Abstracts

Public lecture

Kampf der Geschlechter im Genom: Epigenetik und die Bedeutung der elterlichen Prägung in Biologie und Medizin
Reik, Wolf
Developmental Genetics Programme, The Babraham Institute, Cambridge, UK


Keynote lecture

Proteomics, systems biology and molecular medicine
Superti-Furga, Giulio
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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).
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
Ruden, Douglas M.
Department of Environmental Health Sciences, University of Alabama at Birmingham, Birmingham, USA
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 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 variations. 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. The unifying theme between both trans-generational epigenetic phenomena, in addition to both of them involving Hsp90, is that Hsp90 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 trans-generational effects of DES on the predisposition of breast 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 methyltransferase, Su(var)-3, 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
Antonarakis, Stylianos E.
Department of Genetic Medicine and Development, University of Geneva, School of Medicine, Geneva, Switzerland.
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 % un aligned) that are not transcribed, and we called them 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 than 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 to 1-3 % 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
Deloukas Panos
The Wellcome Trust Sanger Institute, Hinxton, Cambridge CB10 1SA UK
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 involving 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 r.m.f<5%. Phase II will generate data on 2.25M additional SNPs. Raw genotyping data are re-
leased through the Data Coordination Center on a monthly basis (http://www.hapmap.org).

The consortium has released data on circa 1 mil-
ion SNPs across the CEU panel and 0.5 million across the other two panels (YRI and CHB+JPT); phase II will be completed in February (all pan-
els). For the first time we can observe linkage disequilibrium (LD) trends at a genome scale. For example, telemeric regions consistently display low LD whereas centromeric regions are as-
sociated with high LD. The December data-set is used to establish an analysis pipeline to as-
sess common patterns of LD, recombination and natural selection as well as define optimal sets of tag SNPs. The analysis group has selected the work to be carried out 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 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 sys-
tematic validation of putative functional variants. SNP analysis (dbSNP 121) in the context of pro-
tein coding genes found 48,451 non-synony-
umous (ns) SNPs, 1113 SNPs that introduce a
protein coding genes found 48,451 non-synony-
umous (ns) SNPs, 1113 SNPs that introduce a
STOP codon and 104 that disrupt a STOP

The ultimate aim is to apply these resources to
explain the genetic contribution to common dis-
esases of major health impact. We are currently
developing strategies to test large sets of mark-
ers for association in case-control study design,
to confirm positive associations and assess the
impact on gene and/or disease. We have embarked on several pilot studies mainly focusing in regions of linkage.

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 kary-
yotype to be the ancestral one. The chimpan-
zee 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 line-
ages 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 diver-
gence data from colinear and rearranged regions between human and chimpanzee conform to this model. Comparative maps of many mammalian species which are available allow to recon-
struct 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 prevail-
ing and can be used to reconstruct the genome of the common tetrapod 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 an-
cestral genome of all teleost fish. Both these an-
cestral genotypes 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 re-
arrangements, such as inversion and fission are
preferred in the fish lineages, whereas in the
bird/mammal lineage also interchromosomal re-
arrangements did occur. From the two types of
sex chromosomes in vertebrates the 2 chromo-
some of the birds is more conserved. The same
holds true for the whole chicken genome which
despite its fissions into several macro- and mi-
acrochromosomes closely resembles the ances-
tral vertebrate proto-genome.

Disorders of the human mitochondrial

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The true impact of mitochondrial DNA (mtDNA) mutations in human disease remains under-
determined. Until relatively recently, mitochondrial disorders were considered to be obscure, ex-
ceptionally rare diseases affecting perhaps one or two per million of the population. Improve-
ments in referral patterns and the availability of rapid, molecular diagnostic techniques have helped contribute to the expanding clinical phe-
notype associated with mtDNA mutations with rapid epidemiological data suggesting a mini-
mum prevalence of at least 1 in 5000, or even higher.

My talk will focus on the basic aspects of clini-
cal mitochondrial genetics, discussing our expe-
rience in Newcastle upon Tyne of investigating,
diagnosing and managing patients with primary mitochondrial disease, and the approaches we are pursuing towards developing treatments.

Dyskeratosis congenita and telomerases
Dokal, Indreerit
Department of Haematology; Division of
Investigative Science, Imperial College,
London, UK

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 leu-
copekia. A given patient may also have a vari-
ety 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
nuclear protein called dyskerin. Dyskerin asso-
ciates with the H/ACA class of small nuclear
RNAs in small nuclear ribonucleoprotein parti-
cles (snRNPs), which is important in guiding the conversion of uracil to pseudouracil during the maturation of ribosomal RNA. Dyskerin also associates with the RNA component of telom-
erase (TERC), which is important in the mainte-
nance of telomeres. Mutations in TERC were re-
cently demonstrated in patients with autosomal dominant DC and in a subset of patients with aplastic anaemia (AA) and myelodysplasia
Abstracts

S3 01

Disruptions of the Histone Methylase Machinery in Cancer Pathogenesis
Cleary, Michael L.
Stanford School of Medicine, Stanford, CA, USA

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 oncogene 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.

S3 02

Role of telomere acquisition in the formation of chromosome rearrangements in human leukemic cells
Sabatier, Laure
CEA - Life Science Division, Radiobiology and Oncology Unit, Fontenay-aux-Roses, France

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 chromosomal 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. Telomere having lost its telomere... Telomere fusion. We performed an extensive study of the stability of the cell. 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 chromosomal 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. Telomere fusion. We performed an extensive study of the stability of the cell. 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 chromosomal 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...
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 quantification assay we say could show that ducal 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
Hochadelinger, Konrad
Whitehead Institute, Cambridge; USA
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
Ross, Mark T.
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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. Togeth-er 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 the evolution of sex chromosome 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 re-arranged 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 de-generation in non-recombinant regions.

In this presentation, I will discuss our evolution-ary studies of the human sex chromosomes, our observations on sequence composition and 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
McErlavey, Ken
Reproduction, Fertility and Populations, Dept of Developmental Biology, Institut Pasteur, Paris, France
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 con-tain genes expressed specifically in the testis. These paralogous repeats may be associated with the Y- Y gene conversion events and they ren-der the chromosome more able to form interstitial deletions by non-allelic homologous recombina-
tion. Many of these deletions cause spermatogenic failure (AZFa, AZFb and AZFc deletions), some are apparently non-inconsequential polymor-phisms (g1/g3, 1212) and for others the precise relationship with fertility needs to be defined (g1/g9, b1/b3). I will review the 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 line-
egages is mainly characterised by geographical-ly structured drift rather than language or ethnic affinities. 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 patholo-
gies. Recently a number of studies have at-
tempted to associate Y lineages with various phenotypes including blood pressure, prostate and testicular cancer, autism, longevity and in-
fertility/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.
Avner, Phil
Unité Génétique Moléculaire Murine, Institut Pasteur, Paris, France
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-in-
activation centre (Xic) which contains the Xist gene. Some of the genes, approxi-
mately 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 the evolution of sex chromosome 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 re-arranged 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 de-generation in non-recombinant regions.

In this presentation, I will discuss our evolution-ary studies of the human sex chromosomes, our observations on sequence composition and 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.
Beyond 22q11 Deletions: Uncovering the Aetiology of DiGeorge Syndrome-Like Heart Defects in Man.
Scambler, Peter
Molecular Medicine Unit, Institute of Child Health, London, UK

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 (DGVS/VCFS). When mouse gene targeting studies revealed Tbx1 to be haploinsufficient and (DGS/VCFS). When mouse gene targeting studies revealed Tbx1 to be haploinsufficient and (DGS/VCFS). When mouse gene targeting studies revealed Tbx1 to be haploinsufficient and (DGS/VCFS). When mouse gene targeting studies revealed Tbx1 to be haploinsufficient and (DGS/VCFS). When mouse gene targeting studies revealed Tbx1 to be haploinsufficient and (DGS/VCFS). When mouse gene targeting studies revealed Tbx1 to be haploinsufficient and (DGS/VCFS). When mouse gene targeting studies revealed Tbx1 to be haploinsufficient and (DGS/VCFS). When mouse gene targeting studies revealed Tbx1 to be haploinsufficient and (DGS/VCFS). When mouse gene targeting studies revealed Tbx1 to be haploinsufficient and (DGS/VCFS). When mouse gene targeting studies revealed Tbx1 to be haploinsufficient and (DGS/VCFS).
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 Nbn null 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 during Ig class switching and probably in 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.

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 interval 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. Wild-type and mutant peptides were incubated with Abi-SH3- and Src-family SH3 protein. While wild-type and mutant CMT2B2 strongly bind Abi-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 Pmp22 overexpressing 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-activated human CMT1 rats revealed a tremendous increase of 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.

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


1) University of Regensburg, Institute of Human Genetics, Regensburg, Germany
2) University of Wuerzburg, Institute of Human Genetics, Wuerzburg, Germany
3) School of Biosciences, Birmingham, UK
4) University Eye Hospital, Electrodiagnostics Research, Tuebingen, Germany
5) University of British Columbia, Department of Biochemistry, Vancouver, Canada
6) University of Florida, Center for Immunology and Transplantation, Gainesville, Florida

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 Rs1th, 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 Rs1th-deficient mouse serves as a valuable peripheral system to develop possible therapeutic interventions for human RS.
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

Tönnies H. (1), Paul D. (1), Müller A.-S. (1), Neitzel H. (1)

1) Charité, Universität Medizin Berlin, Institute of Human Genetics, Berlin

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 rotation microscope equipped with a 8x8 slide scanning stage controlled by an adaptive scanning software (Metasystems). During the scanning process interphase cell metaphases and 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.


1) Medizinische Universität Wien, Abteilung für Humangenetik, Klinisches Institut für Medizinische und Chemische Labordiagnostik, Wien
2) Ludwig-Maximilians- Universität München, Medizinische Klinik III, Klinikum Grosshadern, München
3) Maternité – CHUV, Service de genetique medicale, Unité de cytogenetique du cancer, Lausanne
4) Max Planck Institute for Molecular Genetics, Dept. Ropers, Molecular Cytogenetics Group, Berlin

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 in 11q23-24 and/or 11q13.5 we have co-amplified with 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 BAH77 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 (ODD4) gene, but not thyroid hormone responsive (THRSP) and p21/Cdc42/Rac1-activated kinase 1 (PAK1). By this way we narrowed down the minimal amplified region of overlap bordered by clones bA715 and bA1536F 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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6) KH St. Pölten, First Clinic of Internal Medicine, Division of Oncology, St. Pölten

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 89kDa nucleoprotein 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 17 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 MLL-M5a and a non-recurrent t(3;11)(p24;q15). The cases with previously identified gene fusions comprised one childhood AML with a t(15;11)(q35;p15) and a NUP98/NSD1, with another a NUP98/DDX10 and another one with a t(11;20)(p15;q12) and a NUP98/TOPO1 gene fusion. Moreover, we also succeeded to identify topoisomerase 2 B (TOP2B) as the new partner gene and, thus, also to characterize the resulting fusion gene 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 is 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 (MSPCR), which allows the simultaneous quantitative of differentially methylated polymorphic DNA sequences. However, quantification with MSPCR 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 (X-inactive specific transcript). We successfully 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 aneuploidies, 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-
Recurrent chromosomal breakpoints in the immunoglobulin heavy chain gene locus in
Reed-Sternberg cells of classical Hodgkin lymphoma
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5) University of Duisburg-Essen, Institute for Cell Biology (Tumor Research), Essen
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 FISH, 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 hyperdiploid 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. Supported by the Deutsche Krebshilfe (Verbundprojekt “Molekulare Mechanismen bei malignen Lymphomen”, 70-3173-Tr3/B1)
Abstracts

ctCHDs, while atypical congenital heart defects are very uncommon in patients with those. This statistical significant differences in the atypical distal 22q11.2 deletion in any of the patients with typical VCFS phenotype revealed only unspecificity of the atypical distal deletion. As most types of small supernumerary marker chromosomes (sSMC) are present 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) uni-parental disomy (UPD) of the sSMC's homologous chromosomes, and/or (iii) different degrees of mosaicism. We did our studies on >170 cases with sSMC and performed a review of the literature (i.e. presently 1628 cases with sSMC) available at http://mi-ri.mito.uni-jena.de/~huw woll/MOL_ZYTO/sSMC.htm. A first genotype/phenotype correlation for sSMC was deduced from that. Thus, small proximal trisomies of Xp, Xq, 1p, 1q, 2p, 4q, 6q, 7q, 8p, 12p, 14q, 17q, 18q, 19q, 20q, 20p, 21p, 22q may not be associated with significant clinical symptoms. No general correlation could be found in more than 100 patients with typical VCFS phenotype correlating with the deletion of 3 Mb. Therefore, we performed an in silico comparative genomic approach, comparing all genes from the candidate interval with those from Chlamydomonas reinhardtii 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#121000). An insertion of four nucleotides (AAGA) in the 5'-UTR causes a frameshift that introduces a premature stop codon. Consequently, 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 cilium.

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

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Small supernumerary marker chromosomes (sSMC) are present 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) uni-parental disomy (UPD) of the sSMC's homologous chromosomes, and/or (iii) different degrees of mosaicism. We did our studies on >170 cases with sSMC and performed a review of the literature (i.e. presently 1628 cases with sSMC) available at http://mi-ri.mito.uni-jena.de/~huw woll/MOL_ZYTO/sSMC.htm. A first genotype/phenotype correlation for sSMC was deduced from that. Thus, small proximal trisomies of Xp, Xq, 1p, 1q, 2p, 4q, 6q, 7q, 8p, 12p, 14q, 17q, 18q, 19q, 20q, 20p, 21p, 22q may not be associated with significant clinical symptoms. No general correlation could be found in more than 100 patients with typical VCFS phenotype correlating with the deletion of 3 Mb. Therefore, we performed an in silico comparative genomic approach, comparing all genes from the candidate interval with those from Chlamydomonas reinhardtii 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#121000). An insertion of four nucleotides (AAGA) in the 5'-UTR causes a frameshift that introduces a premature stop codon. Consequently, 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 cilium.

W02 06

Understanding computer-based decisions of syndrome diagnosis using facial traits

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2) Ruhr-Universität Bochum, Institut für Neuroinformatik, Bochum

Classification rates in syndrome diagnosis rely on computer based analysis of facial traits. A data set consisting of 121 standardized photographs of patients out of 10 different syndromes was investigated (MPS III, CDL, FraX, PWS, WBS, 22q, Noonan S., Sotos S., SLO). Each individual was described by a normalized 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 pa-
rameterized 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 validat-
ed tool for including facial information into the
intricate process of finding a syndrome diagno-
sis.

