untersuchung (MammaPrint) erfolgt anhand einer Tumorprobe der Patientin mit Hilfe von Microarrays. Dabei wird das Genexpressions-Profil von 70 Prognose-Genen bestimmt, sowie von 1.000 Kontroll-Genen. Die Untersuchung erlaubt eine hohe Prognose-Genauigkeit: Bei Lymphknoten negativen Patientinnen unter 55 Jahren im Tumorstadium 1 oder 2 beträgt die Überlebensrate nach 10 Jahren 97%, wenn eine gute Prognose ermittelt wurde. Ein hoher Anteil von 40% der Patientinnen wird aufgrund der Untersuchung der Gruppe mit guter Prognose zugeordnet und kann potenziell von einer systemischen Therapie ausgespart werden. Die Zuverlässigkeit des Genexpressions-Profils wurde in externen Validierungs-Studien bestätigt. Eine neue Herausforderung ist jetzt die Entwicklung von Profilen für weitere Patientengruppen und die Erschliessung anderer Anwendungsgebiete, wie z.B. die Vorhersage von lokalen Rezidiven oder die Lokalisierung des Primärtumors bei CUP-Patienten (carcinoma of unknown primary).

P132

Earlier age at diagnosis in patients with MLH1 versus MSH2 mutations. Results of the German HNPCC Consortium study

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Hereditary nonpolyposis colorectal cancer (HNPCC) accounts for 2%-5% of the colorectal cancer burden. This autosomal dominantly inherited syndrome predisposes to colorectal cancers (CRC), but also to endometrial, gastric, hepatobiliary, urinary tract, and ovarian cancers as well as brain and skin tumours. Criteria (Amsterdam, Bethesda) to identify patients and their families have been deviced. Since the effectiveness of surveillance programmes have been demonstrated to reduce the incidence and mortality of CRC in HNPCC patients early identification of cases is mandatory. A large nationwide multicenter study was started by the German HNPCC Consortium in 1999 aiming at establishing competent clinical management and advice as well as genetic counselling and testing in an interdisciplinary approach.
We report on phenotypic aspects in patients with deleterious mutations in MLH1 and MSH2 selected from families satisfying the Amsterdam and Bethesda criteria. The frequency of CRCs was 78% in patients with MLH1 and 65% with MSH2 mutations. Rectal cancers were in 20% the first colorectal tumor event. Gastric cancer (4%-5%) was the third most common cancer. Urinary tract malignancies were seen more frequently in MSH2 mutation carriers. Skin tumours were predominantly, and CNS tumours and prostate cancer were exclusively seen in MSH2 patients. Syn-/metachronous cancers occurred in about 20% of cases. We detected significantly earlier age at diagnosis in MLH1 versus MSH2 mutation carriers both in regard to first cancer (41 vs. 44 years, p=0.0042) and to first CRC (43 vs. 46 years, p<0.0001). There were no differences between genes with respect to survival. Age at death was significantly earlier in MLH1 as compared to MSH2 mutation carriers.
The identification of genotype-phenotype correlations could provide a basis for an enhanced efficiency of surveillance taking the individual organ and age at risk into account.
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P133

Germinal mutations of *NBS1* gene as a risk factor in childhood acute lymphoblastic leukemia.

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Biallelic *NBS1* gene mutation and chromosomal abberrations are etiopathogeneticaly associated with Nijmegen breakage syndrome (NBS) along with high incidence of lymphoproliferative diseases. Product of *NBS1* gene, nibrine is genome guard and is functionally involved in DNA double strand breaks repair. Heterozygous germinal mutations 657del5 and R215W have been assosciated with increase susceptibility to malignancies including breast and ovary cancer, melanoma malignum and medulloblastoma. So far reports on heterozygous NBS1 mutations in lymphoproliferative diseases are rare and controversial. The aim of the present study was to analyse the mutations in all 16 exons of *NBS1* gene in children with acute lymphoblastic leukemia (ALL). To discriminate between germinal and somatic mutations DNA was isolated from either leukemic or normal oral epithelium cells. The *NBS1* mutations have been detected by PCR-single strand conformation polymorphism and confirmed by direct sequencing. Out of 113 ALL cases the following four *NBS1* gene mutations in 6 patients have been identified: in exon 5 I171V (3 cases), in exon 6 R215W, V210F and 657del5. In addition in 23 children novel intron variants: IVS14-30A/T, IVS15+88C/G, IVS7-18G/A, and IVS8-42 G/C have been detected. In 100 controls (unused Guthrie card) none *NBS1* gene mutation has been found. All the above *NBS1* mutations have been identified at the time of diagnosis and during remission both in peripheral blood and oral epithelium cells. This indicate that the observed mutations in NBS1 gene are of germinal origin. On the basis of obtained result *NBS1* gene germinal mutations can be considered as a risk factor in the development of acute lymphoblastic leukemia.

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P134

***hTERT* and *hTERC* gene amplification in selected childhood malignancies**

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Telomerase, a ribonucleoprotein complex, consists of reverse transcriptase (*hTERT*), telomerase associated protein (*TP1*) and RNA template for telomeric DNA synthesis (*hTERT*C). Telomerase allows cells to grow indefinitely and it is believed that reactivation of telomerase plays an important role in cell immortalization and carcinogenesis. In this study high telomerase activity in selected solid tumours (Wilms tumour, neuroblastoma) and in acute leukemias in children has been observed allowing to distinguish neoplastic cells from normal ones. All studied telomerase components were consistently expressed in cancer cells. Neoplastic RNA produced consistently very strong amplification signals either for *hTERC*, *hTERT* and *TP1*. The expression of *hTERC*, *TP1* and *hTERT* was also observed in some normal bone marrow cells and peripheral blood lymphocytes. The limiting dilution experiments indicated that the cancer cells have at least 100-fold higher telomerase activity and at least 25-fold higher *TP1* and *hTERT* expression in comparison to normal cells. FISH analysis revealed amplification of *hTERT* and *hTERC* genes in malignant cells. It can be concluded that all cancer cells tested have higher telomerase expression and activity, as compared to normal cells. The high expression and activity of telomerase in cancer cells can be explained by amplified *hTERT* and *hTERC* genes.
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P135

Cytogenetic and Molecular Cytogenetic analysis of a 10;11 rearrangement in adult acute leukaemia

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A recurrent chromosome aberration involving 11q23/MLL and the short arm of chromosome 10 is a well-recognised but rare abnormality in acute leukaemia. Molecular studies have shown that the AF10 gene at 10p12 is consistently a partner gene in cases with 10;11 rearrangement. This rearrangement appears to fuse the 5' end of MLL and the 3' region of AF10 through a mechanism that is more complex than a reciprocal translocation, implicating an inversion of one of the two genes to permit the formation of the MLL/AF10 transcript. Here we report the cytogenetic and molecular cytogenetic analysis of a 57 year old patient with a clinical diagnosis of AML M5 with inverted insertion of chromosome 11 in chromosome 10p12. In contrast to other reported cases, in our patient this insertion is associated with a 3'MLL deletion.

P136

Allelic alterations in comparison to morphological grading characteristics in premalignant laryngeal lesions

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Classic histological grading and molecular genetic analysis of epithelial hyperplastic laryngeal lesions (EHLL) were correlated in an attempt to elucidate, which classic marker reflects best the gradual progression of laryngeal premalignant lesions as determined by an increasing number of molecular genetic aberrations. Thirty-two EHLL were grouped according to four grades of nuclear atypia, four degrees of epithelial maturation and three groups of overall and suprabasal mitotic activity. Overall and suprabasal proliferative activity were measured by MIB-1 immunostaining. Allelic imbalance was determined by PCR using 32 microsatellite loci at nine chromosomal arms comprising 3p, 6p, 6q, 8p, 9p, 9q, 13q, 17p, and 18q. Of the six classic criteria, only progressive mitotic indices correlated with allelic alterations in a remarkable variety of microsatellite markers, especially at chromosome 17p. Using the number of allelic alterations as standard for assessment, this preliminary study gives first evidence that certain morphological criteria could reflect differently the gradual progression of premalignant laryngeal lesions with mitotic index being the most promising marker. Further studies with long-term follow-up are required to prove the predictive value of these criteria in daily practice.

P06 Cytogenetics

P137

Is Silver-Russell syndrome associated with maternal Duplication of 11p15?

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Silver-Russell syndrome (SRS) is a heterogeneous malformation syndrome characterised by intrauterine and postnatal growth retardation (IUGR/PGR) (<3rd percentile). SRS patients show numerous additional dysmorphisms such as relative macrocephaly, small triangular face, downturned corners of mouse, clinodactyly V, and asymmetry of head, limbs and trunk. So far, the cause of SRS remains unknown in most cases, however in approximately 10% of SRS patients a maternal uniparental disomy (UPD) of chromosome 7 or chromosomal aberrations can be detected. Recent examinations reveal an involvement of genes in 11p15 in the aetiology of SRS: In two different studies four growth retarded children associated with maternal duplication of 11p15 have been described. Two of these children showed SRS-like features. Interestingly the Beckwith Wiedemann syndrome (BWS) critical region is located in this particular area. In BWS patients paternal UPD11 and genomic disturbances within 11p15 can be observed. Giving the involvement of this genomic region in BWS, we postulated that SRS – with an opposite phenotype to BWS – might also be caused by genomic disturbances in 11p15. By short tandem repeat typing we detected two SRS patients with duplications of maternal 11p material in our study population (n=46). In patient SR46, the duplicated region covered at least 9 Mb; FISH analysis revealed a translocation of 11p15 to 10q. In patient SR90, additional 11p15 material (approximately 5 Mb) was translocated to the short arm of chromosome 15. In conclusion, the search for maternal duplication of 11p15 will shed more light on the aetiology of SRS. Since SRS is a heterogenous condition, we think that carriers of 11p15 disturbances are a subgroup of SRS. We suggest that a diagnostic testing for duplication in 11p15 should be offered to patients with severe IUGR and PGR in combination with clinical signs reminiscent to SRS.

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Paleopathological diagnostic and ancient DNA - molecular cytogenetic investigations of teratological samples of the Meckel Anatomical Collection (Halle/Saale, Germany)

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Between the middle of the 18th to the early 19th century, the study of human congenital malformations, especially those dramatic examples designated as 'monsters', attracted special attention. One of the finest European collection in this field was the teratological section of the Meckel Cabinet, the collection of Johann Friedrich Meckel the Elder (1724-1774), his son Philipp Friedrich Theodor Meckel (1755-1803) and his grandson Johann Friedrich Meckel the Younger (1781-1833). The collection increased substantially during Meckel the Younger's activity as professor of anatomy and physiology (1805-1833) at Halle University. Between 1812 and 1818, J. F. Meckel the Younger published a three-volume "Handbook of pathological anatomy", which became the standard work on teratology in the 19th century. Most of the anomalous samples of the Meckel Collection were described in his books and his articles as well as in the medical doctor theses of Meckel's students. These samples are still an integral part of the Anatomical Collections of the Department of Anatomy and Cell Biology in Halle an der Saale. During the last years the collection of human congenital anomalies was re-catalogued. Moreover, all dried specimens (i.e. pathological skeletons, skulls) were re-described according to contemporary syndromological views. The collection contains also many alcohol or formaldehyde preparations which represent rare samples of human and animal congenital malformations. In order to diagnose these rare anomalies, we developed a research project in which molecular cytogenetic investigations (CGH) as wells as radiographical techniques, computed tomography (CT), spiral CT and magnetic resonance imaging (MRI) play an important role. The value of the comparative genomic hybridization-(CGH) based strategy for the analysis of ancient DNA (aDNA) samples extracted from fetuses preserved in the Meckel Anatomical Collection in Halle is discussed in this paper.

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Situs inversus and heart defect in a girl with a chromosome translocation t(X;1) and involvement of ZIC3

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We report on a girl of 2 years with situs inversus and a complex heart defect. Apart from a slight anal ectopia, no other abnormalities have been detected. Chromosome analysis revealed a de novo balanced chromosome translocation 46,X,t(X;1)(q26;p13.1). Molecular cytogenetic investigations identified a breakpoint spanning X-chromosomal BAC clone containing the ZIC3 gene. Mutations in ZIC3 are known to cause situs inversus and heart defects in males. This is the first report of a live born girl with an X-autosome translocation involving the ZIC3 region.

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Towards a better understanding of the heteromorphic patterns in chromosome 9

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Among the non-acrocentric human chromosomes, chromosome 9 presents with the highest degree of morphological variations. Variants, also called heteromorphisms, like 9qh+, 9qh- or inv(9)(p11q13) are common findings in routine cytogenetics. In a previous study we characterized in summary thirteen molecularcytogenetically different heteromorphic pattern of the pericentric region of chromosome 9 (Starke et al., 2002; Eur J Hum Genet 10:790-800). In that study we used a set of three fluorescence in situ hybridization (FISH) probes: a chromosome 9 specific alpha satellite probe, a chromosome 9 specific classical satellite III DNA probe and a microdissection probe specific for 9p12/9q13-21.1. This probe set was enlarged in the meantime by so-called subcentromeric BAC-probes located in 9p12 and 9q13, respectively (probes are specified in Starke et al., 2003; Hum Genet 114:51-67). Thus, we were able to characterize 3 additional, previously unreported heteromorphic pattern of chromosome 9 and to describe variants like '9ph+' (acc. to Starke et al., 2002) in more detail. The biological and/or clinical significance of chromosome 9 heteromorphisms is still unclear. Connection with reproductive failure, mentally retardation, schizophrenia, the Walker-Warburg syndrome, the oculo-auriculo-vertebral (Goldenhar) spectrum and even with cancer predisposition were suggested throughout the literature. The now available possibility to distinguish by FISH between the different heterochromatic patterns hidden behind the cytogenetic finding of a 'heterochromatic variant of chromosome 9' will lead to a clearer genotyp-phenotype correlation of chromosome 9 heteromorphisms in future.

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Are there different ways to build chromosomal inversions during evolution and in so-called heteromorphisms in human?

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To map the breakpoints of the well-known constitutional pericentric inversions in human chromosomes 2 and 9 high resolution multicolor banding (MCB) and human Bacterial Artificial Chromosomes (BACs) located in the corresponding pericentric regions were applied. Four cases, each, were studied with, according to banding cytogenetics, the same inversions on chromosome 2 or 9. Applying the aforementioned molecular cytogenetic methods we obtained evidence for the existence of 3 different breakpoints, each, for the pericentric inversions in human chromosomes 2 and 9. Based on the molecular cytogenetic data for the variants found in 2/4 cases sequence analysis were performed using the NCBI database. Thus, the one analysed breakpoint region, each, of the pericentric inversion of human chromosomes 2 and 9 is characterized by gene destitution, multitude repeats as well as pseudogenes and a high degree of homologous sequences to the breakpoint on the corresponding other chromosome arm. In contrast, evolutionary inversion breakpoints usually do not have such extensive cross hybridizing regions and are often associated with known fragile sites of the (human) genome. According to these molecular characteristics it can be speculated, that evolutionary inversions do not originate from constitutional inversions like the here studied ones of human chromosomes 2 and 9.

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High resolution molecular cytogenetic studies of chimpanzee chromosomes

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We applied multitude multicolor banding (mMCB – Weise et al., 2003 Cytogenet Genome Res 103:34-39) in combination with a novel FISH DNA probe set including subcentromeric, subtelomeric and whole chromosome painting probes (subCTM) to characterize a Pan paniscus (PPA) cell line. These two high resolution FISH-techniques allowed to refine the breakpoints of a pericentric inversion on chimpanzee chromosome 4, and discovered a novel cryptic pericentric inversion in chimpanzee chromosome 11. Additionally, we did the first high resolution analysis of breakpoints on PPA chromosome 4, which are within a long terminal repeat (LTR) and surrounded by segmental duplications as well as the integration/expansion sites of the interstitial heterochromatin on chimpanzee chromosome 6 and 14. Furthermore, the pericentric inversion breakpoints on PPA chromosomes 16 and 17 could be refined and a proposed pericentromeric inversion on PPA chromosome 18 could be disproved. Moreover, by application of glass neadle based microdissection, we found evidence for different types of heterochromatin in the chimpanzee genome. Finally, karyotypes of three chimpanzee subspecies were studied by mMCB and no cytogenetic differences were found although the phylogenetic distance between these subspecies is suggested to be 2.5 million years.

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New centromere-near and subtelomeric rearrangements detected in Pongo pygmaeus supspec.

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The phylogeny of human and ape chromosomes is not yet fully established, although previous studies on that topic have been done by banding cytogenetics as well as molecular cytogenetics. To refine the established comparative maps human probes specific for chromosome-arms, chromosome bar code, multicolor banding (MCB), locus-specific YAC/BAC or cosmid probes and microdissection-derived probes of different species have been used. For the more detailed molecular-cytogenetic characterization of Pongo pygmaeus pygmaeus (PPYp) in comparison to Pongo pygmaeus abelii (PPYa) and Homo sapiens (HSA) we applied a method called subCTM-FISH for which 24 human chromosome-specific probe sets were created as follows: a whole chromosome paint, the corresponding subcentromeric probes (as described in Starke et al. 2003; Hum Genet 114:51-67) and subtelomeric probes (Knight and Flint, 2000; J Med Genet 37:401-409) were combined in a five-color-FISH approach. Applying all 24 subCTM-FISH probe set, we found new cryptic rearrangements in HSA compared to PPY. HSA has a pericentric inversion in #1 compared to PPY; PPY has a deletion of the subtelomeric probe derived from HSA 17p13.3; the PPY chromosomes homologous to HSA #20 show a colocalization of subcen 20q and subtel 20p, which suggests a possible double inversion. Additionally, hints on more complex events in regions homologous to HSA 1p36.1, #3 and #17 can be suggested. Moreover, PPYa seams to have an additional inversion in the pericentromere of chromosome 1 compared to PPYp. In summary, the systematic application of subCTM-FISH is highly suitable to detect up to present overlooked cryptic rearrangements in Hominidae.

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Multicolorbanding applied in 3D-preserved interphase and metaphase nuclei

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Human cytogenetic preparations obtained by the airdrying procedure of chromosome preparation

are good targets for molecular cytogenetics, in general. However, this standard procedure leads to a flattening of the originally spherical interphase and metaphase nuclei. Thus, when interphase or metaphase architecture shall be studied this flattening leads to questionable results. Recently we reported an approach called suspension-FISH (S-FISH) where the whole FISH-procedure is performed on cell suspension. We proved that it is possible to do 3-D-analyses on totally spherical interphase nuclei or even on three-dimensional metaphases (Steinhaeuser et al., J Histochem Cytochem 2002, 50:1697-1698). Here we present a revised S-FISH protocol that enables three major improvements compared to the published one: (i) the high loss of cells (~70%) during the S-FISH procedure was reduced to ~30%, (ii) the cells are embedded now as a final step of the protocol in a 0,5% DAPI-Vectorshield agarose-gel, thus, cells cannot move during fluorescence microscopic evaluation and (iii) multicolor-FISH experiments can now be performed easily. We did 3-D-analysis of FISH-banded chromosomes X and 5 in metaphase and interphase using multicolor banding (MCB). This data, especially on the localization of the X-chromosomes shall be compared with the localization of derivative X-chromosomes like der(X)t(X,12) or der(X)t(X;12).

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ECARUCA: a new online clinical database for rare chromosome abnormalities

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During recent years a considerable improvement in diagnostic techniques has enabled cytogeneticists to find more and smaller chromosomal aberrations. However, accurate clinical knowledge about rare chromosome disorders is frequently lacking, mostly due to a significant decline in publishable cases. On the other hand, there is an increasing demand from parents and physicians for reliable information about the disorder of their child or patient.
Therefore, we established the European Cytogeneticists Association Register of Unbalanced Chromosome Aberrations (ECARUCA); http://www.ecaruca.net/ . This internet-accessible database collects cytogenetic and clinical data of (non-) published cases with a rare chromosome abnormality. This includes microscopic visible aberrations, as well as micro deletions and –duplications.
Cytogenetic results and clinical data of patients can be submitted and the database can be queried online.
Currently the database contains over 5000 chromosomal aberrations from over 4000 patients. Searches in the database using either clinical features or chromosomal aberrations can be performed trough the Internet after receipt of an account. Submission of data to retain the up to date quality of the collection can be done trough the website as well. The latter will allow the exchange of knowledge in single cases, with either wise not publishable, clinically relevant features. The collection and exchange of cytogenetic results combined with clinical features will allow for accurate information on clinical aspects of rare chromosome disorders that can be used by professionals involved. Additionally, the making of detailed correlations between chromosome aberrations and their phenotype is of invaluable help in localizing genes for mental retardation and congenital anomalies.

P146

Problems with karyotypes due to ambiguous ISCN 1995 nomenclature - a CyDAS based analysis

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Thorough analysis of grammar, syntax and meaning of ISCN elements and their combinations was required during development of a software system for the analysis of ISCN data. Though ISCN 1995 is said to have been developed with consideration of computerized analysis, there are some inconsistencies and some features which are prone to cause errors by the user of ISCN.
The designation of bands involved in rearrangements is optional and bands are included in an extra pair of brackets separated from their chromosomes. Thus chromosomes and bands may be put into non-consistent positions, and give raise to hardly detectable errors.
The use and positioning of multiplicators is purely chaotic, sometimes they precede the aberration, sometimes they follow the aberration with a multiplication sign inbetween, sometimes the aberration is to be shown twice or their duly use remains unclear.
The short nomenclature is often regarded as the ideal solution, but it is severely incomplete from a mathematical point of view. All its operators, i.e. the symbols describing aberrations, actually expect non-derivative chromosomes as input, and short nomenclature boldly fails as soon as an aberration spans a junction in a derivative chromosome. The long nomenclature still copes with most of such rearrangements and its use ought to be encouraged.
The symbol 't' had many different meanings in older ISCN, and with ISCN 1995 it still could mean either a translocation or - when preceded by a 'der' clause - the formation of a dicentric chromosome. Such dicentric chromosomes impose severe extra efforts for calculation, and are very often erroneous obviously because of the ambiguity.
The description of involvements of centromeric fragments in rearrangements is totally missing in the ISCN, e.g. duplication of pericentric regions or insertion of a centromeric fragment.
In summary, the project caused a new view on the nomenclature of cytogenetic findings which may lead to an improved ISCN.

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Pure maternal trisomy of distal 11p as a result of a non-reciprocal translocation characterised by microdissection

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We report on a 11 months old girl with pure partial trisomy of the distal short arm of chromosome 11. As clinical manifestations, our patient showed severe intrauterine and postnatal growth retardation with measurements corresponding to a newborn when last seen, major problems to thrive, and developmental delay. Her face was asymmetric, dysmorphic, and suggestive of a craniostenosis syndrome. Conventional chromosome analysis was done on the patient and her parents and revealed an altered chromosome 5 with extra material on the short arm. By the application of FISH with chromosome 5-specific library, with a subtelomeric probe set and with a probe generated by microdissection of the aberrant chromosome 5p the patient's chromosomal imbalance could be interpreted as de novo partial trisomy 11p, which resulted from a non-reciprocal translocation 5/11. A coexisting deletion of chromosome 5p could be ruled out by FISH with the subtelomeric probe of 5p. Molecular genetic investigation confirmed duplication of a region of at least 5 Mb, which is implicated in Beckwith Wiedeman syndrome (BWS), and showed this rearrangement being maternal in origin. Only a few cases of pure partial trisomy 11p of maternal origin have been reported. Whereas, paternal duplications of the distal region of 11p result in the overgrowth characteristic of BWS, the maternal derived duplication of terminal 11p is in particular associated with growth retardation. The delineation of a distinct phenotype of maternal duplication of distal 11p needs further reports. Nevertheless, all patients described show marked growth retardation and share some dysmorphic features, which might resemble the opposite phenotype to BWS and contributes to the aetiology of Silver-Russell syndrome (SRS). This case further demonstrates the advantages of Micro-FISH and FISH with subtelomeric probes in the characterisation of chromosomal aberrations and the identification of breakpoints.

P148

Familial occurrence of partial trisomy 12q leading to different phenotypes

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We report on a familial case of an unbalanced translocation consisting of partial trisomy 12q and minimal monosomy 5q. The index patient was a female newborn with macrosomy and dysmorphic features including cleft palate, broad nasal bridge, retrognathy, epicanthal folds, low set ears and a deep crease between hallux and second toe. Later on, febrile seizures occurred and mental retardation is obvious. NMR analysis revealed demyelinisation of the paraventricular white matter and mild atrophy of the cortex. The karyotype is 46,XX,der(5)t(5;12)(q35;q24.3). This karyotype was confirmed by comparative genomic hybridization (CGH) followed by FISH with

a wcp 12 probe and a 5q subtelomeric probe which revealed loss of the distal end of 5q. Unexpectedly, the same unbalanced karyotype was detected in the mother who only exhibits a learning disability without dysmorphic features. In conclusion, we describe a new partial trisomy 12q with broad phenotypic variability.

P149

The hierarchically organised splitting of chromosome bands into sub-bands analysed by multicolour-banding (MCB) and chromosome stretching

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The splitting of chromosome bands into their sub-bands has its implications for the precise mapping of DNA probes at the sub-band level and for the understanding of the chromosome architecture. Surprisingly, there have been nearly no scientific investigations dealing with that process. Here we investigated the hierarchically organised splitting of bands in detail using the multicolour banding (MCB) probe set of different human chromosomes (#5, 6, 18,19 and X) hybridised to normal human metaphase and prometaphase chromosomes at the ~850-, ~550-, ~400-, and ~300-band level. The analysis were performed by comparing the disappearance and appearance of pseudo-colour bands of the four different band levels. The regions to split first are telomere- and centromere-near. The directions of band splitting towards the centromere or the telomere could be assigned to each band separately. In contrast to the GTG-band ideograms published in ISCN 95 at the 850-, 550-, and 400-band level pseudo-colours assigned to GTG-light bands are resistant to band splitting. GTG-dark bands split into their dark and light sub-bands because inside dark bands light sub-bands appear which are rather resistant to further elongation. This confirms the results obtained by stretching of GTG-banded chromosome 6 published by Hliscs et al. (Cytogenet Cell Genet 79:162-166, 1997). In this respect the nomenclature of the ideograms of GTG-banding patterns for normal human chromosomes should be reassessed. Furthermore, the results indicate to fundamental doubts on the well established concept of chromosome condensation during mitosis which should be replaced by the recently proposed concept of chromosome region-specific protein swelling.

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Molecular cytogenetic characterization of constitutional structural aberrations involving 14q32 reveals breakpoints in the lymphoma-associated BCL11B and IGH loci

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Structural chromosomal aberrations affecting chromosome band 14q32 are frequent events in malignant lymphomas but are only rarely observed as constitutional changes. This band harbors the immunglobulin heavy chain (IGH) gene locus, translocations of which are the cytogenetic hallmark of several B-cell lymphomas, and the BCL11B locus, which is rearranged in T-cell leukemias. Here, we describe the molecular cytogenetic characterization of two cases with unbalanced constitutional chromosomal changes affecting 14q32.

Case 1: A 3 year-old girl presented with developmental delay, inborn malformations (corpus callosum hypoplasia, ventriculomegaly, ventricle septum defect and diastasis recti) and facial dysmorphism. Chromosomal analysis on blood lymphocytes revealed the karyotype 46,XX,dup(14)(q32.3q32.2). Fluorescence in situ hybridization (FISH) showed an inverted duplication in 14q32 including the BCL11B gene in 14q32.2 as well as the proximal part of the IGH locus in 14q32.3. The distal part of the IGH gene was deleted, mapping the telomeric breakpoint of the duplication within the IGH gene. The partial IGH deletion was confirmed to be constitutional by additional FISH analyses on buccal swaps.

Case 2: In a 34 week fetus, cerebral moderate ventriculomegaly, short femur and a single umbilical arteria were observed. Chromosome analysis on amniotic cells revealed an interstitial de novo deletion del(14)(q31q32.2). FISH identified loss of the TCL1 gene in 14q32.1 as well as loss of the centromeric part of the BCL11B locus, mapping the distal breakpoint of the deletion within the BCL11B gene.

To the best of our knowledge, this is the first time that FISH-proven disruptions of the lymphoma-related genes IGH and BCL11B are reported in the constitutional cytogenetic setting. Although clonal rearrangements of both genes are shown to be involved in tumorigenesis, none of the two patients described herein showed signs of leukemia, lymphoma or other neoplasia so far.

P151

Characterization of a terminal deletion of the long arm of chromosome 4 using FISH

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We report on a 3 years old girl with a de novo terminal deletion of the long arm of chromosome 4. Standard cytogenetic banding techniques map the breakpoint to 4q33, resulting in the karyotype 46,XX,del(4)(q33). The patient presents with developmental delay, facial dysmorphism and pathognomonic features of the fifth finger. Several cases with similar deletion breakpoints within 4q with a likewise severe phenotype including mental retardation, facial dysmorphism, abnormal fifth finger, displacement of toes and cardiac defects have been reported previously. From these cases the critical region for the 4q terminal deletion syndrome was supposed to be within 4q33-4qter. For narrowing down the deletion breakpoint in our case we used a panel of DNA probes from the critical 4q region for FISH. Our preliminary FISH results show that the breakpoint may be located in 4q34. Thus our results indicate that the critical region for the typical pathognomonic features of "4q- syndrome", especially the abnormal fifth finger, might be located distal to 4q33.

The breakpoint will be further refined and the results in our case will be discussed in the context with previously published cases.

P152

Absence of characteristic features of 18p-syndrome in two sibs with partial monosomy 18p / partial trisomy 20p due to a familial cryptic translocation

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The 18p- syndrome is a well-known haploinsufficiency disorder with a variable expression of typical dysmorphic features, malformations and mental retardation (MR) which was first described by de Grouchy et al. in 1963. Some of the characteristic features with a high penetrance in 18p- syndrome are microcephaly, round face, dysplastic and low-set ears, carp mouth, hypertelorism, muscular hypotonia, congenital heart defect and MR. Here, we report two sibs (one female, one male) with partial monosomy 18p and partial trisomy 20p detected by FISH subtelomeric screening. The balanced translocation was diagnosed in the sibs' mother and older sister. The typical facial characteristics of 18p- syndrome were absent in both affected sibs. Features present in at least one of the two sibs included small stature, microcephaly, epilepsy, syndactyly, hip luxation and scoliosis. The male patient showed severe MR and the female patient only mild MR. Ongoing breakpoint analyses of the female patient by FISH with panels of large insert clones showed a partial monosomy 18p of at least 7.5 Mb (at least 18p11.23 -> pter) and a partial trisomy 20p of 5.1 to 5.3 Mb (20p12.3 -> pter). Most typical features of 18p- syndrome including malformations and partial trisomy 20p (such as round face with high cheekbones, dental anomalies including tooth decay and hypertelorism) were absent in our patients in spite of their relatively large imbalances. This lack of typical clinical signs demonstrates - even in seemingly well known chromosomal disorders - the benefit of exact

breakpoint analyses to improve karyotype/phenotype correlations.

P153

Sensorineural hearing loss in a girl with a de novo terminal 10q26.1 deletion

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Terminal and interstitial deletions with proximal break-points in 10q25 or 26 cause genital abnormalities of various degree in male patients. Further clinical features characteristic for partial distal monosomy 10q are anomalies of the urinary tract, growth retardation and psychomotor delay.
We report on a girl presenting with profound mental retardation, growth retardation, ear dysmorphy and wide spaced nipples. At the age of four, auditory brainstem response showed a bilateral sensorineural hearing impairment (early acoustic evoked potentials until 50 dB right, until 80 dB left). The patient did not cry as a baby and did not develop speech even after clear improvement of hearing using hearing aids.
GTG banding analysis of cultured lymphocyte metaphase spreads revealed a terminal deletion of the long arm of one chromosome 10 with break-point in the chromosomal band 10q26.1. Multicolor Banding and Comparative Genomic Hybridization confirmed this finding. FISH with YAC probes revealed the break-point within YAC 937a06.
Up to now, only 5 of 40 published cases with terminal deletions in 10q demonstrated sensorineural hearing impairment in the wide range from mild impairment to deafness. As the break-point locations differ between these patients, different extent of deletion, positional effects or unmasking of heterozygosity may explain the variable manifestation of hearing impairment.

P154

A Further Case of a Novel Microdeletion Syndrome: Subtelomeric Deletion 9q

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Following the introduction of FISH with subtelomeric probes, a growing number of subtelomeric deletions have been recognized associated with distinct clinic features.
Recently Stewart et al. (Am J Med Genet 2004,128A:340-351) reported on 12 patients with subtelomeric deletion 9q and phenotypic abnormalities that constitute a unique and recognizable microdeletion syndrom.
We report on a further patient with this syndrome. The 21 month old old girl (height P50. weight P90, head circumference P25-50) presented the typical clinical features: a distinctive facial appearance with a slightly coarse face and tented upper lips, striking muscular hypotonia, and mental retardation. No heart defect or other organ malformations were diagnosed.
Screening for subtelomeric aberrations reveled a deletion in 9q. By FISH with bacterial arteficial chromosome (BAC) probes the deletion was shown to be localized within the commonly deleted region.