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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2) Ruhr-Universität Bochum, Institut für Neuroinformatik, Bochum

Complex traits are thought to have complex ge-
ic 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 represent-
ing the face. Investigations are under way to
conduct association and linkage analyses with
respect to the phenotype to answer fundamen-
tal questions about the development 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 aim-
ing 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 pro-
cedures in conjunction with other dimensionali-
ty reduction methods like principal component
analysis. 3. Heritability analysis for individual di-

dimensions. This procedure yields a low (20 to 40)
dimensional representation that captures rele-
vant information of the data set. The parameters
are the amount of variation captured, classifica-
tion rates, and heritabilities. They allow further
analysis in standard association or linkage set-
ings. The knowledge gleaned from monogenic

disorders substantially improved the character-
analysis in standard association or linkage set-

ting rates, and heritabilities. They allow further
dimensional representation that captures rele-


W03 02

Increased frequency of CFTR heterozygosity in infertile males
1) Otto-von-Guericke University, Institute of Human Genetics, Magdeburg
2) Otto-von-Guericke University, Clinic of Reproductive Medicine and Gynecologic Endocrinology, Magdeburg
3) Otto-von-Guericke University, Institute of Biometry and Medical Informatics, Magdeburg

Congenital bilateral aplasia of the vasa deferen-
tia (CBAVD) is associated with a high frequency of
cystic fibrosis transmembrane regulator (CFTR)
mutations. However, there are contradic-
tory results concerning the frequency of CFTR
mutations in infertile males without CBAVD. In
the present study, we examined a total of 597 in-
ferile males for CFTR mutations prior ICSI ther-
apy. A heterozygous CFTR mutation was ob-
served in 34 of 597 patients (5.69%). Given that
our mutation panel recognises about 82% of
heterozygotes, it can be assumed that the fre-
quency 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 heterozy-
gosity of 3.42 % can be estimated. This indi-
cates that in our cohort of infertile males the fre-
quency of CFTR heterozygosity is two-fold high-
er than in the general population (p<0.0001).

W03 03

Cardiovascular morbidity and mortality in dialysis patients: a 10 year follow-up
1) Innsbruck Medical University, Division of Genetic Epidemiology, Innsbruck
2) Hospital Feldkirch, Department of Nephrology, Feldkirch
3) Innsbruck Medical University, Department of Nephrology, Innsbruck
4) Hospital Vienna-Lainz, Internal Medicine, Vienna
5) St. Pölten Hospital, Internal Medicine, St. Pölten
6) Wilhelminenspital Vienna, Internal Medicine, Vienna

Objectives: Lipoprotein(a) [Lp(a)] is a genetically
determined risk factor for atherosclerosis. About 30-70% of the Lp(a) plasma concentra-
tions are determined by the apolipoprotein(a) size polymor-
phism. 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) apolipoprotein(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 apolipoprotein(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 recruit-
et between 1991 and 1996 in five Austrian dial-
isis centers. By the end of 2004 we will have fin-
ish 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 follow-
ing 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 (0-1, 1-yes) 1.79 (1.45-2.22) <0.001
Lipoprotein(a) phenotype (1=HMW, 2=LMW) 1.46 (1.17-1.89) 0.001

Conclusions: From the pathogenic standpoint the
HMW apolipoprotein(a) phenotype is mainly associated
with atherosclerotic complication. Therefore, we con-
clude that the association of the apolipoprotein(a)
phenotype with atherosclerosis in dialysis patients is
so strong that we can even observe an associ-
tion with total mortality to which cardiovascular
events might contribute 50%. The ongoing val-
idation 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
1) German Cancer Research Center (DKFZ), Molecular Genetic Epidemiology, Heidelberg
2) Institute of Transfusion Medicine and Immunology, Red Cross Blood Service of Baden-Württemberg-Hessia, Mannheim
3) University of Heidelberg, Institute of Human Genetics, Heidelberg
4) University of Cologne, Division of Molecular Gynaeco-Oncology, Köln

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 test-
ed eight annotated single nucleotide polymor-
phisms for occurrence in familial breast cancer
cases. The confirmed variants Ile654Val, Ile654Val and
Ala1170Pro 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 associ-
ation of both adjacent polymorphisms Ile654Val
and Ala1170Pro resides either. The ERBB2 variant Ile654Val,
however, revealed an increased risk for carriers

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-1-
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 kina
case activity and cell transformation. These find-
ings, for the first time, reveal ERBB2 Val654 as an oncogenic variant which might, in addition, influence clinical outcome and predict worse prognosis.

W03 06
Genomewide Linkage Scan of Idiopathic Generalised Epilepsy reveals Oligogenic Susceptibility Loci on 5q31, 13q32, 16q22 and 19q13
1) Max Delbrück Center, Gene Mapping Center, Berlin
2) on behalf of the European Consortium on the Genetics of Idiopathic Generalised Epilepsy, Europe
Idiopathic generalised epilepsies (IGE) affect about 0.3% of the general population and ac-
count for 30% of all epilepsies. The aetiology of common IGE syndromes, such as juvenile my-
oclonic epilepsy (JME) and idiopathic absence epilepsies (IAE), is genetically determined, but the complex pattern of inheritance suggests an oligogenic predisposition. The aim of the pres-
ent collaborative European genome scan was to map IGE loci and to dissect its oligogenic aeti-
ology. Our linkage study included 96 European families ascertainment through a proband with ei-
ther IAE or JME, and one or more siblings affect-
ed 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, re-
vealed suggestive evidence (P < 0.01) for novel IGE loci in the chromosomal regions 5q31 (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 conforms sus-
ceptibility 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 muta-
tion analyses.

W04 02
Genome-wide identification and functional characterization of novel factors regulating cellular sterol metabolism
1) European Molecular Biological Laboratories, Cell Biology / Biophysics Programme, Heidelberg
2) European Molecular Biological Laboratories, Gene Expression Programme, Heidelberg
3) Dept. of Human Genetics, Heidelberg
Here we describe the establishment of a combi-
ined expression profiling and microscope-based functional screening approach that al-
lows us to systematically identify novel candid-
ate genes which are regulated by sterols and 
therefore 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 statistical-
ly significant changes in expression in HeLa cells at different time points in response to sterol de-
pletion. 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-domi-
inant familial hypercholesterolemia confirmed the specificity and significance of our results. A positive function of the novel identified candi-
date genes is now being further characterized by using cDNA/-RNA-based human live cell arrays and high-content screening microscopy. Our study contributes an important step toward a more comprehensive understanding of the mo-
lecular basis of cellular sterol regulation, with our methodology being suitable for addressing a wide range of biological and medical questions.
Application of High Resolution genome-wide DNA Array CGH analysis within a diagnostic setting

The strategy to use array CGH analysis in a 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 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.

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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 05

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

The 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 orthologues 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 a gene that results in preferential expression of 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 the USP9Y- or USP9X, respectively only the DBY- or DBX-protein. Our results strongly suggest a translational control for DBX 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 tests tissue sections, DBY and USP9X proteins were found predominantly in spermatagonia, whereas DBX and USP9Y proteins were found in pachytene to diploid spermatids. We conclude that although USP9Y/USP9X and DBY/DBX genes 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 06

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

Germline 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 cytologically 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 distribution of the major macro- and microchromosomal 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 asynchromous 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 we 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 asynchromous 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


1) Institute of Human Genetics and Genetics, Heidelberg
2) University of Heidelberg, Institute of Human Genetics, Bonn
3) University of Bonn, Department of Medicine, Bonn
4) Ciphergen Biosystems GmbH, Göttingen

**Methods:** We performed a systematic search for MUTYH-associated polyposis (MAP) in a large series of patients with colorectal adenomas (63 atypical FAP, 14 typical FAP, 16 unknown phenotype). 3 Patients had been detected by conventional mutation analysis. The results show that: (1) proteins identified by SELDI after precleared 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 heterogeneous distribution of the analysed proteins in the tissue probes underlying the indispensability of tissue microdissection prior to all analyses. This work was supported by the IZKF-Jena and the BMBF.

**W05 02**

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

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**Results:** 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 (TFRY8 and J84). Transient transfection studies showed that CATS is localized mainly to the nucleoli 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 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 and calgizzarin in head and neck cancers


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**Background:** MUTYH-associated polyposis (MAP) is a recently described precancerous adenomatous polyposis syndrome of the coloreum (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 atypical 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:** 12 mutations in MUTYH were identified in 12 of 96 patients (12.5%). In 7 patients biallelic mutations for the hot-spot mis-sense change Y165C and/or G382D were identified, in one proband a 777X mutation in homogzygous state was found, in 4 patients were either homozygous or compound-heterozygous for yet unclear variants (P143L, P291L, 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-
ite, in 2 cases vertical segregation was suspected. Monallelic 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.

The study was supported by the Deutsche Krebshilfe.

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 (dBMs) are histologically characterized by two distinct tumor components, the so-called pale islands and the desmoplastic areas. Previous molecular studies have shown that dBMs frequently carry PTCH1 mutations. However, little is known about other genetic and chromosomal aberrations associated with these tumors. We investigated total genomic DNA of 23 sporadic dBMs 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 (2/23 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 dBMs, 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 dBMs using matrix-CGH confirmed amplification of several genes on 17q23 in three dBMs and the JMJD2C gene on 9p24 in one dBMB, 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 dBMs and implicates RPS6KB1 and JMJD2C as novel proto-oncogenes that are aberrantly activated in these tumors.

W05 06


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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 invasion 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 taken, together our results indicate that leupaxin could serve as a putative candidate gene involved in invasive properties of prostate carcinoma cells.

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 Skaltsky 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 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 hybrised 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 encompassing 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 chromosome 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 (unidentified miscarriages and stillbirths/early newborn death). To improve the cytogenetic characterization of der(13;14) the distinction among dicentric and monosomic translocations was introduced. Additionally we performed UPD studies in a selected group of children. Deletions with abnormal phenotypes and balanced der(13;14). Probabilities rates for occurrence of the unfavorable 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 fœtuses at prenatal diagnosis is estimated as 2.2/4 i.e. 8.5±6.8% 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 aneuploidy was similar for the couples with dicentric translocations carriers comparing to monosomic 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. 

W06 04

L1 elements facilitate X-inactivation spreading onto trisomic chromosome 15q in an unbalanced translocation (X;15)(q23.2;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 new 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;15)(q23.2;q11.2) with nearly complete trisomy 15 and a normal karyotype in her male twin. Copynumber testing by FISH 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 SNP confirmed the breakpoint was mapped to within two overlapping BAC clones RP11-575K24 and RP11-483F6 and the 15q break-point 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.
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 tests differentiation. In addition to these and other effects, SOX9 is also expressed in cranial nerve crest cells (CNCCs), embryonic multipotent cells which delaminate from the neural tube and migrate dorsally, 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 and 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 homozgyously 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 embyros show complete exencephaly and deformities in the development of all craniofacial structures. Analysis of early CNCC molecular markers, migrate dorsally, and contribute to a variety of craniofacial tissues including the head and limb, shows 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
Trismethylaminuria, an important cause of body malodor
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Trismethylaminuria (TMA-uria) is an autosomal recessive disorder caused by a deficiency of flavin-containing monoxygenase isofrom 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 odour 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 malodor. Considering additional functions of this enzyme, mild FMO3 deficiency may play a role as susceptibility factor in pharmacological and other pathological conditions.

W07 03
Infantile-lethal non-lysosomal heart glycogenosis caused by activating mutation in the γ2 subunit of AMP-activated protein kinase (PRKAG2), not by phosphorylase kinase deficiency
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Massive, infantile-lthal, 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, missense mutations. PRKAG2 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 γ2 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-lthal non-lysosomal cardiac glycogenosis which can hence be seen as a clinically distinct, particularly severe variant of hypothyic congenital cardiomyopathy. The existence of a heart-specific primary phosphorylase kinase deficiency is questionable.

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

W07 04

Shox2 acts upstream of Bmp4 in regulation of sinovenus 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 fetal heart malformations in Shox2 homozygous mouse 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 before 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 of the heart and suggest an involvement in the formation of the cardiac conduction system.

W07 06

THE NEUROLOGICAL PHENOTYPE OF HUMAN LAMININ β2 DEFICIENCY

EVIDENCE OF GENOTYPE-PHENOTYPE CORRELATIONS IN PIERSON SYNDROME
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Piersson syndrome (PS; OMIM 609049) is a new autosomal-recessive ocular syndrome caused by LAMB2 mutations leading to laminin β2 deficiency. Previously reported patients died in the newborn period or early infancy due to respiratory 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 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 splicing activity.

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.

W08 Neurogenetics

W08 01

The possible role of Ca2+ in the pathophysiology of BSC2L2 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 homozygous missense mutations in the Berardinelli-Seip congenital lipodystrophy gene (BSC2L2), 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-EFPP constructs. Subsequently we quantified the Ca2+ concentration in the cytoplasm of single cells with the Fura-2 technique in time lapse 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 decreased [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 in Oprüfodiscordant families. 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 promotive region is in progress. However, an in vivo protein interaction with SMN was shown for one candidate so far. These interesting findings open new perspectives to better understand the mechanism of pathology and the regulation of the SMN protein, as well as to develop additional therapies for SMA.