P155

Breakpoint characterization of a 2q deletion in a young girl with epilepsy

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We report a 2 year old girl with an unbalanced de novo translocation between chromosomes 2 and 15, identified by conventional cytogenetics. The girl was born in the 38th week as first child of a 45 old father and a 40 year old mother. ICSI had been performed because of male sterility. Both pregnancy and family history were unremarkable. No invasive prenatal diagnosis was done and fetal ultrasounds in first and second trimester showed no morphological abnormalities. Physical examination at the age of 4 days revealed trigonocephaly, prominent nasal bridge, dysmorphic ears, short palpebral fissures with antimongoloid slant, microphtalmia, coloboma, blepharophimosis and micrognathia. In addition, hypotonia was noted. The girl suffered from frequent epileptic seizures accompanied by severe central apnea. The cranial X-ray showed no synostosis of the metopic suture. Ultrasound of brain, abdomen and heart was normal.
Initial banding analysis was already suggestive for a deletion on chromosome arm 2q, however, the size of the deletion could not be determined. Subsequent hybridizations with a panel of BAC clones from the critical region allowed to map the deletion to the chromosomal region 2q24.1-2q31.1. The deletion has a size of about 13 Mb and contains ca. 30 genes. The function of some of these genes is known and can explain the occurrence of the clinical findings described here. Most importantly, this region contains several ion channel genes, such as SCN1A, SCN2A, SCN3A, which have been described to be involved in idiopathic epilepsy. Furthermore, the region contains TBR1, a transcription factor gene relevant for the differentiation of cortical neurons. Thus, the frequently occurring seizures in our patient can most likely be explained by the loss and subsequent haploinsufficiency of several genes involved in ion transportation and neuron differentiation. This case will extent our knowledge about the underlying genetic causes of epilepsy.

P156

Benefits and limitations of CGH in prenatal and perinatal diagnosis and pathology

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Comparative genomic hybridization (CGH) as a method for genome wide screening for changes in DNA copy number is helpful if classical cytogenetic results are to be reviewed or karyotype analysis cannot be performed. But CGH can cause ambiguous results as well. The aim of this study is to evaluate this method in pathology routine. In a series of 17 cases of prenatal and perinatal diagnosis and pathologic examination, the benefits and limitations of CGH were assessed.
In 11 cases with previous karyotype analysis, CGH profiles allowed the:
Confirmation of numerical aberrations like common (trisomies13, 18, and 21, monosomy X) and rare (trisomy 4) aneuploidies (n=7).
Confirmation of unbalanced structural aberrations by the delineation of enhanced and diminished regions (n=4).
Refinement of breakpoints (n=2).
Problems observed: Additional unspecific gains or losses, mostly located in telomeric or centromeric regions.
In 3 out of 6 cases without previous karyotype analysis, irregular CGH profiles were found:
With gains, indicative of numerical or partial aneuploidies (n=2).
With combined gain and loss, indicative of unbalanced translocation (n=1).
Problems observed:
Normal profile in a fetus with striking morphology highly conspicuous for chromosome abnormality (n = 1).
No result at all in cases of poor DNA quality (n=2).
Discrimination between significant aberration and artifacts. Especially the comparison of fetal or neonatal morphology with expected clinical characteristics of the considered aberration might be helpful.
Additional FISH studies are required (e.g. with locus specific, pcp or wcp probes).
Overall, despite the stated restrictions, CGH is a valuable addendum to classical cytogenetics and is very supportive in combination with fetal or neonatal pathology when no karyotype analysis is possible. CHG in combination with FISH-tests might give conclusive findings regarding chromosome aberration as a cause of phenotypic anomalies.

P157

Interstitial deletion 2q22-q24 associated with retardation and apraxia of speech but without most features of Mowat-Wilson syndrome

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To date more than 40 patients with microdeletion for chromosome segment 2q22-q23 have been described. Most of them are associated with features of Mowat-Wilson syndrome (MWS) which is characterized by a specific fazial gestalt, mental retardation, microcephaly, seizures, multiple congenital anomalies and often by Hirschsprung disease (HSCR). Our patient is the 2nd child of nonconsanguineous young parents. At birth only paroxysmal tachycardia was noted. At 5 months of age facial dysmorphism, microcephaly, growth retardation,

general muscular hypotonia and lymphoedemata of upper and lower limbs were recorded. At 3 yrs of age additional features were short stature and severe developmental retardation; he was not able to walk and did not speak any word. Brain MRI and CT provided normal results. Karyotyping revealed a de novo deletion 46,XY del(2)(q22.3q24.1). Genotyping of 14 microsatellite markers identified hemizygosity of 2 markers with loss of the paternal allele. FISH using RP11-BACs confirmed the interstitial deletion of approx. 12 Mb and mapped the breakpoints (BP) between 147.8 Mb and 148.2 Mb (2q22.3-q23.1) as well as between 160.4 Mb and 161.9 Mb (2q24.1).The ZFHX1B gene causing Mowat Wilson syndrome maps about 2.5 Mb proximal to the deletion which explains missing of many of the MWS features such as HSCR, epilepsy and heart anomaly. Dyslalia and the inability to speak has not been reported so far with MWS due to interstitial 2q22-q23 deletions. This could mean that this feature of our patient is related to the more distal nonoverlapping deletion segment. The recent observation by several genome scans (Bacchelli E et al. 2003) that chromosome segment 2q21-q33 is likely to contain an autism susceptibility locus is remarkable in the context with the speech disorder of our patient.

P158

Erfahrungen mit der Polkörperdiagnostik bei Translokationsträgerinnen.

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Die Polkörper können zur indirekten Diagnostik auf chromosomale Fehlverteilungen der Eizelle im Rahmen der künstlichen Befruchtung (IVF/ICSI) herangezogen werden. Beide Polkörper werden dazu mittels Fluoreszenz in situ Hybridisierung (FISH) auf Chromosomen (Aneuploidiediagnostik) und / oder auf Chromosomenabschnitte (bei Translokationspatientinnen) untersucht.
Bisher wurden bei uns insgesamt 109 erste oder zweite Polkörper von 75 Eizellen bei 11 Translokationsträgerinnen (10 mit reziproken Translokationen und eine mit einer Robertsonschen Translokation) untersucht. Alle Patientinnen hatten bisher kein lebendes eigenes Kind, 10 Patientinnen hatten rezidivierende Fehlgeburten, eine Patientin hatte bisher 5 frustrane IVF/ICSI-Zyklen. Die Polkörper wurden mit 2 oder 3 kommerziell erhältlichen Sonden untersucht.
Von 64 eindeutig auswertbaren Eizellen (83%) waren 27 balanciert (43%) und 37 nicht balanciert (57%). Insgesamt wurden 14 Embryonen in 9 Zyklen transferiert. Drei Frauen wurden schwanger, abortierten jedoch im ersten Trimenon.
Im Vergleich zum altersentsprechenden Patientinnenkollektiv (Durchschnittsalter 33,6 Jahre) erscheint die Anzahl der untersuchbaren Eizellen bei Translokationsträgerinnen reduziert (Durchschnitt 6 Eizellen pro Patientin). Die Untersuchungsmöglichkeiten der Polkörper in Bezug auf seltene, jedoch bei reziproken Translokationen typische Verteilungsmuster sind mit kommerziellen Sonden limitiert. Interchromosomale Effekte, die zu einer höheren Aneuploidierate führen, sind nicht auszuschliessen.

P07 Genotype and Phenotype

P159

Identification of 14 novel mutations in the DHCR7 gene causing the Smith-Lemli-Opitz Syndrome and delineation of the DHCR7 mutational spectra in Spain and Italy

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Objectives: The Smith-Lemli-Opitz syndrome (SLOS) is a phenotypically variable metabolic malformation and mental retardation syndrome for which more than 80 mutations in the DHCR7 disease-causing gene have been described. The DHCR7 mutational spectra differ significantly in different areas of Europe, and several common putative founder mutations account for a substantial fraction of all mutations in some ethnic groups.
Material and Methods: Sequencing of the complete coding sequence and of exon-intron bounderies of the DHCR7 gene in more than 200 SLOS patients revealed 14 not yet published mutations in 18 SLOS patients of Ashkenazi Jewish, Austrian, British, German, Irish, Polish, Portuguese, and Spanish origins. SNP haplotypes (8 intragenic DHCR7 SNPs) and phenotypic variation were associated with these mutations. The data set from all SLOS patients was analysed regarding Spanish and Italian mutation spectra.
Results: Half of the new mutations are in the transmembrane domains of the protein. In addition, there were two null mutations, one mutation in the 4th cytoplasmic loop, mutations in the first and last codons, and three mutations in other regions such as the second cytoplasmic loop and the first endoplasmic loop. The T93M mutation is the common Italian SLOS causing mutation (45% of SLOS alleles), the IVS8-1G>C is the common mutation in Spanish SLOS patients (30%).
Conclusions: The novel mutations described here are mostly leading to phenotypic variation predicted according to their protein localisation or functional effect. The common haplotype A was the most frequently associated with these mutations. The analysis of 20 Spanish and 12 Italian SLOS patients revealed very different mutation spectra compared to previously described patients from Czechoslovakia, Germany, Poland, and the UK and implicated p.T93M on the J haplotype as the most frequent Mediterranean founder mutation.

P160

A novel laminopathy combining progeroid phenotypes with cardiac involvement and partial lipodystrophy

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Based on the wide phenotypical range, laminopathies have recently been divided into such with preferential involvement of skeletal and cardiac muscle, and peripheral nervous system, with lipodystrophy, with premature aging and with mixed phenotypes. Here we report a patient with a novel LMNA mutation showing phenotypical overlaps between the entities.
The patient is a 26 year old female presenting at age 15 with growth retardation, squeaky voice, beak like nose, micrognathia, joint contractures and a holosystolic heart noise. Echocardiography showed calcification of aortic and anterior mitral valves leading to a moderate aortic stenosis, aortic insufficiency grade II and sclerosis of the anterior mitral valve finally requiring prosthetic exchange of the valves at age 18. Left ventricular and atrial hypertrophy was observed. Until age 18 she developed scleroderma, scalp hair thinning and greying, skin naevi, generalized subcutaneous lipodystrophy leaving out the face, venal patterning of the thin skin, insulin resistant diabetes mellitus, premature atherosclerosis, and hypogonadism. The maximum body height was 1.55 m. CK serum level was normal. A novel LMNA mutation P485R, was found, which is placed in the gene's region clustering R482 mutations associated to 80% of all FPLD patients. The patient represents a novel mixed phenotypes connecting the group with premature aging to those partial lipodystrophy. This finding supports the view that laminopathies represent a group of contiguous but clinically extremely heterogeneous diseases. The careful clinical differentiation of the laminopathies might finally provide clues to resolve the complexity of the pleiotropic pathogenetic effect of LMNA mutations.

P161

Clinical relevance of ACVRL1 mutations in patients with intrahepatic hereditary haemorrhagic teleangiectasia

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Hereditary haemorrhagic teleangiectasia (HHT) is an autosomal dominantly inherited disorder characterised by cutaneous and mucosal telangiectasias, epistaxis and arteriovenous malformations in lung, liver, central nervous system and gastrointestinal tract. Until today, mutations in the genes for endoglin and for activin A receptor type II-like kinase 1 (ACVRL1) have been identified to cause HHT. Intrahepatic manifestation in HHT occurs to 8-31% and, if severe, might lead to the requirement of liver transplantation. We report here on 8 liver transplanted patients with intrahepatic HHT, in whom both

genes were sequenced. Mutation analysis in all patients revealed the presence of mutations in ACVRL1. This result is of prognostic value concerning the need of liver transplantation in HHT patients.

P162

Human genetic analysis of two families with Tetralogy of Fallot (TOF)

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Tetralogy of Fallot (TOF, MIM # 187500) is the most common cyanotic conotruncal heart malformation and accounts for 1 among 3.000 live births. Genes involved and molecular pathogenesis are largely unknown. Syndromic TOF has been observed with various chromosomal disorders such as trisomy 21 or microdeletions 22q11.2 Few isolated nonsyndromic TOFs, either sporadic or familial, have been found to be associated with single gene mutations such as within ZFPM2/FOG2 and NKX2.5. In addition, recent observations identified the JAG1 gene mutated in Alagille syndrome (AGS, MIM #) as a candidate for TOF. We report about two families of european caucasian origin with several individuals showing TOF and being affected by truncating frame shift mutations within the 3'- terminal coding region of JAG1. In family A with 3 sisters with TOF, the youngest deceased, a segregating maternal frame shift mutation within exon 24 (3021-3022insGC) was detected. None of the sisters demonstrated features characteristic for AGS with the exception of butterfly vertebrae and an additional rib reported for the older sister. The mother carrying the mutation is clinically normal and even does not show any evidence for heart malformation. In family B with 3 out of 6 sibs with TOF (1 sister, 2 brothers) and one affected son a truncating frame shift mutation within exon 26 (3599-3600insG) was detected. Also in this family none of the individuals met the diagnostic criteria for AGS. Our data indicate that even truncating mutations within the 3'-coding region of JAG1 may be causally related with familial nonsyndromic TOF. Further studies in sporadic and familial cases have to reveal whether JAG1 has a significant impact on TOF.

P163

Constitutional deletion of the hSNF5/INI1 gene telomeric of the 22q11 TDR in a patient with 22q11 microdeletion syndrome phenotype and a rhabdoid tumor

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Microdeletions in chromosome band 22q11 are associated with cardiac defects, hypoplasia of the thymus and the parathyroid, velopharyngeal dysfunction, learning difficulties, and characteristic facial features. The expression of these traits is highly variable among patients, despite the fact that a deletion of 3Mb (typically deleted region, TDR), whose frequent de novo occurrence is facilitated by low copy repeats at its end points, is present in ~90% of the cases.
Here we report a patient who presented with a ventricular septal defect, dextroposition of the heart, recurrent respiratory tract infections, hypacousis, chronic otitis media, developmental retardation, and dysmorphic features suggestive of a syndromal disorder. At the age of 9 months, a malignant rhabdoid tumour of the kidney was diagnosed, of which he died at the age of 19 months.
Cytogenetic analysis revealed a normal male karyotype, and FISH with the Tuple1/Arsa probe, designed to detect the typical 22q11 microdeletion, did not indicate the presence of such an aberration. However, a probe for the BCR locus, which served as a chromosome identifier probe in a subsequently applied subtelomere FISH panel, was absent from one of the chromosomes 22. The BCR gene is located ~2 Mb telomeric of the 22q11 TDR, and ~0.5 Mb centromeric of INI1, a tumor suppressor gene that is frequently inactivated in rhabdoid tumors. To determine the exact nature and extent of the atypical chromosome rearrangement in this patient, and to investigate the possible involvement of INI1 in the emergence of his tumor, FISH with a panel of PAC/BAC probes, matrix CGH, microsatellite analysis, and INI1 mutation analysis were performed. We found a complex de novo constitutional rearrangement of chromosome band 22q11, which included deletion of the INI1 locus, in the patient's peripheral blood cells, as well as a somatic 2bp deletion in the second INI1 allele in the patient's tumor.

P164

Rate versus efficiency in a disposable soma.

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In this communication the trade-off between rate and efficiency - exemplified by the linear free energy converter (Kedem and Caplan 1965) as a simple quantative model of a metabolising organism – is put in relation to the trade-off between reproduction and self-maintenance as described by the disposable soma theory (Kirkwood 1977). It is shown that for maximal proliferation efficient but slow metabolisers invest a higher fraction of resources in self-maintenance than fast but less efficient metabolisers.
The optimal trade-off between reproduction and self-maintenance is determined by life history conditions such as external mortality. Consequently, external mortality may also have an influence on the trade-off between rate and efficiency.
Although the above model is semi-realistic the predicted connection between self-maintenance, i.e. life span, and energy metabolism is supported by empirical data. In yeast cells the life-extending effect of caloric restriction has been shown to involve a switch to oxidative metabolism and to be transmitted by the *SIR2* gene product that reacts to the NAD/NADH ratio and influences aging (Lin et al 2002, 2004).
Oxidative metabolism is slower but more efficient than fermentation. Pfeiffer et al (2001) investigated a yeast model *in silico* in order to show how organisms may come to cooperate in efficient, that is, oxidative use of resources. Frick and Schuster (2003) showed that these organisms may be in a prisoner's dilemma where defecting against cooperative oxidation by the use of fermentation has the highest payoff. Thus, translated to the reasoning that I present here, self-maintenance, i.e. life-span may be enhanced by cooperation but challenged by a prisoner's dilemma. Furthermore, genes that are involved in social behaviour may also have an influence on aging.

P165

Renal malformations in deletion 22q11.3 patients

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The microdeletion 22q11.2 syndrome occurs in 1/4000 live births. A wide spectrum of clinical findings have been described in patients carrying the 22q11.2 deletion. The main symptoms are heart defects, particulary conotruncal anomalies, immune deficiency and characteristic facial features. Hearing loss, renal malformations, growth failure and seizures have been described in some cases.
We performed Fluorescence in situ hybridization (FISH) in metaphase chromosomes using DNA probes from the DiGeorge critical region, DGCR [TUPLE1, N25; Abbott/Vysis] in patients with conotruncal heart defects for 22q11-13 deletion screening, if there was a normal karyotype in routine cytogenetic analysis. In 5 patients a microdeletion 22q11.2 was detected. The patients were diagnosed for relevant cardiovascular malformation, e.g. ventricular or atrial septal defect. They showed typical facial dysmorphic features such as dysplastic ears and micrognathia. In 4 of 5 patients (80%) renal dysplasia or single kidney has been recognized. This rate of renal anomalies in our group is much higher than expected. Earlier studies demonstrated a percentage up to 40% for renal malformations in patients with a microdeletion 22q11. We suggest that in patients with a microdeletion 22q11.2 atypical malfomation of the kidneys should be searched for systematically and excluded by ultrasound examination.

P166

Genotype-phenotype correlations in patients with BIGH3 linked corneal dystrophies

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Purpose: Different missense mutations in the BIGH3 gene cause granular (Groenuoew CDGG1, Avellino CDA, Reis-Bücklers CDB1) and lattice (Biber-Haab-Dimmer CDL1) corneal dystrophies and in some reports corneal dystrophy Thiel-Behnke (CDB2). We report on the mutation spectrum and the genotype-phenotype correlations on the basis of clinical and histopathological examinations of 10 German families with BIGH3 linked corneal dystrophies.

Methods: In 10 undependend families and patients with different corneal dystrophies DNA was extracted from leucocytes of the peripherial blood. Mutation analysis was performed by direct sequencing of the BIGH3 gene. Clinical and histopathological findings were compared with the moleculargenetic findings for genotype-phenotype correlation.

Results: In the two families with clinical and histopathological CDL1 we found a missense mutation Arg124Cys and in the family with clinical and histopathological CDA we found a missense mutation Arg124His in the exon 4 of the BIGH3 gene. In the three families with clinical and histopathological CDGG1 we found a missense mutation Arg555Trp in the codon 12 of the BIGH3 gene. In all 3 families with clinical and histopathological CDB2 we could not find any mutation in the BIGH3 gene. In one patient with exceptional clinical and histopahtological findings we found a missense mutation Ala546Asp, wich was reported before only one time in connection with CDL1.

Conclusions: In comparison of our clinical and histopathological findings and the molecular genetic results
we found a strong genotype-phenotype correlation in patients with BIGH3 linked corneal dystrophies. Rare mutations leed to exceptional clinical and histopathological findings wich cannot be classified into the different groups of corneal dystrophies. In our patients with CDB2 we could not find any moleculargenetic correlation to the BIGH3 gene.

P167

Identification of a new 3 kb deletion in the arginine vasopressin receptor 2 gene (AVPR2) in a patient with X-linked nephrogenic diabetes insipidus

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Congenital nephrogenic diabetes insipidus (NDI) is characterized by polyuria and polydipsia due to the inability to concentrate urine, despite normal or elevated plasma concentrations of the antidiuretic hormone arginine vasopressin (AVP). In 90% of families the disease (MIM304800) is inherited in an X-linked recessive manner, and is caused by mutations in the AVPR2 gene on Xq28. Most of the mutations detected are nucleotide substitutions resulting in a truncated receptor protein. Gross deletions involving the AVPR2 gene have been observed in few patients with NDI. This study describes the presence of a new deletion of 3 kb spanning exons 2 and 3 of the AVPR2 gene and the analysis of the proximal and distal breakpoints of the deletion in a patient with NDI. The deletion results in the loss of almost the complete coding region of the gene. Family analysis revealed the deletion in the patient's unaffected mother. Documentation of the diversity of mutations in NDI will assist genotype-phenotype correlation and may contribute to early diagnosis and treatment of the disorder, thereby preventing serious complications such as growth and mental retardation.

P168

A variant form of potassium-aggravated myotonia (PAM) in a big German family harbouring a new mutation in the SCN4A gene

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Objectives: Mutations in the SCN4A (voltage sensitive adult muscle sodium channel, alpha subunit) gene cause three different allelic forms of myotonia: hyperkalemic periodic paralysis (HyperPP), paramyotonia congenita (PMC) and potassium-aggravated myotonia (PAM). Rarely PAM also presents as myotonia fluctuans or myotonia permanens. We describe a family with 14 affected members suffering from a distal form of myotonia, which is cold sensitive and has variable expression from day to day.

Material and Methods / Results: At least one of the patients in the family was clinically suspicious for PROMM (DM2). Both forms of myotonic dystrophy could be excluded, and a linkage analysis for candidate genes gave a significant coupling to DNA markers from the SCN4 locus. Sequencing the whole SCN4A gene detected a mutation in exon 24: Ala1481Asp, which is not yet described to our knowledge and segregated in the family. The oldest patient had a biopsy at the age of 71 years and showed a progressive vacuolic myopathy using routine microscopy and electron microscopy, which could be very rarely seen in HyperPP.

Conclusions: This observation confirms the broad phenotypic spectum of myotonias caused by specific mutations in the SCN4A gene.

P169

Functional evaluation of Dent's disease-causing mutations: Implications for ClC-5 channel trafficking and internalization

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ClC-5 is a member of the CLC family of voltage-gated chloride channels. Loss-of-function mutations of its corresponding gene (CLCN5) cause Dent's disease, an X-linked kidney disorder, characterized by low-molecular weight proteinuria, hypercalciuria, nephrocalcinosis/nephrolithiasis and progressive renal failure. Here, we examined the effect of different mutations on function and cellular trafficking of the recombinant protein. Mutant CLCN5 cDNAs were generated by site directed mutagenesis for three premature stop codon variants (R347X, M571IfsX528 and L521RfsX526), and several missense mutations: C221R, L324R, G462V and R516W (identified in our patients), as well as mutants G506E and R648X (previously reported by others).

After heterologous expression in Xenopus oocytes, ClC-5 channel activity and surface expression were determined by two-electrode voltage clamp analysis and ClC-5 surface ELISA, respectively. Except for the R516W and R648X variants, none of the mutated proteins induced functional chloride currents or reached the plasma membrane. This is readily understandable for the truncation mutations however, the tested missense mutations are distributed over different transmembrane regions implying that correct channel structure and orientation in the membrane is not only a prerequisite for proper ClC-5 function but also for Golgi exit. Interestingly, the R648X mutant although functionally compromised, displayed a significant increase in surface expression. This finding might be explained by the deletion of a ClC-5 carboxy-terminal PY-like internalization signal which in turn impairs channel removal from the membrane. Our observations further imply, that recruitment of ClC-5 to alternative routes (plasma membrane or early endosomes) in the trans-Golgi network is mediated via different signal sequences.

P170

Genome-wide autozygosity mapping and linkage analysis in consanguineous Iranian families with non-syndromic autosomal recessive mental retardation

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Mental retardation (MR), defined by an intelligence quotient (IQ) of less than 70, affects approximately 2% of the general population in the world. Etiologically, MR is a very heterogeneous condition, which in over two-third of the cases is caused by chromosomal rearrangements and/or single gene defects, including X-linked (XLMR), autosomal dominant (ADMR) as well as autosomal recessive (ARMR) forms. So far, next to nothing is known about the genes that play a role in ARMR, because in western civilizations, small family sizes preclude the mapping and gene identification. In contrast, very large and highly consanguineous families are common in Iran, which greatly facilitate autozygosity mapping.
Recently, we have recruited over 30 Iranian families with ARMR. Detailed clinical and cytogenetic examinations of affected individuals have ruled out the fragile X syndrome and chromosomal abnormalities as being the cause of MR. Pedigree structures and consanguinity in these families suggest an autosomal recessive mode of inheritance, i.e. affected individuals should inherit the same autozygous region containing the disease causing defect. Using the Human Mapping 10K SNP-array (Affymetrix GeneChip technology), we have scanned 84 affected and 70 unaffected individuals from 29 families for autozygous regions. SNP genotyping data have been checked by GRR and PedCheck to verify the relationship between individuals, and subsequently analyzed by different software including VARIA (SiliconGenetics), GeneHunter and Merlin. We have identified several candidate (autozygous) regions with a significant Lod Score and compatible haplotypes. The detailed evaluation of these regions revealed several strong candidate genes, which are presently screened for mutations. Perspectively, we will analyze more than 100 families with ARMR, which may result in the identification of common autozygous regions and major genes for ARMR.

P171

Novel mutation in the SDH-B gene presenting with different types of paragangliomas in 3 family members

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Sporadic and familial paragangliomas are rare, slowly growing tumours of the para- and sympathetic nervous system. Parasympathetic paragangliomas usually arise in the head and neck region, are hormonally silent. Medullary and extra-adrenal pheochromocytomas secrete catecholamines and usually are located in the lower abdomen.Malignant transformation has been described for the latter only in familial paragangliomas.
Medullary pheochromocytomas as part of the vHL disease, MEN-2 and neurofibromatosisare are caused by mutations in the vHL-, RET- or NF-1 gene, respectively.Dominant mutations of the genes coding for the subunits of the mitochondrial succinate-dehydrogenase complex (SDH-B, -D,-C)have lately been characterized in familial paragangliomas, including medullary pheochromocytomas.
We identified a novel splice-site acceptor mutation in intron 5 of the SDH-B gene ,IVS5-2A>G. The mutation was detected in a 40year-old woman presenting with an extra-adrenal pheochromocytoma at the posterior bladder wall as a by chance finding at routine gynecological investigation. Her sister was also diagnosed as a carrier. In her the disease had already manifested at age 29 years with neurological symptoms of an an infiltrative,endocrinologically silent glomus tumour. The now 67year-old mother of both sisters was diagnosed as a carrier now and will be specfically investigated now for a known hypertension.
We discuss the mutation, clinical manifestation and intrafamilial variability, as well as clinical follow-up for affected patients, including predictive diagnosis in the 10year-old carrier grandchild.

P172

Spectrum of molecular defects and mutation detection rate in patients with severe haemophilia A

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Haemophilia A (OMIM 306700) is the most frequent X-linked bleeding disorder affecting 1 to 2 per 10 000 males worldwide. Various types of mutations in the factor VIII gene are causative for this condition. The common intron 22- and intron 1- inversions account for about 45% of the severe haemophilia A cases, whereas different types of large rearrangements and point mutations are responsible for the disease in the rest of the patients.
Here we report on the spectrum of mutations and their distribution through the factor VIII protein in 87 patients with severe haemophilia A, previously tested negative for the common intron 22-inversion. The pathogenic molecular defect was identified in all patients, thus, our detection rate is virtually 100%. Thirty six of the mutations identified in the present study are described for the first time. The newly detected amino acid substitutions were scored for potential gross or local conformational changes and influence on molecular stability for every single factor VIII domain with available structures, using homology modelling.

P173

Genotype-phenotype analyes of AZF gene deletions in Yq11: Development of novel PCR-Multiplex protocols for the rapid analysis of single AZF gene deletions and estimation of AZF deletion borderlines

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Critical evaluation of the comprehensive literature on AZF deletions in Yq11 and the described associated testicular pathologies suggests that there is no association of an AZF deletion with a distinct pathological phenotype. This is unsatisfactory for a clinically application, especially when one wants to predict the success rate of TESE (testicular sperm extraction) by the nature of the diagnosed AZF deletion. One reason for this is the fact that most diagnostic AZF PCR-assays are still based on the deletion analyses of a variable number of anonymous genomic Sequence Tagged Sites (STS) and not on the STSs of the 14 AZF genes now mapped to the three AZF intervals: AZFa, AZFb, AZFc by sequence analyses (Skaletsky et al. 2003:Nature 423: 825-837). Another reason is that it is not yet generally acknowledged that only “complete” AZFa and AZFb deletions as defined in the original publication of Vogt et al. (Hum. Mol. Genet. 5: 933-945, 1996) can be associated with a specific pathological phenotype, and that partial AZF deletions can be found even on the Y chromosome of men with normal fertility. We therefore set out to improve the current genomic AZF-PCR diagnostic scheme by establishing novel PCR-multiplex protocols which score the deletion of single AZF genes and of complete AZFa,b,c deletions, respectively, according to the guidelines of the EMQN (European Moleculargenetics Quality Network: http://www.emqn.org). Screening now more than 100 patients with severe oligozoospermia and azoospermia and a control population of men with normal fertility we found a distinct testicular pathology in all men with the deletion of all AZFa genes or all AZFb genes. Partial AZF gene deletions were observed in the AZFc interval where deletions of different DAZ gene copies were found in men with severe oligozoospermia and with normal fertility, some AZFc deletions could be associated with a specific Y chromosomal haplogroup (Fernandes et al. 2004: Am. J. Hum. Genet. 74: 180-187).

P174

d-matrix - exploration, visualization and analysis of molecular and phenotypical data

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Today the analysis of genome, transcriptome and proteome is becoming increasingly widespread, and the resulting data must be merged to generate a molecular phenotype. Moreover, the correlation between molecular and phenotypical data requires acquiring both with comparable profoundness leading to the development of databases holding both informations. Query-

ing and analyzing the stored data to uncover the valuable information hidden in the databases, however, are difficult tasks.
Therefore, we aimed to develop a generic database front-end with embedded knowledge discovery and analysis features. Having a cardiovascular clinical and molecular genetic database (CVGdb) from our laboratory in mind, we focused on human-oriented representation of the data and the enabling of a closed circle of data query, exploration, visualization and analysis (BMC Bioinformatics 2004, 5:170).
Here we introduce d-matrix, a non-task-specific database front-end with new visualization strategy and built-in analysis features. d-matrix is web-based and compatible with a broad range of database management systems. The graphical outcome consists of boxes whose colors show the quality of the underlying information and, as the name suggests, they are arranged in matrices. The granularity of the data display allows consequent drill-down. Furthermore, d-matrix offers context-sensitive categorization, hierarchical sorting and statistical analysis.
To summarize, d-matrix enables data mining, with a high level of interactivity between humans and computer as a primary factor. We believe that the presented strategy is very effective in general and especially useful for the integration of distinct data types such as phenotypical and molecular data.

P175

Genetic and clinical heterogeneity of congenital cataract: A challenge to nomenclature

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Congenital Cataract (CC) is one of the most common cause of vision-loss world-wide and responsible for nearly one third of childhood blindness. Its incidence is about 2.3 per 10,000 live births. Nearly one third of CC cases show a positive family history, with all Mendelian modes of inheritance being documented for non-syndromic CC. In non-consanguineous populations, autosomal dominant is the most common mode of inheritance, with 23 loci mapped and 14 genes identified so far. No clear genotype-phenotype correlations exist: the same type of cataract can be due to mutations in different genes (Vanita et al., Clin Genet 56: 389, 1999), on the other hand, one allele can also lead to phenotypically different types of CC (Vanita et al., J Med Genet 38: 392, 2001). In addition, wide inter- and intra-familial variability exists. We have collected more than 700 cases of congenital cataract, representing over 100 distinct phenotypes, of which more than 20 are novel - not reported in the literature so far.
The great clinical heterogeneity observed for CC phenotypes is paralled by a rather heterogeneous nomenclature, either based on the family or the author's names or the morphology and histology of the cataract. The latter, however, cover only a few classical cataract phenotypes like coralliform, polar, nuclear, central, sutural, cerulean or total cataract. Faced with this complexity there is a need for a uniform and universal classification of non-syndromic CC. In this context, it is of relevance, that the acronym, which was first established as a name for a specific disorder, is often changed after identification of the underlying gene. Here, we will discuss how many genes might be involved in CC and whether a nomenclature, based on the affected genes, is a realistic approach to solve the present confusion in nomenclature.

P176

An internet-based database on the analysis of autosomal recessive congenital ichthyoses and other inherited disorders of keratinization

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Autosomal recessive congenital ichthyosis (ARCI) is a clinically and genetically heterogeneous disorder characterized by generalized scaling of the skin and erythema. Color and shape of the scales and extent of erythema are highly variable, as well as a number of further features. A consistent genotype/phenotype correlation was not identified so far. In order to store and provide data from patients with ARCI and other related skin disorders, we have now developed a concise database service. Different phenotype variables, family history, pedigree, biochemical and histopathological data are stored for each sample. The family history will be automatically transferred to linkage compatible data formats and graphical output of the pedigree, clinical pictures will be included and presented as thumbnails. The service runs on an Apache 2 web server and is powered by a MySQL database management system and accessible through the web using an HTTP interface. For data protection reasons, sample names are stored on a separate server connected with the database by anonymous 32bit sample IDs. Data requests are handled semi-automatically via email using temporary IDs, each valid for immediate access and one dataset. The database stores genotyping data for indirect analysis of all known ARCI loci as well as mutation data obtained by direct sequencing. Further data are being collected within the German "Network for Ichthyoses and Related Keratinization Disorders", which aims at comprehensively recording and characterizing such families. The service is open to external users on a collaborative basis and includes different user levels. Since ARCI and other keratinization disorders are rare, the database not only provides a mutation survey but is valuable for systematically collecting dedicated data. Thus, it helps to generate criteria for analyzing a potential genotype/phenotype correlation and further characterization of the phenotypic outcome of specific mutations.

P177

Two new loci for Non-syndromic X-linked Mental Retardation (MRX) map to Xq22.1-Xq22.3 and Xq23-Xq26.1

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We report on clinical and molecular studies of two large MRX families. All affected males show moderate to severe mental retardation without any conspicuous dysmorphic features. Metabolic tests, cytogenetic studies and molecular screening for the fragile X syndrome revealed no abnormalities. We performed an X chromosome wide scan using 68 biallelic single nucleotide polymorphism markers provided by Applied Biosystems as Assays-on-Demand, and 6 additional microsatellite markers for fine-mapping of one candidate region.
The first family contains in 3 generations five affected males and 7 obligate carrier females, who don't show any mental impairment and other abnormalities. Significant linkage was found to marker DXS8055 with a maximum LOD score of 2.62 in Xq23-Xq26.1. The 26 cM linkage interval contains three known MRX genes: FACL4 (ASCL4), PAK3, and AGTR2. These candidate genes have been screened, but no mutation was found.
The second, also three-generation family consists of five affected males and five obligate carriers. All female carriers examined were of normal intelligence and clinically indistinguishable from their non-carrier sisters. Two- and multipoint parametric analysis yielded significant linkage between the causative gene and the marker DXS8020 with a maximum LOD score of 2.13. Using 6 adjacent microsatellite markers, we could narrow down the candidate region to a 3.1 cM linkage interval between markers DXS8034 and DXS1106. The linkage interval does not contain any known MRX gene. We have selected and analysed several candidate genes, but no abnormalities have been identified so far.
Our results suggest that unknown MRX genes exist in Xq22.1-Xq22.3 and Xq23-Xq26.1, respectively, which are causative for the phenotype in the two MRX families.

P178

Usage of a TT 5' splice site in the Fanconi Anemia gene FANCC

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Objective: Fanconi anemia (FA) is a rare bone marrow failure syndrome with genetic instability and increased risk of neoplasia. 70% of patients are severely affected with congenetical abnormalities including radial ray defects and short stature, and these patients suffer from childhood bone marrow failure. However, 30% of patients display normal growth and appearance with only mild or adult onset hematological changes. A mild clinical course of FA has been attributed to either revertant mosaicism or to mutations with residual protein function.

Results: We identified a G>T splice donor (SD) mutation at position IVS1 +1 of the FANCC gene, resulting in a mild clinical and cellular phenotype. Against all odds, the disruption of the canonical SD dinucleotide was not detrimental. RNA/cDNA analysis showed usage of the TT dinucleotide as a splice donor albeit at a reduced rate. In addition, two cryptic splice sites are used as SDs. One of them is a splice donor located 22 nt upstream. The other cryptic splice site lies 225 nt downstream and reveals a GC dinucleotide instead of the canonical GT. However, the TT site is the only SD that leaves the reading frame intact. Studies are underway to quantitate TT usage. Data base searches are undertaken to identify other examples of functional TT SDs. Additional studies aim at identifying splicing enhancers which facilitate the usage of the mutated and the cryptic splice sites.
Conclusions: Our example shows that contrary to expectation mutational disruption of a canonical splice donor site may have only mild consequences.

P179

Identification of 29 Novel and 9 Recurrent Fibrillin-1 (*FBN1*) Mutations and Genotype-Phenotype Correlations in 76 Patients with Marfan Syndrome

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Marfan Syndrome Type I, (MFS1, MIM# 154700) is an autosomal dominant disorder of the fibrous connective tissue, caused by mutations in the gene coding for fibrillin-1 (*FBN1*). The clinical spectrum of Marfan syndrome is highly variable affecting the cardiovascular, skeletal, ocular and other organ systems, but genotype-phenotype correlations are not well developped. The majority of mutations have been reported in patients displaying classical features of MFS and various screening methods lead to the identification of at present about 600 different mutations (FBN1-UMD; http://www.umd.be/). In this study we performed SSCP and/or direct sequencing to analyze all 65 exons of the *FBN1* gene in 116 patients presenting with classical MFS or related phenotypes. We identified 29 novel and 9 recurrent mutations. The mutations comprised 18 missense (47%), 8 nonsense (21%) and 5 splice site (13%) mutations. 7 further mutations (18%) result from deletion, insertion or duplication events, 6 of them leading to a frameshift and subsequent premature termination. Additionally we describe new polymorphisms and sequence variants. Comparing SSCP and direct sequencing, the detection rate of mutations is almost identical.
On the basis of our data presented here and earlier, we were able to establish highly significant correlations between *FBN1* mutation type and MFS phenotype. Most strikingly, there is a significantly lower incidence of ectopia lentis in patients carrying a mutation that leads to a premature termination codon (PTC) or a missense mutation without cysteine involvement in *FBN1*, as compared to patients whose mutations involved a cysteine substitution or splice site alteration. 54 mutations were identified in 92 (59 %) patients fulfilling or nearly fulfilling diagnostic criteria of Ghent nosology, whereas 7 mutations were found in 63 (11 %) patients not fulfilling established clinical criteria.

P08 Neurogenetics

P180

In vitro modelling of paraganglioma by iRNA mediated knock-down of SDHC/SDHD in PC 12 cells

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Hereditary paraganglioma can be caused by mutations in components of complex II of the mitochondrial respiratory chain. This complex is composed of four subunits which are encoded by the nuclear genome. The two catalytic subunits SDHA (flavoprotein subunit) and SDHB (iron-sulfur protein subunit) are anchored in the inner mitochondrial membrane by subunits SDHC and –D. Mutations in paraganglioma have been observed in the genes coding for subunits SDHB, -C, and –D. Both SDHC and SDHD function as tumor suppressor genes (Baysal et al., 2000; Niemann and Müller, 2000). A distinct mutation is present in constitutive DNA and loss of heterozygosity at the SDHC and SDHD locus, respectively occurs in tumors. In order to study the consequence of functional loss of complex II of the respiratory chain in tumorigenesis we modelled SDHC and SDHD deficiency in PC12 cells, a neuronal adrenal-derived cell line, by iRNA mediated knock-down of either SDHC or SDHD. Efficient knock down of this genes was shown by Northern blot analysis. When grown on soft agar, knock-down cells formed significantly more colonies than controls. We performed array analysis of the expression pattern of genes in knock-down and in control cells. Of mitochondrial complex II genes both SDHC and SDHD were down-regulated when either gene was “knocked-down”. However, the expression level of the genes coding for the catalytic subunits SDHA and –B of complex II remained unchanged. We are currently exploring the role of genes that are components of established pathways and that are dysregulated by loss of either SDHC or SDHD.
Literature: Baysal BE et al., Science 287: 848-850, 2000
Niemann S and Müller U, Nat. Genet. 26: 268-270, 2000

P181

VEGF: Angiogenesis factor with roles in neurogenesis and neuroprotection

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Inactivating missense mutations in the kinase domain of receptors for the vascular endothelial growth factor, VEGFR-2 and VEGFR-3, were found in patients affected with congenital lymphedema and in hemangioma specimen. More recently, impaired hypoxic upregulation of VEGF has been implicated in murine adult-onset motoneuron degeneration reminiscent of amyotrophic lateral sclerosis. In addition, VEGF was shown to rescue motoneurons from death in a mouse model of X-linked spinal and bulbar muscular atrophy. We have used alkaline phosphatase (AP) fusion proteins to unveil non-vascular roles of VEGF since multiple, functionally active binding partners can be recognized simultaneously *in situ* with this approach. The AP-VEGF164 affinity probe predominantly labeled non-vascular nerve tissues such as forebrain, hindbrain, the optic nerve, and the surface ectoderm of the future cornea in murine embryonal tissues. Similar results were obtained *in vitro*. AP-VEGF164 bound strongly to proliferating and differentiated rat pheochromocytoma PC12 cells which are an established model for investigation of neurotrophic factor-mediated signaling pathways. In contrast, the AP-Endostatin affinity probe turned out to be a negative control despite collagen XVIII/endostatin’s neuronal role in the avascular nematode *C. elegans*. Consistent with AP-staining, we have shown by immunofluorescence that proliferating and differentiated PC12 cells express three VEGF receptors, VEGFR-1, VEGFR-2, and Neuropilin-1. Downstream signaling as well as functional effects of VEGF164 on PC12 cell proliferation, differentiation, and survival are currently evaluated. In addition, coimmunoprecipitation experiments with AP-VEGF164 are performed in order to search for a putative novel receptor.

P182

Is the KNS2 gene a candidate for primary torsion dystonia?

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Primary torsion dystonia (PTD) is the most common inherited dystonia, a disabling autosomal-dominant disorder characterized by involuntary muscle contractions, frequently causing twisting and repetitive movements or abnormal postures. PTD has a wide clinical spectrum and its severity is largely determined by the age of onset. A 3 bp-deletion in the DYT1 gene (torsinA), coding for torsin A protein, was identified in about 70% of patients suffering from generalized early-onset PTD, but only in very few single cases of focal or segmental PTD. The majority of genes responsible for the more common localized forms of dystonia (segmental, focal) is still elusive. Low

penetrance (30%) and phenotypic variability lead to the suggestion that additional factors must contribute to the factual manifestation of the disease, i.e., modifier loci that influence phenotypic severity. Recently, the light chain subunit (KLC1) of kinesin-I was identified as an interacting partner for torsin A. To investigate the underlying genetic basis of primary torsion dystonia, we evaluated the contribution of mutations in the KNS2 gene, coding for KLC1 protein, for its pathogenic relevance in a series of about 300 patients with sporadic and familiar forms of PTD of different movement disorder outpatient clinics in Germany. Detection of mutations in the KNS2 gene was performed by means of dHPLC following PCR amplification of the whole coding sequence of the gene (13 exons). Mutational analysis of the coding region of the DYT1 gene and in the SGCE gene has been performed previously and mutation carriers have been excluded. This information might be helpful for the appropriate application of DNA diagnostic testing and for genetic counselling.

P183

Detection by triple PCR of expanded CCTG repeats in the ZNF9 gene of myotonic dystrophy 2

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Myotonic dystrophy 2 (DM2, OMIM 602668) is the most common form of muscular dystrophy in adults and is caused by large expansion of a CCTG repeat in intron 1 of the ZNF9 gene. As the disease alleles are too large to be amplified by PCR across the expanded region containing a TG, a TCTG and two CCTG repeats, Southern analysis is usually applied but remained to be difficult to unambiguously detect the signals of the expanded alleles which are often very faint due to mitotic instability. Triple PCR has been propagated as an alternative to Southern blotting but the reported procedure still required blotting of the PCR products and their detection by probing with a labelled internal oligonucleotide. Based on published methods, we developed two triple PCR protocols which both were able to produce fragments after annealing of primers to the elongated repeat tract, and allowed their visualization as typical stutter signals on a capillary sequencer. In wild-type alleles, proximal and distal ends of the repeat region are seen as characteristic signal profiles whereas no distal end profile is obtained from expanded alleles. One protocol predominantly amplifies the 5' proportion of the hypervariable region and gave positive signals of CCTG expansions (≥ 50 CCTGs) in all patients (> 20) that were previously diagnosed by Southern analysis. The other protocol predominantly amplifies the 3' portion of the repeat region and gave positive signals in most but not in all patients. False positive results have not yet been obtained. The protocols allowed for some calculation of both the size and the structure of the hypervariable repeat region.

P184

Parkin mutations and polymorphisms in Parkinson's disease: Comparison between German and Norwegian cohorts

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Mutations in the Parkin gene coding for an E3 ubiquitin-protein ligase cause autosomal recessively transmitted early-onset Parkinson's disease (PD). Recently, strong indications were worked out for a possible role of Parkin mutations in the late-onset form of PD (Oliveira et al. 2003).
We compared a German (95 patients) with a homogeneous Norwegian cohort of 132 patients (Kurz et al. 2003) suffering from primary late-onset PD by investigating mutation and polymorphism frequencies with SSCP analysis. Comparing the frequencies of known single nucleotide polymorphisms (SNPs; IVS2+25T>C; IVS3-20T>C; IVS7-35G>A; IVS8+48C>T; Val380Leu), a significant difference could be observed between the two populations. Val380 (p=0.0006) and IVS2+25T (p=0.0005) were significantly more frequent in the Norwegian cohort. A positive family history for PD was documented in ~15% of the patients (German PD patients 16.5% and Norwegian PD patients 12.6%). Yet, mutations were rare in our cohorts: Only one missense mutation (Arg256Cys, 0.75%) was identified in heterozygous state in the Norwegian patients. Two mutations (Arg402Cys and Thr204Met, 2.1%) in heterozygous state were found in the German cohort. None of these 3 mutations was observed in our control samples. The results support the hypothesis (Foroud et al. 2003) that mutations in the Parkin gene in heterozygous state may act as susceptibility alleles for late-onset form of PD in rare cases. These data do not suggest a major role of point mutations in the Parkin gene, especially not for the Norwegian population.

Foroud et al. Heterozygosity for a mutation in the parkin gene leads to later onset Parkinson Disease. Neurology 2003;60:796-801.
Kurz M, Alves G, Aarsland D, Larsen JP. Familial Parkinson's disease: a community-based study. Eur J Neurol. 2003;10:159-63.
Oliveira et al. Parkin Mutations and Susceptibility Alleles in Late-Onset Parkinson's Disease. Ann Neurol 2003;53:624-629.

P185

Spinocerebellar ataxia type 4: analysis of candidate genes and polymorphic microsatellites

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The spinocerebellar ataxias (SCAs) are a clinically and genetically heterogeneous group of autosomal dominant neurodegenerative disorders. At least 21 different loci have been identified for this condition. The SCA4 locus at chromosome 16q22.1 has been first described in an Utah family of Scandinavian origin with cerebellar ataxia and axonal neuropathy and further delineated in a German kindred with a similar phenotype. Furthermore, Japanese families with a pure cerebellar ataxia show linkage to the same region. The corresponding SCA4 mutation was narrowed down to 7.94 Mb for the two European and to 1.25 Mb for the Japanese pedigrees. Due to phenotypic differences between European and Japanese patients, it is a moot question whether they share the same underlying gene defect or not. We screened 34 candidate genes in the German SCA4 family for point mutations. With the exceptions of two cSNPs, no segregation of DNA variations with the disease phenotype was found. In addition, we analysed 19 yet unpublished polymorphic microsatellite repeats within the 3.69 cM region. None of the tested repetitive sequences is expanded in this family.

P186

Small Neonatal Head Circumference - A previously unrecognized Manifestation in Niemann-Pick disease Type C that is useful in a Diagnostic Score

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Objectives: Niemann-Pick disease type C (NPC, MIM 257220), a rare, progressive autosomal recessive neurovisceral disorder due to mutations in either the NPC1 gene or the HE1 gene, respectively, was investigated with respect to early manifestations. Based on the new observation of a small neonatal head circumference a diagnostic score to facilitate an early diagnosis was developed. Methods: During a retrospective study of 21 patients the development and clinical course of NPC were examined. The diagnosis of NPC was based on the range of clinical manifestations, the course of illness, histochemical and biochemical data, and in four patients on mutation analysis. Based on a new observation, a reduced head circumference at birth, a diagnostic score (NPC score) was developed, including four other clinical signs of NPC, such as hepatosplenomegaly, prolonged icterus, and muscular hypotonia. The score was applied to patients and compared to an age-machted control group. Results: NPC was confirmed in all patients by clinical and laboratory data, and in four patients by molecular genetic analysis. A reduced neonatal head circumference at or below the 3rd percentile was found in 9 of 17 patients with the neonatal and infantile form of NPC compared to an age-matched control group. These patients showed scores indicative of NPC, in contrast to the control group.Conclusions: Reduced neonatal head circumference is a hitherto unrecognized manifestation of NPC. Integrated into the known clinical manifestations, we developed a diagnostic score (NPC score) which could facilitate an early diagnosis and avoid unnecessary gene tests.

P187

Detection of new variations of the polyalanine stretch in PMX2B in patients with ataxia or Huntington Disease (HD)

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The transcription factor PMX2B (paired-like homeobox 2b gene) is located on chromosome 4p12 and discussed to be involved in diseases with different neurological context. Recently variation of the polyalanine stretch length in PMX2B was associated with schizophrenia with ocular misalignment. Haploinsufficiency of PMX2B caused by a large deletion might be predisposing for Hirschsprung's disease (HSCR). We investigated the PMX2B gene in 150 DNA samples of patients with Huntington Disease that carry the most common repeat expansion in the Huntingtin gene and the same number of individuals that exhibit clinical Huntington symptoms without expansion in the Huntingtin gene. Along with these samples we screened the same number of patient DNA samples with ataxia and 150 healthy control individuals. For the ataxia patients all other known causative genes were tested and excluded. With SSCP analysis and subsequent sequencing we were able to detect a broad range of deletion mutations and SNPs within the polyalanine stretch of PMX2B in all four tested sample groups.

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Late onset ataxia caused by FMR1 premutations

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One of the main causes of mental retardation in males is the fragile X-syndrome. It is caused by an expansion of the (CGG)n repeat in the 5'untranslated region of the FMR1 gene. The involvement of fragile X premutations in patients with a late onset ataxia/tremor syndrome (FXTAS) has been described recently. 270 males and 241 females with spinocerebellar ataxia (SCA) and age of onset > 50 years were tested for fragile X premutations. CAG repeat expansions at the SCA loci 1, 2, 3, 6, 7, 12 and 17 were previously excluded in all patients. One female patient carried 84 triplets and one male patient 96 triplets. Both CGG repeat expansions are in the range of premutations. The 73-year-old women started to notice weakness at age 64. Clinically gait ataxia, tremor and deterioration of memory and concentration followed. The 65-year-old man had increasing tremor of both arms and head, gait disturbances, dysarthric speech and imbalance since eight years. No cognitive or psychiatric disturbances were noticed. In conclusion the FMR1 premutations are a rare cause of late onset ataxia. Testing for the CGG repeat length in patients with late onset ataxia can therefore be recommended.

P189

Recurrent Dardarin (PARK8) mutation in a case with early-onset Parkinson's disease

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Objectives: To evaluate the role of mutations in the Dardarin gene in early- and late-onset Parkinson's disease (PD).
Background: Very recently, a new gene has been identified that plays a role in autosomal dominant, late-onset PD, Dardarin (PARK8, LRRK2), that consists of 51 exons. To date, six different missense mutations have been detected in five exons including two recurrent mutations (c.6096A>G; c.4321C>T) and an additional substitution at the latter position (c.4321C>G).
Patients/Methods: After having obtained informed consent, we included 110 PD patients, 67 early-onset PD (EOPD) cases (age at onset [AAO] </= 50 y.) and 43 late-onset PD (LOPD) cases (AAO > 50 y.). We screened the five mutation-bearing exons for mutations by SSCP analysis.
Results: Patients (54% m) had a mean AAO of 43.9+/-13.9 y. (range 15–73 y.) and 91 (83%) had a positive family history. Patients were mainly of German origin. We identified one German mutation carrier. She carried the same mutation (c.4321C>T) as previously reported in two families. In contrast to these families (AAO 48–78 y.), our patient had an AAO of only 30 years. The first symptoms were tremor and foot dystonia. At age 50 years, she showed resting tremor, bradykinesia, and rigidity (Hoehn & Yahr stage 3). After 20 years of disease duration and five years of L-dopa therapy, she had motor fluctuations and dyskinesias. Family history was positive, however, no relatives were available to test for segregation of the mutation. In addition, we detected two polymorphisms in introns 35 (IVS35+23A/T) and 40 (IVS40-39G/A). The latter was only found in a single EOPD case and not among 100 control chromosomes. The relevance of this intronic substitution remains to be investigated.
Conclusions: Mutations in the Dardatin gene need to be considered also in EOPD cases. It is conceivable that mutational hot spots occur in Dardarin since we found the fourth substitution at the same base pair in an unrelated patient.

P190

Analysis of molecular events underlying the early degeneration of photoreceptors in a mouse model for X-linked juvenile retinoschisis

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Objectives: X-linked juvenile retinoschisis (RS) is caused by mutations in the RS1 gene encoding a 24-kDa protein, termed retinoschisin, which is secreted from photoreceptor and bipolar cells. Although loss of retinoschisin function is underlying the disease mechanism, little is known about the molecular events leading to the loss of cellular architecture and the degeneration in the neural retina. In the present study, we are interested in the apoptotic processes underlying photoreceptor degeneration in the Rs1h-deficient mouse model of RS.
Material and Methods: Retinal cryosections of wildtype, Rs1h-deficient (Rs1h-/Y) and Rs1h-/Y - Casp1-/- mice double knock-out mice were investigated by immunohistochemistry and TUNEL-staining. RNA samples of the retinae from 10, 14, 16, 21, and 28 days old mice were used for quantitative real-time PCR (qRT-PCR).
Results: TUNEL-positive cells were significantly increased in the retinal photoreceptor layer of the Rs1h-deficient mouse with a peak in apoptotic cell degeneration around postnatal day 18. qRT-PCR of genes expressed by activated microglia such as Spp1, Casp1, or IL1ß revealed that microglia activation precedes initiation of apoptotic gene expression as seen in significant upregulation of gene transcription e.g. for Casp8 and Fadd. This Fas-mediated apoptosis may be caused by neurotoxicity of activated microglia mediated by cytokines and free radicals. Most prominently, Casp1 was found upregulated in Rs1h-deficient mice. This protein is required for the activation of Il1ß which plays a role in promoting neuronal cell death. We have now generated double knock-outs of Rs1h- and Casp1-deficiency to further analyze the role of Casp1 in the apoptotic events affecting the photoreceptors.
Conclusions: Activation of microglia may be a key event in the initiation of apoptosis-related photoreceptor loss in Rs1h deficiency. This early event may point to a novel therapeutic approach to prevent neurodegenerative progression of this retinal disease.

P191

Microarray analysis of mouse models of Parkinson's disease

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The presynaptic protein a-synuclein plays a central role in the pathogenesis of Parkinson's disease (PD). Usually, a-synuclein is degraded by the proteasome-ubiquitin pathway. In PD, however, a-synuclein accumulates and aggregates in the Lewy bodies. Thus, the proteasomal degradation of a-synuclein might be altered. To investigate the proteasomal-ubiquitin pathway in PD further, we analyzed mouse models of PD by microarray analysis. First, the expression profile of mice over-expressing a-synuclein, SNCA+/+ (Kahle, et al., 2000), was generated. Furthermore, mice with a reduced activity of UbcM4 (Ubc-30%, Harbers et al., 1996), the mouse homologue of UbcH7, which is a ubiquitin-conjugating enzyme interacting with parkin, were analyzed by microarray analysis. Parkin, an E3-ligase, is mutated in autosomal recessive PD and thought to ubiquitinate glycosylated alpha-synuclein. RNA for chip-analysis was isolated from total mouse brains of a-synuclein over-expressing and of Ubc-30% mice,C57Bl6 mice as controls. Three 3-month-old and three 1-year-old males were analysed using the murine genome U74v2 oligonucleotide chip (Affymetrix). Genes were though to be differentially regulated if at least 7 of 9 comparisons showed a signal log ratio (SLR) of more than +/-0.75. In consideration of these points we found about 60 genes which are differently regulated in the Ubc-30% mice. The most interessing gene, a-synuclein, is highly expressed in 1-year-old Ubc-30% mice.

Histological analysis to identify Lewy body-like a-synuclein-inclusions in transgenic mice brain at different ages are in progress. To find out if Ubc-30% mice have any motor coordination deficits we performed rotarod tests. To get an earlier phenotype we bred heterozygote Ubc-30% mice with homozygote SNCA+/+ mice. In spite of numerous breedings we did not get any double transgenic offsprings. We are now in the process to investigate double-transgenic mice for differences between single- and double-transgenic embryos.

P192

Novel Mitofusin2 (MFN2) mutations and phenotypic variability in Charcot-Marie-Tooth neuropathy type 2A

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Charcot-Marie-Tooth disease (CMT) represents a group of genetically and clinically heterogenous peripheral neuropathies. According to electrophysiological criteria two main CMT forms are distinguished: the demyelinating CMT type 1 with decreased nerve conduction velocities and the axonal CMT type 2. Mitochondrial fusion protein Mitofusin2 (MFN2) was recently described as second causative gene in the CMT2A locus. MFN2 functions as mitochondrial outer membrane GTPase and regulates the mitochondrial network architecture by fusion of mitochondria. It is necessary for structural integrity and mobility of mitochondria. Reduced mitochondrial mobility in axons of peripheral nerves probably contributes to the CMT2 phenotype. We investigated 30 patients with axonal CMT2. Overall we found six novel mutations in MFN2 (I213T, R275W, G298R, S378P, R707W, E744L). This represents 20 % of these CMT2 patients and indicates the importance of this gene for peripheral neuropathies. The typical clinical signs of steppage gait and pes cavus were present. Distal weakness and atrophy was more pronounced in the lower than in the upper limbs. All patients showed normal or only mildly reduced nerve conduction velocity (NCV~ 38 m/s). No or only mildly reduced sensory disturbance was detected. Facial paresthesia in one of the patients was observed for the first time (variation S378P). One patient (R707W) exhibited additionally inner ear deafness since early childhood and nystagmus. She was wheel chair bound at the age of 20. Deafness was described for axonal neuropathies caused by selected Myelin Protein Zero (P0), Connexin32 (Cx32) and PMP22 mutations. Five mutations comprise the functional important GTPase or the fzo_mitofusin domain, two are clinically sporadic novel mutations. The S378P mutation is located in an evolutionary highly conserved interdomain region. The here presented patients and MFN2 mutations give deeper insight in the genotype-phenotype correlation in CMT type 2.

P193

Clinical and genetic analysis of patients with polymicrogyria (PMG)

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Polymicrogyria (PMG) is a heterogeneous cortical malformation, which is characterized by an excessive number of small gyri with disturbed lamination at different cortical regions. PMGs can be unilateral or bilateral, focal or diffuse. There are isolated and syndromic forms associated with other malformations. In some spontaneous cases environmental factors like infections during pregnancy are discussed to cause PMG. In other cases, especially in bilateral forms, the familial inheritance points towards genetic factors being involved in the development of PMG. This is also supported by the observation of chromosomal translocations in some of these families. Up to date 3 different loci are associated with different forms of bilateral PMG. Recently, the first gene leading to autosomal recessive bilateral fronto-parietal PMG (BFPP) could be localized on 16q12 and mutations in the G protein-coupled receptor gene GPR56 were demonstrated to cause one form of BFPP. The functional role of GPR56 and other members of this family of proteins during cortical development is still unknown, no association to typical neuronal migration disorders had been reported so far.
In our cohort of patients with cortical malformationswe have analyzed individuals with isolated PMG as well as pedigrees with different types of PMG. In families with PMG we first performed linkage analysis to the GPR56 locus, which in one family demonstrated two affected sisters to share identical parental alleles. By direct sequencing we identified in this family two novel causal GPR56 mutations, a splice mutation and a nonsense mutation, leading to BFPP. In addition, we present families with different forms of PMG without linkage to the known PMG loci. Our data confirm that inherited PMGs are a heterogeneous group of cortical malformations. Identification of additional PMG genes will be an important tool to improve our understanding of normal and abnormal cortex formation.

P194

Expression analysis of mutant seipin in AtT20 cells

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We recently identified two missense mutations in *BSCL2* associated with dHMN-V (OMIM #600794) and Silver syndrome (OMIM #270685). Both mutations (N88S and S90L) are located in exon 3 of *BSCL2* and destroy the only N-glycosylation site of the gene product seipin. It is well established that missing N-glycosylation sites have an adverse effect on proper folding of proteins often resulting in an altered 3 dimensional conformation.
Cell culture experiments with AtT20 cells expressing mutant forms of seipin resulted in formation of stable protein aggregates which are located mainly around the centrosomal region. In contrast to the definition of aggresomes we could show that the aggregates of overexpressed seipin are not coated by vimentin. Additionally we used antibodies against β-tubulin, γ-tubulin, hsp70, proteasome 19 S subunit and ubiquitin to look for the distribution of these molecules in mutant seipin-EGFP transfected cells. The role of the inclusion bodies during the cell cycle was investigated in time laps experiments. Our results will further contribute to understand the involvement of mutated seipin in the pathophysiology of Silver syndrom / dHMN-V.

P195

Phenotypic spectrum of ARXopathies and functional analysis of a truncated human ARX gene product in neuronal cell cultures

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Distinct hemizygous mutations in the aristaless-related homeobox gene (ARX; Xp22.13), the human orthologue of the Drosophila gene aristaless, have recently been reported to cause a wide spectrum of clinical phenotypes including X-linked mental retardation, Partington syndrome, X-linked West syndrome, X-linked mental retardation with myoclonic epilepsy and X-linked lissencephaly with abnormal genitalia (XLAG). All phenotypes in males consistently include mental retardation, which in the more severe forms of ARX-associated disorders is accompanied by further functional and/or structural brain abnormalities like lissencephaly as seen in XLAG.
Here we report the preliminary results of an ARX mutation analysis in a cohort of 44 unrelated probands presenting with clinical signs of ARX-associated phenotypes. In all 5 independent probands with characteristic clinical features of XLAG a causal ARX mutation could be identified. 4 frameshift mutations all resulted in either partial or complete loss of the aristaless domain, while the fifth proband was found to carry a missense mutation R332C in the highly conserved homeodomain. In one family with X-linked WEST syndrome the characteristic repeat expansion of the first polyalanine tract was identified.
In order to further characterize the function of the normal and abnormal human ARX gene product during neuronal migration and differentiation, neuronal tissue cultures with a hemizygous truncating ARX mutation were established. Polyclonal antibodies were used to analyze the

expression of different proteins known to be involved in cortical development and the differentiation of neuronal precursor cells into the different types of neurons. Our preliminary data indicate, that loss of the aristaless domain in ARX is not abolishing the differentiation into GABAergic neurons in the human in vitro cell culture system.

P196

Wide clinical variability in a German pedigree with Cerebral Cavernous Malformations due to a Krit1 frameshift mutation

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Cerebral cavernous malformations occur with a frequency of about 0.1-0.5% in the general population. Serious clinical complications include seizures, cerebral hemorrhages or focal neurological deficits. The presence of multiple vascular lesions is considered as a genetically heterogeneous autosomal dominant condition with incomplete penetrance and wide clinical variability. A truncating mutation in the Krit1 gene (CCM1) can be identified in up to 47% of the CCM families. About 30% of the CCM1 negative families carry mutations in the CCM2 gene. A third CCM locus was identified in 3q25.2-q27.
Here we report the molecular and clinical data of a German CCM pedigree. The index proband was identified at the age of 18 years after his first epileptic seizure. At the age of 25 years he is currently without neurological symptoms after 5 neurosurgical interventions. MR imaging identified multiple lesions in his younger maternal half sister at the age of 12 years, where she had only presented so far with recurrent episodes of severe headache. She is currently 16 years old and in the mean time had 3 neurosurgical interventions. One maternal cousin of both half sibs was reported to present with complex neurological handicaps after severe complications due to multiple cerebral lesions. Both obligate mutation carriers, the mother of our index cases (48 years) as well as the mother of their severely affected cousin are reported to be clinically unaffected, but so far denied MR imaging to evaluate the presence of asymptomatic vascular lesions. Sequence analysis of the index case revealed a 1bp deletion in the CCM1 gene: c.1813delT, which is also present in his tested half sister as well as his mother.
Identification of causal mutations in CCM families allows to discuss the option of presymptomatic mutation testing of further relatives at risk and the development of adequate diagnostic strategies including early MR imaging and clinical controls in identified mutation carriers.

P197

Wolfram syndrome (WS) caused by compound heterozygosity for transition G>A at the splice donor site of exon 4 leads to a new missense mutation but normal splicing and expression of WSF1 cDNA

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WS, a rare autosomal recessive disorder is mainly caused by mutations of the WFS1 gene at 4p16.1. Patients develop very early onset type 1 diabetes in association with progressive optic atrophy. The WFS1 gene encodes a transmembrane protein of 890 amino acids called wolframin, which recent evidence suggests may serve as a novel endoplasmic reticulum calcium channel in pancreatic beta-cells and neurons. About 100 mutations were reported thus far. Genotype-phenotype studies indicate that causative mutations preferentially occur in exon 4 and 8. Here we report on a 29 year old WS patient (MK), the first of two sons of healthy nonconsanguineous parents. Diabetes mellitus was diagnosed with 4 and optic atrophy at 10 years. He is completely blind now. A mild non progressive deafness was recognized when he was 7. Olfactory function is reduced too. He is smart, will achieve an academic degree soon and is professionally and socially well adapted. DNA sequence analysis of his WFS1 gene revealed a compound heterozygosity for 460 G>A (G154S) at the last codon of exon 4 and 2315ins(T) in exon 8. The latter frame shift mutation was already reported in an Austrian WS patient whereas the missense mutation in exon 4 was not described yet. Calculation of splice site scores using different programs predicted a clear reduction of scores since the last nucleotide of exon 4 is affected. Therefore cDNA obtained by reverse transcription from blood cell RNA was subcloned and subsequent analysis indicated that expression and spicing of this allele is not grossly changed. Parents are heterozygous for 460 G>A (mother) or 2315ins(T) (father) and his brother is not a carrier. Conclusion:This study supports that indeed in exon 4 and 8 there are more often severe WFS1 gene mutations but also shows current limits of splice score calculation programs. Recent reports suggest a clear association of WFS1 gene mutations with psychiatric illness but MK up to now does not show any anomaly of that kind.