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 deletion 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 copy numbers can show variable phenotypes from unaffected to affected indicating the existence of SMN 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 promotive region is in progress. However, an in vivo protein interaction with SMN was shown for one candidate so far. These interesting findings open new perspectives 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 motor neuron 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 showed 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 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 showed 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

Peripherin maps to the PARK8 region and is a new interactome 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 peripherin by yeast-two hybrid screening. Immunohistochemical studies identified peripherin as a component of LB in brains of PD patients. Interestingly, the peripherin 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 peripherin 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, HEC cells stably expressing mutant peripherin appeared to be more susceptible to the nitric oxide donor S-nitroso-N-acetylpenicillanate (SNAP). Since peripherin accumulates under proteasomal inhibition with MG132, it seems likely that this protein is degraded by the proteasome, fitting peripherin in the ubiquitin-proteasome degradation pathway. In summary, our results link peripherin with synphilin-1 and open up new vistas for peripherin 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) is an autosomal dominant disorder characterized by degeneration of cerebellar and motor cortex neurons. Recent advances in this field have shown that a CAG-repeat expansion in the coding region of the ataxin-3 (ATXN3) gene is widespread in SCA3 pedigrees. In this study, we have generated a new mouse model of SCA3 containing 148 polyglutamine repeats in the Atxn3 gene resulting in a 40-fold expansion of the CAG repeat. This model shows behavioral deficits similar to those in affected humans. Furthermore, perinatal lethality is observed, which might correlate with the pathogenesis of SCA3 in humans. Our results provide a novel mouse model for SCA3 that should help to elucidate the pathogenic mechanisms of this disorder.
Photoreceptor cell retinol dehydrogenase, in
Mutations in RDH12, encoding a
retinol dehydrogenase, in patients with childhood-
CCTG, Y226C) in homozygous or compound
heterozygous form in patients with childhood-
hemminus (T49M, R62X, Q189X, 806delC-
L274P , C285Y , and R295X. Each of the missense
variants tested exhibited decreased or aberrant
activity relative to wild-type when assayed for
L274P, C285Y, and R295X. Each of the missense
variants tested exhibited decreased or aberrant
activity relative to wild-type when assayed for
onset severe retinal dystrophy (Janecke et al.
W09 03
An inversion involving the Sonic Hedgehog
domain results in the mouse Short Digits (Dsh)
dominant mouse mutant. Dsh/Dsh mice exhibit
multiple internal and skeletal defects strongly re-
sembling the Shh-/- mouse. In contrast to Shh
mice, Dsh/+ mice, Dsh/+ mutants are characterized by a
limb reduction phenotype with fusion of proxim-
and middle phalanges in all digits, reminiscent
of human brachydactyly type A1. We mapped Dsh to mouse chromosome 5 in a re-
gion 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-reg-
ulatory enhancers. Using microarray-based ho-
bridization analysis and quantitative RT-PCR we
were able to show an almost complete downreg-
ulation of Shh expression during developmental
stages E9.5 to E12.5 in Dsh/Dsh mice, leading
to an almost complete downregulation of Shh
expression during developmental stages E9.5 to E12.5 in Dsh/Dsh mice, leading
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to an almost complete downregulation of Shh
expression during developmental stages E9.5 to E12.5 in Dsh/Dsh mice, leading

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 98% of all NBS patients are homozygous for a 5 bp deletion (657DSI) in exon 6 of the NBS1 gene.

To circumvent the embryonic lethality of null mutation of the murine NBS1 homologue, Nbn, we have generated mutant mice with an inducible null mutation. LoxP-site were inserted 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, Ms1, allowing induction of Cre-mediated exon 6-deletion by injection of poly(I:C). 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.

The impact of ovarian stimulation on imprinted gene methylation in preimplantation stage mouse embryos. Alexandridis A.(1), Großmann B.(1) and Konersmann M.(1), Haaf T.(1), Zechner U.(1)

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5) Development of the human neocortex depends spatially and temporally correct expression of numerous genes. Disturbances of this highly orchestrated 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 genesis of human brain development (cortico-genesis) and cognitive processes.

W09 06

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

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


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Family 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. Deleterious 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 (54%) 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.

The Role of Common Single-Nucleotide Polymorphisms on Exon 9 and Exon 12 Skipping in Wildtype and Mutated 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 (CFTR-associated disorders) and in nonclass 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 transcription 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.1540G>A, 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 very vulnerable to skipping. The number of splice variants in individuals with cSNPs was similar to previously reported values for the T allele, suggesting that cSNPs may enhance susceptibility to CFTR 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.

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 six single case studies had been described. 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” (Kenneklecht et al. 2002, Grütter et al. 2003). Here, we present evidence that hereditary prosopagnosia is a very common cognitive disease. Our results were based on a 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) 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 (Grütter 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 Okhrio and acro-renoc-ocular syndromes and confirm haploinsufficiency as the pathogenetic mechanism. Borozdin W(1), Boeth D(2), Lizpold M.(1), Wilhelm C.J,(1), Graham J. M.G.(1).


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Okhrio (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 Okhrio and acro-renoc-ocular syndrome. However, in a substantial proportion of patients with such phenotypes no SALL4 mutation was found. Segregation analysis of the 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, informative 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 Okhrio 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, Okhrio and acro-renoc-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 01

W11 Prenatal Diagnosis

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 toward 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 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 69 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%: 9,9%; 2-5%: 23%; 6-10%: 17,7%; 11-20%: 32,6%; 21-30%: 30,0% and 31-50%: 7,8%. The risk was underestimated by younger participants (< 20 years, OR 0.73) 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 a 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 40-year-old woman to bear a child with DS?’ indicates a dramatic overestimation with answers according to the given categories: <1%:3,8%; about 1%-3,9%; 2-5%:23%; 6-10%:17,7%; 11-15%:12,0%; 16-20%: 21-30%: 32,6%; 31-40%:8,5%; >41%:5,3%. Thus 86% 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

Herterich S.(2), Schindler D.(1), Hoehn H.(1)

Polar body diagnosis for monogenic diseases has been recognized in the Netherlands since many years. The risks should be given in a much broader context, 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 cultured amniotic fluid (AF) cells. Likewise, 8 negative cases and 2 positive cases were correctly predicted using bivariate flowcytometry of 72 h umbral cord (UC) blood cultures. In contrast, bi- and multivariate 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 prior 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 8 (SLC12A8) 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, whereas bivariate flowcytometry proved unreliable when applied to BrDU-substituted AF-cell cultures. Cases with a low prior 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.

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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 (BDP), and schizophrenia.

Material and Methods: We genotyped three polymorphisms at BDNF (rs6265 leading to Val86Met, rs889748, and a (GT)n repeat) in DSM-IV diagnosed patients with major depression (MDD), schizophrenia, bipolar affective disorder (BDPD), and in healthy controls. There were 465 MDD patients, 533 schizophrenia patients, 281 BPD patients, and 1,097 controls of German origin (Bonn sample). A second sample (312 MDD patients, 444 controls) was recruited in Munich (Sachsenhausen) and included in the present analysis.

Results: Three-marker haplotype rs889748-rs6265 produced significant associations with all phenotypes under investigation.
Background: The SCN5A gene encodes the α-subunit of the cardiac voltage dependent sodium channel. Coding region mutations cause Brugada Syndrome and other familial cardiac and neurological disorders. Recent studies have suggested SCN5A promoter mutations may also contribute to arrhythmias.

Aim: We investigated the influence of common SCN5A promoter and gene variants on ECG parameters in a central European general population and patient cohorts.

Methods: We genotyped 702 individuals from the population based KORA S2000 survey for 55 SNP markers. Haplotypes were inferred by the Haploview software package. We investigated the influence of common SCN5A promoter mutations (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.

Objectives: Sarcoioidosis 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 (BTN2L2) gene. The major disease variant (provisional designation “BTN2L2_SAR,” p = TDT = 3*10^-6, p case/control = 1*10^-8, replication p TDT = 0.0018; p case/control = 1.8*10^-6) represents an independent risk factor from DRB1.

Conclusions: BTN2L2 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 CD2. The G to A transition at “BTN2L2_SAR” leads to a premature termination codon in the splice site and up regulates the 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.

Abstracts

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 conduc- tion disturbances. Recent studies have suggested SCN5A promoter mutations may also contribute to arrhythmias.

Aim: We investigated the influence of common SCN5A promoter and gene variants on ECG parameters in a central European general population and patient cohorts.

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 the SCN5A gene may modulate the electrocardiographic parameters in the population.

Conclusions: This study identifies a 15 kb disease-associated segment of the butyrophilin-like 2 (BTN2L2) gene. The major disease variant (provisional designation “BTN2L2_SAR,” p = TDT = 3*10^-6, p case/control = 1*10^-8, replication p TDT = 0.0018; p case/control = 1.8*10^-6) represents an independent risk factor from DRB1.

W12 05

Genetic association of psoriatic arthritus 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 erythematosus and diabetes mellitus type 1, is also associated with psoriatic arthritus. Three independent cohorts of Caucasians patients with either psoriatic arthritus, psoriasis vulgaris without joint manifestation or rheumatoid arthritus were analysed for this variant as well as for linkage disequilibrium-based haplotypes. All three patient cohorts showed association to same PTPN22 haplotype. In contrast, polymorphism R620W was only associated in the rheumatoid arthritus group (OR=1.806, 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 arthritus (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 analysis 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-08) and the psoriatic arthritus 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 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 seizures and in 30-36% 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 18 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 independent 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.
P001 Cancer cytogenetics

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-GGH 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 referents. 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, genomewide matrix-GGH 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 (Beesdijk E.(1), Huvanandnan G.(2), Greulich K.(3), Rapp A.(3), Arutyunyan R.(2)

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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 technique in DNA damaged by cytostatics was determined.

Results: Involvement of telomere repeats in DNA fragmentation in BL 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 characteristic of the HT1080 cells while the lowest effect was seen in CCRF-CEM cells. MMC at lower doses increased the percentage of migrating DNA dose-dependent, 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 neo-plastic 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 sequence specifically when the assay for 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 neo-plastic 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.


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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 the analyzed mononuclear platelets (mpm) 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% (11%, p<0.01), 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 thrombocytopenia 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. comm.; Dr. E. Gebhart, Erlangen), however, to the

Weise et al., 2003, Cytojenet Genome Res 103:34-39 and subCTM-FISH (i.e. 24 chromosome-specific probe sets including subcentromeric, subtelomere 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)(q18), del(5)(q21)/del(5)(p11.3).(q5), ins (5;7)(q21;p14)(q22)(5;7)(p21;q11.29) and (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(15)(p15.3), del(17)(p13.33) in 3 cases, del(12)(p12), del(13)(q34), t(del(17)(q25) and del(18)(p1dup(18)(q11) were recognized. The most frequent abnormalities were subtelomeric deletions of the short arm of chromosome 1 (2 cases). The highest percentage of cryptic chromosomal losses of chromosome 12p is associated with childhood ALL and several solid neoplasias this suggests the presence of a tumour suppressor locus. This pilot-study demonstrates an unexpected high rate of cryptic chromosomal aberrations of 75% of ALL-cases.
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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Characterization of chromosomal aberrations in glioblastoma multiforme

Gliomas are 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 numbers of chromosomal aberrations of normal cells loss of one sex chromosomes X, Y, and autosomal imbalances +7 -7, -7, -7, and -11. Besides, we found that individuals without cancer 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 this 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)(q21;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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Identification of genetic subgroups and progression markers of oligodendroglialomas 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 genetic distinct subgroups and progression markers of ODGs is still a matter of debate.

Analyzing the detailed CGH results of the 89 ODGs published between 1992 and 2004, in a meta-analysis we could confirm the existence of three distinct subgroups of ODGs, characterized by the existence of the aberration pattern -1p/-19q (n=58, 65%, group A), -7/+7 (n=6, 7%, group B) or the absence of either of the two pattern (n=25, 28%, group C). The distinct aberrations were missed in almost all patients in group C (1p-, -19q, -4q, -11q, 5q, 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, analyzing our database, we found that only the individual events -10, -15q and +7 were significantly correlated with the histopathological 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 demonstrate the urgent 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.
Abstracts

P009
Molecular cytogenetic investigation of t(11;14)(q13.32;p21)-positive B-cell NHL cell lines classifies the sequence of tumor-associated genes on chromosome 1

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Introduction: Translocation t(11;14)(q13.32;p21) 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 CCND1/BCL1/PRRAD-1. CCND1/BCL1/PRRAD-1 rearrangement has been detectable in nearly all mantle cell lymphoma (MCL) cell lines, translocation t(11;14)(q13.32;q23) occurs in only 15-20% of multiple myelomas (MM). The detection of recurrent genomic imbalances at chromosome arm level. Gains at 6p (68.9%) and 12q13, 12q21, 21q21 and 21q22. To achieve a higher resolution, seven 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 the 3 of 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.

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 favorable 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 +1q and -16q 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). 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 the 3 of 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.

P012
Assessment of the reliability of conventional chromosome analysis based on R- and G-banding and M-FISH for the detection of chromosomal changes in high grade B-cell lymphomas

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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 +1q and -16q 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). Amplifications were found in 2 tumors and mapped to 2p23-p25, 4p15, 12q13, 12q21, 21q21 and 21q22. To achieve a higher resolution, seven 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 the 3 of 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.
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 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 un-resolved 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 (84%) 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 more detailed 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.

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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 telangiectasia and Nijmegen Breakage Syndrome patients were examined to determine a common cellular phenotype of increased radiosensitivity. Some causes genetic 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 employed 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 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) 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 this additional karyotypic abnormalities besides the complex translocation involving 8q12 and 3p13.

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 distinct facial features include synophrys, long eyelashes, depressed nasal bridge, an upturned nasal tip with antverted nares, and a thin upper lip. In many patients, diagnosis is easily made on the basis of the characteristic facial gestalt co-occurring 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 (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 development. It is also homologous to a family of chromosomal adheins which have roles in sister chromid 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 relation 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. Heterozygous 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 (Weiland 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 33/37 (91.9%) cases including 26/29 (89.7%) without novel mutations. A recurrent mutation, R66X, was detected in one family and 4 sporadic patients. The majority of mutations (26/33) were located in 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 Gb4 and PDZ-effector proteins involved in reverse ephrinB1 signaling. Comparing the mutations and phenotypes no specific genotype-phenotype 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 mosaicism 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 functioning. 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/ (common in epilepsy treatment), 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. This approach is expected 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 SMN2 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 nosological 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 ISX (infantile spasm syndrome, X-linked), Parlonton 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 tonic-clonic seizures, with or without hypertelorism 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 in 100 MEHMO patients 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.


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 proportionally 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 8 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)(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
P021

Narrowing the candidate region of Albright Hereditary Osteodystrophy-like syndrome by genome-wide 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 metatarsals, especially the 7th and 8th. We analysed 7 patients with a deletion of various sizes of chromosome 2q with a real time-PCR based approach. The smallest deletion found in a patient with AHO-like syndrome has a size of 2.5 Mb, together with a duplication of chromosome 10pter, another patient suffering from mild mental retardation and autism but not affected by AHO-like syndrome has a deletion of 1.5 Mb and an additional 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 eg. gipcian-1, that was already suggested as a candidate gene in other studies. To elucidate the role of gipcian-1 in the AHO-like syndrome, a knockout approach in mice is planned.

P022

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

Genomics and personalized medicine

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Objective: The androgen receptor gene is a critical player in the male reproductive system. Fifty percent of human males are carriers of at least one mutated copy of the androgen receptor gene. The investigation of mutations in the androgen receptor gene is an important diagnostic tool. This study focuses on the mutational analysis of the androgen receptor gene in patients with androgen insensitivity syndromes. Material and Methods: We studied 18 patients with androgen insensitivity syndromes by sequencing and identified 14 mutations in the androgen receptor gene in seven patients with 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 current 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 a 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 cannot be established. Therefore, modifying factors must be effective.