P198

Rare variants in the familial hemiplegic migraine gene ATP1A2 in familial clustering of common forms of migraine

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Migraine is a recurrent neurovascular disease, its most common forms are migraine without aura (MO) and migraine with aura (MA) both showing familial clustering and a complex pattern of inheritance. Familial hemiplegic migraine (FHM) is a rare monogenic subform of migraine which is caused by mutations in the calcium channel gene CACNA1A or the ATP1A2 gene which encodes the α_2 subunit of a Na+/K+ pump.
In order to determine whether ATP1A2 is also involved in the molecular pathogenesis of complex inherited MA, we systematically screened ATP1A2 in families with several members affected by MA and/or MO. We identified two novel missense mutations (E174K and C515Y), which were not found in more than 500 control chromosomes. We performed functional studies of these variants in Xenopus oocytes by two-electrode voltage clamp measurements and radiochemical determination of ATPase activity. Whereas C515Y leads to a complete loss of function indistinguishable from the effect of FHM-mutations, for E174K no functional alteration could be found.
Although the in vitro data could not prove the pathogenicity of E174K suggesting additional effects of “modifying” genes nor could they establish a genotype-phenotype relationship for C515Y versus typical FHM-mutations, we propose that rare variants in ATP1A2 are involved in the susceptibility to common forms of migraine, because of (i) the absence of alterations in controls, (ii) the pattern of segregation, (iii) the high conservation of alterations in Na+/K+-ATPases, (iv) the functional effect of C515Y, and (v) the established involvement of ATP1A2 in a monogenic form of migraine.

P199

The gene LRRK2 (PARK8) which causes autosomal dominant Parkinsonism does not influence common forms of Parkinsons Disease

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We recently showed that mutations in the gene for a leucine-rich repeat kinase (LRRK2) cause autosomal-dominant late-onset Parkinsonism. We identified five missense and one putative splice site mutation in the LRRK2 gene in six families linked to chromosome 12 (PARK8). LRRK2 is a large, multifunctional protein belong-

ing to the ROCO protein family. ROCO proteins include a protein kinase domain of the MAPKKK class and several other major functional domains (smallGTPase domain, protein-protein interaction domains). LRRK2-mutations appear to be a common cause of dominantly inherited Parkinsonism. Currently we are investigating the function of LRRK2 in vitro by cloning the cDNA of the longest open reading frame and by subcloning the kinase and GTPase domain. In different assays we are comparing wildtype to mutant isoforms. In order to find out if this novel gene influences non-mendelian forms of Parkinson´s disease (PD) we genotyped 120 SNPs in a set of 662 Parkinson´s disease patients and 1020 controls from a European population. No case-control partition for allele and for haplotype could be demonstrated until now. No evidence for the existence of a common functional variant in LRRK2 that has a strong influence on PD risk was found.

P200

Functional analysis in Drosophila models for hereditary neuropathies and lipodystrophies

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Mutations in the human gene BSCL2 were known to cause type 2 congenital lipodystrophy and recently it was found to be also responsible for distinct neurological phenotypes. In this project we seek to develop model organisms for the neurological disorders spastic paraplegia syndrome 17 (SPG17) and Charcot-Marie-Tooth disease type 2D (CMT2D) as well as for the metabolic disorders congenital lipodystrophy, type 1 and type 2 (BSCL1, BSCL2) using excision mutagenesis for the Drosophila homologues of the human genes BSCL2, GARS and AGPAT2. In parallel, transgenic flies will be created for "rescue assays" and to also allow spatio-temporal control over gene expression. Additionally transgenic RNAi strategies might be used for controlled spatio-temporal gene inactivation. Antibodies and GFP-fusions will be generated to study tissue and sub-cellular distribution of the proteins in intact Drosophila. This will form the basis to gain additional insight in which cellular and molecular pathways these proteins function in as well provide us with the required lines to analyse the phenotype in flies. We hope that these experiments will strengthen our insights into the primary cell biological scenario underlying the hereditary spastic paraplegia (HSP) and congenital generalised lipodystrophy (CGL) diseases.

P09 Molecular Genetics

P201

The OSTL gene, encodes a RING Finger protein, which is highly expressed in memory B cells and the acute lymphoblastic leukemia (ALL) cell line SEM, interacts with HAX1 and SIVA

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We have cloned and characterized a new gene (OSTL), that is involved in the translocation t(6;12)(q23;p13). This translocation results in the fusion of ETV6 with STL in a childhood B- ALL cell line. OSTL shares the first exon with STL but is transcribed in the opposite direction (OSTL = Opposite STL). Since the ETV6/STL fusion gene encodes only a very small protein, which lacks any known functional domains, we hypothesize that the main leukemogenic effect of this translocation is the deregulation of OSTL. OSTL encodes for a protein of 307 amino acids containing a variant RING2 motif, a modified B-box domain (DRIL) and a RING finger motif. The OSTL protein is highly conserved: the human and mouse proteins are 99% homologous and between the human and puffer fish (Fugu rubripes) protein the homology is 83%. A green fluorescent protein OSTL fusion protein expressed in mouse fibroblast cells (NIH3T3) localized to the cytoplasm. Northern blot analysis showed high expression of human OSTL in testis, ovary and skeletal muscle. Northern analysis of eleven B-cell lines showed expression of OSTL in Karpas (mature B cell line derived from B-NHL) and in several Epstein Barr virus transformed lymphoblastoid cell lines. RT-PCR analysis of cDNA from B cells of different developmental stages showed expression of OSTL in naive, memory and plasma B cells, with strongest expression of OSTL in memory cells and in the ALL cell line SEM. Interestingly, a yeast two hybrid screen identified two OSTL interacting proteins that are important in B-cell receptor signaling and B-cell survival (SIVA, HAX-1). The OSTL - SIVA and OSTL - HAX-1 interactions were confirmed by cotransformation assays in yeast, colocalization assays in mouse fibroblast cells, and coimmunoprecipitation (for HAX1 only). These results support our hypothesis that deregulated OSTL expression can lead to hematologic malignancies and suggests that OSTL might have an important function in memory B cell survival.

P202

Recombination hotspot in the vicinity of the SHOX gene defines a common genetic cause for short stature

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Aside from the influence exerted by environmental and internal factors, growth is orchestrated by a large number of different genes. One of them, SHOX, is believed to play a major role since defects in this homeobox-containing short stature gene on the sex chromosomes lead to syndromal (Léri-Weill, Langer and Turner syndrome) or idiopathic short stature. We have analysed 118 independent patients with Léri-Weill dyschondrosteosis and 1,500 patients with idiopathic short stature for deletions encompassing SHOX. Deletions were detected in 34% of the patients with Léri-Weill dyschondrosteosis and 2% of the patients with idiopathic short stature. For 27 patients with Léri-Weill dyschondrosteosis and 6 with idiopathic short stature, detailed deletion mapping was carried out. Analysis was performed by PCR using pseudoautosomal polymorphic markers and fluorescence in situ hybridisation using cosmid clones. Here we show that the identified deletions vary in size, yet the vast majority of patients share a distinct deletion breakpoint. This breakpoint region is characterised by locus-specific low copy repeats and a high preponderance of Alu and LTR sequences, which create a recurrent deletion breakpoint that occurs in more than 1% of all short individuals. It also represents one of the most frequent deletion breakpoints leading to disease.

P203

CASK is disrupted by a paracentric inversion of the X chromosome in a female with microcephaly, cerebellar hypoplasia, and severe psychomotor retardation

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Disease-associated balanced chromosomal aberrations are powerful tools for gene identification. We report a female with microcephaly, moderate hypoplasia of the brainstem, severe hypoplasia of the cerebellum, moderate vermis hypoplasia, conductive hearing impairment, and severe psychomotor retardation. She carries a paracentric inversion on one of her X chromosomes with breakpoints p11.4 and p22.33. Analysis of DNA methylation at the AR locus and acridin-orange staining of metaphase chromosomes from peripheral blood cells of the patient suggested a unilateral X inactivation pattern with the rearranged X chromosome being always inactive. By FISH, we identified 4 fosmid clones spanning the breakpoint in Xp22.33 and 7 fosmids overlapping that in Xp11.4. The breakpoint in Xp22.33 is in a gene-poor region, proximal to the pseudoautosomal boundary. In Xp11.4, we mapped the breakpoint in intron 5 of CASK that encodes a protein with similarity to Ca2+/calmodulin-dependent protein kinase II and membrane-associated guanylate kinase. Remarkably, CASK intron 5 harbors two small genes, GPR34 and GPR82, both encoding G-protein-coupled receptors. Transgenic male mice with an insertion in Cask show craniofacial abnormalities. In addition, CASK acts as coacti-

vator of the transcription factor Tbr-1 to induce transcription of T-element containing genes, including reelin, a gene involved in cerebrocortical development. Mutation analysis of CASK, GPR34, and GPR82 in eight patients with microcephaly and cerebellar hypoplasia revealed an isocoding mutation in CASK in one patient. This transition (c.915G>A, p.K305) affects the last nucleotide of exon 9. The mutated sequence was not detected as a donor splice site by two computer prediction programs suggesting that this sequence variant affects splicing. The same sense mutation in CASK has not been found in 176 unaffected males. Further studies are being performed to elucidate whether the mutation is causative for the patient's phenotype.

P204

Htra2-ß1 - a splicing modulator of SMN2 transcripts: functional studies in transgenic and knock out mice

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Spinal muscular atrophy is an autosomal recessive disorder caused by homozygous loss of the survival motor neuron gene 1 (SMN1). The loss of the gene leads to degeneration of alpha motor neurons in the spinal cord resulting in a progressive weakness of limbs and trunk. Patients retain at least one of a nearly identical copy of the gene – SMN2 – which differs to SMN1 only in 5 bp. Due to alternative splicing most of the transcripts generated from SMN2 lack exon 7, resulting in SMN protein deficiency. The remaining correctly spliced transcripts are not successful to compensate the loss of SMN1 in patients. We have already identified the SR-like splicing factor Htra2-ß1 to restore the correct splicing of SMN2 exon 7 in vitro. The protein is therefore a promising candidate for an in vivo modulation of SMN RNA processing to serve as a therapeutic tool to prevent SMA. Compared to humans, mice possess only one Smn gene whose loss is embryonic lethal. Transgenic Smn-/- mice carrying the human SMN2 show an SMA-like phenotype, whereas the phenotypic severity like in human is correlating with the SMN2 copy number. We have generated mice overexpressing the Htra2-ß1 neuronal specific. Overexpression of Htra2-ß1 was confirmed via immunostaining of cultured motor neuron cells.The transgenic Htra2-ß1 mice are phenotypically normal. Our final goal is to create Smn-/-, SMN2+, Htra2-ß1+ animals to investigate if Htra2-ß1 shows a rescue effect on the SMA phenotype in vivo. Further we have developed a knock out strategy for the murine homologue Sig41 via the Cre/loxP system to investigate the function of the Tra2-ß1 in mammalians. By using PCR-strategy we have previously excluded splice variants of the mSig41 in all tissues of interest. We have already succeeded in generating the target vector for ES cell transfection. Since reduced SMN protein level also leads to reduced Htra2-ß1 protein level, the construction of Tra2-ß1 knock out mice is of particular interest.

P205

PCC syndrome: The underlying gene defect, cellular and clinical characteristics of the first genetic disorder affecting chromosome condensation

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Chromosome condensation is a fundamental and tightly regulated process during the cell cycle resulting in the folding of interphase chromatin to metaphase chromosomes. Recently, we described the first congenital disorder in man which is associated with a chromosome condensation defect characterized by premature chromosome condensation in the early G2 phase of the cell cycle and delayed decondensation postmitosis (OMIM 606858). Clinically, the syndrome is associated with microcephaly, short stature, and moderate to severe mental retardation. Furthermore, we have shown that the disorder is caused by mutations in MCPH1 (microcephalin), a gene containing three BRCT domains which are often found in proteins implicated in cell cycle regulation. Dividing cells of patients with the disorder are characterized by a high proportion of prophase-like cells detectable in routine cytogenetic preparations. Ionizing irradiation increases the fraction of prophase-like cells and could serve as an additional approach to facilitate the diagnosis. siRNA-mediated depletion of MCPH1-mRNA in Hela-cells is sufficient to reproduce the specific cellular phenotype with misregulated chromosome condensation. So far, two mutations in exon 2 and exon 5 have been described resulting in protein truncation. The affected patients exhibit a severe cellular and clinical phenotype. We report here a new patient with a missense mutation in an evolutionary conserved amino acid of microcephalin having a milder cellular and clinical phenotype.
In conclusion, our findings implicate microcephalin as a novel regulator of chromosome condensation and link the apparently disparate fields of neurogenesis and chromosome biology. Further characterization of microcephalin is thus likely to lead to fundamental insights into both, the regulation of chromosome condensation and neurodevelopment.

P206

Detection by triple PCR of expanded CGG repeats and their AGG interruptions in the FMR1 gene of fragile X syndrome

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Triple PCR has been used successfully to visualize largely expanded microsatellite sequences too large to be amplified by PCR across the expanded gene regions, e.g., in patients with expansion of the CCTG repeat in the ZNF9 gene of myotonic dystrophy type 2, but has never been tried to detect expanded FMR1 CCG repeats in fragile X families. We investigated the FMR1 alleles of patients and carriers, previously ascertained upon Southern analysis, by a newly developed triple PCR strategy allowing detection of PCR products of pre and full mutation alleles on a capillary DNA sequencer. With this technology, we were particularly able to detect the number and position of AGG interruptions in CGG repeats of any size in both male and female individuals, and, thereby, identified a proportion of fragile X patients with AGG-interrupted full mutations. This discovery led us to investigate the haplotype backgrounds of interrupted and non-interrupted full mutation alleles.

P207

Screening of hot spot mutations in the Ryanodine receptor gene for Malignant hyperthermia and Central core disease identifies 8 novel exchanges

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Malignant hyperthermia (MH) is a pharmacogenetic condition that may cause severe and uncontrollable metabolic overreaction during anaesthesia as response to specific anesthetics and muscle relaxants. If not treated immediately MH episodes might have lethal consequences. A form of myopathy closely associated with MH is central core disease (CCD) which is inherited in an autosomal dominant mode. Mutations in the ryanodine receptor 1 gene (RYR1) are the most frequent cause of MH and CCD. RYR1 protein mediates calcium release in skeletal muscle during excitation-concentration coupling. Mutations alter the RYR1 channel kinetics for calcium inactivation and make it hyper- and hyposensitive to activating and inactivating ligands, respectively. RYR1 is one of the largest known proteins with 2.200 kDa corresponding to 5.039 amino acids encoded by 106 exons. Therefore, mutation analysis for both conditions has so far been concentrated on "hot spots" in functionally relevant regions: including parts of exons 2 to 17 and 39 to 46, and especially in CCD in the C-terminal region exons 71 and 95 to 103.
We used denaturing high performance liquid chromatography (DHPLC) to analyse 18 regions of the RYR1 gene. Altogether 208 individuals including fourteen families with 52 members were investigated. 20 mutations causing MH were detected, 7 of which are also associated with CCD. 8 mutations have not yet been published. In addition, 10 (partly novel) polymorphisms were identified during the mutation analysis.
In conclusion the RYR1 mutations were shown to account for ~35% of MH susceptibility and in ~15% of CCD cases. In muscle biopsy with a characteristic CCD phenotype the detection rate could be increased to ~35%.

P208

Junction PCR based genotyping of NF1 microdeletions indicates preferred sites of non-allelic recombination in the NF1 gene region

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Heterozygous NF1 deletions that encompass at least 1-Mb are the most common recurrent mutations in Neurofibromatosis type 1. Clinically, NF1 microdeletions are of interest as they have been reported to be associated with early onset of tumor growth, mental retardation, facial dysmorphism and high risk for malignancy. Thus NF1 deletions promise to identify modifying genes aggravating tumor growth. However, genotype/phenotype correlations in large cohorts of patients identified in unselected screenings have not yet been performed. Molecularly, NF1 deletions are of interest as they are triggered by the genomic architecture of the NF1 gene region. The majority of deletions are caused by aberrant recombination between duplicated sequences flanking the NF1 gene region. Deletion sub-types were identified, which differ with respect to breakpoints and the number of deleted genes. The frequency of the different deletion types has not yet been determined in a collective of NF1 patients ascertained in screenings not biased by the severity of the manifestation. We examined 611 unselected NF1 patients for the presence of deletions and determined the breakpoint sites in most cases. In 4,6% of the screened patients (28/611), a microdeletion was confirmed by FISH and marker analysis. This screen revealed two major types of deletions: Type 1 deletions of 1.4 mb with breaks in the NF1 LCRs were detected in 61% of patients. Type-2 deletions of 1.2 mb have breakpoints in the JJAZ1 gene and were identified in 28% of patients. Two other patients had larger deletions of 2-Mb and 2.7-Mb, respectively, which were non-LCR mediated. We determined the exact breakpoint sites in 18 NF1 microdeletions and identified preferred regions of strand exchange for which deletion-junction PCRs were established. These deletion junction assays will facilitate the mapping of deletion breakpoints in large cohorts of patients and enable detailed genotype/phenotype correlation studies for large NF1 deletions.

P209

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). Further genes are likely to be involved. Forced by the lack of families suitable for positional cloning we started a functional candidate gene approach. We considered such genes as candidates for EDMD that encode (1) functionally related proteins to emerin and lamin A/C including LMNB1, LMNB2, LBR, LAP1, LAP2, NRM and MAN1 or (2) proteins interacting with emerin and/or lamin A/C including Narf, Zmpste24, BAF, PSME3, SREBF1, YT521B. Additionally, a third group of candidates were those, which are expressed specifically in heart and skeletal muscle – the preferentially affected tissues in EDMD (FLNC, SMPX, POP 1-3, AKAP 7, Nesprin1 and Nesprin2). Until now we have studied 21 genes in 110 patients from Germany. We identified five unique variations in Nesprin1α (29A>G, N323H, V572L, E646K, 2368G>A), one in SrebF1 (R812Q), one in Nesprin2α (525G>A), one in YT521B (3100G>A), one in LAP2 (E384K), one in NRM (715C>A) and one in FLNC (6508C>T), which were not detectable in the reference population. None of the DNA variants have so far been associated to EDMD. Interestingly, a Nesprin2α 525G>A variation was also found in the patient's affected son, but not in the healthy son and partly affected daughter. So this variation seems to segregate with EDMD. Our results might be the first hint that Nesprins are involved in the pathogenesis of EDMD. But to validate such an effect, in vitro mutagenesis and transfection experiments are required.

P210

MLPA for screening of large alterations affecting the NF2 Gene

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Neurofibromatosis 2 (NF2) is a genetic disorder caused by the mutational inactivation of the NF2 gene and is characterized by bilateral vestibular schwannomas, spinal tumors, and other benign tumors of the nervous system. Previously, we have found intragenic NF2 mutations in 99 out of a total of 188 unrelated NF2 patients by exon-scanning based methods. Tumor analysis of 22 de novo NF2 patients led to the identification of 12 additional constitutive NF2 mutations. The remaining 77 patients were further examined for gross alterations using the newly developed gene dosage assay multiplex ligation-dependent probe amplification (MLPA). One deletion of a single exon, seven deletions of multiple exons, seven deletions involving the 3'- or 5'- end of the NF2 gene, four deletions involving the whole NF2 gene, and one duplication of three exons were detected. For 47 of the 77 patients, mRNA of adequate quality could be obtained, enabling transcript analysis, which confirmed eight alterations detected by MLPA. In addition, in one family the mRNA analysis detected an insertion of two exons of another gene. Deletions, duplications, and insertions affecting the NF2 gene were thus found in a total of 21 cases. This relates to 11% of the 188 unrelated NF2 patients studied, 16% of the 132 mutations identified, and 27 % of the 77 cases in which no intragenic small mutations were detected by exon-scanning. The combination of multiple screening techniques facilitated a mutation detecting rate of 100% for the 21 inherited cases in this study. Our results demonstrated that a considerable portion of NF2 patients have larger alterations affecting the NF2 gene which can not be detected by exon-scanning based screenings and that MLPA is a suitable method for detecting such alterations.

P211

Position effects at the SOX9 locus: acampomelic campomelic dysplasia with XY sex reversal caused by a novel translocation breakpoint mapping about 900 kb upstream of SOX9

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Campomelic dysplasia (CD; MIM 114290) is a semilethal skeletal malformation syndrome with or without XY sex-reversal. CD is mainly caused by mutations within the *SOX9* gene on 17q and occasionally by chromosomal rearrangements in the vicinity of *SOX9*. The breakpoints in 13 CD translocation or inversion cases analysed in detail map up to 400 kb (11 cases) and as far as 900 kb (2 cases) upstream of *SOX9*. The most likely explanation for this kind of position effect is that the breakpoints interrupt an unusually large *SOX9* control region. We studied a patient with the acampomelic form of CD with the karyotype 46,X,t(Y;17)(q11.2;q23). The patient presented at birth with cleft palate, thoracic kyphoscoliosis and normal female external genitalia. She suffered from recurrent lower respiratory infections and required tracheostomy and gastrostomy at 9 months of age. She has resided in a chronic care facility since that time. Her neurodevelopment is mildly impaired. She is now 1 year and 9 months of age. Fluorescence in situ hybridization (FISH) gave a positive signal for *SRY* and for the centromeric region (*DYZ3*) on the derivative Y chromosome. BAC probes up to 800 kb upstream of *SOX9* always gave signals on the der(Y) only. BAC probe RP11-879D6, however, was found to span the translocation breakpoint on 17q. From the distribution of the FISH signals, the breakpoint was estimated to be around 880-930 kb from *SOX9* and thus in the same region as in the two previously reported cases. Together, these cases indicate the existence of a second, far upstream breakpoint cluster region, in addition to the breakpoint cluster 1-400 kb upstream of *SOX9*. The present case also adds weight to the hypothesis that essential *cis*-regulatory elements are located up to almost 1 Mb from the *SOX9* gene they control. - By FISH and by PCR analysis of somatic cell hybrids, the breakpoint position will be further refined.

P212

Genetic and phenotypic diversity of autosomal, dominant congenital cataracts

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In industrialized countries, approximately 30 infants (out of every 100.000) will be diagnosed with cataract by their first birthday. Since viral infections and consanguinity are rather rare, most of them can be characterized as hereditary, dominant forms (mainly autosomal). Morphologically, these lens opacities can be classified as cerulean, lamellar, sutural, aculeiform, nuclear or cortical depending on their size and shape. During the last years, we and others could molecularly characterize some of the underlying muta-

tions. Based upon our broad experience with mouse cataract models, we showed by a 'functional candidate-gene approach' that mutations in the crystallin genes CRYAA, CRYGC, CRYGD or CRYBB2 are responsible for most of the cataracts (further functional candidate genes include also GJA3, GJA8, MIP or PAX6). Unfortunately, there is no correlation obvious between the particular phenotype and the affected gene, the type of the underlying mutation nor the affected protein domain.
Moreover, the overall number of known mutations in the particular genes is obviously different: there are five mutations known in the CRYGD gene, but none in the CRYGA or CRYGB. The only described change in the CRYGA is an insertion at pos. 43 leading to a frame shift and a premature stop codon after 7 new amino acids. Since the heterozygous situation is without pathological consequences, it might be speculated that the corresponding mRNA is degraded because of nonsense-mediated decay. We observed also a remarkable number of polymorphic sites in these genes but none in the closely linked CRYBA2 nor GJA8. This feature might be explained by the small number of families investigated, however, we made similar observations in the mouse including a larger number of lines. It might be speculated whether particular genes may have a higher mutation frequency than others or if this phenomenon reflects a different importance of some genes for the functional integrity of the particular tissue.

P213

A novel Q49P mutation in the GJA1 (Connexin-43) gene in an 11 year old boy with Oculo-Dento-Digital dysplasia (ODDD)

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Objectives: Oculodentodigital dysplasia (ODDD) is a rare autosomal dominant disorder with highly characteristic craniofacial findings including a thin nose with hypoplastic alae nasi, prominent columella, microcornea, enamel hypoplasia, oligodenty, and microcephaly. Neurologic symptoms are mainly spastic paraparesis and bladder disturbencies. We observed a boy of Turkish-Austrian origin, who was diagnosed with ODDD clinically already in the newborn period. His parents are healthy. The aim of this study was to confirm the clinical diagnosis at the molecular level and to compare the results with previous findings (Paznekas, AJHG 2003).
Methods: The complete coding region of the GJA1(connexin-43) gene was investigated by PCR and bidirectional sequencing and avoidance of amplification of the pseudogene GJA1P1.
Result: A heterozygous missense mutation c.146 A>C was found, causing an exchange of a glutamine residue by prolin in the first extracellular domain of Cx43. Our patient presented with the hallmarks of the syndrome but in addition showed bilateral 4/5 syndactyly and signs of cardiac failure (low voltage ECG).
Conclusions: So far, all ODDD patients who underwent molecular studies revealed mutations in the coding region of the GJA1(Cx43)gene. The glutamine residue in codon 49 is highly conserved among species and a mutation at this codon has already been described in ODDD. However, no obvious genotype-phenotype correlation can be derived.

P214

Search for mutations in the sodium dicarboxylate cotransporter-1 (NaDC1) in the etiology of hypocitraturia as a major risk factor for urolithiasis

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The aetiology of urolithiasis is influenced by environmental as well as by genetic factors. Indeed, the genetic basis for specific metabolic disorders that lead to urolithiasis, such as cystinuria and oxaluria is well established; however only little is known about the genetic basis of hypocitraturia as the major stone forming factors in calcium-oxalate stone disease. Urinary citrate concentration is primarily determined by its rate of reabsorption in the proximal tubule. Citrate reabsorption is mediated by the NaDC1 gene. Recent studies showed that increased NaDC1 expression is associated with a decline in urinary citrate excretion. Aim of our study was to determine the role of mutations of the coding region of NaDC1 as a cause for hypocitraturia. Patient population was evaluated by means of 24h-urinary specimen and citrate load test. Genomic DNA of 13 patients with hypocitraturia less than 1 mmol/d were included in the study, in addition, 5 patients from hypocitraturia families were analysed. The coding sequence (12 exons) as well as the intron/exon boundaries of NaDC1 was screened by single strand conformation analysis (SSCP). To demonstrate the sensitivity of our SSCP approach, 10 of the fragments were additionally analysed by denaturing high-performance liquid chromatography (DHPLC). By SSCP and DHPLC analysis, we detected unusual patterns in the fragments of exons 3, 8, and 12 of the NaDC1 coding sequence. Direct sequencing of these variations confirmed basepair substitutions which correspond to known SNPs (rs11568466 in exon 3, rs11568443 in intron 7, rs11568454 in exon12). All three variants were detected in patients as well as in controls. Thus, our results do not indicate a relevant role of mutations in NaDC1 in the etiology of hypocitraturia.

P215

Insights in the mechanism of targeted nucleotide exchange (TNE) by single stranded oligonucleotides through experiments resulting in the exchange of up to three nucleotides in hprt of V79 hamster cells

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Recently, we demonstrated that a point mutation in the hprt sequence can be repaired by specific single stranded oligonucleotides in V79 cells. In addition, it is also possible to exchange up to two additional nucleotides near by the hprt point mutation. The repair mechanism involved is not understood. According to our investigations one can formulate hypotheses to the mechanism. In case of the finding of a clone with exchanges of all three nucleotides speculation could be done about a total exchange of all nucleotides over the complete length of the oligonucleotides. That is unlikely because we found in the same experiment aside the clones with the exchange of all three nucleotides also clones with the exchange of just one or two nucleotides. According to this result we could also suggest that the exchange of the three nucleotides is an independent exchange of one nucleotide after another. So we had to calculate that the rate of the first nucleotide exchange is about 0,33x10 6 and the probability for two exchanges has to be (0,33x10 6)2. The rates are higher than calculated. So the independent exchange of single nucleotides is not very likely. In our experiments we found exchanges of all three nucleotides in a maximum distance of 15 nucleotides. So we calculate the possibilities assuming a exchanged region of 15 nucleotides and probabilities of the rate of 1, 2 or 3 exchanges and compared them with the experimental data. The rates differed from the calculated prediction. All these hypotheses didn't fit our experimental data. In addition, we found evidences of a preferred orientation of the repair mechanism. First, looking at the exchanges with up to three nucleotides we found that it is more likely to detect clones with the first two exchanges counted from the 5' side than from the 3' side. Second, asymmetric modified oligonucleotides also showed differences in total exchanges rates in the correction of the point mutation.

P216

Identification of two novel germline mutations of the *PROC* gene in two elderly patients with thrombosis

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Introduction: Protein C deficiency is normally inherited as an autosomal-dominant trait associated with an increased risk of venous thrombosis. Heterozygous protein C deficiency is thought to have a prevalence of between 1/16,000 and 1/36,000 in the general population. Several different mutations lead to protein C deficiency, in the majority point mutations.
Patients: We report about two patients with biochemically confirmed protein C deficiency.
A 65-year-old patient from Germany with thrombosis of the right eye, 6 years ago. Two years ago, a second thrombotic event occurred in the left eye.
A 75-year-old patient from Germany with no family history of thrombosis developed a thrombosis in the left wade followed by a pulmonary embolism when he was 67 years old.
Methods: Genomic DNA was extracted from peripheral blood leukocytes, followed by PCR amplification of the 9 exons of the *PROC* gene, including corresponding exon-intron boundaries. PCR products were sequenced directly.
Results: Sequence analysis of the *PROC* gene shows a novel heterozygous germline mutation

in exon 8, codon 199 (GTG>ATG) of the first patient. This mutation results in the substitution of the amino acid valine (Val) for methionine (Met) at amino acid position 199.
The mutation detected in the second patient is located in exon 9 of the *PROC* gene. The T-to-C change at nucleotide number 8743 leads to the substitution of methione (Met) to threonine (Thr) at amino acid position 343.
Conclusion: Differential diagnosis of thrombophilia should be considered not only in young patients with thromboembolic disease but also in older patients with recurrent thromboses. Genetic counseling and careful genetic testing of the *PROC* gene should be carried out for patients with biochemically confirmed protein C deficiency, to confirm the diagnosis and define presymptomatic gene carriers.

P217

Gene expression profile of cultured primary podocytes from Denys-Drash syndrome patients

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Germline missense mutations in the WT1 gene result in Denys-Drash syndrome (DDS), mainly characterized by nephrotic syndrome due to diffuse mesangial sclerosis. The transcription factor WT1 plays an important role in nephrogenesis and podocyte homeostasis.
The aim of our study was to find WT1 specific gene expression differences between primary podocytes from DDS patients and controls. For this we have isolated glomeruli with the sieving method from two nephrectomized DDS kidneys, one adult kidney and one kidney of a patient with finnish nephrosis (CNF) and a NPHS1 mutation. Outgrowing primary cells were positive for podocyte markers like WT1, CD2AP, Podocin, Synaptopodin and Vimentin but were negative for the mesangial cell marker alpha SM-actin, charcterized by immunofluorescence or RT-PCR. Expression analyses were performed with Atlas 3.6 Arrays (Clontech) and data were verified by comparing them to the Gene Chip Array 133A (Affymetrix) data and by real-time RT-PCR, semiquantitative RT-PCR and immunohistochemistry. In total we found 42 genes differentially expressed (>2 fold or <0,5 fold) in DDS compared to CNF or to normal control. Functionally, they belong to extracellular secreted proteins, extracellular matrix proteins, cell adhesion proteins and cytoskeletal proteins. Interestingly, some of the extracellular secreted proteins are known to induce matrix formation in mesangial cells, one of the characteristics of diffuse mesangial sclerosis. In conclusion, this demonstrates that in Denys-Drash syndrome important components of the filtration barrier are disturbed and that podocytes secrete proteins that may influence mesangial matrix formation.

P218

MPP4 – a scaffolding protein in the connecting cilia of photoreceptors

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Objectives: MPP4 (membrane protein, palmitoylated) is a member of the large family of membrane-associated guanylate kinases (MAGUKs). MAGUKs act as scaffolding proteins at various cell-cell junctions by mediating the assembly and anchoring of protein complexes through various conserved protein interaction domains. We have demonstrated that MPP4 is preferentially transcribed in the retina. The current study aims at the elucidation of MPP4 expression in the ciliary organelles of photoreceptors in various mammalian species.
Material and Methods: We have generated a panel of highly specific mono-and polyclonal antibodies raised against various epitopes of the MPP4 protein. Immunofluorescence labelling with antibodies against MPP4 and known molecular markers for connecting cilia was performed on fixed and unfixed frozen retinal sections, on isolated individual photoreceptor inner and outer segments and on intact photoreceptor microtubule-based axonemes. Furthermore, we have prepared cytoskeleton-enriched fractions from photoreceptor extracts by sucrose density gradient centrifugation to investigate an association of MPP4 with ciliary axonemes.
Results: Cellular localization studies consistently revealed that MPP4 is abundantly present in the synaptic terminals of rod and cone photoreceptors. In addition, we provide evidence that MPP4 is a component of the non-motile primary cilia (connecting cilia) of photoreceptor cells.
Conclusions: Defects in cilia-localized proteins have been linked to hereditary diseases including retinitis pigmentosa, Bardet-Biedl syndrome and polycystic kidney disease. This study provides further insights into the role of MPP4 in ciliary function and dysfunction.

P219

The contribution of multiple pericentric inversions to the human/chimpanzee speciation.

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Human and chimpanzee karyotypes differ by nine pericentric inversions from which seven are identified as chimpanzee specific events. The two chimpanzee species Pan troglodytes and Pan paniscus, which separated about 2 million years ago (MYA) share all these seven rearrangements. Therefore it can be concluded that these inversions occurred and were fixed in the homozygous state between 6 and 2 MYA, in the time interval when the human and chimpanzee lineages have separated. This supports the common view that chromosomal rearrangements were instrumental for the genetic divergence and the separation of species. A new model of the chromosomal speciation theory is applied to explain the divergence of human and chimpanzee lineages, which took place in East Africa without local separation (symparty). According to this model in interbreeding populations inversions lead to the suppression of recombination what facilitates the evolution of species-specific haplotypes in the rearranged regions. Since a positive correlation exists between recombination frequency and interspecies nucleotide substitution rates, differences in DNA divergence are indicative of a substantial period of heterozygosity with suppression of recombination. We have determined the human/chimpanzee DNA divergence at 15 loci (total length 299333 bp, average length 19956 bp) in inverted chromosome fragments and at 21 loci (total length 446593 bp, average length 21266 bp) in colinear regions. We compared non-coding sequences as these can be regarded as evolving neutrally. The average divergence was 1.15 % in the regions encompassed by the inversions and 1.37 % in the colinear ones (P = 0.0137, Mann-Whitney-U-Test). Our findings are in agreement with the chromosomal speciation model of restricted recombination within the rearranged regions. Further investigations will address the specific genes within the inverted regions, which could have triggered the separation of the human-chimpanzee lineages.

P220

Microarray analysis of the transcriptome in human testes: overexpression of DNMT3a and MDB4 in spermatogonia

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Male germ cell development is a complex process that involves stem-cell renewal, meiosis and dramatic reorganisation of the resulting haploid genome. Meiosis is the key process for recombination and reduction of the diploid chromosome set to a haploid one. To date, about 100 genes have been found, mainly in knockout mouse models, to be implicated in spermatogenesis. Many of the genes that play a role in meiotic recombination are also important for DNA damage repair. To identify new genes which are relevant for male meiosis and infertility, we have analysed the DNA repair gene transcriptome in human testes. Microarrays allow monitoring the expression of numerous candidate genes in parallel. To this end, we developed a cDNA chip with approximately 500 genes which are involved in different types of DNA repair and/or cell cycle control, along with 100 control house keeping genes. This customized gene chip was used to quantify the mRNA expression levels in four adult human testes, compared to a pool of fibroblast RNAs. Approximately 350 clones showed detectable expression levels in adult human testes, approximately 50 genes were expressed differentially in testicular and fibroblast cells. Microarray results were validated with reverse Northern blots. From the subset of genes that showed at least twofold elevated mRNA levels in testes, the DNA methyltransferase 3a (Dnmt3a) and the methyl-CpG domain binding protein 4 (MBD4) were analyzed in more detail. Immunofluorescence staining localized both proteins in the nucleolus of spermatogonia, whereas other testicular cell types showed only very weak, if any specific staining. A similar nucleolar co-localization was observed in a percentage of nuclei from an exponentially growing fibroblast culture. We hypothesize that DNMT3a and MDB4 are involved in methylation and silencing of ribosomal RNA genes during mitosis and

most likely also during other differentiation processes.

P221

Bacterial expression and analysis of residual argininosuccinate synthetase activity in patients with mild citrullinemia

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Deficiency of the urea cycle enzyme argininosuccinate synthetase (ASS) lead to citrullinemia type I. Most patients with the classical course of the disease present with neonatal acute hyperammonemia leading to metabolic coma. Since the introduction of extended newborn screening programs using tandem mass spectrometry there are also asymptomatic patients with only moderately elevated concentrations of serum citrulline reported. The variable degree of the citrulline elevations is not understood and there is no reliable prognostic marker available to distinguish between the mild or classical course of the disease. Here, we report on the bacterial expression of the single most common ASS mutation in a group of 30 patients with mild citrullinemia, c.535T>C (p.W179R). We measured the ASS activity of the expressed and purified wildtype and W179R mutant protein in a direct enzyme assay using tandem mass spectrometry. W179R protein showed a yield of 37,8% of ASS wildtype activity. In summary, for the first time significant in vitro residual activity of a mutant ASS protein was detected with a bacterial expression system. This result might explain the milder course of affected patients. In the future, determination of the residual ASS activity using bacterial expression systems might serve as a prognostic marker for citrullinemia type I.

P222

Molecular diagnosis of the classical type of Ehlers-Danlos Syndrome (EDS Type I/II)

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The classical type of Ehlers Danlos syndrome is the second most common one with an estimated prevalence of 1:20.000. Major diagnostic features are hyperextensible skin, tissue fragility often recognized by widened atrophic scars, and hypermobility of large and small joints. Classical EDS is primarily classified by clinical diagnosis according to the Villefranche Nosology (Beighton et al., 1998). About half of the cases result from abnormalities in type V collagen, which occurs predominantly as a heterotrimer of two a1 (V) and one a2 (V) chains encoded by the COL5A1 and COL5A2 gene, respectively. The clinical diagnosis of classical EDS can be supported by abnormal ultrastructural dermal architecture recognized by characteristic findings in electron microscopy like composite collagen fibrils with enlarged "flower-like" cross sections and rope-like longitudinal sections.
We report on the molecular analysis of 32 patients fulfilling the major diagnostic criteria of classical EDS. Electron microscopy of a skin biopsy preceeded molecular genetic analysis in 15 cases. Mutation screening of all coding exons of the COL5A1 and the COL5A2 gene by direct sequencing of leukocyte DNA has been finished in 19 patients. Most likely pathogenic COL5A1 mutations were identified in 8/19 patients (42%). Five are translation terminating composed of two frameshift, two splice site and one nonsense mutation. Two missense changes affect a glycin residue and a conserved arginin residue in the triple-helical domain of the proa1 (V) chain, respectively. One patient carries a one amino acid in-frame deletion. Our so far reached detection rate of 42% is in accordance with previously reported molecular genetic studies and supports further genetic heterogeneity of classical EDS. The fact that part of type V collagen in certain tissues occurs as heterotrimer of one a1 (V), one a2 (V) and one a3 (V) chain prompted us to extend mutation analysis on the COL5A3 gene in COL5A1/COL5A2 negative patients.

P223

Haplotype structure and frequencies of the chromosome 15 imprinting center

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Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are neurogenetic disorders caused by the loss of function of imprinted genes in 15q11-q13. Most of the patients have a large deletion of 15q11-q13, uniparental disomy 15, or, in the case of AS, a UBE3A mutation. In 1% of patients with PWS and in 3% of patients with AS the disease is due to an imprinting defect. Approximately 10% of these patients have a microdeletion affecting the imprinting center (IC), which consists of two critical elements (the PWS-SRO and the AS-SRO). By contrast, 90% of patients with an imprinting defect do not have any obvious IC mutation, but the affected chromosome may carry an IC sequence variant that is associated with an increased epimutation rate. Based on DNA sequencing and data base searches we have identified nine single nucleotide polymorphisms (SNPs) and one 4 bp insertion/deletion polymorphism in the AS-SRO, the PWS-SRO and flanking regions. By genotyping patients with a large deletion of 15q11-q13, we have been able to define IC haplotypes experimentally. So far, we have studied 262 normal chromosomes. Using Haploview we have found that the AS-SRO and the PWS-SRO, which map 30 kb apart, lie in two distinct haplotype blocks. For the AS-SRO we have found six different haplotypes, which occur at a frequency of 0.49, 0.23, 0.13, 0.08, 0.06 and 0.004, respectively. The second and third frequent haplotypes differ in two SNPs from the most frequent haplotype. The three rare haplotypes differ in only one SNP from the most frequent haplotype. For the PWS-SRO we have found four different haplotypes; two are frequent (0.6 and 0.35) and two are rare (0.04 and 0.004). The two frequent haplotypes differ in all three SNPs. The two rare haplotypes differ in only one SNP from the most frequent haplotype. We are currently genotyping patients with an imprinting defect and their parents to identify haplotypes associated with an increased epimutation rate.

P224

Identification of protein-protein interactions by co-immunoprecipitation and ProteinChip technology (SELDI-MS)

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Objectives: Aim of the present study was the identification of protein interactions of S100A8 and S100A9 heterodimer, respectively. The S100 proteins are involved in the Ca^{2+} signalling network and have received increased attention because of their involvement in several human diseases such as rheumatoid arthritis, acute inflammatory lesions, cardiomyopathy, Alzheimer's disease and cancer.
In a former protein profiling study of HNSCC (head and neck squamous cell carcinoma) a decrease of S100A8/A9 expression in tumour tissue was detected (Melle et al., Cancer Res. 2004). On that account we started to analyse the interactions of S100A8/A9.
Material and Methods: An immunoprecipitation assay followed by a ProteinChip analysis was performed. The immunoprecipitation assay was done by using specific S100A8/A9 antibodies coupled on IDM beads (Ciphergen) and incubated with protein lysate of the HaCaT cell line. The bead eluates then spotted on a ProteinChip and analysed by SELDI-MS showed a reproducible protein pattern of specific signals compared to protein patterns which were generated using non-specific antibodies. The eluates were subsequently subjected to an appropriate digestion.
Results: Molecular masses of digestion fragments were determined by SELDI-MS and database searches revealed unambiguous identifications of proteins. These results were assessed by co-immunoprecipitation using specific antibodies against the new identified interacting protein, S100A8 and S100A9 respectively.
Conclusions: This approach shows the potential of SELDI mass spectrometry for the identification of protein-protein interactions.
Upcoming, these new interacting proteins have to be analysed for additional interacting partners to get more insight into the corresponding pathways and their influence to the HNSCC.
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P225

Identification of human specific DNA copy-number differences compared to non-human primates as determined by BAC CGH-array.

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Comparative DNA sequence studies between humans and nonhuman primates will contribute to the understanding of the genetic basis of the phenotypic differences between these species. Here we present the results of an evolutionary study using BAC array comparative genomic hybridisation (CGH array), measuring DNA copy-number gains and losses among human, chimpanzee, bonobo, gorilla, orangutan and macaque. The aim of the study is to characterize rearrangements that occurred specifically in the human lineage.

We used an array of 6101 human bacterial artificial chromosomes (BACs), the human 6K RPCI-11 BAC array, encompassing about 30% of the genome. The arrays were simultaneously hybridised with a primate and a human genomic DNA probe, each were pools of ten female individuals. We identified eleven human specific sites of DNA copy-number variation between human and the great apes. All of them are duplications that occurred in the human lineage. From these eleven variant sites, two were human copy-number polymorphisms (CNP), as identified in the study from Sebat et al. (2004).

We divided the eleven sites of variation in two categories: seven are intrachromosomal duplications, three of them were found on HSA1, two on HSA2, one on HSA10 and one on HSA18. The four others are interchromosomal duplications spread through the complete human genome.

In order to find the ancestral position of these copy number differences, we compared them with the mouse genome. Interestingly the ancestral locus of six of these sites is found on HSA1, HSA2 and HSA18, which are those chromosomes that were subject to human specific evolutionary rearrangements. These events occurred after the separation of the human from the chimpanzee lineage.

P226

Searching for mutations in the OTOA gene in a family with deafness

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Mutations in OTOA gene were shown to be one cause of prelingual sensorineural autosomal recessive deafness. The OTOA gene is located on chromosome 16p12.2. So far only one mutation had been reported in this gene. In a consanguineous Palestinian family a T to C transition at the exon 12/intron 12 junction was identified. This mutation is expected to lead to aberrant splicing, such as exon 12 skipping (resulting in an in-frame deletion of 72 amino acids) or the use of a cryptic site. Mutations in consensus splice sites belong to the most common disease-causing DNA changes. Previous studies showed only a rare occurrence of this form in deafness.

In our study family K was mapped to DFNB22 by linkage analysis. In DFNB22, the responsible gene has been already identified as OTOA. Therefore, exon 12, reported to carry the known mutation (see above), was the first one to be investigated using sequence analysis, however, no mutation was detected so far. This result suggests other mutations within the OTOA gene sequence to be present. Currently, a complete mutational analysis is being performed.

P227

Frequencies of mutations in the GJB2 gene in Egyptian and Romanian patients with autosomal-recessive non-syndromic hearing loss

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Mutations in the GJB2 gene (encoding the gap junction protein Connexin 26) are the most common cause of sensorineural non-syndromic hearing impairment worldwide. Presently more than 100 mutations are described within GJB2 in different populations. One specific mutation, 35delG, is responsible for the majority of the mutations in many countries. The allele frequencies in sporadic cases range from 5 % in Northern European countries to 35% in Mediterranean countries. In familial cases, the ratio is much higher (29 % in Great Britain, 33 % in Belgium, 66 % in Hungary, 63 % in Italy). However, there are no data for Egyptian or Romanian patients despite of the fact that GJB2 consists of only one small-sized coding exon and therefore is easy to analyze.

The aim of this study was to determine the prevalence of GJB2 mutations in Egyptian families and Romanian patients with autosomal-recessively inherited non-syndromic sensorineural hearing loss (NSHL). All the probands were examined by clinical evaluation to exclude syndromic forms of deafness. The 35delG mutation was found in 23,53 % (4/17) of the Egyptian families or in 24 of 142 (16.9 %) investigated alleles. Four patients were heterozygous carriers and 10 patients were homozygous for the 35delG mutation.

In our Romanian samples we found 4 (3.6 %) patients heterozygous and 8 (7.21 %) patients homozygous for the 35delG mutation. Thus 9.01 % (20/222) of the analyzed alleles were carrying the 35delG mutation. Our results emphasize the importance of genetic diagnosis, providing early treatment, and genetic counseling of deaf patients in countries all over the world.

P228

A mutation at chromosome 7q22.3-31.1 can lead to autosomal-dominant dilated cardiomyopathy

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Dilated Cardiomyopathy (DCM) is one of the major causes of heart failure. 20-30% of DCM cases are familial, usually showing an autosomal dominant pattern of inheritance that allows identification of the disease gene by linkage analysis and positional cloning.

Several DCM genes have been identified so far. Most mutations affect proteins from the contractile aparatus or cytoskeleton like actin, myosin, troponin or dystrophin. These findings lead to the hypothesis that DCM might be a disease with impairment of force generation and/or force transmission to the extracellular matrix.

Here we report the genetic analysis of a four generation pedigree including 16 individuals affected by dilated cardiomyopathy without additional phenotype. After exclusion of genetic linkage to all known DCM loci we performed a genomwide screen using 379 autosomal polymorphic microsatellite markers from the 10th version of the Marshfield STRP Screening Set. Several microsatellite markers on 7q22.3-31.1 showed co-segregation with the disease status. We obtained a maximum two-point LOD-score of 4.20 at theta = 0.00 for markers D7S471 and D7S501. Fine mapping and haplotype analysis restricted the candidate region to a 9.73Mb interval between markers D7S2545 and D7S2554. This chromosomal region contains approximately 40 genes, none of which encode known cytoskeletal proteins. We already screened several candidate genes for mutations by sequencing the protein-coding exons, but no disease-causing mutation has been detected yet.

Identification of this novel non-cytoskeletal DCM gene can provide substantial new insight into the pathophysiology of human heart failure.

P229

The gene causative for the Zimmermann-Laband syndrome is located in 3p14.3

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Zimmermann-Laband syndrome (ZLS) is a rare disorder characterized by coarse facial appearance including bulbous soft nose, thickened lips, thick and floppy ears, gingival hypertrophy, aplasia or dysplasia of hand- and toenails, various skeletal anomalies, hyperextensibility of joints, and, in some cases, hepatosplenomegaly and mental retardation. Autosomal dominant inheritance has been suggested, however, the genetic basis of ZLS is unknown. We previously reported an apparently balanced chromosomal translocation, 46,XX, t(3;8)(p21.1;q24.3), in an affected mother and daughter. By FISH analysis, we delineated and refined both breakpoint regions and identified breakpoint spanning fosmid clones. Molecular characterization of the 3;8 translocation revealed that the rearrangement occurred by non-homologous end joining and is

molecularly unbalanced with 8 bp deleted on the derivative chromosome 8 and 9 bp on the derivative chromosome 3. The finding of an additional patient with ZLS and a 3;17 translocation with breakpoints in 3p21 and 17q25 indicated that the gene causative for ZLS is located on 3p. Delineation of the 3p breakpoint by FISH showed that the CACNA2D3 gene (in 3p14.3) is disrupted by the breakpoint in the 3;17 translocation patient whereas the breakpoint on 3p of the 3;8 translocation mapped 100 kb downstream of CACNA2D3. The CACNA2D3 gene encodes a member of the alpha-2/delta subunit family, a protein implicated in the voltage-dependent calcium channel complex. Remarkably, calcium channel blockers cause gingival hyperplasia suggesting that CACNA2D3 is indeed a good candidate for ZLS. However, we did not find pathogenic mutations in CACNA2D3 in 12 sporadic patients with ZLS. The finding of two translocation breakpoints that map in or near the CACNA2D3 gene suggests that this chromosomal region might be prone to genomic instability and thus, heterozygous microdeletions encompassing various parts of CACNA2D3 might be present in sporadic patients with ZLS.

P230

Characterization of murine mp28 gene

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The diversity of ciliary and flagellar function depends on the coordinated activity of multiple dynein motors. Several lines of evidence suggest that dynein dysfunction results in Primary Ciliary dyskinesia (PCD), also denoted as Immotile Cilia Syndrome , which is characterized by recurrent infections of the respiratory system, bronchiectasis, and sperm immotility. To elucidate the specific role of mammalian axonemal dynein light chains in the organization of the dynein complex, we started to analyse the mp28 protein in the mouse. The mp28 gene consists of six exons and exhibits significant similarity to p28 dynein light chains of other species. It is expressed in several tissues containing cilia and flagella proved by RT PCR and Northern blot experiments.

To study mp28 at the amino acid level, we generated specific antibodies against the mp28 protein. Using these antibodies, we could localize the mp28 protein along the entire sperm flagella. Moreover, its localization could also be detected along tracheal cilia supporting a fundamental role of mp28 in ciliary motility.

As mp28 is a component of multisubunit dynein complex, we were interested to identify putative interacting partners of this protein. Therefore we performed a yeast two-hybrid screen using a mouse testicular library. Surprisingly, we were not able to identify other axonemal dynein polypeptides, however, our results indicate that mp28 can bind to the C-terminal part of the cytoplasmic dynein heavy chain (cDHC). This interaction was verified by co-immunoprecipitation and colocalization assays. These data suggest that either mp28 is transported by the cytoplasmic transport machinery within the differentiating germ cells or that mp28 itself is required for the retrograde transport of components during flagellar assembly and maintenance, presumably as a subunit of a cytoplasmic dynein motor.

P231

Alteration of DNA binding, dimerisation and nuclear translocation of SHOX homeodomain mutations identified in idiopathic short stature and Leri-Weill dyschondrosteosis

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Haploinsufficiency of the short stature homeobox gene SHOX has been found in patients with idiopathic short stature and Leri-Weill dyschondrosteosis. In addition to complete gene deletions and nonsense mutations, several missense mutations have been identified in both patient groups leading to amino acid substitutions in the SHOX protein. The majority of missense mutations were found to accumulate in the region encoding the highly conserved homeodomain of the paired-like type. In this report, we investigated nine different amino acid exchanges in the homeodomain of SHOX patients with idiopathic short stature and Leri-Weill dyschondrosteosis. We were able show that these mutations cause an alteration of the biological function of SHOX by loss of DNA binding, reduced dimerisation ability and/or impaired nuclear translocation. Additionally, one of the missense mutations (R153L) is defective in transcriptional activation even though it is still able to bind to DNA, dimerise and translocate to the nucleus. Thus, we demonstrate that single missense mutations in the homeodomain fundamentally impair SHOX key functions, thereby leading to the phenotype observed in patients with Leri-Weill dyschondrosteosis and idiopathic short stature.

P232

Identification and characterization of members of a novel family of transmembrane proteins with neuronal function

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Objectives: The evolutionarily conserved TMEM16 family of putative transmembrane proteins has recently been identified by cDNA cloning and bioinformatic analysis of genomic sequence. In human and mouse, the TMEM16 family consists of eight paralogous members, each containing eight putative transmembrane-spanning helices. TMEM16 proteins also share eight cysteines in the extracellular loops throughout evolution. Moreover, amino acid substitution of one of these cysteines in human TMEM16E causes gnathodiaphyseal dysplasia, an autosomal dominant skeletal syndrome. Thus far, the functions of the TMEM16 proteins are still unknown. The goal of this study is to further characterize this protein family with respect to tissue distribution, cellular localization and biochemical properties.

Material and Methods: RT-PCR analysis in adult tissues was performed to investigate the mRNA expression profiles of the TMEM16 genes. GST fusion proteins corresponding to different regions of the TMEM16 proteins were used to immunize animals for antibody production. The TMEM16B cDNA was expressed in several cell lines to study subcellular localization and the formation of disulfide bonds.

Results: Among the eight TMEM16 proteins, TMEM16B, TMEM16C and TMEM16D were found to be preferentially expressed in neuronal tissues including various brain regions and the retina and were therefore chosen for further analysis. So far, polyclonal antibodies against TMEM16B have been demonstrated to specifically detect the TMEM16B protein when expressed in bacteria or mammalian cells. Immunocytochemistry of heterologous expressed TMEM16B suggests an integration of the protein into the plasma membrane.

Conclusions: Expression of TMEM16B-D specifically in neuronal tissues makes them interesting candidates for a possible role in neurological disorders and/or retinopathies. Knowledge about their cellular localization and biochemical properties provides important steps towards a functional characterization of these novel proteins.

P233

Identification of valosin-containing protein (VCP) with 2-D gel electrophoresis (2-DE) and SELDI-MS (surface enhanced laser desorption/ionization - mass spectrometry) as a putative marker for glucocorticoid resistance of human leukemia cells

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Objectives: Prednisone response (PR) is a clinical phenomenon in childhood acute lymphoblastic leukemia (ALL) indicating in vivo glucocorticoid (GC) response. Patients resistant to glucocorticoids (prednisone poor responders, PPR), have a significantly poorer event-free survival compared to glucocorticoid-sensitive patients (prednisone good responders, PGR).

Methods: The proteome of the blasts was analysed with 2DE in combination with SELDI-MS. Bone marrow leukemic blasts from 12 in vivo glucocorticoid-sensitive (n=8) and resistant (n=4) patients with childhood B-cell precursor ALL were analysed. 2DE was performed with a linear pH gradient of 3-10. Differentially expressed protein spots were identified with SELDI MS.

Results: Proteins overexpressed in PPR were catalase, ring finger protein 22 alpha, VCP and a G-protein-coupled receptor (family C, group 5, member D (GPRC5D)). In addition, 5 proteins were found that were more frequently present in PGR than in PPR. Two of the proteins (G1 and G5) were identified as protein kinase C beta1 and malate dehydrogenase 1. The valosin-containing protein (VCP) was chosen for validation and quantification by Western blot analysis. In a second independent case-control group of B-

cell precursor ALL patients (cases: 10 PPR, controls: 20 PGR), Western blot analysis confirmed the 2-DE results: median VCP expression (P25-P75) was 0.15 (0.11 - 0.28) in PGR and 0.34 (0.14 - 0.99) in PPR patients.
Conclusion: VCP appears to be a marker protein for GC resistance and, therefore, a putative predictor for multi-agent chemotherapy resistance in childhood ALL patients. Since VCP is a key molecule of the NFΩB- and the proteasome degradation pathways, it may be speculated that the overexpression of VCP induces an enhanced cell proliferation. Functional studies now have to be performed to prove the biological importance of differential VCP expression in childhood ALL.

P234

Therapy of spinal muscular atrophy: Hydroxamic acids increase survival of motor neuron protein levels
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Spinal muscular atrophy (SMA) is an autosomal recessively inherited alpha-motoneuron disorder. The disease determining survival of motor neuron gene 1 (SMN1) is homozygously lost in 95% of SMA patients, while intragenic SMN1 muations are correspondingly rare. Within the SMA region on chromosome 5, the SMN exists in two almost identical copies, SMN1 and SMN2, which are ubiquitously expressed and encode identical proteins. The functional difference between both genes is due to a silent exonic nucleotide variant, affecting the processing of primary SMN transcripts. As a consequence, the SMN1 gene produces full length transcripts (FL-SMN) only, whereas the majority of SMN2 transcripts lack exon 7 due to alternative splicing. Since all SMA patients lacking SMN1 carry at least one SMN2 copy, transcriptional activation of the disease modifying SMN2 gene is likely to be clinically beneficial.
The fatty acids butyrate and valproic acid (VPA) transcriptionally activate human SMN2 and restore the correct splicing pattern, resulting in increased FL-SMN levels. Butyrate and the anticonvulsant VPA possess histone deacetylase (HDAC) inhibitor properties. We experimantally characterized a highly potent second generation class of HDAC inhibitors (hydroxamic acids) as potential drugs for SMA treatment and identified four novel compounds to increase SMN protein levels in a time- and dose dependent manner using several experimental paradigms, including fibroblasts derived from SMA patients as well as rat and human organotypic hippocampal brain slice cultures. Analysis of HDAC inhibitor activity of fatty vs. hydroxamic acids revealed that both groups address diverse histone deacetylases, giving rise to different mechanisms of action. One of the new drugs is already under clinical phase II investigation for cancer treatment and represents a promising compound for SMA treatment due to its low in vivo toxicity, its good oral bioavailability and its capacity to penetrate the blood/brain.

P235

Diagnostic screening for MeCP2 mutations in patients with suspected RETT syndrome
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Classic Rett syndrome is an X-linked dominant progressive neurodevelopmental disorder that affects approximately one in 15 000 girls. Following a normal birth and apparently normal psychomotor development during the first 6-18 months of life, the girls enter a short period of developmental stagnation followed by rapid regression in language and motor skills. The hallmarks of the disease are repetitive stereotyped hand movements, loss of social contact and language use. Up to 80% of female cases with typical Rett syndrome harbor loss of function mutations in the gene encoding the methyl-CpG-binding protein MECP2 (Xq28), whereas such mutations are lethal in males already in utero. Within the last years we have screened DNA from 165 females with a clinically suspected RETT syndrome for disease causing mutations by directly sequencing the three coding exons of the MeCP2 gene. The vast majority of patients (158) came from Austria, three were from Bulgaria and four from Slovenia. We identified 14 different mutations in 41 girls (25%) that were already previously described in the literature. They comprised missense and protein-truncating mutations as well as single nucleotide deletions, which clustered in the two functional MeCP2 regions, the methyl-cytosine-binding and the transcriptional repression domain. The most frequent recurrent mutation in seven patients was the nonsense mutation R168X. In addition to these already known mutations, we also detected novel sequence variants in six additional patients, whose potential relevance for the disease could so far only be resolved in two cases with certainty. These consisted of a truncating mutation (658_725 del ins A) and a nucleotide change (IVS2+4 A>G) that had occurred de novo. Whether the other sequence variants were inherited or had also arisen de novo is currently under investigation. Of particular interest is also one case with two rare amino acid exchanges (R106W and T197M), whose significance is also not yet known.

P236

The role of Pelota (Pelo) during the cell cycle
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Mutation in either the Drosophila pelota (Pelo) or the S. cerevisiae homologe, dom34 cause defects of spermatogenesis and oogenesis in Drosophila, and delay of growth and failure of sporulation in yeast. Both phenotypes are suggestive a requirement of Pelo for normal progression of the mitotic and meiotic cell cycle. To explore the function of Pelo in mammals, we have disrupted the mouse Pelo gene and shown that the gene is essential for normal mouse embryonic development. Development of homozygous embryos arrests about 6.5-7.5 days after conception. The failure of mitotic active inner cell mass (ICM) of the Pelo-/- blastocysts to expand in growth after 4 days in culture and survival of mitotic inactive trophoblast indicate that the lethality of Pelo null embryos is due to defect in cell proliferation. Increase of percentage of cells exhibiting polyploidy at E7.5 can be directly responsible for the arrested development and suggests that the Pelo is required for the maintenance of the genomic stability. Approaches to establish Pelo-/-cells by culture of Pelo+/- ES cells in medium containing high concentration of G418 failed to detect a Pelo deficient cells. These results suggest that Pelo is essential for cell viability and or cellular proliferation. Analysis of cell line containing the GFP/Pelo fusion allele revealed that the Pelo is sublocalised at the stress actin filaments. To overcome the early embryonic lethality of the Pelo deficient mice, generation of conditional knock-out mice is underway.

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Role of the Fas-associated protein factor (Faf1) in germ cell and embryonic development
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The Fas system is a receptor-ligand signalling system, in which Fas ligand (FasL) binds to and activates the Fas receptor (Fas) to initiate a cascade of intercellular events that leads to the elimination of the Fas-bearing cells via apoptosis. Fas-mediated signalling system participates in the regulation of germ cell apoptosis in testis. After the activation of the Fas receptor, several proteins binds to the intercellular domain of the receptor. The Fas-associated protein factor-1 (Faf1) was identified in the Fas associated protein complex. Expression analyses demonstrated that the Faf1 is widely expressed in murine tissues. The Faf1 expression is restricted to haploid germ cells of adult testis, while in cryptorchid testis, the Faf1 gene is highly expressed in apoptotic germ cells. To determine the role of Faf1, we characterised a gene trap mutant mouse line (line 40) and found that the gene trap vector is inserted in intron 8 of the Faf1gene. Heterozygous animals appeared normal, however 15% of heterozygous males are infertile. All seminiferous tubules in testes of infertile heterozygotes showed a reduced number of spermatogonia and karyolysis of most of primary spermatocytes. Genotyping of the progeny of heterozygous intercrosses shows the absence of Faf1 homozygous mutant and suggests an embryo-lethal phenotype. These results suggest theat the Faf1 is essential for germ cell and embryonic development.

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Novel mutations in BCOR in three patients with oculo-facio-cardio-dental syndrome, but none in Lenz microphthalmia syndrome
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Oculo-facio-cardio-dental syndrome (OFCD syndrome) is a rare X-linked dominant condition with male lethality characterized by microphthalmia, congenital cataracts, facial dysmorphic features, congenital heart defect, and dental anomalies. Mutations in *BCOR* (BCL6 co-repressor) located in Xp11.4 have been shown to cause OFCD syndrome. Here we report three novel mutations, c.2488_2489delAG, c.3286delG, and a deletion of approximately 60 kb encompassing at least *BCOR* exons 2-15 in three patients with OFCD syndrome. Lenz microphthalmia syndrome is an X-linked recessive trait comprising microphthalmia/anophthalmia, mental retardation, malformed ears, digital, skeletal, and urogenital anomalies (synonym: microphthalmia with associated anomalies [MAA]). One locus for MAA has been mapped to Xq27-q28, whereas linkage analysis in a large family with presumed Lenz microphthalmia syndrome has suggested that a second locus (MAA2) is in Xp11.4-p21.2. Recently, a missense mutation (p.P85L) in *BCOR* has been found in the original MAA2 family. Therefore, we sequenced all coding exons of the *BCOR* gene in eight male patients with Lenz microphthalmia syndrome, but no disease-relevant mutation was detected. On one hand, our data confirm that *BCOR* is the causative gene for OFCD syndrome. On the other hand, the failure to identify any mutation in patients with Lenz microphthalmia syndrome together with the oligosymptomatic phenotype of the patients in the original MAA2 family suggest that *BCOR* is not the major gene for Lenz microphthalmia syndrome.

P239

Detection of Protein-Protein-Interactions by ProteinChip Technology (SELDI)

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For a functional characterisation of the proteome interaction studies are of particular interest, because it is known that most of the proteins usually work interconnected. In this work we used the E2F-transcription factors as a model system to investigate whether the ProteinChip technology can be used for protein-protein-interaction studies. The E2F-transcription factors play an essential role in the regulation of DNA replication, the nucleotide biosynthesis or cell cycle and seemed to be an ideal model system because of their already known interacting partners.
The cell lines U-2 OS and MCF-7 were used to detect endogenous E2F and possible binding partners by the ProteinChip technology SELDI (surface enhanced laser desorption/ionisation). For a sufficient precipitation we tested several affinity surfaces for coupling of specific E2F antibodies and their compatibility with the ProteinChip technology just like protein A/agarose, IDM-affinity beads, the PS10/PS20 ProteinChips and the RS100 ProteinChips. Only the IDM-Affinity beads lead to conclusive findings. To analyse interactions, an immunoassay was done by using specific E2F antibodies coupled on IDM beads. After incubation with U-2 OS or MCF-7 lysate, an H50 ProteinChip was loaded with the eluate. The protein pattern generated by SELDI-MS showed differential signals compared to assays using non-specific antibodies.
To identify these specific signals, immunoblots against already known interaction partners, correlating to the SELDI data, where done with the same eluate used for SELDI-analysis. With this procedure we identified pRB in both cell lines and another E2F1-interaction partner in the U-2 OS cell line.
For further identification of other specific signals, the eluates have to be subjected to other processes. The resultant data may give us more insight into the corresponding pathways and their influence on DNA replication or cell cycle regulation.

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Quantification of Allele-Specific Expression by Pyrosequencing identifies three novel imprinted transcripts in the mouse transcriptome

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A proportion of genes in the mouse and human genome is expressed from only one allele in a parent-of-origin-specific manner. This proportion includes imprinted X-chromosome inactivation and autosomal imprinted loci. We are aiming at a systematic identification of novel imprinted genes using Quantification of Allele-Specific Expression by Pyrosequencing (QUASEP), a highly accurate method to detect allele-specific expression differences. Based on the Pyrosequencing technology, QUASEP can quantify allele-specific expression by analyzing heterozygous exonic SNPs in transcripts from fetal or adult tissues of F1 hybrid mice and humans. We started with a total of 66 candidate imprinted transcripts from recent mouse and human microarray studies and focused on genes that map to imprinted chromosomal regions. Up to now, three novel imprinted transcripts encoding putative nonprotein-coding RNAs have been identified on the basis of monoallelic expression in d11.5 p.c. (C57BL/6J x Cast/Ei)F1 embryos. The results were confirmed in embryos derived from the reciprocal cross. Experiments to verify imprinted expression in adult tissues of hybrid mice are in progress. Two of these transcripts showed paternal-allele expression and map to the imprinted regions on proximal and distal mouse chromosome 7, respectively. The third transcript was maternally expressed and maps to distal mouse chromosome 12. The three transcripts are located in close vicinity of the known imprinted genes Usp29, Lit1 and Gtl2. Further experiments will elucidate if the newly identified imprinted transcripts are independent genes not belonging to the transcription units of either Usp29, Lit1 or Gtl2. The novel imprinted transcripts may be good candidates for imprinting related disease phenotypes on the respective mouse and human chromosomes.