P023

Novel and Recurrent Mutations in Patients with Androgen Insensitivity Syndromes


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Objective: 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 nonsense 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 current 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 a 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 cannot be established. Therefore, modifying factors must be effective.

P024

THREE UNRELATED PATIENTS WITH NICOLAIDES-BARAITSER SYNDROME


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Objective: We report on three unrelated boys with concordant clinical features 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.

Material and Methods: We studied three boys with 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 stature (about – 2 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.

Results: 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.

Conclusions: 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 DGE and MLPA in Sotos patients

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Objective: Sotos syndrome is an autosomal dominant overgrowth syndrome 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

SMC(22) using DNA probes corresponding to these intervals.
point mutations of the NSD1 gene are found in most patients, whereas submicroscopic dele-
tions have been found in the majority of Japan-
ese Sotos patients.

The NSD1 gene consists of 23 exons. Point mu-
tations are scattered throughout 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 amplifica-
tion) to identify submicroscopic deletions of NSD1 exons.

Till now there have been 12 patients with sus-
p ected Sotos-syndrome genotyped in our lab-
 oratory. We were able to detect 10 different se-
quence alterations in our ongoing study demon-
strating that DGGE is a reliable technique.

In a 12 year old boy with classical Sotos symp-
toms and a novel nonsense mutation (929X3) was detected. No deletions were found in our patients. Our first data confirm the observation that haploinsuffi-
ciency in caucasian patients is mostly the result of nonsense mutations rather then the result of dele-
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quen...
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 deep 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 patient 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 2770g (< 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-band es 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 et al. (Birth Defects Orig Artic Ser 1979; 15(SB): 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.

PO31
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 patients 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 couple. At birth the propositus was 50 cm (50th centile), OFC 33 cm (3rd centile), head circumference 36 cm (10th centile). Postnatal development was normal, as were FISH 22q11.2 investigations.

Cytogenetic analysis in the propositus was normal, as were FISH 22q11.2 investigations. Cytogenetic analysis was performed on peripheral blood 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, demonstrated 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 hypointensities (T1) seen in MRI caused by TSC2 missense mutation

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Tuberous sclerosis is an underdiagnosed multi-system 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 suggestive diagnosis of Neuf-1 fibromatosis due to multiple hypertense (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 Neuf-1 fibromatosis nor for tuberous sclerosis. Skin biopsy revealed fibromatous naevoid 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 TSC2 genes revealed a c.325G>C (Thr107Ile) 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 aminoacid.

Both parents of the boy were initially unconspicuous 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.

The data presented shed light on the possible connection between heterochromatin localization and repression.
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 cytogenetically characterized by G-banding and further fluorescent in situ hybridisation (FISH) has been performed to narrow the breakpoints to the deletion of the aberrant chromosome 4. After all 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 Bac 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 proximal one could be defined between Bac clones RP11-265M21 and RP11-326N15 and the distal breakpoint between RP11-43SP6 and RP11-1062K20. The phenotypic expression not characterized by G-banding and further fluorescein isothiocyanate (FITC) labeled 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 mediate 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.

P040

A new locus for brachydactyly 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 polymeric 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 mediate 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.

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 positive-ly 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 process.

Conclusions: 1. Unrealistic or missing expecta-tions 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 sup-plemented by open questions it may fail to show the ‘real’ picture and thus to serve as a valuable means of feedback and quality control.

P041

Clinically atypical Brachydactyly A2 with a missense mutation in BMPR1B

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Isolated brachydactyly (BD) are phenotypical-ly and genetically heterogeneous and have been classified into at least 5 different groups A-E (Bell 1951). Subgroups BD-A3 are considered as autosomal dominant traits. Linkage analysis in 2 unrelated German families mapped the BD-locus to 4q21-q25. Mutation analysis of the postional candidate gene BMPR1B identified the first 2 BD-A2 causing missense muta-tions, 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 BD-A2 families, fingers III on both hands instead of fingers II are severely af-fected 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 in stead 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 BD-A2. Mutation analysis identified a transition 1457G>A resulting in a missense mutation R486Q in exon 10 of BMPR1B 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 ap-pears that in our patient the arginine-to-glutamin substitution is related with a slightly different manifestation compared with the arginine-to-glutamin substitution of the other missense mutations within the highly con-served 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 dysmorphism, and mild growth retardation


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Most cases of Neurofibromatosis type 1 result from a point mutation and some represent chromosomal microdeletions at NF1. Patient was born from the 2nd pregnancy to young, healthy, non-consanguinous but endogamic parents (no contact to mutagens, teratogenes). There are no relavates with a similar phenotype. The pregnacy, delivery and newborn period were normal. At the age of 1 year he had an enlarged with elastic microphaty, prominent supraorbital arches, convergent strabismus, long nose, crowded teeth, and retromandibula. She had several large cafe-au-lait spots described at the age of 1 year she showed a coarse face and was not able to speak. At the age of 3 years (first seen by E.S.) she was eurthopic, euartic, and tall (97th percentile) with mild thoracal scoliosis. The face was elongated with relative microcephaly (25th percentile), prominent supraorbital arches, convergent strabismus, long nose, crowded teeth, and retromandibula. She had several large cafe-au-lait spots, numerous pigmented lesions and retinal telangiectasias. She had several large cafe-au-lait spots, numerous pigmented lesions and retinal telangiectasias. She had several large cafe-au-lait spots, numerous pigmented lesions and retinal telangiectasias. She had several large cafe-au-lait spots, numerous pigmented lesions and retinal telangiectasias.
yielded diminished signals: ish del(17) (q11.2q11.23) [p53]+, RH465601, RH71187+dim, RH71187_ -NFI/RH106225, RH70003+ , RH26307_ -RH55147/RH26307+dim). During the introduction of FISH on the 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(7)(q14)dup(7)(q14)(G31934+). 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-Prerger syndrome and other lamin A/C related disorders, some chromosome instability syndromes, Werner syndrome, Wiedemann-Rautenstrauch syndrome, some types of Ehlers-Danlos syndrome, Gerodera osteoysplasium 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 very rare entities. Chromosome analysis revealed a 46,XY, inv(11p12.1q13) 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 palmpaternal 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/Velo-cardio-facial syndrome. The presence of chromosome specific low copy repeats LCRs predispose the region to genomic rearrangement. Eight copies of such LCRs 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 LCR's 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 cosmides 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 of the chromosome. The 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 extend 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 palmpaternal 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 paracetamol, 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 351 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 fluorescent dyes. In total a result in a colour banded chromosome with a greater number of pseudo-colour bands than the number of libraries used. In order to illustrate the advantages and limitations of MCB, we re-investigated ten clinical cases with different aberrations previously identified by conventional banding techniques; these included deletions (three cases), duplications (four cases), inversions (two cases) and complex rearrangement (one case). MCB defined 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 hybridization (FISH) failed to reveal the precise karyotype, MCB was able to resolve the structural aberration in detail. One of these cases was an insertional duplication in the p arm of chromosome 4. Another was a complex rearranged X-chromosome showing a terminal deletion of Xp and an additional duplication of the terminal Xq regions 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, downsloping palpebral fissures, prominent nose, left half of face is more prominent than the right one, head slightly inclined to the right, high arched palate, clindactyly. 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 1½ years and talking at 3½ years. Y is mildly developmentally 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, downsloping 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, hypoplasia, cryptorchidism and mild mental retardation. We will discuss the clinical features of our patient and the two brothers described by Carnevale et al.

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 -4.8Mb 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 inconspicuous 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 chromosone 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 demonstrates the variable phenotypical expression and the difficulties in clinical diagnosis of this syndrome.

P051

Molecular cytogenetic characterisation of a terminal 2q37 microdeletion


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Mutations in the lamin A/C gene (LMNA) lead to a variety of phenotypes including Emery-Dreifuss-muscular dystrophy (EMDM2), limb-girdle muscular dystrophy (LGMD1B), partial lipodystrophy type 2 (FPLD2), and mandibulocutaneous dysplasia type A with partial lipodystrophy (MADA).

In addition, familial dilated cardiomyopathy associated with conduction system disease (CMD1A) and atrial fibrillation has been correlated 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 polyamines” 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 lamin A/C, presumably leading to a protein truncated in the 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 polyamines”.
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)(8;22)(p22.1;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 congenital heart defect (truncus arteriosus communis type I), 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 abdominal auries are reported in both, 9p- and 22q11.2 deletion syndrome. Some distinct anomalies are 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.

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 heterogeneous disorder characterised by prenatal 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, 17r and 17l in FISH analysis using probes derived from Cosmid chromosomes so establishing a refined clone and PAC contig region. According to the mutation analysis of the candidate region for hereditary neurologic 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 ended resulted in localising the breakpoint within 81.5 kb in the 5'-region of SEC14L1.

Corresponding FISH analysis resulted in the identification of a PAC clone RP1-232m11 spanning the breakpoint on chromosome 20 including parts of the Receptor Protein Tyrosine Phosphatase Rho gene (PTPRD) considered to be another SRS candidate gene. In order to establish a precise physical map of the region of interest we located one Xhol fragment of RP1-232m11 distal to the 20q13 breakpoint. Several BanHI 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 break-point region and giving more insight into the genetic background of SRS.
glucosuria occurred. Mild gestational diabetes mellitus was not treated.

Radiographic examination of the affected newborn revealed multiple vertebral segmentation defects, cloverleaf cranial vault, coarctation of the aorta, bilateral club foot deformity and bilateral cubitus valgus. Multicystic renal dysplasia and hydrenephrosis were detected prenatally by ultrasound. Post-mortem examination showed additionally 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 5
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The short-rib-polydactyly syndromes (SRPSs) are a hetrogenous group of skeletal dysplasias characterized by short-limbs dysplasia, narrow hypoplastic thorax with short ribs, polydactyly and associated visceral abnormalities, mainly polycystic kidneys. To date a responsible muta- tion in a defined gene could be identified only for the Ellis-van-Creveld syndrome (EVC). This EVC gene was excluded as causative for other SRPSs, as demonstrated by linkage analysis. In order to further elucidate the genetic background of SRPSs we compared phenotypic features of af- fected individuals with developmental defects of knockout models in mice. Previous studies ana- lyzed the histological changes in endochondral ossification in SRPSs, mentioning mainly a disor- ganization and irregularity of the growth plate in long bones. Our study showed that changes in endo- chondral ossification represent a partly arrested orthotopic ossification at the stage of the hyper- trophic chondroblast and heterotopic pericon- dral ossification with accessory ossicles in the lower leg of one case. Impaired renal development is indicated by polycystic kidney disease associated with dilated collecting tubules and glomerulo- cystic changes in the other case. Excessive polycystadynctyly and median cleft palate was ap- parent in both cases. Due to apparent phenotypi- cally analogies to the knockout mouse model for BMP-7 we excluded impaired expression or muta- tion of this morphogen as a causative factor for SRPSs in the two clinical cases by immunohistochemical methods and analysis of genom- ic sequence.

P058
Clinical and Mutational Spectrum of Mowat-Wilson Syndrome
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Mowat-Wilson syndrome is a recently delineat- ed mental retardation syndrome usually associ- ated with multiple malformations and a recogniz- able facial phenotype caused by defects of the transcriptional repressor ZFHX1B. To address the question of clinical and mutational variabi- lity, we analysed a large number of patients with suspected Mowat-Wilson syndrome (MWS). Without prior knowledge of their mutational sta- tus, 70 patients were classified into “typical MWS”, “ambiguous” and “atypical” groups ac- cording 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 associated with sev- eral mental retardation and variable mal- formations such as Hirschspring disease and congenital heart defects. Our findings indicate that structural eye anomalies such as microoph- thalmia should be considered as part of the MWS spectrum. We also show that agenesis of the corpus callosum and urogenital anomalies (especially hypoplasias) are significant positive predictors of a ZFHX1B defect. Based on our observation of affected siblings and the number of MWS cases previously reported, we associate a recur- rence risk of around 1%. The lack of mis- sense mutations in MWS and MWS-like patients suggests there may be other, as yet unrecog- nised 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 pa- tients with NF1. The two major types of NF1 deletions encompass 1.4- and 1.2-Mb, respec- tively, and have breakpoints in the NF1 LCRs or in the JAZZ 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 preferen- tially occur on the maternal allele, the deletion pattern here affects the paternaly inherited chromosome. Precise breakpoint mapping re- vealed that the proximal deletion breakpoint oc- curred in an L1-element located 1.3-Mb prox- imal to the NF1 gene. The distal breakpoint bound- ary is located in a single copy segment between an AT-rich segment and an AluSx-element in in- tron 15 of the JAZZ1 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 recur- rent, it is conspicuous that one breakpoint is lo- cated in the JAZZ1 gene. Paralogous recombi- nation between the JAZZ1 gene and its pseudo- gene has been described as being responsible for the “atypical” NF1 deletion of 2-Mb. In contrast to 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 preferen- tially occur on the maternal allele, the deletion pattern here affects the paternaly inherited chromosome. Precise breakpoint mapping re- vealed that the proximal deletion breakpoint oc- curred in an L1-element located 1.3-Mb prox- imal to the NF1 gene. The distal breakpoint bound- ary is located in a single copy segment between an AT-rich segment and an AluSx-element in in- tron 15 of the JAZZ1 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 recur- rent, it is conspicuous that one breakpoint is lo- cated in the JAZZ1 gene. Paralogous recombi- nation between the JAZZ1 gene and its pseudo- gene has been described as being responsible for the “atypical” NF1 deletion of 2-Mb. In contrast to

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 dif- ference in metabolic capacity and, therefore, might contribute to therapeutic failure. The vari- ability of the CYP3A4 activity has been de- scribed 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 (c.11375delinsTGT;DelTA- GT). 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 poly- morphisms.