P241

Variability pattern of the NF1 gene in Europe: traces of population subdivision, or signature of balancing selection?

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The pattern of variability observed in the human genome can be exploited to answer questions of both academic interest and medical relevance. Up to now the demographic history of populations, a prerequisit for the interpretation of results obtained in studies to identify genes which have been under selection or in association studies to identify risk alleles for common diseases, has been inferred from the analysis of only a few genomic loci. To improve knowledge about the historical background of the European population, we analysed the pattern of variability at the NF1 gene region, as a further suitable locus for population genetic studies, due to the observed long range LD over 300 kb. For this purpose 25 kb of noncoding DNA from the NF1 gene region of 24 German probands was sequenced. The haplotypes, which could unambigously be deduced from the genotypes at alltogether 45 polymorphic sites, showed a clearcut division into two subgroups with large pairwise differences in intergroup comparisons and little variability within the subgroups. These pairwise differences, together with the results of the summary statistics of Tajima and Fu and Li, applied to the data, demonstrated a relative excess of medium frequency variants, a hint on balancing selection or population subdivision. Because no coding variant could be detected in the whole relevant region, which may have served as a target of balancing selection, we prefer the latter explanation. In this case the two haplogroups may represent sequences brought to Europe by two genetically homogeneous but well separated groups of immigrants. Two waves of immigrations of anatomically modern humans into Europe are documented, one of a group of paleolithic hunters and gatherers and a second of neolithic farmers. The question whether the two NF1 haplogroups indeed represent the genetic heritage of these two founder populations can be answered by the inclusion of further polulation samples in the analysis.

P242

The divergence of DNA sequences between and within species is interdependent with the structure of the mammalian genome.

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The mammalian genome is a highly ordered structure composed of long stretches of DNA with a defined GC content different from the genomewide average, called isochores or GC content domains. Isochores are basic for the chromosomal banding pattern which is tightly correlated with a number of structural and functional features, e.g. distribution of repetitive elements, gene density, recombination frequency

and timing of DNA replication. We have recently demonstrated that the transition from a GC rich isochore to a GC poor one in the NF1 gene region is sharp, occurring within 5 kb, and is well conserved between human and mouse. The transition zone separates regions with low (GC poor) and high (GC rich) recombination frequencies and was shown to be a boundary between replication time zones, with GC rich sequences replicating early and GC poor sequences late in S phase. Now we report on the divergence of DNA sequences located around this boundary in the human and the chimpanzee genome. On average the GC poor sequences showed 7.37 divergent sites per 1000 bp, whereas 11.15 divergent sites per 1000 bp were found in the GC rich isochore. Thus, the isochore boundary was found to be also the boundary between sequences with high and low interspecies divergence. In addition sequence analysis of 25 kb of noncoding DNA from the GC poor and 20 kb from the GC rich isochores in a sample of 24 German probands revealed a striking difference in the degree of variability found within the population. In the GC poor part 1.8 variable sites per 1000 bp were found in the 48 analysed chromosomes, whereas 3.55 variable sites per1000 bp were detected in the GC rich part. In summary the results of the analyses of inter- and intraspecies variation demonstrate that the isochore boundary demarcates sequences showing large differences in their mutation rates, in addition to differences in the recombination frequency and replication timing.

P243

Interchromosomal segmental duplications of the pericentromeric region on the human Y chromosome

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Basic medical research critically depends on the finished human genome sequence. Two types of gaps are known to exist in the human genome: those associated with heterochromatic sequences and those embedded within euchromatin. We have identified and analysed a euchromatic island within the pericentromeric repeats of the human Y chromosome (Kirsch et al., Genome Research, in press). This 450 kb island, although not recalcitrant to subcloning and present in 100 tested males from different ethnic origin, was not detected and is not contained within the published Y chromosome sequence. The entire 450 kb interval is almost completely duplicated and consists predominantly of interchromosomal rather than intrachromosomal duplication events that are usually prevalent on the Y chromosome. We defined the modular structure of this interval and detected a total of 128 underlying pairwise alignments (≥90% and ≥1kb in length) to various autosomal pericentromeric and ancestral pericentromeric regions. We also analyzed the putative gene content of this region by a combination of in silico gene prediction and paralogy analysis. We can show that even in this exceptionally duplicated region of the Y chromosome putative genes with open reading frames reside, including fusion transcripts formed by the splicing of exons from two different duplication modules as well as members of the homeobox gene family DUX.

P244

Rare partial deletions in SHOX gene of three patients with Leri-Weill syndrome, identified by MLPA

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Leri-Weill syndrome (LWS) is a well characterised dominant inherited short stature syndrome associated with mesomelic shortening of the lower legs and the forearms. Patients are often affected by Madelung deformity of the forearms. Phenotypic inter- and intrafamilial heterogenity is a frequent finding in LWS. Skeletal manifestations are more severe in females than in males. This heterogeneity is well documented but the cellular mechanism is about to be discovered.
It was suggested that a gene for short stature and skeletal features resides in the pseudoautosomal region (PAR1), a 2,6 Mb segment of the distal ends of the short arms of the X and Y chromosome that are identical. In 1997, the SHOX gene (short stature homeobox-containing gene) was cloned from the distal part of the PAR1. Like all the genes within the PAR1, SHOX escapes from X inactivation, so that there are two active copies of this gene in males as well as in females. In addition, SHOX protein shows a distinct tissue distribution, whereby it is predominantly present in bone marrow fibroblasts and acts as a transcriptional activator.
In most studies, mutations in the SHOX gene were identified in more than 60 % of LWS patients. Thereby, submicroscopic deletions, encompassing the whole gene, are more prevalent than point mutations. Patients with partial deletions of the SHOX gene are rarely described in the literature. With the use of MLPA, we were able to quantify all of the SHOX exons and the adjacent regions of the gene simultaneous in one single multiplex PCR reaction. We present the finding of three cases with deletions of exon 3, exon 4 to 5 and exon 6b respectively, which were not recognized in the previous FISH analysis. Our experiences show, that MLPA is a efficient and sensitive method for the detection of deletions in the SHOX gene – this in combination with sequencing analysis of the coding region provides an exhaustive molecular screening for Leri-Weill syndrome.

P245

Attrition of telomeres as consequence of telomerase knockdown by a specific shRNA in a colorectal cancer cell line

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Telomerase is the enzyme that catalyzes the synthesis of telomeres, the chromosomal ends. In humans the minimal subunits required for telomerase activity are the hTERT reverse transcriptase and the RNA template hTR. Maintenance of telomere length by telomerase reactivation is a major event in the tumor progression of most human cancer types. Thus, inhibition of telomerase would be an interesting goal in cancer studies. Recently, we designed a siRNA molecule directed against hTERT that is able to inhibit transiently telomerase activity very efficiently in the immortal colorectal adenocarcinoma cell line HT29. Now, for long term inhibition studies HT29 cells were transfected with this siRNA cloned as short hairpin (sh)RNA into a mammalian expression vector. A nonsense shRNA construct was used as negative control. Thus, we were able to establish a cell clone which stable expresses shRNA and subsequently produce siRNA molecules directed against hTERT mRNA by the intrinsic Dicer enzyme. Telomerase inhibition and hTERT mRNA knockdown in these cells were confirmed by TRAP-assay and RT-PCR analysis, respectively. In contrast with the control subclone the telomere lengths became markedly shorter after 20 cell passages in the telomerase negative subclone as measured by Southern Blot analysis. As lack of telomerase activity and telomere erosion are sometimes associated with genetic instability we performed microsatellite instability (MSI) analysis at several terms of cell passages. But none of five microsatellite markers displayed MSI. Our results show that it is possible to knockdown telomerase activity by the ectopic expression of shRNA directed against hTERT in HT29 cells. Thereby telomere erosion but not MSI occurred within the successive cellular divisions. The established telomerase silenced cell line could be used for further enlightenment of the role of telomerase in tumor progression by comparative protein and RNA expression profiling analysis.

P246

Characterization of Scapinin in mouse and human

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Scapinin has been found to bind to cytoplasmic actin and furthermore being a putative regulatory subunit of protein phosphatase-1 (PP1). It is attached to the nuclear matrix-intermediate filament (NM-IF) and is being down regulated by differentiation of tumor cells. In this study we analyzed several anonymous ESTs with respect to their localization in the vicinity of the area known to be syntenic to imprinting region 1 on mouse chromosome 2 (MMU2) as part of a comparative physical and transcriptional mapping project on the human chromosome 20q13 segment (HSA20q13). As a result two of these ESTs (A005Z17 and H17739) emerged to be part of the human scapinin gene. We have analyzed the genomic structure and tissue specific expression of human scapinin as well as of the orthologous mouse gene. Both the genes showed a highly conserved complex 16 exon structure with four different leader exons, alternative splicing of human exon 5 and variable polyadenylation which was limited to mouse transcripts only. In both species expression seems to occur predominantly in the brain. By Northern blot analysis two

major signals of 3.0 kb and 2.7 kb were found in human. Three murine transcripts were detected sized 3.3 kb, 2.9 kb and 2.5 kb. Detailed expression analysis in the mouse revealed a tissue specific complex expression pattern in the brain as well as a developmental specific pattern during embryogenesis displaying weak signals from day 10 pc and strong signals from day 15 pc onwards to adult mice. This complex spatial and temporal regulation of scapinin indicates an essential biological function in the developing brain.

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Mutant TRPS1 proteins exert a dominant negative effect on the activity of the wild type protein.

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The transcription factor TRPS1 is a potent repressor of GATA-mediated gene transcription. This repression is dependent on the presence of the GATA type zinc finger and the IKAROS-like double zinc finger domain. Mutations in the TRPS1 gene lead to the tricho-rhino-phalangeal syndrome types I and III (TRPSI/III), which present with a broad spectrum of facial abnormalities, brachydactyly and short stature. Mutations in the GATA zinc finger cause the most severe TRPS III. We analysed the repressional activities of four different mutant TRPS1 proteins mimicing the mutations Q1038, C1217R and C1217Y, which we identified in patients with TRPS I, as well as T901P, which was found in a patient with TRPS III.

Wild-type (wt) and mutant TRPS1 constructs were expressed in COS-7 cells and activity was assessed by luciferase reporter assays. The truncated TRPS1 (Q1038X), which misses the entire IKAROS-like domain, has no significant repressional activity and does not affect repressional activity of the wt protein upon co-expression. Mutants affecting the first zinc finger of the IKAROS-like domain (C1217R, C1217Y) have significant residual repressional activity (72-85 %), and even TRPS1-T901P, which affects the GATA zinc finger, is capable of repressing reporter gene expression (55 %). Interestingly, co-expression of the wt –TRPS1 with either C1217R or C1217Y leads to lower repressional activity, and co-expression of wt-TRPS1 and T901P even results in a strong reporter gene activation.

Our results clearly prove that missense mutations in the GATA- and IKAROS-like zinc fingers exert a dominant negative effect on the activity of the wt protein in the heterozygous state.

P248

Comprehensive mutation analysis in the NF1 gene achieves mutation detection rates higher than 90% and confirms a high percentage of splicing mutations

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Neurofibromatosis type 1 (NF1 [MIM 162200]) is one of the most common autosomal dominant disorders, affecting approximately 1 in 3500 individuals. A hallmark of this pleiotropic and progressive disorder is the extreme heterogeneity in clinical expression. Clinical diagnosis is usually readily achieved in most adult and adolescent patients due to the presence of at least two of classical signs of NF1. However, the absence of many of the disease-defining features in young children renders definite diagnosis frequently impossible in this age-group. Thus, there is a need for a reliable and sensitive genetic testing to help resolve diagnostic dilemmas in patients not fulfilling the NIH diagnostic criteria, especially young children but also atypical patients, to determine the affection status of family members of an affected person and to perform prenatal diagnosis, if desired. However, even 14 years after cloning the NF1 gene molecular-genetic diagnostics is still considered a major challenge in this large and complex gene. Nevertheless, approaches have been developed during the recent years that reach mutation detection rates of up to 95% (Messiaen et al. 2000). It appears to be essential for the success of these approaches that they combine an RNA-based assay with additional supplementing methods to identify missense mutations as well as whole gene and multi-exon deletions. Therefore, we established a comprehensive NF1 mutation detection approach in Vienna that includes improved protein truncation testing, direct cDNA sequencing and FISH analysis. We have applied this mutation detection scheme in more than 100 cases so far and our results confirm mutation detection rates of over 90 % with this approach. The analysis of the mutational spectrum found in our laboratory further confirms that a significant fraction of the NF 1 mutations cause aberrant splicing and many of them are due to alterations outside the canonically conserved splicing sequences.

P249

Stable siRNA mediated conditional knock-down in myoblast cells

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Short interference RNAs (siRNAs) provide a powerful tool to induce loss-of-function phenotypes by post-transcriptional gene-silencing. siRNA technology saves substantial time and money compared to gene targeting approaches by homologous recombination and has been successfully applied for conditional knock-down in cell-based assays and the generation of mouse models. Driven by our motivation to analyse downstream targets of cardiac transcription factors that had been differentially expressed in our array analysis of cardiac samples from patients with tetralogy of Fallot (Circulation 2003; 107:2467-74), we established a vector based siRNA technology for the C2C12 and the HL-1 myocyte cell lines. We will present the technology set-up with its pitfalls for the generation and analysis of stable siRNA mediated knock-down cell lines. Firstly, we selected gene specific sequences suitable for siRNA through a bioinformatic analysis using different software tools from Invitrogen (Block-it RNAi designer), Promega (siRNA target designer), GenScript (siRNA target finder) and the Sonnhammer bioinformatics group (siSearch). Secondary, we analysed the siRNA efficiency by reporter gene assays in HEK293 cells. Reporter constructs were generated expressing either green fluorescent protein (GFP) or Renilla luciferase and the target gene in the 3'UTR. While the GFP reporter system was hard to quantify reliably, the Renilla luciferase approach proved to be a highly reproducible, easy quantifiable and high-throughput applicable tool. Finally, siRNA constructs showing a down-regulation of more than 40% in the luciferase reporter gene assay were integrated in C2C12 and HL-1 cell lines. We established stable transfected cell lines since transfection efficiency of the selected cell lines is around 30 %. Real-time PCR was applied to uncover respective down-stream targets in addition to the monitoring of phenotypical changes induced by the depletion of gene activity.

P250

A bipartite nucleus localisation signal is responsible for the nuclear transport of the bHLH transcription factor TWIST

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TWIST, a member of the basic helix-loop-helix transcription factor family, acts as a master regulator in embryonic morphogenesis and in oncogenesis. Mutations described in TWIST are causing Saethre-Chotzen syndrome (MIM 101400), an autosomal-dominant disorder. To characterise TWIST in more detail, we performed an evolutionary alignment of vertebrate TWIST proteins to determine additional conserved domains.

In result we could identify alongside the highly conserved bHLH domain, two potential nuclear localisation signals (NLS1, 37RKRR40 and NLS2, 73KRGKK77), a highly conserved 5 aa region, called NSEEE-domain, and a WR-domain in the C-terminus of the protein.

To answer the question whether the TWIST protein undergoes an active transport to the nucleus or cross over into the nucleus by diffusion we altered the Lysine residues in both motifs by "site-directed mutagenesis". The amino acid Lysine in NLS motif is essential for the interaction with the importin protein, which is responsible for the active transport through the nuclear pore. Human U2OS cells were used for transient transfection with c-myc-tagged TWIST constructs and the localisation were analysed by immunofluorescence microscopy. The alteration of the 38 Lysine in NLS1 resulted in the mislocalisation of the protein to the cytoplasm of the cells.

To understand the role of the second nucleus localisation signal, we modified the aa pos. 73, 76 and 77, respectively. The subcellular distribution of mutant K76R TWIST*NLS2 was similar to that of the wt protein. In contrast the K73R and K77R alterations inhibit the correct localisation of the protein in the cells examined.

As a control we proved the combination of mutated NLS1 (38Lys) and mutated NLS2 (73Lys, 76Lys, 77Lys), in all cases the signals for TWIST-constructs were detected in the cytoplasm. In conclusion, our results indicate that the aa Lys, present in the NLS1 at pos.38, and aa Lys present in the NLS2 at pos.73 and 77 are of utmost significance.

P251

The Glaucoma risk associated Gln368X Myocilin variation impairs the mitochandrial architecture in insect cells

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MYOC variations are associated with some forms of primary open angle glaucoma (POAG). An insect cell expression system comprising insect cell specific promoters, multiple cloning site adaptation, a V5 epitope and in frame GFP fusion has been constructed to trace heterologous expressed Myocilin (MYOC). Myocilin wildtype and the Gln368X variation have been introduced in this expression system. MYOC carries a leucin-zipper motif, a myosin-like domain and an olfactomedin-like domain; a secreted and a cytosolic form were described. The Gln368X protein truncates the protein by the olfactomedin domain. Heterologous overexpression of MYOC-GFP wildtype revealed a cytosolic localisation; no secretion was observed. However, insoluble MYOC aggregates are formed and collected in vacuoles resulting in cell death. Electron microscopy revealed ribosomes directly associated with the membrane of these vacuoles. This indicates that in a late growth stage MYOC protein synthesis is not any more cytosolic but directed to the steadily growing vacuoles. In contrast to the wildtype the Gln368X mutation has obviously some impact on the mitochondrial function. The number of mitochondria as well as their size was increased compared to the MYOC wildtype expression. This may indicate that the intracellular needs for energy are increased, possibly for the protein degrading pathway. The expression of the Gln368X variation without GFP resulted furthermore in high molecular weight aggregates in a Westernblot analysis. These aggregates were not detected for the wildtype. Our observations indicate that not only the overexpression of wildtype and mutant MYOC has pathogenic effects, but furthermore the nature of the mutation is important for the altered intracellular function and biochemical properties. The carboxyterminal fusion of GFP may have unexpected, but sometimes positive effects depending on structure and nature of the investigated protein.

P252

Transcriptional control of GLI3 gene expression

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Limb defects present an excellent model for the study of signaling pathways in humans. Molecular clues involved in limb patterning are similarly used to direct the development of other segments or organs of the body. The products of the GLI gene family, translate signals of the sonic hedgehog protein (SHH) into specific patterns of gene expression. Their co-ordinated function appears to determine a GLI-code, which, in the limb, directs pattern formation in anterior-posterior direction. Factors controlling the localized and timely expression of GLI genes and their targets are unknown.
We report the identification and functional analysis of cis-regulatory elements controlling expression of GLI3.
The genomic sequence upstream of the experimentally determined start of exon 1 of human GLI3 is predicted to contain a promoter sequence. By deletion analysis we identified a minimal promoter region with a high capacity for transcriptional activation of a luciferase reporter gene in cell culture in a 300 bp element located 70 bp upstream of the transcription initiation site. To assay the involvement of trans-active factors, predicted binding sites within this region will be modified by mutagenesis.
Comparison of the human, mouse and fugu genomic GLI3 sequences showed regions of very high conservancy residing in intronic regions. Three such segments were tested for their potential to regulate luciferase expression in cell culture. Two segments differing in these properties were further analyzed for their ability to control time and localization of beta-galactosidase reporter gene expression in transgenic mouse embryos. Localization and time course of the reporter gene expression in the mouse are compared with the established GLI3 expression patterns.
The detection of sequence elements controlling in cis the expression of GLI3 contributes to the understanding of the pattern formation and addresses the question of highly conserved non-coding DNA sequences in vertebrate genomes.

P253

Establishment and characterization of a primary testicular cell culture from a patient with Frasier Syndrome

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Frasier syndrome is characterized by a slow progressive nephropathy, XY gonadal dysgenesis and an increased risk to develop gonadoblastoma. The underlying causes are heterozygous germline mutations in the WT1 gene, disrupting splicing at the second alternative splice donor site and resulting in a reduced amount of the +KTS protein isoform. The transcription factor WT1 plays a crucial role in nephrogenesis and gonadogenesis in males. Gonadal expression is restricted to Sertoli cells in testis and to granulosa cells in the ovary.
The aim of our work was to establish a primary Sertoli cell culture from a patient with Frasier syndrome, enabling further studies regarding the molecular pathomechanisms in this disease. The patient presented with hypergonadotropic hypogonadism caused by testicular atrophy, hypospadia, and end-stage renal disease at the age of 15 years and underwent kidney transplantation two years later. WT1 mutation analysis revealed a heterozygous germline mutation at the second alternative splice site (c.1228+5G>A). Based on these findings, he was diagnosed as having Frasier syndrome. Prophylactic gonadectomy was performed and histological analysis identified a severe tubular atrophy with spermatogenesis arrest and a focal intratubular germ cell neoplasia with a diffuse increase of Sertoli cells. From this material we have established a primary cell line in culture with a normal 46 XY karyotype and approximately 100 cell doublings up to now. Cells have prominent nucleoli and show a criss-cross growth pattern, however they do not show anchorage independent growth in soft agar. We have further characterized these cells by immunohistochemistry and RT-PCR and could show that they express typical Sertoli cell markers like WT1 (reduced amount of +KTS), Sox 9, Vimentin and MIC2 and are negative for c-kit, a germ cell marker.
Using these cells we have an in vitro system to analyze molecular pathomechanisms regarding Sertoli cell dysfunction in Frasier syndrome.

P254

Cohen syndrome: Mutational and transcriptional analysis of COH1

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Cohen syndrome is a rare autosomal recessive disorder, clinically highly variable and mainly characterized by developmental retardation, craniofacial dysmorphism, retinal dystrophy, and neutropenia. In 2003, a novel gene, *COH1*, on chromosome 8q22 was described, and we and others have identified mutations in patients with Cohen syndrome therein. Here we describe new molecular findings in twelve patients with Cohen syndrome, descending from 7 families originating from France, Germany, Poland, Turkey, and the U.K., with mutations in *COH1*. We have found nine different novel mutations, including four nonsense mutations, three frame shift mutations, and two potential splice site mutations. Our data contribute to further confining the phenotypic spectrum of Cohen syndrome; a consistent genotype/phenotype correlation, however, has not been established so far. All data released until now indicate that Cohen syndrome is mainly caused by mutations in *COH1* that result in a defective *COH1* protein through frame shift or nonsense sequence alterations. The lack

of a second pathogenic mutation in some patients points to the existence of further alternative exons and/or other transcripts of *COH1*. Therefore, we have embarked on a detailed analysis of *COH1* transcript variants by RT-PCR and Northern hybridization. Furthermore, we are studying the expression of *COH1* in humans and mice with respect to the different splice forms. Localization and specificity of the protein, which is similar to VPS13p from yeast and therefore supposed to be involved in intracellular protein sorting, are being characterized in order to shed light on the molecular pathology of Cohen syndrome.

P255

The pericentric inversion of chimpanzee chromosome 11 homologous to human chromosome 9 is associated with repositioning of the centromere

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Human and chimpanzee karyotypes differ by nine pericentric inversions, which might have contributed to chromosomal speciation during the early human evolution. In this study we characterized the breakpoints of the pericentric inversion which distinguishes chimpanzee chromosome 11 (PTR 11) and the homologous chromosome 9 in humans (HSA 9). The break of homology between PTR 11p and HSA 9p12 maps to low-copy repeats or segmental duplications, whereas the breakpoint region orthologous to 9q22.1 is located in single copy sequences. In the chimpanzee, alpha-satellites are located close to the q-arm breakpoint. This indicates the occurrence of the centromere at a region orthologous to HSA 9q22.1. Thus the chimpanzee specific inversion of PTR 11 was associated with neocentromere formation close to the inversion breakpoint and the inactivation of the ancient centromere. Over evolutionary time, this neocentromere has acquired alpha satellites, thereby evolving into a conventional centromere.

P256

Functional analysis of the transcription factor-like nuclear regulator (TFNR) protein by Y2H and generation of a conditional knock-out mouse

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The transcription factor-like nuclear regulator (TFNR) is a gene that maps on 5q13, distal to the duplicated region that includes SMN1, the spinal muscular atrophy gene. In rare cases SMA patients can present additional atypical features such as axonal neuropathies or cerebral atrophy. These phenotypes correlate in patients with large deletions including SMN1, SERF1, NAIP, GTF2H2t and the TFNR gene. We hypothesized that haploinsufficiency of TFNR may cause brain atrophy and neuronal disfunction. The 9.5 kb TFNR transcript is expressed in all tissues but mainly in cerebellum. TFNR encodes a protein of 2254 amino acids and contains nine repeats of a 55 amino acid motif of yet unknown function. The coding region is organized in 32 exons. The TFNR protein is present exclusively in the nucleus, where it is concentrated in several nuclear structures. It has been shown that the first third of the protein is part of the transcription factor TFIIIB.
The function of TFNR was investigated by yeast-2-hybrid. The TFNR interacts with ZNF297B, a zink finger protein. It was shown that ZNF297B is mainly expressed in the nucleus in many tissues. The N-terminus of the protein contains a BTB/POZ domain that interacts with other proteins. Three zink finger domains are located at the C-terminus. These results implicate that ZNF297B could be as well a transcription factor. Antisense RNA experiments in cell cultures showed that the TFNR gene is of major importance for cell survival. To further investigate the role of the TFNR protein in mammals we are generating a conditional knock-out mouse by using the cre/lox P system. The human sequence of the TFNR is 98% homologous to the mouse sequence. Using the cre/lox P system, exon 2 will be floxed in the F1 generation and after crossing with a cre transgenic mouse in the F2 generation, TFNR will be deleted. These animals will proof whether TFNR has indeed an essential role in brain development.

P257

Functional analysis of murine Foxq1

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We have isolated a mouse genomic and cDNA clone that belongs to the family of the Fox transcription factors (previously called *HNF-3/forkhead* transcription factors). The 2.7-kb transcript of the murine *Foxq1* gene is expressed prominently in stomach and kidney. Expression of *Foxq1* was detected in stomach and kidney during pre- and postnatal development. Immunohistologic analysis revealed that the expression of *Foxq1* is located to parietal cells in the gastric unit. Parietal cells are responsible for the output of hydrochloric acid in the gastric lumen. To determine the function of *Foxq1*, we have generated knock out mice by deleting the whole coding region of *Foxq1*. *Foxq1* deficient mice are viable and fertile. No apparent histological abnormalities can be observed in stomach and kidney. Using RT-PCR assays and Northern-Blot analysis we found altered expression of genes which are involved in regulation of gastric acid secretion. Injection of histamine, an agonist of gastric acid secretion, do not stimulate gastric acid secretion in *Foxq1* deficient mice. In addition *Foxq1* -/- mice exhibit a silky shiny skin. This silky skin results from a lack of medullary structure in the hair shaft. Radiation-induced mice mutants, called satin mice, exhibit the same phenotype according to the hairs. Satin mice harbour an intragenic deletion in the *Foxq1* gene.

P258

Leukemia Inhibitory Factor Receptor (LIFR) Mutations in Patients with Stüve-Wiedemann Syndrome

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Stüve-Wiedemann syndrome (SWS) is a severe autosomal recessive condition characterized by neonatal feeding and swallowing difficulties, unexplained hyperthermic episodes, bowing of the long bones, and distal arthrogryposis. The clinical, radiological, and histological similarities between SWS and 'neonatal' Schwartz-Jampel syndrome 2 (SJS2) led to the suggestion that SWS and SJS2 are a single entity. Through a study of a series of 19 patients with either SWS or SJS2 Dagoneau et al. (2004) have shown (i) that SWS and SJS2 are allelic disorders, and (ii) that both conditions are caused by null mutations in the leukemia inhibitory factor receptor (LIFR) gene. The LIFR gene maps to chromosome 5p13.1, consists of 19 coding exons and codes for a protein which is made up of 1097 amino acids.
With app. 50 cases worldwide, SWS/SJS2 is a very rare disorder. Most of the mutations reported so far occurred in consanguineous families and were homozygous. Thus, information concerning the spectrum of mutations within the LIFR gene is very limited and phenotype-genotype correlations are not yet evident.
In order to provide additional data, we have sequenced the LIFR coding exons from six patients with congenital bowing of the long bones including three that were diagnosed with SWS. One patient was homozygous for the 1798C>T (R597X) mutation which has been found before, mainly in families with consanguineous parents. Another patient was compound heterozygote and both mutations (the paternal H116Y mutation as well as the maternal S133T mutation) have not yet been described. Furthermore, these mutations do not a priori lead to premature termination of translation, which is in contrast to most of the mutations reported so far. These are either nonsense mutations or frame shift mutations. Therefore, the mutations reported here may provide new insight into the function of LIFR. Investigations concerning the effects of these mutations on the corresponding mRNA are on their way.

P259

A novel ncRNA gene adjacent to human *LMX1B*

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Loss-of-function mutations in the *LMX1B* gene, encoding a member of the LIM homeodomain protein family, cause Nail-Patella syndrome (NPS). NPS is an autosomal-dominant disorder characterised by dysplastic nails, absent or hypoplastic patellae, dysplasia of the elbows, and

nephropathy in approximately 50% of the cases.
In an attempt to identify modifying factors of *LMX1B* action we started to characterise the promoter region of human and murine *LMX1B*. We mapped the transcription start sites of *LMX1B* and performed extensive computer based analysis and interspecies sequence comparison. In addition to the known *LMX1B* exons the data revealed several conserved regions upstream of exon 1 and within intron 2 of *LMX1B*. From EST-data and RT-PCRs we concluded that some of these regions correspond to a novel gene adjacent to *LMX1B* in reverse orientation. A corresponding gene has recently been described in chicken (Holmes et al., 2003) and was named *Alc* (adjacent to *Lmx1* in chicken). The human gene (provisionally called *ALH*) showed a broad expression pattern and complex alternative splicing. The gene spans a region of at least 32 kb and overlaps with exon 1 of *LMX1B* in antisense orientation, indicating a putative function in the regulation of *LMX1B* gene expression. Remarkably, *ALH* shows no significant sequence similarity to *Alc* or any other sequence in the databases. In addition, no longer ORFs can be found, which suggests a classification of *ALH* as a non coding (nc) RNA gene. We performed transient transfection assays to identify possible regulatory regions. We could show that a short 476 bp region upstream of the *ALH* transcription start site has basal promoter activity in a variety of different cell lines. We are currently isolating the corresponding murine gene and gene promotor in order to perform detailed expression studies.

P260

Nijmegen breakage Syndrome: Apoptosis in patient cell lines and its relevance for the clinical phenotype.

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The gene mutated in Nijmegen Breakage Syndrome, NBS1, is implicated in two major aspects of cell viability: DNA repair and cell cycle checkpoint assembly. However, the role of NBS1 in the p53-mediated onset of apoptosis is still controversial. In consequence, the relative contribution of DNA repair, cell cycle checkpoints and/or induction of apoptosis for the clinical phenotype in NBS remains unclear.
We have instigated a pilot study using seven cell lines selected randomly from our large collection of patient lymphoblasotid cell lines and using two methods for measuring apoptosis by flow-cytometry: the propidium-iodide, sub-G1 Nicoletti method and the propidium-iodide/Annexin-V assay.
Amongst the seven patient cell lines, two groups became obvious in terms of apoptosis: a group responding to DNA damage comparably to normal cells and a group that was apoptosis-resistant.
Whilst apoptosis-competence did not correlate with cellular parameters such as cell growth, chromosomal breakage or sensitivity to DNA damaging agents, there was a remarkable correlation with a clinical parameter: the occurrence of malignancy. All four patients whose cells belong in the apoptosis-resistant group have developed lymphomas typical for NBS. In contrast, two of the three patients in the apoptosis-competent group are malignancy-free while the third has suffered from a meningioma, a generally benign tumour untypical for NBS. Although it is clearly too early to draw final conclusions, it is already clear that failure to enter appropriately into apoptosis is critically involved in the NBS phenotype. Since all seven patients examined here are homoallelic for the same founder mutation, it seems likely that clinical expression is modulated by other factors, particularly those that contribute to the regulation of apoptosis. Examination of further patient cell lines well allow us to test this novel hypothesis.

P261

Nijmegen Breakage Syndrome: NBS1 functions in immunoglobulin class switch recombination

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Immunoglobulin (Ig) class switch recombination (CSR) is a region–specific DNA recombination, induced in B lymphocytes upon activation. During CSR, the upstream switch-regions of two different heavy chain genes recombine, changing the class of antibody produced. The initiation of CSR requires switch-region-specific transcripts, the B-cell-specific protein, activation-induced-cytidine-deaminase (AID) and uracil-DNA-glycosylase (UNG). DNA double strand breaks, presumed to be produced at the switch regions, are first bound by the non homologous end joining repair factors, Ku70/80 and DNA-PKcs, and subsequently realigned and ligated. Nijmegen breakage syndrome (NBS) is a rare chromosomal-instability syndrome associated with defective DNA repair. Most NBS patients have normal IgM but decreased IgG and IgA serum levels. This deficiency could be due either to a defect in the survival of activated B lymphocytes or a defect in immunoglobulin class switch recombination. So far, it has been unclear, whether nibrin, the product of the NBS1 gene, is involved in switch recombination. Here we show, by conditional inactivation of the murine homologoue (Nbn) of the human NBS1 gene in activated B lymphocytes, that switch recombination is impaired in null-mutant B cells, demonstrating that NBS1 is indeed involved in CSR. Survival of B cells in the time frame examined is only marginally affected, however, the frequencies of cells switched to IgG3, upon LPS stimulation, or IgG1, in response to LPS plus IL-4, are consistently reduced by over 50%. This shows that nibrin is directly involved in the process of switch recombination, probably as part of the mammalian non-homologous end-joining DNA repair system.