Material and Methods: DNA has been isolated from whole blood or mouth swabs of healthy volunteers. Four allele-specific PCRs were de- veloped which allowed for specific amplification from whole blood or mouth swabs of healthy volunteers. Four allele-specific PCRs were de- veloped which allowed for specific amplification from whole blood or mouth swabs of healthy volunteers. Four allele-specific PCRs were de- veloped which allowed for specific amplification from whole blood or mouth swabs of healthy volunteers. Four allele-specific PCRs were de- veloped which allowed for specific amplification from whole blood or mouth swabs of healthy volunteers. Four allele-specific PCRs were de- veloped which allowed for specific amplification from whole blood or mouth swabs of healthy volunteers. Four allele-specific PCRs were de- veloped which allowed for specific amplification from whole blood or mouth swabs of healthy volunteers. Four allele-specific PCRs were de- veloped which allowed for specific amplification from whole blood or mouth swabs of healthy volunteers. Four allele-specific PCRs were de- veloped which allowed for specific amplification from whole blood or mouth swabs of healthy volunteers. Four allele-specific PCRs were de- veloped which allowed for specific amplification from whole blood or mouth swabs of healthy volunteers. Four allele-specific PCRs were de- veloped which allowed for specific amplification from whole blood or mouth swabs of healthy volunteers. Four allele-specific PCRs were de- veloped which allowed for specific amplification from whole blood or mouth swabs of healthy volunteers. Four allele-specific PCRs were de- veloped which allowed for specific amplification from whole blood or mouth swabs of healthy volunteers. Four allele-specific PCRs were de- veloped which allowed for specific amplification from whole blood or mouth swabs of healthy volunteers. Four allele-specific PCRs were de- veloped which allowed for specific amplification from whole blood or mouth swabs of healthy volunteers. Four allele-specific PCRs were de- veloped which allowed for specific amplification from whole blood or mouth swabs of healthy volunteers. Four allele-specific PCRs were de- veloped which allowed for specific amplification from whole blood or mouth swabs of healthy volunteers. Four allele-specific PCRs were de- veloped which allowed for specific amplification from whole blood or mouth swabs of healthy volunteers. Four allele-specific PCRs were de- veloped which allowed for specific amplification from whole blood or mouth swabs of healthy volunteers. Four allele-specific PCRs were de- veloped which allowed for specific amplification from whole blood or mouth swabs of healthy volunteers. Four allele-specific PCRs were de- veloped which allowed for specific amplification from whole blood or mouth swabs of healthy volunteers. Four allele-specific PCRs were de- veloped which allowed for specific amplification from whole...
ed positive for the mutant allele (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.

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 heterogeneous group of rare genetic diseases with patients located all across Germany. Due to the rare occurrence 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.net, providing access to the SKELNET member 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 a 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 muscular hypotonia, 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 1q24.1. A CCG-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. The missense mutation has not been seen in > 600 sequenced chromosomes of control.

The myotonic dystrophies (DM) subdivided into DM1 and DM2/PRO1 share common pathogenic mechanisms and result in abnormal RNA splicing. DM1 women face a considerable risk in pregnancy which can partly be attributed to congenital 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 DM1 for a retrospective evaluation of their obstetric history. The participants complet-
ed 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 parases, myalgia, myotonia and cata
caracts. Seven women (21%) 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 remaining 68 women, 5 of whom were multiparous, 36 (53%) had a normal pregnancy, 14 (21%) had complicated by fetal loss, prematurity or in
terupted pregnancies, 4 (6%) had a pregnancy loss did not reach term, 1 was twins and 1 was triplet pregnancy. Of these, 7 deliveries resulted in premature births and 36 deliveries resulted in normal births. The obtained results indicated on relatively low prevalence of DM2 in Polish gestational dia
betic patients. This work is supported by Ministry of Sciences and Informatics grant no P0PO5E9326.

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 these 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 AQP4 for 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 chro
matography method and sequencing, we found novel mutations in the PMP22, MPZ and Cx32 genes. These mutations were confirmed by co-
segregation 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/PRO1 share common pathog
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genital 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.

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caracts. Seven women (21%) 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 remaining 68 women, 5 of whom were multiparous, 36 (53%) had a normal pregnancy, 14 (21%) had complicated by fetal loss, prematurity or in
terupted pregnancies, 4 (6%) had a pregnancy loss did not reach term, 1 was twins and 1 was triplet pregnancy. Of these, 7 deliveries resulted in premature births and 36 deliveries resulted in normal births. The obtained results indicated on relatively low prevalence of DM2 in Polish gestational dia
btic patients. This work is supported by Ministry of Sciences and Informatics grant no P0PO5E9326.

P067
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 secre
ion. Glucokinase-related MODY 2, autosomal dominant disorder, is detected in children with mild hyperglycemia and in women with gesta
tional diabetes and positive family history. Glu
cokinase mutations resulted in reduction of the beta cells sensitivity to glucose and in distur
bance of glycolysis synthesis in the liver. The aim of this study was to estimate the prevalence 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 glu
cose tolerant test and a family history of type II or gestational diabetes in a first or second-de
gree relatives. The analysis involved the coding regions of the 12 exons and the intron-exons boundaries of the GCK gene, which were ampli
died by PCR and electrophoresed on gycerol-containing free denaturing polyacrylamide gel at room temperature or 4°C. Abnormal fragment PCR products were se
quenced directly using a dyeoxyribonucleotide-cy
cle 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 previous
ly unreported intronic variants: IVS2-12C>T, IVS3-8G>A, IVS7-13A>G have been identified. The obtained results indicated on relatively low prevalence of MODY 2 in Polish gestational dia
btic patients. This work is supported by Ministry of Sciences and Informatics grant no P0PO5E9326.

P03 Genetic Epidemiology

Die Analyse des Oberflächen-EGKs in Familien einer Isolatpopulation offenbart hohe Heritabilitäten
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The signature of Oberflächen-EGKs (QT-, ORS-Intervall etc.) widerspiegeln den Ablauf der kardialen Elektrophysiologie in physiologischen und pathologischen Zuständen. Die Bedeutung einzelner Signatur für die nichtinvasive Risikostratifizierung ist, nach anfänglichen Über
bewertungen, in den letzten Jahren genauer ver
standen und definiert worden, z.B. der prädik
ptive Aussagegehalt der QRS-Verbreiterung für Tachycardien und Mortalität in der MUSTT-Studie (Zimbaum et al., 2004). Viele dieser Signu
are weisen neben bekannten exogenen Ein
flussfaktoren hohe Heritabilitäten auf. Um zugrun
delegenden Gene zu identifizieren und Genvari
anten auf ihre potentielle Bedeutung als genetis
che Risikomarker zu testen, sind Familienstudi
en von zentraler Bedeutung. Wir haben in einer familienbasierten Studie mit 500 Personen die Heritabilitäten vieler dieser Signatur, darunter auch innovativer Signatur aus dem signalver
stärkten EKG mit Hilfe der Varianzkomponenten

Abstracts
We will give a demonstration of the software by come an important tool in clinical genetics. particularly suitable for clinical application where providing all of its features offline, CGHPRO is simplify the identification of "weird" clones. By the definition of shortest regions of overlap and simultaneously comparative analysis of different tial data in a back-end database allows the si- mosomal breakpoints. The storage of all essen- algorithms facilitate objective detection of chro- nation and unsupervised Hidden Markov Model al- ments. The exponential growth of array CGH (array CGH) is a high resolution screening tool for genomic 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 versa- tile 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 im- age analysis data formats are covered by the im- port 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 ex- plore the characteristics of each clone, such as the involvement of the clones sequence in seg- mental duplications. Circular Binary Segmenta- tions and recompiled runtimes for the linkage programs under a Windows environment easyLINKAGE boosts the use of most known linkage programs can be set interactively. The program generates input files for pedigree/haplotype drawing software such as HaploPainter. easyLINKAGE provided the program PedCheck, which does an automated Mendel check prior performing linkage analyses. To run the program, the user must provide mark- er files containing subject IDs and genotypes; a pedigree structure information file, and a mark- er database with chromosomal positions. Geno- types 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 position on either autosomes or the X chromosome. All options for the cov- ered 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 au- dience. All windows binaries including instruc- tions and recompiled runtimes for the linkage programs can be found at http://www.uni- wuerzburg.de/nephrologie/molecular_genetics/download.htm.

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 tradition- ally 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 out- puts were further limits of those programs. Nowadays, scientists prefer to perform their 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 perform- ance 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 postprocess plots of LOD scores, NPL scores, P values and other parameters. The program generates input files for pedigree/haplotype drawing software such as HaploPainter. easyLINKAGE provided the program PedCheck, which does an automated Mendel check prior performing linkage analyses. To run the program, the user must provide mark- er files containing subject IDs and genotypes; a pedigree structure information file, and a mark- er database with chromosomal positions. Geno- types 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 position on either autosomes or the X chromosome. All options for the cov- ered 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 au- dience. All windows binaries including instruc- tions 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 Himmelbauer H.(1), Wurst W.(2)

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Himmelbauer H.

The sequencing of the genomes of man and mouse has led 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 pro- duced 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 muta- genesis, or be gene targeting. Chemical muta- genesis of mouse embryonic stem (ES) cells combines the random approach with gene target- ing. The exposure of ES cells to a mutagen (e.g. ethynitrosourea; ENU), randomly intro- duces mutations in the genome of ES cells. Dur- ing NGFN1, we have established libraries of chemically mutagenised ES cells. Currently, li- braries of 40.000 clones in pools (MPI) and 10,000 single clones (GSP) are available. In par- allel, we have developed and optimised screening- strategies to retrieve mutant clones harbour- ing specific gene defects from our libraries. Screening of ES cell libraries is carried out using DNA-based single-base resolution approaches. 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 pro- tein are affected. The clones that contain inter- esting mutations within the gene under study can be used to generate a mouse mutant. Li- braries of chemically mutagenised ES cells are therefore valuable tools for the analysis of genes, and in particular so in a biomedical con- text.

P072

Analysis of a conserved long-range sox9 notochord enhancer in zebrafish Heid U.(1), Rastegar S.(2), Dohrmann U.(1), Strähle U.(1), Scherer G.(1)

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Heterozygous de novo mutations in and around the human SOX9 gene on 17q cause campomel- onia (CD; MIM 184250), 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 chromoso- some rearrangements in the vicinity of SOX9. The large 5’ intergenic region of SOX9 contains several sequence elements highly conserved be- tween human, mouse, and Fugu (Bagheri-Fam et al. 2001). In CD translocation and inversion cases, some or all of these elements are sepa- rated from SOX9. A 516 bp fragment containing the 130 bp conserved element E1, located 28 kb 5’to human SOX9, directs expression of a re- porter 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 sys- tem. Because of a large-scale genome screen- ing event in ray fin fish phylogeny, zebrafish has two soxx homologues, termed soxxa and sox9b, both of which contain the conserved element E1. We as- sayed the regulatory potential of the 130 bp core sequence of the soxxa E1 element, located 7.8 kb 5’ to the gene, in transient transgenic ze- brafish embryos, using a gfp reporter gene driv- en by the sonic hedgehog (shh) promoter. We

P04 Functional Genomics, New Technologies

CGHPRO - A software tool for array CGH
data analysis

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P069

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 versa- tile 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 im- age analysis data formats are covered by the im- port 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 interac- tive graphical interface offers the chance to ex- plore the characteristics of each clone, such as the involvement of the clones sequence in seg- mental duplications. Circular Binary Segmenta- tion and unsupervised Hidden Markov Model al- gorithms facilitate objective detection of chro- mosomal breakpoints. The storage of all essen- tial data in a back-end database allows the si- multaneously 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 be- come an important tool in clinical genetics. We will give a demonstration of the software by analyzing a male-to-female co-hybridisation onto a 14000 BAC array.
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 (HntDB). Mutations in and around this binding site cause a simultaneous loss of notochord and floor plate expression, indicating that FoxA may 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 Koebele 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 protein 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 leukemia virus (ALV-A) fused to various endostatin domains 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-trg2, 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 bond TVA-Endostatins can also mediate ALV-A entry, cells are infected with ALV-A virus. In untransduced cells the fluorescence signal is low. Approximately 72 hours after viral challenge, the cells are either visualized under an in vivo microscope or isolated by flow cytometry. From these experiments, 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.

**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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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 assay, 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 the development of novel therapies in the treatment of sight-threatening complications such as choroidal neovascularization.

**P075**

Extensive amplification of exon 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 cloning we have identified two new isoforms 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 non-syntenic human centromeric 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.
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 C1-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 proteins associate 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 other family members. Subsequent analyses of mutant bestrophin species will address a possible influence of the mutations on functional subunit assembly.

Transgenic studies on physiological function of testicular insulin


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Transgenic studies in our group revealed that the Leydig insulin-like hormone (InsI3) produced in pancreatic β-cells is able to restore the cryptorchidism phenotype in InsI3-deficient mice. These results demonstrate regulated secretory mechanisms of processed proinsI3 to mature InsI3 by proteolytic cleavage of C-peptide in β-cells of the pancreas. In this report, we investigate the efficiency of testicular Leydig cells to secrete mature insulin and the functional consequence 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 InsI3 promoter (I3I2). Northern blot analysis showed that more than ten times insulin mRNA is present in testis than in pancreas of transgenic mice. Immunohistochemical 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 Pdx4-deficient mice resulting from the developmental impairment of β-cells, we introduced the I3I2-transgenic allele in the genome of Pdx4/- mice. Analyses of I3I2: Pdx4 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 as well as that 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.

The forkhead transcription factor Foxi1 regulates AE4 expression


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As promoters 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 β-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 more than 100-fold activation in a cell-based reporter–gene assay. Transcription 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.

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, oligonucleotides, 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 imbalances (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 focuses 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 investigat-
ed.

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) 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 sensiti-
vity 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%.

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 multi-step 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 diagnostics.

In our study, we comparatively analysed microdissected normal and tumorous colorectal ep-
ithelium by ProteinChip technology to detect and localize proteins specific for the tumor di-
rectly in the tissue which would certainly be of a very low concentration in serum. By this ap-
proach 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 epitheli-
um, which could also be confirmed by immuno-

histochemistry (IHC). Detection of these pep-
tides 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 suc-
cessful strategy in this study. So we can con-
clude that these promising markers would not have been found in serum without the informa-
tion gained through the analysis of microdissect-
ed tissue by ProteinChip technology.

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P084

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


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Evaluation of an unclassified variant of the BRCA1-Genes in a 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 Segregationsana-
lysen durch.

Methoden: Zur Mutationssuche in den Genen BRCA1 und BRCA2 erfolgte ein Vorscreening mittels DHPLC-Methode mit anschließender di-
rekter Sequenzierung der auffälligen PCR-Frag-
mente. Blutproben wurden von der Indexpati-
entin, ihrer ebenfalls erkrankten Tochter und ein-
er nicht erkrankten, erstgradigen Verwandten mit fortgeschrittenem Lebensalter zur Durchführung einer Segregationsanalyse abgenommen. Es standen zusätzlich Paraaffinblöcke von Tu-
morgewebe zweier Mammakarzinome und eines Ovarialkarzinoms zur Verfügung. Im Anschluss an die DNA-Präparation wurde die LOH-Analyse mittels Fragmentanalyse durchgeführt.

Ergebnisse: Die Segregationsanalyse zeigte das Voreignen der unklassifizierten Variante bei beiden der an Mamma- und/o oder Ovarialkarzinom erkrankten Frauen, während bei der gesunden, 76-jährigen Verwandten die Genveränderung nicht nachweisbar war. Das Ergebnis der LOH-
Reveals HOX Gene Deregulation in AML and ALL; Gene Expression Profiling

These results emphasize the significance of DICE1 promoter region in prostate cancer cells. We have analyzed samples from 12 patients with different types of leukemia and a (10;11) translocation suggesting a CALM/AF10-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 108 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 HOX4, HOX5, HOX7, HOX9, and HOX10. 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 (e.g. HOX gene deregulation) for these diverse leukemias.

Process of genetic diagnostics at cancer patients - analysis of medical records

Purpose: A number of 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 diagnosis (molecular analysis). The detection of a mutation at a diseased person (diagnostic testing) is on the one hand a pre-condition for offering special surveillance for this person and on the other hand a good pre-condition for genetic diagnostic in relatives (predictive testing). Therefore purpose of investigation was to describe the implementation of gene 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-reactant method of measuring variance was conducted – a retrospective analysis of medical records. In doing so existing medical records from cancer patients that requested a human geneticist between 2002 and 2004 in two settings were analysed. So the total sample (n=96) consist 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 sample sub (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 cases of colorectal cancer (FAP and HNPCC) and breast cancer. Further investigations are required e.g. in order to derive appropriate modes of cooperation between primary care physicians and human geneticists.

Molecular-cytogenetic analysis of two cases of leiomyosarcoma

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 a small number of patients 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 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 different 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 withIGH/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;q2), 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 interacts with CALM

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The balanced t(10;11)(p13;q14) translocation results in the CALM/FHL2 fusion transcript. This translocation is found in 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.

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 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 heterozygosity (LOH) analysis have revealed that mammary 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 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. The proto-oncogene PTEN (protein tyrosine phosphatase type 1A) and PPP2R3B are both involved in the PTEN/PI3K/AKT signaling cascade. PTEN is involved in apoptosis and growth control, while PPP2R3B is a member of the PP2A (protein phosphatase 2A) family and a putative tumor suppressor gene. PTEN has been shown to act as a tumor suppressor, and several of its mutant 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. Se- quencing 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 tumors.

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Methylation profiling of transforming growth factor beta (TGF-β) receptor genes by methylation specific PCR (MSP) has demonstrated that the aberrant methylation of these genes might represent a marker for the histological subtype in thyroid tumors. Our results showed a significant higher methylation rate compared to normal thyroid tissue. We assessed the methylation of 17 cancer-related genes by MSP in nine thyroid cancer cell lines. 12 of 17 genes (RASSF1A, p16INK4A, TSH, MGMT, DAPK, EEA, ERB, RBT, PTEN, CD26, SLC5A8 and UCHL1) showed significant 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 FTC, UTC and 1 poorly differentiated thyroid cancer PTC). The most frequently methylated genes were: RASSF1A (77,8%), p16INK4A (75,7%), TSH (57,9%), MGMT (46,2%), DAPK (38,5%), EEA (38,5%), ERB (35,3%), RBT (29,1%), PTEN (16,2%), CD26 (12,2%), SLC5A8 (10,5%) and UCHL1 (7,8%). Overall, we found a high variability in the methylation pattern of these genes in the examined thyroid cancers. The significant higher methylation rate compared to normal thyroid tissue was found in papillary FTC (80,0%) and follicular FTC (65,9%) compared to follicular adenoma (58,3%) and normal thyroid tissue (47,6%). Our results suggest a significant correlation between the aberrant methylation of these genes and the histological subtype of thyroid tumors. Further studies should be performed to determine the clinical significance of this aberrant methylation pattern.
Identification of a novel germline mutation of the MEN1 gene in a family with MEN1

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Abstract: 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 transsphe- roidal adenectomy was performed four years ago (choromaphic 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 presympto- mated gene carriers. Periodic screening should be carried out for endocrine tumor manifesta- tion, as we do with the son of the patient, for whom pHTP was diagnosed in the meantime.

P096

Different molecular pathways in the development of pericuocular sebaceous gland carcinomas

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Pericuocular sebaceous gland carcinomas (SGC) are rare malignant skin tumors occurring sporadically or as a phenotypic feature of the Muir-Torre syndrome (CMMR18320).

The fragile his- tone triad genes, encompassing the most active common human chromosomal fragile region, FRA3B, was proposed as a tumor suppres- sor gene for many human cancers. Fhit deficient knockout mice develop Muir-Torre-like symp- toms including SGC. In the current study, peri- ocular sebaceous gland carcinoma obtained from three patients (male, *1944; male *1960; male *1969) were analyzed for Fhit, hMSH2, hMLH1 and hMSH6 expression by immuno- chemistry (IHC). Polymerase chain reaction (PCR)-based analysis of the markers BAT25, BAT26, BAT40, D2S123, DSS346 and D17S250 was performed for microsatellite instability (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 FHT as revealed by IHC, demonstrated micro- satellite stability (MSS). To explore the mecha- nism responsible for loss of FHT, we studied loss of heterozygosity (LOH) at 3p14.2 by utiliza- tion of a panel of intragenic FHT specific CA- markers, genomic multiplex PCRs for combina- tion specific primers to perform a highly sensitive real- time-PCR analysis also for this molecular aberration. We identified nine patients (8%) with FHT-TKD mu- tations (5 pts. D835V, 3 pts. D835H, 1 pt. DelI836), while none of the 122 patients we investi- gated had a FHT-V592A mutation. Screen- ing for FHT-TKD mutations with fluorescent probes is equivalent with conventional screen- ing using standard PCR followed by EcoRV re- striction. We present a real-time PCR protocol that can be used for MRD analy- ses based on individual FHT-TKD mutations. Ex- amples for MRD analyses are presented for all three subtypes of FHT-TKD mutations that were identified in this study. In summary, we demon- strate new methodological approaches for rap- id screening of FHT point mutations as well as the detection of MRD based on patient specific FHT-TKD mutations.

P097

Genetic 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 ER alpha was investigated in 84 primary soft tissue sarcomas (STSs), including 22 liposarcomas, 18 malignant fibrous histi- ocytomas (MFHs), 18 leiomyosarcomas, 6 hab- domyosarcomas, 6 neurogenic sarcomas and several other sarcoma entities. RASSF1A hyper- methylation 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 li- posarcoma the methylation frequency was high- er (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 ana- lysed 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 alter- ation of K-ras was detected. The samples have been analysed for the occurrence of the RASSF1A polymorphism 133 (GQT-TCT), 8 out of 69 soft tissue samples showed this base change. Interestingly this substitution was de- tected more frequently in liposarcomas (4 out of 15; 26.7 %) compared to leiomyosarcomas (1 out of 16; 6.3 %). In an univariate Cox propor- tional-hazards regression model, we found that the risk of a tumor-related death for STS pa- tients with methylated RASSF1A was significant- ly 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 Sach- sen-Anhalt and DFG.
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 standardized 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.

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


Objective: 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 Familienbreast 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, and 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 186-bp of perfect base pair matches (del exon 1 -2, del exon 5) while the breakpoint regions of the exons 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.

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: MLH1 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 7. We have characterized 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 minigene 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, possibly working in concert with other MMR genes. In this context, we analyzed the involvement of CAV-1 in familial and non-familial prostate cancer. 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 intron- and four single nucleotide polymorphisms. Resulting haplotypes were tested for association using age at diagnosis, tumor grade, TNM stage, and response to gefitinib. 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

HNPPC based on MSH6 mutations is characterized by late age of disease onset and lower incidence of colorectal cancer compared to MLH1 or MSH2 mutations: The German HNPPC-Consortium
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3) Medical Genetics, University of Munich
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7) Molecular Pathology, University of Heidelberg
8) Pathology, Klinikum Kassel

Results: HNPPC cases were identified in 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-HNPPC-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 HNPPC. 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. This work was supported by the Verbundprojekt "Familiärer Darmkrebs" of the Deutsche Krebsshilfe.

P104

Matrix-CGH enables the identification of genomic imbalances in childhood MDS with monosomy 7
Meeting of the German HNPPC-Consortium
1) Surgical Research, University of Dresden
2) Institute for Medical Informatics, Statistics and Epidemiology, University of Leipzig

Objectives: MDS is believed to originate from a hematopoietic stem cell, but it is not known which myeloïd progenitor is targeted by 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 was to analyse genomic abnormalities in addition to monosomy 7 in a first with the monosomy 7 solely, a second with the monosomy 7 plus +18, +21, +22, +1p which were also detected by cytogenetics and a third group displaying several genomic abnormalities in addition to monosomy 7. Results: 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 different etiology of childhood MDS and have to be correlated with clinical data.

Project funded by the BMBF Contract N01GM0307

P105

EGFR mutations in head and neck cancer
1) Medical University Innsbruck, Department of Internal Medicine, Innsbruck
2) Medical University Innsbruck, Department of Medical Biology and Human Genetics, Innsbruck
3) Medical University Innsbruck, Institute of Pathology, Innsbruck

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) [Lynch T., et al., N Engl J Med 350, 2004; Paez J.G., et al., Science 304:1497-1500, 2004]. Inhibition of activated tyrosine kinases with target- ed small molecule drugs has emerged as an ef- fective approach to cancer therapy. It was also observed in about 10% of head and neck squa- mous cell carcinoma (HNSCC) that gefitinib can lead to a rapid and often dramatic clinical re- sponse [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 [Grands CR, Can- cer 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 includ- ed. The DNA was extracted from paraffin em- bedded tissue samples. PCR and sequencing was performed with primers already described in [Lynch T., et al., N Engl J Med 350, 2004].

Results: None of the already published mutations described to be associated with a re- sponse to gefitinib had been detected in this co-hort 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 exon 18 (IVS18-2/4delG) was ob- served. In exon 21 R836R occurred with 0.2 allele frequency. In one patient a 1 bp deletion in intron 18 (IVS18-2/4delG) was ob- served.

Conclusions: In contrast to non-small cell lung cancer activating EGFR mutations were not found in head and neck squamous cell carci- noma 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 cy- togenetics and a third group displaying several genomic abnormalities in addition to monosomy 7. Interestingly, the highly genomic instability ob- served in the third group was more extensive in MNC compared to granulocytes.
ma. The relevance of the intron 18 mutation has to be determined. Other molecular mechanisms than EGFRT-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 resistance 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 of three of the resistant cell line and is potentially linked to the drug-resistance of KCL22 R1. Protein identification by trypsin digestion in currently in progress to get closer to the function of the 19 kDa protein in the mechanism of drug-resistance to Imatinib.

This work is supported by the IZKF Jena and the BMBF.

P107
Mutation screen and association study of the EZH2 gene in PCa patients
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2) University of Ulm, Department of Human Genetics, Ulm

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 resistant and sensitive 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 this 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 technology
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2) Fukushima Medical College, Fukushima
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7) University of Ulm, Ulm

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 (Granata-519, NCEB1, JeKo-1, Rec-1, SP-53 and HBL-519) 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 4p21.3, 2p11.2, 2q35.1-2q35.2, 1p21.1-p31 in 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 13;1827-37). Up to now, losses of 2p11.2 (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
Haplootype-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 haploype-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 57% 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 chromophobe renal cell carcinomas
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2) Memorec biotech GmbH, Mediacal Molecular Research Cologne, Cologne

Gene expression analysis of clear cell and chromophobe renal cell carcinomas
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 analyses. 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%), DBC1 (18%), p16 (66%), P16(14)F1A (71%) and EGF-like domain 7 (56%), but methylation was rarely observed for MLTI1 (18%), DBC1 (18%), P16 (10%). In primary lung tumor, methylation of SOX18 (100%), CD105 (69%), SEMA2 (93%), SLIT2 (100%), TIMP3 (13%), TIMP4 (84%), DBC1 (61%), p16 (41%), P16(14)F1A (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.

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

P113

Detection of translocations and amplifications of the MLT/MALT1 gene in Non-Hodgkin’s Lymphoma using FISH

Murga Penas E(1), Becker C(2), Ott G(3), Harder L(2), Lampe W(1), Hinz K(1), Zickov T(1), R ser K(4), Copie-Bergman C(5), Pott C(6), Copi M(7), Ailhaud G(8), Hansmann M(9), M Ier P(10), M Ier-Hermelin H(3), Stein H(11), Gaulard P(5), L ning T(4), Parwaresch R(12), Possfeld D(1), Siebert R(2), Diehlmann J(1)

1) University Hospital Hamburg-Eppendorf, Department of Oncology and Hematology, Hamburg
2) University Hospital Kiel, Institute of Human Genetics, Kiel
3) University of Wuerzburg, Department of Pathology, Wuerzburg
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The t(1;18)(q21;q21) and the t(14;18)(q22;q11) 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 (PAG 117BS and 597N 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 lymphomas (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 AP2 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); these translocations and/or 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 (8;14)-positive high grade Non-Hodgkin’s Lymphomas

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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, is downregulated in breast cancer cell lines. Analysis of 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.

AKAP12/Gravin is down regulated in breast cancer

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2) Signature diagnostics AG, Potsdam

We identified AKAP12 (A-kinase anchoring protein) as a candidate tumor suppressor gene in breast cancer. AKAP12, also known as gravin, is downregulated in breast cancer cell lines. Analysis of 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.

P111

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 chromophobe 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 chromophobe RCC. We found DNA methylation at a total of 341 genes, predominantly at CpG islands in the hot spot region of loss of heterozygosity (LOH) in breast cancer. AKAP12 is also expressed at low levels in normal kidney tissue. Although the results are pointing towards possible marker genes for diagnostic purposes, more samples are needed to validate the study.

This work was supported by the BMBF and the IZKF, Jena.
Frequent loss of differential methylation at malformities and cryptic translocations, which are the aberrations of 17p (4 cases, 21%) and 6q (3 cases, 42%), as well as deletions and structural duplications (5 cases, 26%) and structural aberrations. The most frequent secondary abnormalities were der(14)t(X;14)(p11;q32).

The translocation t(11;18) distinguishes two groups of aggressive lymphoma with different IG VH and BCL6 mutational status. Furthermore, in 75% of diffuse large B-cell lymphomas (DLBCL), 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 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 hypomethylation as well as unmethylated 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 neuroblastosomas 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.

The translocations t(6;18;11)(q24;q21;21) and t(11;14;18)(q21;q32) represent two novel variant translocations of the t(11;18)(q21;q21) associated with extranodal MALT lymphomas. The translocation t(11;18)(q21;q21) is frequently observed in both primary tumors and in tumors with retention of heterozygosity of 1p. 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 hypomethylation as well as unmethylated 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.

The translocation t(8;14) distinguishes two groups of aggressive lymphoma with different IG VH and BCL6 mutational status. Furthermore, in 75% of diffuse large B-cell lymphomas (DLBCL), 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 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 hypomethylation as well as unmethylated 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.