P262

Nijmegen Breakage Syndrome: Variation in the expression level of truncated NBS1 protein in patient cell lines and peripheral blood lymphocytes

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Nijmegen breakage syndrome (NBS) is a rare autosomal recessive genetic disease belonging to the group of cancer-prone chromosomal-instability syndromes. The NBS1 gene is located on human chromosome 8q21 and encodes the nibrin protein product. Approximately 90% of NBS patients are homozygous for the founder mutation, 657del5, which is hypomorphic and leads to the expression of amino- and carboxy-terminal nibrin fragments of 26kDa and 70kDa, respectively. These partially functional proteins may be responsible for the viability of NBS patients whilst null-mutation of the murine homologue is embryonically lethal.
In this study the relative expression levels of the 70kDa protein fragment in EBV-transformed lymphoblastoid cell lines (LCLs) of 30 NBS homozygotes were measured by immunoprecipitation with anti-nibrin antibody. As an internal control, lysates were simultaneously precipitated with anti-Mre11 antiserum. We found the NBSp70 protein in all 657del5 homozygous LCLs tested, however, its amount varied 10-fold between the different LCLs. Cells have been categorized into three groups: 15 patients with intermediate expression levels, 11 patients with low expression and two with high levels of the 70kDa fragment. These differences in the levels of the truncated protein may contribute to the extensive variation in the clinical phenotypes of homozygous NBS patients. Correlations of p70 expression level with clinical parameters are currently being evaluated.
The expression of truncated nibrin was also investigated in PHA-stimulated lymphocytes of 10 heterozygous 657del5 individuals. Surprisingly, we observed here a slightly larger 75kDa protein, although lymphoblastoid cell lines established from the same individuals expressed the 70kDa fragment. We propose an alternative splicing mechanism, which could theoretically explain the existence of this 75kDa protein.

P263

Infantile Hypophophatasia due to a new compound heterozygous mutation in the *ALPL* gene - functional evidence for a hydrophobic side-chain

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Infantile hypophosphatasia (IH) is an inherited disorder characterized by defective bone mineralization and a deficiency of alkaline phosphatase activity. The disease is due to mutations affecting the *ALPL* gene comprising 12 exons, localized on chromosome 1p36-1p34, and at least 129 mutations have been found in IH. We report on a 4-week old female infant with craniotabes and failure to thrive. Alkaline phosphatase was reduced to 41 U/l and serum calcium reached concentrations of up to 4.0 mmol/L. X-rays of wrist, skull, and thoracic wall showed severe defects of ossification. Urinary calcium excretion was increased to 3.5 mol/mol creatinine and renal ultrasonography confirmed nephrocalcinosis. Vitamin D was withdrawn and the patient was started on calcitonin, hydrochlorothiazide and feeding by nasogastral tube. Nonetheless, the girl's overall clinical condition further deteriorated and the patient died at the age of 5 months from respiratory failure. Sequence analysis of all regions of functional significance of the *ALPL* gene revealed a compound heterozygous mutation in the infant [T653C (I201T), C1171T (R374C)]. Transfection studies of the so far unknown I201T variant in COS7 cells yielded a mutant ALP protein with only a residual enzyme activity (3.7%) compared with wild-type cDNA, whereas the R374C variant was previously shown to reduce normal activity to 10.3%.
3D-modeling of the mutated dimeric protein showed that I201T resides in a region that does not belong to a known functional site (calcium binding site, crown domain and homodimer interface). We note that I201, which has been conserved during evolution, is buried in a hydrophobic pocket and therefore, the I>T-change should affect its so far unknown functional properties. Residue R374C is located in the interface between monomers and it has been previously suggested that this mutation affects dimerization. These findings explain the patient's clinical picture and severe course.

P264

Abundance of splice site mutations in FANCD2

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Objectives: The conserved FANCD2 gene serves as a central effector in the evolutionarily novel Fanconi anemia/ BRCA caretaker pathway. The gene is embedded in pseudogene regions and rich in repetitive elements. In order to establish the mutation spectrum for FANC2, we sequenced DNA from 26 patients belonging to 22 families.
Results: Exon 22 skipping is frequent in patients of German and Turkish origin caused by two different base substitutions in the pyrimidin-rich consensus signal of the acceptor. Both mutations are likely to weaken the 3'splice site of intron 21. In order to substantiate these effects, we applied an acceptor splice site calculation program based on the maximum entropy model. Both types of exon 22 splice acceptor mutations reduce the score relative to wildtype, indicating weakening of the acceptor. In addition, one of the two intron 21 mutations leads to the loss of two exonic splicing enhancer(ESE)-motifs and to the loss of two putative ESEs, whereas one new putative ESE is created. Three other patients showed skipping of exon 5 caused by insertion of an Alu repeat into the preceding intron sequence. Except for 36 nucleotides, the inserted Alu element is identical to Yb9. Alu integration occurs in reverse orientation and is flanked on both sides by 13 duplicated nucleotides of the target sequence. A single of our patients exhibits skipping of exon 10 resulting from a base substitution in the splice donor site. Another homozygous mutation results in exonization of an intron 9 fragment. According to the splicefinder programme, a single base substitution in the newly created donor sequence results in a change of donor strength from low complementarity to high complementarity, and thus in the recognition of a new exon.
Conclusions: In our series of patients mutations in FANCD2 predominantly resulted in aberrant splicing causing exon skipping, exonisation of intronic sequence, activation of cryptic and creation of new 3' splice sites.

P265

Mutation analysis in hereditary haemorrhagic telangiectasia in Germany reveals novel mutations including large genomic rearrangements

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Hereditary haemorrhagic telangiectasia (HHT, or Rendu-Osler-Weber syndrome) is an autosomal dominant disease characterized by recurrent epistaxis, mucocutaneous telangiectasias and visceral arteriovenous malformations. Mutations in Endoglin (ENG) and activin A receptor type II-like kinase 1 (ACVRL1 or ALK1) have been found in patients with HHT. We have screened of a total of 51 German index cases with the suspected diagnosis of HHT. We identified a mutation in 32 cases (62.7%) by direct sequencing. Among these mutations, 11 of 13 ENG mutations and 12 of 18 ACVRL1 mutations were not previously reported in the literature. An analysis of the genotype-phenotype correlation confirmed that pulmonary arteriovenous malformations are more common in patients with ENG mutations than in patients with ACVRL1 mutations. In the 20 patients without detectable mutation we performed a deletion analysis by real-time PCR. We were able to identify two families with a large deletion of the ACVRL1 gene. To our knowledge, these are the first reported cases of large genomic rearrangements in ACVRL1 in patients with HHT. The consequences for mutation screening strategies in HHT are discussed.

P266

Molecular and biochemical analysis of PTPN11/SHP2 in Noonan Syndrome

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Mutations in PTPN11, coding for the non-receptor tyrosine phosphatase SHP-2, are causative for Noonan Syndrome (NSI) and for Leopard Syndrome (LS).
We screened 114 patients with clinically suspected NSI for mutations in PTPN11 and detected mutations in 35 patients (30.7%).
As SHP2 is known to dephosphorylize phosphorylated STAT1 protein, a major signal transduction factor, we sought to demonstrate the biological effect of deregulated SHP2 phosphatase activity on intracellular signaling pathways in Noonan Syndrome. SOCS1 is an acute phase protein which acts as a negative feedback regulator of IL6 and IFN induced stimuli and is mainly regulated via the JAK/STAT pathway, namely STAT1. In a promoter/receptor assay we showed that cotransfection of SOCS1 promotor/luciferase plasmid together with mutant SHP2 plasmid results in a downregulation of SOCS1 promoter activity. This downregulation of SOCS1 activity by mutant SHP2 can be reduced with the cotransfection of SHP2 small interfering double stranded RNA (siRNA). However, an allele specific effect could not be achieved.
Because the transcriptional regulation of PTPN11 is still not known, we studied the homology between the mouse and human promoter region. By a promoter/reporter-constructs assay we showed that the promoter probably extends only 300 bp 5'of the ATG. Electrophoretic mobility shift assay (EMSA) showed that SP1 is one of the principal transcriptional factors that regulate PTPN11.

P267

Spectrum of new mutations found in Waardenburg syndrome type 1 and type 2 patients

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Waardenburg syndrome (WS) is the most frequent syndromal form of human deafness usually inherited in an autosomal dominant manner. Type 1 is characterized by sensorineural deafness, heterochromia iridis, partial hypopigmentation of skin and scalp hair, dystopia canthorum as well as additional facial features. Type 2 presents with the same clinical picture as WS1 but without dystopia canthorum. Type 3 is similar to WS1 but includes upper limb abnormalities. Type 4 has WS symptoms in combination with Hirschsprung disease.
The genetic basis of WS is complex. WS1 and WS3 are associated with loss of function mutations in the PAX3 gene. WS2 is heterogenous, the majority of cases being due to mutations in the MITF gene. Additional candidate genes include SLUG, EDN3, EDNRB, and SOX10 which are primarily associated with the rare WS4 variant.
The molecular analysis of PAX3 and MITF by direct sequencing in 19 patients with suspected Waardenburg syndrome revealed in 15 cases mutations in the PAX3-gene and in 4 cases mutations in the MITF-gene. Of these alterations 9 are novel mutations in the PAX3-gene and 4 are novel mutations in the MITF-gene. The spectrum

of mutations includes nonsense mutations, deletions, insertions, missense mutations, and splice site mutations.
Molecular analysis of WS depends on accurate clinical data to target the molecular study to the genes most likely involved in a specific WS type. Eventually, it could be difficult to distinguish between WS1 and WS2. In these cases the analysis of PAX3 and MITF could help to classify the syndrome. In case of negative results, the diagnostic strategy could then be extended by inclusion of additional candidate genes. The identification of the genetic basis of WS in a given family could provide valuable infotmation, on the other hand, the genetic counsellor should take into account possible restrictions due to the high inter- but also intra-familial variability of the WS phenotype.

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Recombinant Expression of the C1-Inhibitor Protein in Human Cells

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Objectives: Hereditary angioedema (HAE) (OMIM: 106100) is an autosomal dominant disease due to mutations of the C1 inhibitor gene (C1INH). Clinically, HAE presents as edemas of the extremities, face, trunk, airways or abdominal viscera, often triggered by psychological and/or physical stress. Laryngeal edema may cause suffocation and if not treated properly can be fatal.
Material and Methods: We have expressed the C1INH protein in human cells. The activity of the recombinant protein was measured by a chromogenic assay. C1INH is a pseudosubstrate of C1-esterase to which it binds irreversibly. Cell culture supernatants were incubated with an excess of C1-esterase and residual C1-esterase activity was measured photometrically. C1INH antigen was detected by Western blotting and hybridisation with an anti-C1-INH-antibody.
Wildtype C1INH showed an activity and antigen comparable to diluted human plasma. Mock transfected cells showed neither activity nor antigen thus ruling out endogenous expression of C1-INH in HEK293 cells.
Results: By site-directed mutagenesis we studied the effects of mutations that had been identified in HAE patients. Replacement of arginine444 by either histidine or cystein is a mutation frequently found in HAE Type II. These patients have no C1INH activity but are positive for C1INH antigen. Measurements of the supernatant of transfected cells showed no activity but the antigen could be detected by Western blotting. We have further investigated after site-directed mutagenesis the role of other amino acid substitutions that were observed in patients. All substitutions studied so far resulted in a significant decrease of the activity of the recombinant protein.
Conclusions: The recombinant expression of mutated C1INH protein is an useful tool to characterize the role of individual amino acid residues for C1INH activity.

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Site-directed mutagenesis of VKORC1, the target protein of coumarin-type anticoagulants

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Coumarins are antagonists of vitamin K, an essential cofactor for the posttranslational γ-glutamyl carboxylation mainly of vitamin K dependent coagulation factors. As vitamin K hydroquinone is converted to vitamin K epoxide in every carboxylation step, it has to be recycled by the vitamin K epoxide reductase complex (VKOR). Recently, we have identified the first subunit of this protein complex, VKORC1, which is the target protein for coumarin drugs such as warfarin. Mutations in VKORC1 result in two different phenotypes: warfarin resistance (WR) and multiple coagulation factor deficiency type 2 (VKCFD2).
We have investigated the role of individual amino acids of VKORC1 after site-directed mutagenesis and recombinant expression in HEK 293 cells. All seven cysteine residues which may be involved in the formation of disulfide bonds or the provision of reducing equivalents were substituted by serine or alanine residues. A highly conserved Ser/Thr residue at position 57 was replaced by alanine. The recombinant proteins showed a varying decrease of VKOR activity. Mutations of the supposed thioredoxin motif C132-X-X-C135 destroyed VKOR acitivity completely. Mutations at Tyr 139 which were detected in warfarin resistant rats retained good VKOR activity and conferred insensitivity towards warfarin. Tyr 139 is embedded in a hydrophobic sequence context Thr-Tyr-Ala and may be part of the warfarin binding site. Mutation R98W (found in patients with the VKCFD2 phenotype) abolished VKOR activity completely. However, when Arg98 was substituted by structurally related amino acids recombinant proteins showed 20 to 120 % of wildtype activity.
This study supports the hypothesis of different binding sites for vitamin K epoxide and its antagonists and underlines the crucial role of the thioredoxin motif CXXC in VKORC1. Understanding the structure and function of the VKORC1 protein is the basis for the development of new anticoagulants with an improved efficacy/side effect profile.

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Identification of a new mutation in the CYLD gene in Brooke-Spiegler syndrome

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A 75-year-old woman consulted the dermatologist presenting with multiple skin tumors of the head and neck. She recognized that the tumors were growing over 15 years. The partially ulcerated, erythematous and teleangiectatic tumors with a hemispheric shape were identified histologically as cylindromas and trichoepithliomas. The occurrence of these special skin lesions is known as Brooke-Spiegler syndrome. To date there are reported about 40 families with this rare condition. The patient has three children and one daughter was identified as also clinically affected with cylindromatosis. There is no history of skin tumors in past generations or in the siblings of the patient. Mutations of the CYLD gene, a tumorsuppressor gene which localizes to the long arm of chromosome 16 (16q12-q13), have been identified as the underlying cause of Brooke-Spiegler syndrome. There is a mutation cluster in the C-terminal region of the gene (exon 16-20), but genetic alterations were also described in other regions (exon 9-13). The CYLD gene encodes for a deubiquitinating enzyme, which plays a major role in tumor protection by regulation of NFkB. In vitro studies could demonstrate compensative effects of acetyl-salicylate in cells with a loss of enzyme activity. Mutation analysis of the CYLD gene in genomic DNA of the patient was performed by using PCR, DHPLC and automated sequencing. We could identify a previously unknown genetic alteration in exon 14 of the CYLD gene, which was classified to be pathogenic in our patient. The mutation is predicted to result in a premature termination of enzyme biosynthesis due to a translational frameshift. The mutation was not observed in healthy controls and in the non-affected family members. Brooke-Spiegler syndrome is associated with a high risk of malignant transformation of single tumors. Therefore a special prevention program should be established for mutation carriers. The prophylactic application of acetyl-salicylate needs to be discussed.

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Mild Nijmegen Breakage Syndrome (NBS) Phenotype, Possibly Due to Alternative Splicing

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Hypomorphic mutations of the NBS1 gene are responsible for Nijmegen breakage syndrome (NBS), which is characterised by microcephaly, chromosomal instability, radiosensitivity, immunodeficiency and high cancer predisposition. Over 90% of NBS patients are homozygous for the truncated 657del5 mutation and 9 further mutations have been identified in other patients. Partially functional proteins produced by alternative initiation of translation and possibly diminishing the severity of the NBS phenotype have been described for the NBS1 mutations 657del5, 834del4 and 900del25. Here, we report on an NBS patient (52 years old) , homozygous for the 742insGG NBS1 mutation in exon 7 and presenting with a very mild phenotype. In an attempt to find a potential molecular explanation for the mild phenotype observed, we carried out a conventional RT-PCR analysis which revealed two transcripts in almost equal amounts in the patient and her parents – the expected 742insGG mutation carrying transcript and a second one with skipped exons 6 and 7. The latter transcript was not present either in the control sam-

ples nor in NBS patients with other mutations analysed. Further quantitative RT-PCR analysis was able to detect the aberrant transcript even in the controls and NBS patients with other mutations, but in amounts 100 times less than in the patient with the 742insGG mutation. The skipping of exons 6 and 7 does not lead to a frame-shift, suggesting that the corresponding transcript will encode a partially functional protein. The existence of the alternative transcript and its high expression in the 742insGG patient is unclear, but it might be responsible for the mild patient's phenotype. However, to asses further the nature of the alternatively spliced transcript found here, functional experiments are in progress.

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Species-specific variation of VKORC1-activity and resistance to Warfarin

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Vitamin K serves as a cofactor for the post-translational modification by ^{a}-carboxylation of several proteins with regulatory function, the group of Gla-proteins. Vitamin K-dependent proteins include the coagulation factors II, VII, IX, and X and proteins S, C and Z, illustrating the therapeutic importance of vitamin K-metabolism. Inhibitors of the coumarin-type like warfarin reduce coagulation activity by interfering with the vitamin K 2, 3-epoxide reductase enzyme complex (VKOR). They are the most used oral anticoagulants for antithrombotic prophylaxis. The same VKOR complex is also targeted by coumarin derivatives used in rodent pest control worldwide.

In humans, mutations in the VKORC1 gene lead to combined deficiency of vitamin-K-dependent clotting factors type 2 (VKCFD2; OMIM: 607473) or warfarin resistance. Mutations in VKORC1 were also reported for warfarin resistant strains of mice and rats.

Natural susceptibility to anticoagulants varies widely between species. Based on LD50 values house mice are about 20 times less susceptible to warfarin than brown rats. Some rodent species, e.g. Acomys cahirinus, (Egyptian spiny mouse), were found to be relatively tolerant to anticoagulant compounds. Compared to rats chicken are completely resistant against warfarin whereas pigs (Sus scrofa) are extremely sensitive.

The coumarin-sensitive activity of species-specific VKORC1 was measured in liver microsomal preparations and in HEK 293 cells after cloning and recombinant expression of VKORC1-cDNAs and was found to vary widely between species.

Conclusions: Since the VKORC1 protein is highly conserved among mammals, the few amino acid differences between the studied species already give hints to functionally important residues. Complete understanding of the Vitamin K-cycle could lead to the development of novel coumarin derivatives without the often lethal side-effects of warfarin-therapy in humans.

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Organization and expression of muride *TSPY*

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TSPY (**t**estis-**s**pecific **p**rotein, Y encoded), is conserved in placental mammals and its expression is restricted to the testis. Within the testis *TSPY* is expressed almost exclusively in spermatogonia. Topology and timing of *TSPY* expression in premeiotic germ cells, the strong *TSPY* expression in testicular seminoma and carcinoma-in-situ, and also the *TSPY* homology to members of the TTSN-family that play a role in cell cycle regulation suggesting a role of *TSPY* in regulation of germ cell proliferation. Since it was first discovered in humans, *TSPY* orthologous gene families have been subsequently characterized in many other mammalian species including the primate, artiodactyl, perissodactyl and rodent lineages. In contrast to the situation in cattle and primates, where *TSPY* is organized in a moderately repetitive cluster, including functional members and pseudogenes, a peculiar situation is observed in the rodent order. Whereas *TSPY* is functionally conserved and organized as one or two copies in the genera Apodemus and Rattus, *TSPY* lost the struggle of survival within the male specific region of the Y chromosome (MSY) in species of the Subgenus Mus and degenerates as a single copy. We speculate that the functional or non-functional status of *TSPY* in different murine species represents a snapshot of the decay of a repetitive gene family in the non recombining portion of the Y chromosome. In order to elucidate further the organisation and evolution of rodent *TSPY*, we isolated and analysed the *TSPY* gene in the Syrian hamster (*Mesocricetus auratus*) and the Mongolian gerbil (*Meriones unguiculatus*). Whereas *Mesocricetus auratus TSPY* resembles the human and bovine orthologs in almost all aspects of structure and expression the closely related gerbil gene, g*TSPY*, has clearly become non functional. Hamster *TSPY* is functional conserved, organized in multiple copies, and testis-specifically expressed. The closely related Mongolian gerbil possess a single-copy pseudogene that is unable to generate a functional transcript.

P10 Genetics of Mendelian Traits

P274

The genetics of hypogammaglobulinemia

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Defects in the humoral immune system are the most frequent primary immunodeficiencies in humans. Whereas several genetic causes of primary agammaglobulinemia due to defects in early B cells development been discovered during the last decade, causes for hypogammaglobulinemia with B cells present in peripheral blood, were obscure. Patients with primary hypogammaglobulinemia may present in childhood or in adult life. The phenotype is known as common variable immunodeficiency (CVID).

Candidate gene analysis led us to the identification of four molecular causes for primary hypogammaglobulinemia:

1. The loss of the CD19 molecule on the B cells surface leads to an impaired signaling via the BCR and thus seems to influence the activational status of B cells. The phenotype mimics the one of common variable immunodeficiency.
2. The homozygous loss of BAFF receptor, transmitting an important survival signal to transitional B cells to proceed into the naïve B cell stage, leads to an increase of transitional B cells. The consequence is hypogammaglobulinemia with retained IgA levels.
3. Mutations in TACI, another TNF-like ligand for BAFF also lead to a phenotype with hypogammaglobulinemia, but in contrast to BAFF receptor deficiency, patients with mutations in TACI suffer from lymphoproliferation and autoimmunity. Interestingly, also heterozygous mutations display a phenotype.
4. The homozygous loss of the inducible costimulator (ICOS) on T helper cells also leads to the CVID phenotype with prominent hypogammaglobulinemia and severely reduced B cell memory but only subtle anomalies in the T cell compartment. We will demonstrate that ICOS ligation is important in the germinal center reaction.

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Mild forms of Börjeson-Forssman-Lehmann Syndrome are caused by triplet deletions in the isoform I of PHF6

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Objectives: Börjeson-Forssman-Lehmann Syndrome (BFLS) is an X-linked mental retardation syndrome with trunk obesity and facial dysmorphisms. The disorder is caused by mutations in the PHD protein family gene PHF6. Sequence variants in the gene have been reported as single base nonsense mutations leading to premature termination of translation or to missense mutations, and thus to a rather severe phenotype in boys. We have described recently a triplet deletion in PHF6 isoform I in a family with four affected boys. Here we report about another unrelated family with very mild symptoms of BFLS and again a triplet deletion in PHF6 isoform I, however at a different position than in the first case.

Material and Methods: DNA was obtained of all family members from peripheral blood lymphocytes. All coding PHF6 exons 2-10 were amplified by PCR and sequenced. A putative skewing of X-inactivation in the unaffected mother was determined by quantitative PCR before and after methylation-sensitive restriction digestion at the AR gene (androgen receptor).

Results: We detected another 3 base pairs deletion c.1009_1011delGAA in the isoform I of PHF6, which leads to the loss of one amino acid p.337delGlu on the protein level. The mutation was found in two affected boys, but not in their healthy brother. The mother is a heterozygous carrier and does not show any skewed X-inactivation.

Conclusions: This is the second report on a family with Börjeson-Forssman-Lehmann Syndrome with mild symptoms and a three base pairs deletion in isoform I of PHF6. We conclude

that severe forms of BFLS are attributed to missense and nonsense mutations in the common part of isoforms I and II, whereas mild phenotypes are generally caused by mutations in the unique sequence part of isoform I. In addition, there is evidence that mild forms show no preferential inactivation of any X chromosome.

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No evidence for a role of SLC1A5 in 19q13 in the aetiology of cystinuria

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Cystinuria is an inherited metabolic disorder characterised by the abnormal urinary excretion of cystine and dibasic amino acids and results in the formation of cystine kidney stones. Two genes involved in cystinuria have been identified: Mutations in the SLC3A1 (2p16) gene cause cystinuria type I, whereas mutations in the SLC7A9 (19q13) gene can be detected in non-type I as well as in type I cystinuria patients. The mutation detection rate for both genes in cystinuric patients does not reach more than 80% and is influenced by several factors (screening techniques, ethnic origin, classification of patients). Therefore, the role of further genes in the aetiology of cystinuria has been postulated. Interestingly, linkage analysis in cystinuria families does so far not indicate the existence of more than the two cystinuria loci in 2p16 and 19q13. Thus, the localisation of further genes encoding amino acid transporter subunits within these regions is conceivable. One candidate is SLC1A5 in 19q13 which is expressed in both kidney and intestine and encodes a B0 neutral amino acid transporter in humans. To further elucidate whether SLC1A5 is involved in the aetiology of cystinuria, we screened for mutations in two non-type I cystinuria families compatible with linkage with 19q13 but without detectable mutations in SLC7A9. Despite strong evidences for an involvement of SLC1A5 in the aetiology of cystinuria, we could not identify any mutation in SLC1A5 in the two families. Nevertheless, there remains the possibility that other genes are involved in cystinuria. Further molecular studies will clarify the complex nature of this disorder.

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Primary failure of eruption of permanent teeth – a genome-wide linkage analysis

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Objectives: Tooth eruption is a complex, localized, bilateral symmetric and timed process. Eruption disturbances are due to various reasons and include ectopic position of the tooth germ, mechanical interferences and failure in the eruption mechanism caused by systemic factors or local eruption disturbances. Although disturbances of tooth eruption are associated with many systemic disorders (e.g. ectodermal dysplasia with adrenal cyst, Rutherfurd syndrome or Gapo syndrome), there is only a single report of a non-syndromatic, autosomal dominant inheritance of this condition (Shokeir, Clin. Genet. 5: 322-326, 1974; OMIM 125350). We have now ascertained and clinically characterized a three generation family of German descent segregating an autosomal dominant form of primary failure of eruption and aim at identifying the genetic basis of this disease.
Material and Methods: Genome-wide linkage analysis is performed with 200 polymorphic markers selected from the Marshfield STRP screening set 12. Average marker distance is 17.5 cM at an average heterozygosity of 0.78 (between 0.64 and 0.92).
Results: Approximately 40% of the genome scan has now been completed including the entire chromosomes 1 to 6. Thus far, there is no evidence for genetic linkage. Best fit modelling predicts a maximum LOD score of 2.4 within the given family constellation. Additional affected family members are being ascertained at present in an effort to reach LOD scores over 3.0.
Conclusions: The full genome scan will be completed shortly and should provide a novel gene location for this condition. Identification of the molecular defect will be crucial to further our understanding of the molecular processes underlying this rare disorder.

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Autosomal dominant retinitis pigmentosa: Linkage analysis in a large German pedigree excludes known loci

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Retinitis pigmentosa (RP) is the most common form of inherited retinal dystrophies, with a prevalence of about 1/5.000. It is characterized by early night blindness, loss of the peripheral visual field, and can lead to blindness in late stages. RP is phenotypically and genetically heterogeneous. In 20% of cases, family history indicates autosomal dominant inheritance (ADRP). Twelve ADRP loci have been mapped to date, with genes identified in all cases. However, mutations in these genes only account for half of all ADRP cases, suggesting the existence of additional disease genes. We studied a large German pedigree with ADRP. Both onset of symptoms and severity of the disease show intrafamilial variation: Some of the nine affected family members noticed visual impairment in the early 3rd decade, others not before early 4th decade. Progression of visual impairment ranges from rapid to moderate. Linkage studies using microsatellite markers for all known ADRP loci excluded *NRL* (RP27), *CRX*, *RP1*, *PIM1K* (RP9), *IMPDH1* (RP10), *CA4* (RP17), and *FSCN2* as causative genes in this family. Where markers were not informative (loci for *PRPF31*, *RDS*, and *RHO*, respectively), the entire coding regions of the corresponding genes were sequenced. In case of *PRPF8* and *HPRP3*, mutations have only been described in restricted parts. We found no mutations in either gene by sequencing these regions. As there is no male-to-male transmission in this family, and since mutations in *RPGR* (underlying X-linked RP3) have been described in families with apparent ADRP, markers flanking the RP3 locus where also typed and showed no linkage. Genome-wide linkage analysis will be performed in order to identify the ADRP locus in this family.

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Primary congenital glaucoma:Identification of 2 ancient SNP haplotypes associated with pathogenic variations in the CYP1B1 gene

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Mutations in the cytochrome P450 1B1 (CYP1B1) gene, a member of the cytochrome P450 gene family, have been shown to cause primary congenital glaucoma (PCG). At least 14 different mutations are frequently (>60%) associated with the known intragenic SNP haplotype 5'-CCGGTA-3'. HapMap data indicate that the CYP1B1 gene and the 5'-CCGGTA-3' SNP haplotype is located within one linkage disequilibrium (LD) block comprising approximately 55kb. To investigate the evolutionary origin of CYP1B1 mutations we have analysed 7 additional SNPs located within the preliminary 55kb CYP1B1 LD block and 6 CYP1B1 flanking microsatellites were included. The 9 analysed PCG patients carry CYP1B1 mutations, either in homozygous or compound heterozygous form and are furthermore homozygous for the intragenic 5'-CCGGTA-3' haplotype. One PCG patient belongs to a Costa Rican PCG family, one to a German PCG family and 7 are clinically sporadic PCG patients. The microsatellites flanking CYP1B1 varied much more as the intragenic 5'-CCGGTA-3' SNP haplotype. Since this observation hampers the hypothesis of a founder effect based on the intragenic SNP haplotype we have analysed 7 new assigned high frequency SNPs within or close to the CYP1B1 gene. Although the above mentioned mutations are within the identical intragenic 5'-CCGGTA-3' SNP haplotype, the additional SNPs allowed us to order the mutations in two subgroups. The Arg355Stop, Arg368His, 7901del13 and 622delC mutations are all within one SNP haplotype (CAGCCGCGTAATT), while the Trp57Stop, Gln42Stop, 1209insC and 8037dup10 are embedded in a second SNP haplotype (TAGCCGAGTACGA). It is highly probable that almost all of the CYP1B1 mutations associated with the intragenic 5'-CCGGTA-3' SNP haplotype can be assigned to one of these two ancestral haplotypes. This indicates a founder effect and may explain the high frequency of compound heterozygous mutation carriers compared to homozygous PCG patients.

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Autosomal Recessive Mesoaxial Synostotic Syndactyly with Phalangeal Reduction Maps to Chromosome 17p13.3

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Non-syndromic syndactyly is a common congenital malformation showing webbing of fingers and/or toes. The malformation can be unilateral or bilateral, and the fusion within the web may be cutaneous or bony. The majority of syndactylies show autosomal dominant mode of inheritance except Cenani-Lenz syndactyly which follows the recessive model. Previously we reported a novel syndactyly in a consanguineous Pakistani family segregating as an autosomal recessive entity with a unique combination of clinical features: mesoaxial reduction of fingers with synostosis of 3rd and 4th metacarpal bearing single phalanges, clinodactyly of 5th fingers and preaxial webbing of toes. A literature search for this distinguished phenotype revealed three similarly affected patients in a large inbred Turkish family. In the present study we localize the phenotype in the Pakistani and Turkish family to chromosome 17p13.3 (multipoint lod score 5.23). The identification of a single locus for a similar complex hand-foot malformation in two families with distinct ethnical backgrounds gives evidence for a new form of syndactyly. We propose to name this phenotype mesoaxial synostotic syndactyly with phalangeal reduction (SDMS, type IX syndactyly, Malik-Percin type). The mapping of this locus would aid the clinical and genetic delineation of syndactyly. The recruitment of more families with similar phenotype might help to narrow down the candidate region and the eventual cloning of the gene would provide insight into the complex process of limb development.

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Balanced (9;11) translocation in a patient with non-syndromic craniosynostosis disrupts the SOX6 gene (11p15) and a conserved non-transcribed region (9q32).

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Craniosynostosis is a congenital developmental disorder involving premature fusion of cranial sutures resulting in an abnormal shape of the skull. While significant progress has been made in understanding the molecular basis of syndromic craniosynostosis, little is known about the various forms of non-syndromic craniosynostosis. Here we report on a male infant with non-syndromic craniosynostosis presenting at birth with Crouzon-like brachycephaly, proptosis, midfacial hypoplasia and low set ears. Three-dimensional cranial computer tomography showed fusion of the lambdoid sutures and distal part of the sagittal suture with a gaping anterior fontanelle. Mutations in the genes for FGFR1-3 were excluded. Standard chromosome analysis revealed a de novo balanced translocation t(9;11)(q32;p15). We have cloned the DNA fragment containing the chromosome 9 and 11 breakpoints. The breakpoint on chromosome 11p15 disrupts the SOX6 gene, a gene known to be involved in skeletal growth and differentiation processes. Consequently, we have screened the complete SOX6 gene in 102 patients with non-syndromic craniosynostosis. No causative mutation was found. Nevertheless, we can not rule out that the translocation may have lead to a truncated SOX6 protein exhibiting a dominant negative effect. The breakpoint on chromosome 9 is located in a region without any known or predicted gene but, interestingly, disrupts patches of evolutionary highly conserved non-coding, non-transcribed DNA. Assuming a regulatory function of these sequences, we suggest that the translocation could have led to a dysregulation of flanking genes on chromosome 9 or 11 involved in cranial suture formation. Candidate genes have been evaluated.