The translocation t(6;18;11)(q24;q21;21) and t(11;14;18)(q21;q32) represent two novel variant translocations of the t(11;18)(q21;q21) associated with extranodal MALT lymphomas. The translocation t(11;18)(q21;q21) is frequently observed in both primary tumors and in tumors with retention of heterozygosity of 1p. 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 hypomethylation as well as unmethylated 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.

Frequent loss of differential methylation at the imprinted ARHI locus in uveal melanoma Geschmick M.1), Müseler T.1), Eggert A.2), Narechania G.3), Horshtemeke B.1), Lohmann D.1)

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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 1q21 and the MLT gene on 18q21 resulting in a activation of NF-κB. Recently, we reported the first variant translocation of the t(11;18), a t(11;12;18)(q21;q13;p21) 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 novel variant translocations of the t(11;18), the t(6;18;11)(q24;q21;21) and t(11;14;18)(q21;q32);21), occurring in MALT lymphomas of the stomach and the lung, respectively. By conventional cytogenetic analysis revealed: 1.46.XXY(6;18;11)(q24;q21;21)[7], II.46.idem.del(1)(q43)[10], III.46.XY[3] in the first case, and 46.XY[11], t(8;14)(q21;q32)[23] in the second case. In all cases, fluorescence in situ hybridization (FISH) with API2 (PAC166G16) and MLT (PAC5397) specific probes showed API2-MLT fusion encoded on the der(11) as in API2-MLT fusion and that due to an addition-
ent VH and BCL6 mutation status independent from the histologic subtype. Ongoing studies in- vestigate whether hypermutation patterns can also identify different subgroups within the (8;14) positive lymphomas.

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P118

Amplification of the MLT/MALT1 gene in a hrs(18) occurring in a case of diffuse large B-cell lymphoma of the tongue involving 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 a pathogenetic mechanism in non-Hodgkin’s lymphomas (NHL). Amplifications in 18q21 frequently involve the BCL2 gene, which lies about 5 Mbp 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 1989, this 60-year-old male presented with tonsil enlargement without other organomegalias and normal levels of lactate dehydrogenase. Bone marow 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 cytochemically characterized by a complex karyotype including a homogeneous staining region in chromosome 18p (hrs(18)(p11)). The karyotype was described as follows: 47,XY,add(1)(p35-36),add(2)(p21),add(4)(q25-26),del(6)(q12),11q23(+)x2,-8,-12,del(13)(q11-21),13p11(+)x1,+3mar[20]. The karyotype was described as 12,del(13)(q21q25)x2,-8,-36),add(2)(p21),add(4)(q25-26),del(6)(q12),11q23(+)x2,-8,-12,del(13)(q11-21),13p11(+)x1,+3mar[20]. The karyotype was described as:

Results:

P119

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

1) The German HNPPC-Consortium(10)
2) Dresden University of Technology, Department of Surgical Research, Dresden
3) University of Leipzig, Institute of Medical Informatics, Statistics and Epidemiology, Leipzig
4) University Hospital Bonn, Institute of Human Genetics, Bonn
5) University of Heidelberg, Institute of Molecular Pathology, Heidelberg
6) University of Munich, Department of Medical Genetics, Munich
7) Heinrich-Heine-University Duesseldorf, Department of Surgery, Duesseldorf
8) Ruhr University Bochum, Medical Department, Knappschaftskrankenhaus, Bochum
9) Klinikum Kassel, Institute of Pathology, Kassel
10) Deutsche Krebshilfe, German Cancer Aid, Germany

Background: The polymorphic variants at codon 72 of the p53 gene were shown to be strongly associated with the age of onset of sporadic colorectal cancer. In addition, the arginine (arg) variant induces apoptosis more efficiently than the proline (pro) variant. Due to the evidence that the DNA mismatch repair system and the MLT gene were shown to be involved in maintaining genomic integrity, we hypothesized that the codon 72 variation may influence the age of onset of disease in HNPPCC 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 254 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 HNPPCC.

P120

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

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2) P.A.L.M. Microlaser Technologies AG, Bernried

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 (ca. 1cm²) from tumor 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 PSSII 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 β-Catenin mutations. We have analysed 106 tumors for β-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 β-Catenin protein (Thr41 and Ser45) and His36 is next to Ser37, another phosphorylation site in the same 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 β-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 B-Catenin mutations. Both mutations were simultaneously present in 15, i.e. 88% of the tumors with WT1 mutations also had a B-Catenin mutation. This extends and confirms a previous observation of a significant correlation of WT1 and B-Catenin mutations, suggesting that the presence of both mutations is important for the development of a specific subtype of Wilms tumors. B-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 B-Catenin mutations of the blastemal type had a higher expression of B-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 nucleolated areas was found in limited areas of the tumor. Activation of the wnt signaling pathway by mutations in B-catenin does not result in a nuclear localisation of this protein in all cells.

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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2) University of Kiel, Institute of Pediatric Pathology, Kiel
3) University of Heidelberg, Children's Hospital, Heidelberg

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.

P124

Fusion of the H4/D10S170 to the PDGFIR gene in a patient with chronic myelomonocytic leukemia (CMLM) and responsiveness to Imatinib


1) University Hospital Hamburg-Eppendorf, Department of Oncology and Hematology, Hamburg, Germany

Rearrangements affecting band 12p13 are recurring aberrations in hematologic 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 cosmids 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)(p)12 and iso(12)(p)q28; p11), respectively. Only 2 of the cytogenetically described t(3;12)(p21; p13), t(4;12)(p12; p13), and t(5;12)(p13; p12). 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, 2q73, and 17q25. Summarizing cytogenetic and FISH results, the revised partial karyotypes were determined as follows: inv(12)(p13q24), previously del(12)(p13); t(12;7)(q33;p13), previously del(12)(p12); and del(3)(12)(p21;p13), respectively. Furthermore, a translocation occurred in patients with acute myeloid leukemia and the breakpoints within ETV6 were located as follows: 12q24 in intron 1, 2q73q3 distal to exon 5, and 1q25 distal to 2. The molecular cloning of these translocations is in progress.

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P126 Improving for-client letters in routine genetic counseling

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Genetic counseling should help clients to arrive at well-reasoned 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 tape-recording of the counseling session. Standard and enriched letters were randomly and double-blindly 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 maintained continuity and coherence of the genetic counseling. They 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 relatively 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.

P127 Clinical manifestation of non-hereditary retinoblastoma is influenced by a parent-of-origin effect in a subset of patients

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Breast cancer occurs at a relatively high frequency 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 occurs 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, J) 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 genetic and expression level in our collective of sporadic breast tumors. In this report we present the results of the mutational screening in FANCE, FANCL and FANCB genes. 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 Investigation of alterations in fanconi anemia genes FANCE, FANCL and FANCB in human breast cancer.

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1) University of Hamburg, Institute of Human Genetics, Hamburg

Breast cancer occurs at a relatively high frequency 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 occurs 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,J) 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 genetic and expression level in our collective of sporadic breast tumors. In this report we present the results of the mutational screening in FANCE, FANCL and FANCB genes. 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.

P129 Exhaustive mutation analysis of RNASEL in a hereditary prostate cancer family with high linkage to 1q25

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2) University of Ulm, Department of Urology, Ulm

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 peptidase sequence, (2) splice pattern of (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 SNP 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 Deletious germline mutations of the MSR1 gene in prostate cancer families from Germany


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2) University of Ulm, Department of Urology, Ulm

The short arm of chromosom 8 was indicated by linkage analyses to harbour a gene predisposing to hereditary prostate cancer. Germline mutations were found subsequently, in the gene encoding the Macrophage Scavenger Receptor MSR1.
Genexpressions-Profile zur Prognose-
controls (n = 210) is in process, and will help to further deleterious variants of MSR1 apart from our knowledge, our study is the first to provide H235Y, P286S, P392R, A398G and K430R. To two further deleterious germline mutations were reading frame. In the ongoing screening we re-
used in the present study in order to (1) clarify, if 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 suscept-
ability 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 screening we re-
covered the known R293X variation in two of our familial prostate cancer cases. Moreover, two further deleterious germline mutations were identified each in a single family: a nonsense mutation 84X, and a splice site mutation IVS8- 1. Rare missense mutations include the variants H235Y, P286S, P392R and A398G. To date however, the role of MSR1, as of other suggested hereditary evidence for linkage. To date however, the role of MSR1, as of other suggested hereditary evidence for linkage.

University, Department of Medicine, Bochum 3) University of Leipzig, Institute of Medical Informatics, Statistics and Epidemiology, Leipzig 4) Ludwig-Maximilian-University, Institute of Human Genetics, Munich 5) University Hospital, Institute of Human Genetics, Bonn 6) Dresden University of Technology, Department of Surgical Research, Dresden 7) University of Heidelberg, Department of Molecular Pathology, Heidelberg 8) Ruhr-University, Institute of Human Genetics, Bochum 9) Technical University, Department of Surgery, Munich 10) Technical University, Institute of Pathology, Munich 11) University Ruhnsburch, Institute of Pathology, Regensburg 12) Klinikum Kassel, Department of Pathology, Kassel

Abstracts

1 P132

Germinale mutations of NBS1 gene as a risk factor in childhood acute lymphoblastic leukemia. Mosor M.(1), Januszkiewicz-Lewandowska D.(1). Zi‡kowska I.(1), Nowak J.(1) 1) University of Medical Sciences, Institute of Human Genetics Polish Academy of Sciences, Poznan, Poland

1 P133

hTERT and hTERC gene amplification in selected childhood malignancies Nowak J.(1), Januszkiewicz-Lewandowska D.(1), Zawada M.(1), Pernak M.(1), Maękowski P.(1), Nowicka K.(1), Rembowska J.(1), Lewandowski K.(1) 1) University of Medical Sciences, Institute of Human Genetics Polish Academy of Sciences, Poznan, Poland

1 P134

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P131

Genexpressions-Profile zur Prognose-
Bestimmung beim Mammakarzinom
Wilke G.(1)
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Nach einer Umfrage ist die Möglichkeit einer in-
dividualisierten adjuvanten systemischen Therapie die wichtigste Herausforderung bei der Be-
handlung von Brustkrebs. Dabei sind die bish-
everen klassischen Kriterien nur unzureichend in der Lage, Patientinnen zu identifizieren, ohne adjuvante Therapie Metastasen entwickeln wür-
den und daher eine adjuvante Therapie benötigen. Um solche Patientinnen zu identifizieren, ist eine genaue Bestimmung des Metastase-Risikos notwendig. Die dazu durchgeführte Unter-
suchung (MammaPrint) erfolgt anhand einer Tu-
morpho-cells of the Patientin with Hilfe von Microar-
yrays. 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 Tu-
morjahr 1 oder 2 beträgt die Überlebensrate nach 10 Jahren 97%, wenn eine gute Prognose ermittelnt 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 ausgenützt werden. Die Zuverlässigkeit der Genexpressions-Profile wurde in externen Vali-
dierungs-Studien bestätigt. Eine neue Heraus-
forderung stellt derzeit die Entwicklung von Profilen für weitere Patientengruppen und die Erschließ-
ung anderer Anwendungsgebiete, wie z.B. die Vorhersage von lokalen Rezidiven oder die Lokalisation des Primärtumors bei OUP-Patien-
ten (carcinoma of unknown primary).
Abstracts

Poetsch M. reported cases, in our patient this insertion is as-11 in chromosome 10p12. In contrast to other AML M5 with inverted insertion of chromosome genetic and molecular cytogenetic analysis of a MLL/AF10 transcript. Here we report the cyto-

10 is a well-recognised but rare abnormality in A acute leukaemia Cytogenetic and Molecular Cytogenetic PO5/2003.

studied telomerase components were consis-
tulerness and cell immortalization. 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 distin-
guish neoplastic cells from normal ones. All studied telomerase components were consist-
ently expressed in cancer cells. Neoplastic RNA produced consistently very strong amplification signs either for hTERT, hTERT and TP1. The expression of hTERT, TP1 and hTERT was also observed in some normal bone marrow cells and peripheral blood lymphocytes. The limiting dilu-

dilution experiments indicated that the cancer cells have at least 100-fold higher telomerase activ-
y and at least 25-fold higher TP1 and hTERT ex-
pression in comparison to normal cells. FISH analysis revealed amplification of hTERT and hTERT genes in malignant cells. It can be con-
cluded that all cancer cells tested have higher telomerase expression and activity, as compared to normal cells. The high expression and activi-
ty of telomerase in cancer cells can be explained by amplified hTERT and hTERT genes. This work is supported by Ministry of Sciences and Informatics grant no PBZ-KBN-090/ POS/2003.

Cytogenetic and Molecular Cytogenetic analysis of a 10:11 rearrangement in adult acute leukaemia Türkmen S.(1), Bommer C.(1), Rackwitz S.(2), Robinson R.(1), Mühlau S.(1)

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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 cyto-
genetic and molecular cytogenetic analysis of a 37 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 associ-
ated with a 3’ MLL deletion.

Allelic alterations in comparison to morphological grading characteristics in prema
dern laryngeal lesions 

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2) Ernst-Moritz-Arndt-University, Institute of Pathology, Greifswald

Classic histological grading and molecular ge-
netic analysis of epithelial hyperplastic laryngeal lesions (EHL) 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 EHL were grouped according to four grades of nuclear atypia, four degrees of epithelial matu-
ration 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, 8q, 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 al-
terations as standard for assessment, this pre-
liminary study gives first evidence that certain morphological criteria could reflect differently the gradual progression of premalignant laryn-
geal 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


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Silver-Russell syndrome (SRS) is a heteroge-
neous 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 mouth, clinodactyly V, and asymmetry of head, limbs and trunk. So far, the cause of SRS remains unknown in most cas-
es, however in approximately 10% of SRS pa-
tients a maternal uniparental disomy (UPD) of chromosome 7 or chromosome 15 malformations. Genetic analysis revealed amplification of 11p15 in 11p15 in the aetiology of SRS. In two different studies four growth retard-
ated children associated with maternal duplication of 11p15 have been described. Two of these children showed SRS-like features. Interesting-
ly the Beckwith Wiedemann syndrome (BWS) critical region is located in this particular area. In BWS patients paternal UPD11 and genomic dis-
turbances within 11p15 can be observed. Given the involvement of this genomic region in BWS, we postulated that SRS – with an opposite phe-
notype to BWS – might also be caused by gen-
etic 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 du-
plicated 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 heterogeneous 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 pa-
tients 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) Göbel L.(1), Schultka R.(1), Künker R.(1), Gerlach A.(2), Tönnes H.(2)

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Wittenberg, Institut für Anatomie und Zellbiologie, Halle/Saale 2) Charité Universitätsmedizin Berlin, Campus Virchow-Klinikum, Institut für Humangenetik, Berlin

Between the middle of the 18th to the early 19th century, the study of human congenital malfor-
mations, especially those dramatic examples designated as ‘monsters’, attracted special at-
tention. 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 activ-
ity as professor of anatomy and physiology (1805-1833) at Halle University. Between 1812 and 1818, J. F. Meckel the Younger published a monographic volume “Handbook of external human anatomy”, which became the standard work on tera-
ology in the 19th century. Most of the anom-
alous samples of the Meckel Collection were de-
scribed in this book and the Meckel Collection as well as in the medical doctor theses of Meckel’s stu-
dents. 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. More-
over, all dried specimens (i.e. pathological skele-
tons, skulls) were re-described according to con-
temporary syndromological views. The col-
lection contains also many alcohol or formalde-
hyde 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 molecu-
lar cytogenetic investigations (CGH) as well as radiographical techniques, computed tomogra-
phy (CT), spiral CT and magnetic resonance im-
aging (MRI) play an important role. The value of the comparative genetic hybridization (CGH) based strategy for the analysis of ancient DNA (aDNA) samples extracted from fetuses pre-
served in the Meckel Anatomical Collection in Halle is discussed in this paper.
Are there different ways to build chromosomal inversions during evolution and in so-called heteromorphisms in human? 