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Autosomal dominant perniosis maps to chromosome 3p

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Familial perniosis is a novel autosomal-dominant genodermatosis. The clinical picture consists of painful purple-red inflammatory lesions in acral locations such as fingers, toes, nose, and cheeks induced by a combination of cold temperature and moisture. The lesions may ulcerate and may be associated with arthralgias. Histologically, lesions are characterized by unspecific vasculitic changes with deposits of complement and immunoglobulin and absent hyperkeratosis. The disease onsets in early childhood and tends to improve in late adulthood. Thus, the clinical and histological findings are consistent with either perniosis or Chilblain lupus, a rare cutaneous form of lupus erythematodes. Extensive investigation of 3 affected individuals of a multigenerational nonconsanguineous German kindred could exclude the presence of antinuclear antibodies, complement deficiency, cryoglobulinemia, cold agglutinins, infections, traumatic injury, keratolytic winter erythema, or lupus pernio.
Whole-genome linkage analysis was carried out on 25 family members including 16 affected individuals using the Affymetrix GeneChip Human Mapping 10K Array version Xba131. PedChek was used to detect Mendelian errors. Parametric linkage analysis was carried out with GeneHunter 2.1 and SimWalk 2 using a stepwise analysis of non overlapping marker sets covering 25-100 SNPs at a time. Assuming a fully penetrant autosomal-dominant trait the disease gene was localized within a 15 cM interval on chromosome 3p21-3p14 with a maximum LOD score of 2.2 for GeneHunter 2 and a location score of 4.7 for SimWalk 2 analysis.
Identification of the gene responsible for familial perniosis may shed light onto the pathogenesis of common forms of collagen vascular disease such as vasculitis or lupus erythematodes.

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Genetic Model of Duchenne / Becker muscular dystrophies (DMD/BMD)

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When doing risk calculation in DMD/BMD, a genetic model has to be chosen that describes the genetics of the sex linked trait and other parameters such as frequencies of the different mutation types (deletion, duplication and "point mutations"). These are determined by the mutation rates, which depend on the sex and the type of mutation. Their influences are fertility and the possibility of germ line mosaicism.. In defining all these parameters it is important to remember that under the assumption of a mutation selection equilibrium, the values of these parameters are interdependent.
In the literature there are good data for the estimation of the following parameters: frequencies of the different mutation types; sex ratio of mutation rate in deletions and "point mutations", parameters of germ line mosaicism. There are no data about the sex ratio of mutation rate in duplications.
Because all parameters of the genetic model of DMD/BMD are interdependent unknown parameters can be estimated from those where data are available. Using this approach we arrive at the following estimates:
In "point mutations" the sex ratio of mutation rates is about k = 5; in deletions it is k = 0.5 and in duplication k = 0.5. This means that deletions and duplication have their origins mainly in oogenesis and "point mutations" mainly in spermatogenesis.

P284

Interactions of proteins associated with Bardet-Biedl syndrome

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Bardet-Biedl syndrome (BBS; MIM #209900) is s a genetically heterogeneous, pleiotropic disorder. 8 Genes (BBS1 – 8) have been mapped and identified, so far. The trait is characterized by learning disabilities, pigmentary retinopathy, polydactyly, obesity (caused by hyperphagy), renal malformations and hypogenitalism in male. Although BBS is considered to be a recessive disorder, heterozygotes of BBS mutations show carrier effects like increased risk of obesity, diabetes mellitus, and hypertension. Furthermore there is a high prevalence of Clear Cell Renal Cell Carcinoma (CC-RCC) and renal malformations among unaffected relatives of BBS patients.
There exists limited structural similarity between several BBS proteins. However, this information is neither sufficient to understand the normal function of these proteins nor the reason why deficiencies or anomalies in each of them cause a similar spectrum of phenotypic anomalies. The cellular localization of several BBS proteins indicates that they might interact physically, and

that defective targeting and anchoring of pericentriolar proteins as well as disorganisation of microtubules might contribute to the BBS phenotype.
To clarify if some of the BBS proteins interact directly or indirectly to form a multisubunit complex, we subjected the proteins BBS1, BBS2, BBS4, BBS6 and BBS7 (short isoform) to direct interaction assays.
Mutational and linkage analysis had suggested that there might exist even more BBS loci. To search for novel BBS candidate genes or to contribute to the functional understanding of the known ones, we performed yeast two hybrid screens for BBS1 and BBS4. Successively, BBS1, BBS2, BBS4 and BBS7 (short isoform) were tested for interaction with candidates detected in BBS4 and BBS1 screens.
To find new interacting partners of BBS proteins may lead to a better understanding of biochemical pathways involved in common complex disorders such as obesity, diabetes mellitus and hypertension.

P11 Prenatal Diagnosis

P285

Cytogenetic discrepancies in prenatal diagnosis: Tissue specific mosaicism in extraembryonic and embryonic cells
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Chorionic villi sampling has become a reliable method in invasive prenatal diagnosis, representing an acceptable alternative to second trimester amniocentesis. Nevertheless, mosaicism and discrepancies between chorionic villi and fetal chromosomal constitution generate problems in genetic counselling. In most cases, the chromosomal abnormalities are confined to the placenta and do not affect the fetus. Nevertheless, in some instances, the placental karyotype is normal, while fetal cells show an abnormal karyotype.
Here, we report on two cases with rare mosaicism showing an extreme expression of tissue specific extraembryonal/embryonal discrepancies. In the first case, the diagnosis of a false positive pure trisomy 4 in chorionic villi short term as well as in long term culture could not be confirmed in the amniotic fluid and in the fetus itself. In opposite, the second case showed a false negative normal chromosomal set in chorionic villi short term as well as in long term culture, whereas, the newborn demonstrated a partial trisomy 10p due to an unbalanced translocation (4;10) in the lymphocytes. Both cases demonstrate a complete discrepancy by comparing the karyotypes of the extraembryonic and embryonic tissue.

P286

Healthy child born after polar body diagnosis for Mucopolysaccharidosis Type I
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In Germany the only possibility to perform preimplantation genetic diagnosis is the analysis of polar bodies (PBs). We performed a PB analysis in a consanguineous couple that had an affected child who died at the age of 3 ½ years by a severe form of Mucopolysaccharidosis type I (MPS I). The couple also had two pregnancy terminations after prenatal diagnosis of homozygous fetuses. Polar body diagnosis was performed using a multiplex PCR assay for five linked polymorphic markers. 16 first PBs were analysed in the first cycle. Three PBs were carrying the mutation allele of the mother and one the corresponding oocytes was fertilised. This oocyte was transferred and a pregnancy was established. Prenatal diagnosis during pregnancy showed a heterozygosity of the fetus for the mutation. Further investigations identified the mutation as inherited from the father. Thus the PB diagnosis could be confirmed. A healthy child was born.

P287

The QF-PCR in routine prenatal diagnosis of the common human trisomies (1 1/2 years of practice)
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A rapid prenatal test for the common human trisomies is offered to pregnant women in many genetic laboratories. Most labs rely on FISH hybridisation of selected chromosome-specific DNA probes to interphase chromosome preparations on uncultured amniotic fluid cell nuclei. Since counting of labelled nuclei is very labour intensive, we decided to establish an alternative method, the (semi)quantitative fluorescence PCR (QF-PCR).
This test is based on a semiquantitative PCR with fluorescently labelled primers for known tetranucleotide microsatellite markers located on chromosomes 13, 18, 21, X and Y. To minimize PCR-efforts, 18 microsatellite markers are amplified in two multiplex reactions, employing five markers from chromosome 13, six markers from chromosome 18 and five markers from chromosome 21, as well as the amelogenin locus for sex discrimination and HPRT on the X chromosome.
Despite the relatively high heterozygosity rates of applied markers it is recommendable to analyse a large number of loci from each chromosome. The protocol with fewer markers, which we applied in our first experiments, did not meet the criteria for reliable diagnosis (minimum of two markers informative) in a number of cases.
Experiences with the robustness of our test protocol, heterozygosity rates of markers and selected cases will be presented. Five percent of 220 cases were not interpretable, mostly due to maternal contamination of amniotic fluids.

P288

Molecular confirmation of complete mole
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Objectives: To confirm paternal uniparental diploidy at molar pregnancies.
Material and Methods: 15 different highly polymorphic STR markers (Ampfestr Identifiler PCR Amplification Kit -Applied Biosystems, USA) together with amelogenin marker were co-amplified from DNAs isolated from molar pregnancy tissue with karyotype 46,XX and mother's blood.
Results: Paternal uniparental diploidy was confirmed if all 15 STR polymorphisms of product of conception were monoalelic and more than one polymorphism could not be inherited from mother.
Conclusions: Our method can reliably confirm paternal origin of 46,XX karyotype at molar pregnancies with significant clinical consequences. Partial and complete mole can be distinguished by ultrasound and histological appearance but cytogenetic and molecular genetic evaluation should be considered whenever there is a question of the diagnosis particularly to prevent malignancies.

P289

Prenatal diagnosis of femoral-facial syndrome
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We describe a case of femoral-facial syndrome (FFS) detected prenatally on second-trimester sonography. Fetal abnormalities shown by sonography included micrognenia, bilateral hypoplastic femurs and bilateral talipes. The diagnosis was confirmed after birth. FFS is a rare sporadic syndrome with femoral hypoplasia and unusual facies. The facial features include upslanting palpebral fissures, short nose with broad tip, long philtrum, thin upper lip, micrognenia, and cleft palate. The femora are mostly bilaterally affected and they are short with lateral bowing. Upper limb involvement is possible. In one third of cases the mother has diabetes mellitus. Mental development in FFS is normal. Stature is short due to short legs. There are therapeutic options for micrognenia and short femurs. If micrognenia and short, bowed femora are found on prenatal sonography FFS should be suspected.

P290

Cytogenetic discrepancies in prenatal diagnosis: Tissue specific fetal mosaicism
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Chromosomal mosaicism diagnosed in chorionic villi or in amniotic fluid cells present a main problem in prenatal diagnosis and genetic counselling. Pathological cells might be restricted to the extraembryonic tissue, but might also indicate a true fetal mosaicism. Therefore, the question remains: "What is the correct fetal karyotype?" Fetal blood sampling is the assay of choice to get the answer, because only fetal cells are analysed by this method. Nevertheless, because of tissue specific fetal mosaicism, fetal blood analysis can also result in an incorrect karyotype (e.g. i12p-mosaicism).
Here we report two cases of fetal blood analysis which gave a false negative result, while amnion cells indicated the real fetal karyotype. Results have been proven by cytogenetic analyses of cells from other fetal tissues. In the first case, a trisomy 16 mosaicism diagnosed in amniotic fluid cells was confirmed by conventional and molecular cytogenetic analysis of fetal cells gained by puncture of an pleural effusion. In contrast, all fetal blood cells analysed showed a normal karyotype. In the second case a trisomy 6 mosaicism was proven by the analysis of cells gained by fetal bladder puncture. The chromosomal aberration was also detected in amniotic fluid cells, while fetal blood cells showed a normal chromosome set (1). In summary, in contrast to the common assumption and general experience, in both cases, the amniotic fluid but not the fetal blood was the specimen representing the fetal karyotype correctly.

(1) Wegner et al. (2004) Prenatal diagnosis of fetal trisomy 6 mosaicism and phenotype of the affected newborn. Am J Med Genet 124: 85-88.

P291

Prenatal diagnosis of a derivative Y chromosome der(Y)t(X;Y) in a female fetus

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Prenatal cytogenetic analysis revealed a karyotype with a derivative Y chromosome in a female fetus. We were able to narrow down the breakpoints to der(Y)t(X;Y)(Ypter->Yq11.21::Xq13->Xqter).
STR-marker analysis showed that SRY (Yp11.3) and AZFa (Yq11.21) are present. AZFb (Yq11.222) and AZFc (Yq11.223) are deleted. FISH analysis identified several Xq –specific regions including the Xist-locus in Xq13.2.
Although SRY is present, ultrasonografic investigation at 16+1 and 18+1 weeks showed a female fetus. Interestingly, BrdU incorporation studies demonstrated that the derivative Y chromosome is late-replicating and due to the present Xist locus largely inactivated. Because of the partial monosomy Xp (Xpter->Xq13) and the apparently inactive status of the derivative Y chromosome Ullrich-Turner-like stigmata are expected and may explain the normal female genital in ultrasonographic findings.
To our knowledge this is the first report of the described unbalanced translocation between a X and Y chromosome in a female fetus. Because of the risk of an incomplete inactivation of the derivative chromosome and, therefore, an uncertain outcome for the fetus, this unique case posed a considerable challenge for the genetic counselling of the parents.

P292

Familial arhinia, choanal atresia und microphthalmia

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We describe three females (aunt and nieces) with variable manifestations of arhinia, choanal atresia, microphthalmia and hypertelorism with normal karyotyp 46, XX.In the literature there are few reports of this syndrome. We hypothesize autosomal dominant inheritance with reduced penetrance.
The mother of the two sisters is currently pregnant with a male fetus without abnormal sonographic findings.

P12 Complex Disease

P293

Protein biosynthesis and infection – is the elongation factor 2 the possible link?

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In the healthy heart, the last step of the elongation cycle, catalyzed by the elongation factor 2 (EF2) is considered to be a major regulatory step of the protein biosynthesis. In recent studies it could be shown that the availability on biological active EF2 in the heart is decreasing with age, associated with a decrease in overall protein biosynthesis. Assuming a negative metabolic effect for the proper function of the heart, the decrease in EF2 availability may also lead to different heart diseases. For this reason, investigations regarding the influence of factors, involved in the different metabolic pathways leading to heart diseases, on the EF2 regulation are under debate. However, up to now there is no evidence of an transcriptional regulation of cardiac EF2 activity. In the present study we focused on the investigation of the influence of infectious and inflammatory markers including TNFa (20ng/ml), Ps. exotoxin A (10ng/ml), endotoxin (1µg/ml), and IL-1b (100U/ml), on mRNA expression of the cardiac EF2. For this investigation neonatal rat heart myocytes (app. 80% purity at day of preparation) were cultivated under standard conditions and incubated for 24h with the appropriate factors. The mRNA expression of EF2 was measured by means of Real-Time-PCR (Rotor-Gene) in relation to cells not treated with any mediator. No different expression pattern of EF2 could be detected under the influence of IL-1b or TNFa. However, the two exogenous toxins, Ps. exotoxin A and endotoxin, reduced the transcription rate of EF2 dramatically by 50% ($p<0.001$) and 40% ($p<0.001$), respectively. These results suggest, that bacterial toxins may influence the biological availability of EF2 at least at transcriptional level and could therefore directly modulate the protein biosynthesis. One could assume, that patients who suffered from bacterial infections may exhibit, among others, an unbalanced protein biosynthesis possibly leading to a worse adaptation to changed conditions.

P294

Association of common SNPs with LDL- and HDL-cholesterol in two independent population-based samples from Switzerland and Germany - A comparative analysis -

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Introduction: The use of SNPs as a predictive and diagnostic tool for complex traits requires reproducible and replicable results from association studies across different population samples. We therefore analysed two population-based samples from Switzerland and Germany for allelic association with LDL-C and HDL-C.
Population and Methods: Two population-based samples from Germany and Switzerland (Geneva) were independently drawn. Individuals were selected based on atherogenic (high LDL-C & low HDL-C) or atheroprotective (low LDL-C & high HDL-C) blood lipid phenotypes. 196 German and 371 Swiss individuals were genotyped for common SNPs in 16 lipid relevant genes. Association and regression analyses were used to test for allelic association.
Results: The phenotype distributions, allele frequencies, and contributions of single genes were very similar in both samples. ApoE and CETP contributed the most, while ApoB, ABCA1, and LDL-receptor, LPL, HL, and PLTP contributed the least to lipid variation. Variations in ApoA1, LCAT, and SRBI were not associated with lipid variations. In contrast, individual SNP associations were different between the two populations.
Summary: Common SNPs in 16 lipid relevant genes contribute in different extents to LDL-C and HDL-C variation in these two central western European populations. SNPs, except within ApoE, showed different association signals and did not allow a prediction of atherogenic or atheroprotective lipid phenotypes. We can only speculate on whether these findings are due to complex gene-gene interactions, to genetic drift of neutral variants even in two geographically neighbouring populations, or to methodological differences between the two studies.

P295

A repeat polymorphism in the CLCN7 gene influences bone density in patients with autosomal dominant osteopetrosis (ADO) type II and in postmenopausal women

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Heterozygous mutations in the ClC-7 chloride channel cause autosomal dominant osteopetrosis type II (ADO II), a disorder characterized by a highly variable phenotype. We hypothesized that polymorphisms in the CLCN7 gene could be associated with the variability of bone density in ADOII and in the general population. Therefore, we investigated two exonic SNPs and a repeat polymorphism (VNTR) in intron 8 on the non-mutated CLCN7 allele in a large family with ADO II with an exceptionally low penetrance. No association between the phenotype of the carriers and the SNPs was observed. In all affected carriers, however, we detected exclusively 3 repeat units in the intronic VNTR, whereas the frequency of this allele was significantly lower either in non-affected carriers and other family members (p=0.02). We were further interested if this repeat polymorphism could also be associated with a higher bone density in 391 postmenopausal women. After adjustment for age, height, weight, years since menopause and hormone replacement therapy, women with 3 repeat units on both alleles (3/3) had a higher BMD at the femoral neck (p<0.01) than individuals with higher repeat numbers. In a multiple linear regression analysis the intron 8 genotype was a significant determinant of BMD at the femoral neck and explained 0.9% of the population variance of femoral neck BMD. In addition, we found a significant association of the 3/3 genotype with lower deoxypyridinoline/creatinine levels (p=0.04). In conclusion, a VNTR polymorphism in intron 8 of CLCN7 has a small impact on BMD in ADO II and in the normal population.

P296

A whole-genome scan in 155 German sib pairs with attention-deficit/hyperactivity disorder substantiates linkage to chromosome 5p

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Objectives: Attention-deficit/hyperactivity disorder (ADHD) is one of the most heritable disorders in child and adolescent psychiatry; heritability is estimated at approximately 0.8. In the current study we report the results of a genome-wide scan performed in a German sample of 102 families encompassing a total of 229 affected children (155 sib-pairs).
Material and Methods: The families were ascertained and phenotypically characterized by physicians in the outpatient units of the Departments of Child and Adolescent Psychiatry of the Universities of Aachen, Marburg and Würzburg and Regensburg. Families were included if they comprised at least two affected children with ADHD, according to DSM-IV criteria. In 80, 19 and three families two, three, or four affected children were ascertained, respectively.
We performed a whole genome scan with initially 404 autosomal and X-chromosomal microsatellite markers (average distance 10cM). In total, 425 individuals were genotyped. Additional markers on chromosome 5p at the DAT1 (SLC6A3) locus were subsequently genotyped: D5S2005, the DAT1 VNTR, rs6347 (in exon 9) and rs11564774 (in exon 15).
Results: The highest multipoint LOD score in the initial analysis was obtained on chromosome 5p at the marker D5S807 (LOD 2.2). Other multipoint LOD scores exceeding 1 were obtained for chromosomes 8, 12 and 17. Fine mapping on chromosome 5 revealed that two DAT1 variants (the VNTR and rs6347) are not associated with ADHD in our sample (PDT p-values of 0.97 and 0.57, respectively). The linkage peak cannot be explained by these variants. One of the DAT1 markers (rs11564774) might have a role in the observed linkage, as the families contributing to the linkage peak show a weak association with ADHD (PDT p-value of 0.096).
Conclusions: We detected suggestive linkage to ADHD on chromosome 5p. The linkage peak could not be explained solely by one of the investigated DAT1 polymorphisms.

P297

Mutational analysis and association study of the human angiotensinogen gene in German patients with preeclampsia

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The aim of this study was to evaluate the influence of genomic variants in the angiotensinogen gene (AGT) on the individual predisposition to preeclampsia (PE). The AGT gene was screened for mutations in patients with PE, subsequently we performed association studies for the detected AGT variants. The study population comprised 82 Caucasian primi- and multigravid patients with PE (blood pressure ^{3}140/90 mmHg after 20 gestational weeks; proteinuria ^{3} 1 g/l) or superimposed preeclampsia (blood pressure ^{3} 140/90 mmHg before 20 gestational weeks; proteinuria ^{3} 1 g/l) and 100 controls with uncomplicated pregnancies. The mutation screening was carried out in 46 patients by single strand conformation polymorphisms (SSCP) and direct sequencing. In the case of identification of a genomic variant, the total study cohort was analysed. Allele frequencies for the identified variants were analysed in the total study population by SSCP, restriction fragment length polymorphism analysis (RFLP) and mutagenically separated PCR (MS-PCR). In total, 17 genomic variants were detected. A heterozygous missense mutation L43F [L10F] was found in one patient but in none of the controls. This finding is in accordance with previous studies suggesting that L43F [L10F] has a predisposing effect in a small subgroup of PE patients. The other 16 genomic variants in AGT were classified as single nucleotide polymorphisms (SNPs). A general association of AGT-variants with preeclampsia could not be observed in our study population except the polymorphism g.2576G>A which was found to be significantly associated with PE (p = 0.047). This observation has to be confirmed in larger study cohorts but may provide evidence for a role of AGT in the pathogenesis of PE.

P298

Genetic Linkage and Association Studies in Sarcoidosis

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Objectives: Sarcoidosis is a complex inflammatory disease of unknown cause that primarily affects the lung and the lymph system. An approximately 20-fold increased recurrence risk in close relatives documents the contribution of predisposing genes to the aetiology of the disorder. We have established an extended DNA and data bank from approximately 2500 German sarcoidosis patients and their relatives and have investigated functional and positional candidate genes in the search for sarcoidosis susceptibility genes.
Material and Methods: Eighty-three affected sib pair families and 183 single patients together with their parents (trios) were drawn from the sarcoidosis DNA and data bank. They were genotyped for flanking microsatellite markers and intragenic single nucleotide polymorphisms of NOS3, B-7, CD28 and CTLA4. Non-parametric linkage (NPL) analysis and transmission disequilibrium tests (TDT) were performed using GENEHUNTER 2.0.
Results: NOS3, the nitric oxide synthase 3 gene is located on chromosome 7q36, close to a moderate linkage peak of a previous genome-wide linkage scan. The analysis of two flanking and one intragenic microsatellite markers, together with one functional single nucleotide polymorphism (SNP) moved the NPL peak towards the intragenic NOS3 markers, with a single point NPL score of 2.3 (p = 0.01) from the NOS3 microsatellite. Study of the B-7, CD28 and CTLA4 loci is close to completion and results will be presented.
Conclusions: The samples of sib pair families and trios from our sarcoidosis DNA bank provide an informative resource to quickly check candidate genes for genetic linkage or association with sarcoidosis. Positive results can readily be studied in detail in the remaining approximately 2000 single patients of the DNA and data collection.

P299

Periodic catatonia: systematic analysis of a schizophrenia locus on chromosome 15q15

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Periodic catatonia (MIM 605419) is a psychiatric disorder with a strong genetic component and 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. In genome-wide linkage studies on multiplex pedigrees segregating for periodic catatonia we previously identified a major disease locus on chromosome 15q15 in a sample of 12 extended pedigrees, and replicated the chromosomal locus in an independent set of four pedigrees.
Linkage and haplotype analyses in three exceptionally large pedigrees linked to chromosome 15q15 disclosed an 11 cM critical region between marker D15S1042 and D15S659. In our efforts to reveal the disease gene we performed linkage-disequilibrium mapping (LD) and haplotype analyses in multiplex pedigrees and parent-offspring trios with microsatellite markers and SNPs. Simultaneously we carried out a complete systematic mutation scan of candidate genes annotated in this region by semi-automated sequencing of DNA fragments of individuals from the linked pedigrees and controls. So far no disease-causing mutation was identified among the brain expressed genes. All detected SNPs are included in ongoing linkage-disequilibrium mapping as well as in family-based and case-control association studies with 831 individuals, including 27 multiplex pedigrees, 40 parent-offspring trios, 31 affected sib pairs (ASP), 144 cases and 230 controls.

P300

SNP discovery in the Cited 2 gene and risk evaluation for congenital heart defects

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Congenital heart defects (CHD) account for the largest number of birth defects in humans, with an incidence of about 8 per 1000 live births. The heterogeneity of CHDs associated with single-gene defects points to a complex genetic network with modifier genes, genetic polymorphisms and the influence of environmental factors. Mice with a disruption of the Cited2 gene, encoding a CBP/p300 interacting transcriptional modulator of HIF-1α and TFAP2, die in embryonic stages due to significant defects in heart development. To study the potential impact of polymorphisms in the Cited2 gene for CHD we screened a population of 160 patients using the WAVE technology. This method relies on the differential elution of polymorphism carrying heteroduplexes and homoduplexes during a hybridisation protocol. Resequencing of analysed samples detected 11 polymorphisms thereof 5 previously unknown variations. Results of regenotyping using the Amplifluor method were well in accordance with the WAVE data. Moreover, Amplifluor based comparative genotyping between the CHD and an equal sized control population showed a higher frequency of 3 new identified polymorphisms in the CHD patients, but risk estimations are imprecise owing to the low frequency of the studied variants. One of the discovered variations in the Cited2 gene leads to the deletion of 1 Histidine. Functional analysis, however, revealed that this mutation neither alters the ability of the protein to modulate HIF-1α and TFAP2 transcriptional activity nor the intracellular localisation. Therefore the role of the new mutation remains to be elucidated. Finally, data presented here show that WAVE SNP discovery and Amplifluor genotyping are a valuable tool to study candidate gene polymorphisms in complex diseases.

P301

Determination of the linkage disequilibrium (LD) structure for a putative glaucoma locus on chromosome 14q11 in German patients

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Glaucoma is a clinically and genetically heterogeneous group of ophthalmologic disorders leading to visual impairment and blindness worldwide. The most common form of this complex trait is primary open angle glaucoma (POAG), characterized by late onset and elevated intraocular pressure (IOP) in most cases. Several loci have been linked to POAG, but with the classical positional cloning approach only two genes have been identified until now, myocilin and optineurin, accounting for about the 5% of the cases.
The aim of our ongoing project is to identify new glaucoma genes through systematic LD analysis and association studies in previously described susceptibility POAG loci. We report here the reconstruction of the LD pattern and haplotype structure in a region spanning 3,3Mb in 14q11. A total number of 15 candidate genes were selected based on their pattern of expression in affected tissues and on the presence of structural or functional domains shared with the known glaucoma causing genes. 90 single nucleotide polymorphisms (SNPs) were identified until now in these genes by direct sequencing of exons, 5'-3' UTRs and flanking intronic regions in 46 unrelated German POAG patients. 20 variants were found within the coding sequences of ZNF219, RPGRIP1, DAD1, BCL2L2, NRL, ISGF3G and ADCY4. These were further investigated for functional relevance, evolutionary conservation and presence in a control group. None of them could be classified as disease-causing. For future association studies, haplotypes will be reconstructed using those SNPs with minor allele frequency (MAF) over 0.2 and tagging-SNPs (htSNPs) will be selected with haplotype coverage of 90%. These studies are currently underway.

P302

Genetics of retinal drusen formation in rhesus macaques.

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Objectives: Macular drusen formation is a hallmark of ARMD (Age Related Macular Degeneration), but the mechanism underlying their formation is not well characterized. It is known that rhesus macaques (*Macaca mulatta*) present a natural animal model for retinal drusen, and in both, man and monkey, genetic factors are implicated. We tried to identify associated genes in rhesus macaques.
Material and Methods: A search using 42 microsatellite markers, linked to 7 different autosomal loci implicated in retinal pathology in humans, was performed in a single rhesus matriline, followed by a positional candidate gene approach. Several genes implicated in the etiology of ARMD with and without drusen formation have been mapped to the 6q region in humans. Two of the candidate rhesus orthologs, *ELOVL4* (Elongation Of very Long Chain Fatty Acids 4) and *IMPG1* (Interphotoreceptor Matrix Proteoglycan-1) were searched for mutation using PCR, SSCP (Single Stranded Conformational Polymorphism) and sequencing.
Results: An association between two alleles at heptallelic marker D6S1036 and the severity of drusen formation was found (P combined: 0.012). For *ELOVL4*, no sequence changes were found in the coding region, but a polymorphism was found for the 3' UTR region which was not associated with the drusen pathogenesis and could play a protective role. In *IMPG1*, 6 SNPs were identified. Haplotype frequencies of these six SNPs were found to differ significantly between affected and non-affected animals (chisquare=10.102, 3 d.f., p=0.018). This difference was mainly due to a haplotype which was found on 17% of chromosomes in animals with drusen, but not in controls.
Conclusions: Our data demonstrate that one or several genes on the rhesus homologue of human 6q are likely to play a role in retinal drusen formation.

P303

Mutation analysis of the PlGF gene and the FLT1 gene in pregnancies with IUGR and ARED or PED flow

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The development of the fetoplacental system, i.e. capillary sprouting as well as the switch from branching to non-branching angiogenesis, in human pregnancy is essential for the birth of a healthy infant at term. This angiogenic process is controlled by growth factors such as bFGF, VEGF and PlGF. The interaction of PlGF with FLT1 seems to induce non-branching angiogenesis and this type of angiogenesis predominates in pregnancies with intrauterine growth retardation (IUGR) and ARED (absent or reversed enddiastolic)-flow (postplacental hypoxia). In pregnancies with IUGR and PED (preserved enddiastolic)-flow in the presence of a bilateral abnormal uterine artery Doppler waveform (uteroplacental hypoxia), histology of the placenta shows a netlike arrangement of capillaries, forming multiply branched terminal villi. To study a possible role of mutations in the PlGF and the FLT1 genes in the pathogenesis of placental dysfunction, we analysed the PlGF and FLT1 genes in an IUGR/ARED-flow group (18 mothers and 18 fetuses) and an IUGR/PED-flow group (14 mothers and 14 fetuses). DNA was extracted from ve-

nous blood samples (mothers) and umibilical cord blood samples (fetuses). The coding sequences of PlGF (7 exons) and FLT1 (30 exons) and their intron/exon boundaries were screened by single strand conformation polymorphism analysis (SSCP), restriction assays and direct sequencing. Apart from established SNPs, we identified one unknown polymophism in exon 1 of the non-coding sequence of PlGF and two novel variants in exons 1 and 6 of FLT1. The variations were detected in similar frequencies in the two groups. However, we could not identify a pathogenic mutation neither in the PlGF gene nor in the FLT1 gene, thereby providing no evidence for a relevant role of both genes in the aetiology of IUGR/ARED or IUGR/PED.

P304

Extended mutation analysis and association study of TSPYL gene in sudden infant death syndrome (SIDS)

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Sudden Infant Death Syndrome (SIDS) is defined as the sudden death of an infant which remains unexplained after a careful autopsy, death scene investigation, and review of the medical history have been performed. With an incidence of 0.46 per 1000 life births in Germany in 2002, it constitutes the most frequent cause of death in the postperinatal periode. Genetic (long QT syndrome genes, IL-10 and serotonin transporter gene etc.) and environmental factors (prone sleep position, maternal smoking, early weaning from breastfeeding etc.) seem to contribute to SIDS. Recently a lethal phenotype characterized by sudden infant death with dysgenesis of the testes syndrome (SIDDT) was identified to be caused by loss of function mutations in TSPYL gene. To reveal a possible role of TSPYL in SIDS, we investigated DNA samples of 126 affected children from the German study on sudden infant death (GeSID). Five sequence variations in the TSPYL gene have been detected and their frequencies will be compared to German control subjects. The results of the mutation analysis and the association study will be presented.

P305

The age related changes in the profiles of the MTHFR genotype combinations in CAD patients are modified by the cigarette smoking and the co-ocurrence of the diabetes.

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The MTHFR 677 C>T and 1298 A>C polymorphisms were studied in 170 male CAD patients in whom the diagnosis was confirmed by angiography (>50% stenosis of at least one epicardial coronary artery). Previously the increased risk of CAD has been associated with the MTHFR 677 TT genotype (Brattstrom, 1998) and the early occurrence of CAD (age <50y) with the MTHFR 1298 AC and CC genotypes (Szczeklik, 2001).
The analysis of the MTHFR genotypes combinations was performed in groups differing in the age of the CAD diagnosis. In the group of patients in whom the CAD occurred up to the age of 60 years, the combined genotypes of MTHFR 677/1298: CC/AC, CT/AC and CC/CC occurred with the higher frequency (69,8%; n=116) as compared to the frequency noted in the group of the older patients (55,6%; n=54) (p=0,05). In the nonsmoking patients (n=59) the same MTHFR genotype combinations occurred at the higher frequency (83,3%) in the younger patients (n=36) as compared to the older group (60,9%) (p=0,053).
In the younger patients group (n=102), differentiated for the smoking habit, the different MTHFR genotypes combinations (CC/AA, CT/AC, CC/CC and TT/AA) occurred at the higher frequency (54,5%) in the smoking patients (n=66) as compared to the nonsmoking group (27.8%; n=36) (p= 0,0008).
In the younger patients group differentiated for the diabetes (T2DM), the other MTHFR genotype combinations CC/AA, CT/AA and TT/AA occurred at the higher frequency (62,5%) in the T2DM patients (n=32) as compared to the non-T2DM patients (41,7%, n=84).
The reported finding fit with the assumption that both the cigarette smoking and the co-ocurrence of the T2DM accelerate the occurrence of CAD by the mechanisms differing from these operating in the nonsmoking and the non T2DM patients.
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