1) Institut für Humangenetik und Anthropologie, Jena

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 chromosomes 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 analyses 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.

Supported by the Dr. Robert Pfleger-Stiftung.

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, centromeric, pericentromeric, euchromatin and/or heterochromatin are necessary. However, molecular cytogenetics offers the advantage of being able to detect evolutionary inversions in animal genomes.

Therefore a project was started, which compares pericentric inversions in human chromosomes 2 and 9 with those in homologous chimpanzee chromosomes 2 and 9. Based on the information gained in this project a chromosome translocation t(X;1)(q26;p13.1) was characterized. This case was detected in a girl with partial situs inversus, heart defects, no other abnormalities.

P134 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, centromeric, pericentromeric, euchromatin and/or heterochromatin are necessary. However, molecular cytogenetics offers the advantage of being able to detect evolutionary inversions in animal genomes.

Therefore a project was started, which compares pericentric inversions in human chromosomes 2 and 9 with those in homologous chimpanzee chromosomes 2 and 9. Based on the information gained in this project a chromosome translocation t(X;1)(q26;p13.1) was characterized. This case was detected in a girl with partial situs inversus, heart defects, no other abnormalities.

P144 Multicolorbanding applied in 3D-preserved interphase and metaphase nuclei

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Human cytogenetic preparations obtained by the air-drying procedure of chromosome preparation...
Abstracts

Currently the database contains over 5000 chromosomal visible aberrations, as well as micro deletions and –duplications. Cytogeneticists need information on the 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.

P145


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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.ecaruka.net/. This internet-accessible database collects cytogenetic and clinical data of published cases with rare chromosomal aberrations. It includes microscopically 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 through the Internet after receipt of an account. Submission of data to retain the up to date quality of the collection can be done through the website as well. The latter will allow the exchange of knowledge in single cases, with either wise not publishable, clinically relevant features. The combination 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 Hiller B.(1), Rieder H.(1,2)

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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 chromosome. 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 between, 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 badly 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 ‘d’ - the derivation of a dicentric chromosome. Such dicentric chromosomes impose severe extra efforts for calculation, and are very often erroneously obvious because of the ambiguity.

The description of involvements of centromeric fragments in rearrangements is totally missing in the ISCN, e.g. description 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.

P147

Pure maternal trisomy of distal 11p as a result of a non-reciprocal translocation characterised by microdissection Bell L.(1), Rudnik-Schöneborn S.(1), Eggermann T.(1), Schüler H.M.(1)

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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 craniosenosis syndrome. Conventional chromosomal 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 a non-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. Nevertheless, maternal 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 this distinct phenotype of maternal duplication of distal 11p needs further reports. Nevertheless, all patients described showed marked growth retardation and 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 Wümmel P.(1), Muschke P.(1), Tönnes H.(2), Stumm M.(2), Voelte M.(1), Wiescker P.(1)

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We report on a familial case of an unbalanced translocation consisting of partial trisomy 12q and minimal monosomy 5p. The index patient was a female newborn with macroscopy and dysmorphic features including cleft palate, broad nasal bridge, retrognathia, epicantial 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)(5;12)(p53;q24).3. This karyotype was confirmed by comparative genomic hybridization (CGH) followed by FISH with
the recently proposed concept of condensation during mitosis which should be reassessed. Furthermore, chromosome 6 published by Hliscs et al. (Cytogenet Cell Genet. 61:17–26, 1993) shows 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.
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 genitale abnormalities of various degree in male patients.

We report on a girl presenting with profound mental retardation, growth retardation, ear dysmorphism, and wide spaced nipples. At the age of four, auditory brainstem response showed a bilateral sensorineural hearing impairment (early acquired hearing loss) with 50 dB loss right, unilateral 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 907E6. 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 masking 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 syndrome.

We report on a patient with this syndrome. The 21 month old girl (height 55.75 cm, weight 9.23 kg, head circumference 49 cm) 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 revealed a deletion in 9q. By FISH with bacterial artificial 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 year 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 ear, short palpebral fissures with antimongolid slant, microphthalmia, 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 ambiguus 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 trisomies 13, 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).

Discrimination between signifcant 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 morphology 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

Intersitial 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 facial 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 6 months of age, facial dysmorphism, microcephaly, growth retardation,
general muscular hypotonia and lymphoedema 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(q22.3-q23.1). 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 ZFXH1B 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. Dysplasia and the inability to speak has in all reported so far with MWS other than 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 microarray scanners (Baccelli 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.

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 boundaries 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 a common Italian SLOS causing mutation (45% of SLOS alleles), the IVS8-1G>C is the first endoplasmic loop. The T93M 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 data set 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, beard like nose, micrognathia, joint contractures and a holosystolic heart noise. Echocardiography showed calcification of anterior and mitral valves leading to a moderate aortic stenosis, aortic insufficiency grade II and sclerosis of the anterior mitral valve finally requiring prosthetic valve exchange 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 ACVR1. 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) Gläser C., Zöller D., Caliebe A., Kottke R.(1), Schulz S.(1), Schweigmann U.(4), Hansmann I.(1)
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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 NX2O.5. In addition, recent observations identified the JAG1 gene mutated in Alagille syndrome (AGS, MIM #118120) as a candidate for TOF. We report 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 and one Turkish boy, 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

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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 quantitative model of a metabolising organism - is put in relation to the trade-off between reproduction and self-maintenance as described by the disposability soma theory (Kirkwood 1977). It is shown that for maximal proliferation efficiency 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-ex-tending 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 deferring against cooperative oxidation by the use of fermentation has the highest payoff. Thus, translated to the reasoning that I present here, cooperation, i.e., self-maintenance, is 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, particular 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, FISH with a 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 chromosomal 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.
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 (Gruenouew CDG1, Avelino CDA, Reis-Bückers CDB1) and lattice (Biber-Haab-Dimmer CDL1) corneal dystrophies and in some reports corneal dystrophy Thiel-Behnke (CD2B2). We report on the mutation spectrum and the genotype-phenotype corre- lations on the basis of clinical and histopathological examinations of 10 German families with BIGH3 linked corneal dystrophies.

Methods: In 10 undependent families and pa- tients with different corneal dystrophies DNA was extracted from leucocytes of the peripher- al blood. Mutation analysis was performed by direct sequencing of the BIGH3 gene. Clinical and histopathological findings were compared with the molecular genetic 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 mis- sense mutation Arg124His in the exon 4 of the BIGH3 gene. In the three families with clinical and histopathological CDGG1 we found a mis- sense 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 histopathological find- ings we found a missense mutation Ala546Asp, which was reported before only once in con- nection with CDL1.

Conclusions: In comparison of our clinical and histopathological findings and the molecular ge- netic results we found a strong genotype-phenotype correla- tion in patients with BIGH3 linked corneal dys- trophies. Rare mutations lead to exceptional clinical and histopathological findings which can- not be classified into the different groups of corneal dystrophies. In our patients with CDB2 we could not find any molecular genetic correla- tion 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 nor- mal or elevated plasma concentrations of the antidiuretic hormone arginine vasopressin (AVP). In 90% of families the disease (MIM034800) 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 nu- cleotide substitutions resulting in a truncated re- ceptor 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 prox- imal 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 in genotype-phenotype correlation and may con- tribute to early diagnosis and treatment of the disorder, thereby preventing serious complica- tions such as growth and mental retardation.

P168

A variant form of potassium-aggravated myotonia 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 flu- tuans or my- otonia permanans. We describe a family with 14 affected members suffering from a distal form of myotonia, which is cold sensitive and has vari- able 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 myoton- ic dystrophy could be excluded, and a linkage analysis for candidate genes gave a significant coupling to DNA markers from the SCN4A 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 vacuolar myopathy using routine microscopy and electron microscopy, which could be very rarely seen in HyperPP.

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

P169

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

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CIC-5 is a member of the CLC family of voltage- gated chloride channels. Loss-of-function muta- tions of its corresponding gene (CLCN5) cause Dent’s disease, an X-linked kidney disorder, characterized by low-molecular weight protein- uria, hypercalciuria, nephrocalcinosis/nephrolithiasis and progressive renal failure. Here, we examined the effect of dif- ferent mutations on function and cellular traffick- ing of the recombinant protein. Mutant CLCN5 cDNAs were generated by site directed mutage- nesis 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, CIC-5 channel activity and surface ex- pression were determined by two-electrode volt- age clamp analysis and CIC-5 surface ELISA, re- spectively. Except for the R516W and R648X variants, none of the mutated proteins induced functional chloride currents or reached the plas- ma membrane. This is readily understandable for the truncation mutations however, the tested missense mutations are distributed over differ- ent transmembrane regions implying that correct channel structure and orientation in the mem- brane is not only a prerequisite for proper CIC-5 function but also for Golgi exit. Interestingly, the R648X mutant although functionally compro- mised, displayed a significant increase in sur- face expression. This finding might be explained by the deletion of a CIC-5 carboxy-terminal PY- like internalization signal which in turn impairs channel removal from the membrane. Our obser- vations further imply, that recruitment of CIC-5 to alternative routes (plasma membrane or ear- ly endosomes) in the trans-Golgi network is med- iated via different signal sequences.

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

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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-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 gene 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 analyses 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 borders


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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 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 some 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 EMGN (European Molecu-largenetics Quality Network; http://www.emgn.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 AZFa,b interval and the deletions of different DAZ gene copies were found in men with severe oligozoospermia and with normal fertility, some AZF deletions could be associated with a specific Y chromosomal haplogroup (Fernandes et al. 2004: Am. J. Hum. Genet. 74: 180-187).

P174

d-counter - 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-
pleness there is a need for a uniform and univer-
sal classification of non-syndromic CC. In this
context, it is of relevance, that the acronym, which
was first established as a name for a spe-
cific disorder, is often changed after identifica-
tion 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 pre-
sent 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 (ARC) is a clinically and genetically heteroge-
nenous 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 cor-
relation was not identified so far. In order to
store and provide data from patients with ARCI
and related skin disorders, we have now
developed a concise database service. Different
phenotype variables, family history, pedigree,
bioclinical and histopathological data are
stored for each sample. The family history will be
automatically transferred to linkable compatible
data formats and graphical output of the pedi-
gree, clinical pictures will be included and pre-
seminated as thumbnails. The service runs on an
Apache 2 web server and is powered by a
MySQL database management system and ac-
cessible through a known ARCI interface. For
data protection reasons, sample names are stored on a separate server connect-
ed with the database by anonymous 32bit sam-
ple IDs. Data requests are handled semi-auto-
matically 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 muta-
tion data obtained by direct sequencing. Further
data are being collected within the German
“Network for Ichthyoses and Related Keratiniza-
tion Disorders”, which aims at comprehensively
recording and characterizing such families. The
service is open to external users on a collabora-
tive basis and includes different user levels. Since
ARCI and other keratinization disorders are
rare, the database not only provides a muta-
tion survey but is valuable for systematically col-
lecting dedicated data. Thus, it helps to gener-
a criteria for analyzing a potential genotype/
phenotype correlation and further characteriza-
tion of the phenotypic outcome of specific mu-
tations.

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. Metabol-
tic 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 addi-
tional microsatellite markers for fine-mapping
of one candidate region.
The first family contains in 3 generations five af-
acted males and 7 obligate carrier females, who
don’t show any mental impairment and other ab-
normalities. 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), FAK3, and ATRG2. These candidate
genes have been screened, but no mutation was
found. The second, also three-generation family con-
ists of five affected males and five obligate car-
riers. All female carriers examined were of nor-
nal intelligence and clinically indistinguishable
from their non-carrier sisters. Two- and multi-
point parametric analysis yielded significant link-
age 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 con-
tain 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
could narrow down the candidate region to a 3.1
cM linkage interval between markers DXS8034
and DXS1106. The linkage interval does not con-
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analysed several candidate genes, but no
abnormalities have been identified so far.
Our results suggest that unknown MRX genes
could narrow down the candidate region to a 3.1

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 congenital abnor-
malities including radial ray defects and somat-
us defects, and these patients suffer from childhood
bone marrow failure. However, 30% of patients
display normal growth and appearance with only
mild bone and/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.

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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 function- al TT SDs. Additional studies aim at identifying splicing enhancers which facilitate the usage of the mutated and the cryptic splice sites.

Conclusions: Due to the fullle shown, contrary to expectation mutational disruption of a canonical splice donor site may have only mild consequences.


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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 developed. 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.umdb.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.

In vitro modelling of paraganglioma by RNA mediated knock-down of SDHC/SDHD in PC 12 cells, Troidl C.(1), Klein-Hitpass L.(2), Müller U.(1)

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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 have generated knockdown PC12 cell lines expressing three VEGF receptors, VEGFR-1, VEGFR-2, and Neuropilin-1. Downstream signaling as well as functional effects of VEGF164 on PC12 cell proliferation, differentiation, survival and gene expression are currently evaluated. In addition, coimmunoprecipitation experiments with AP/VEGF164 are performed in order to search for a putative novel receptor.


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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 actual manifestation of the disease, i.e., modifier loci that influence phenotypic severity. Recently, the light chain subunit (KL1C) of kinesin-1 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 KN52 gene, coding for KL1C 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 KN52 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 DTY1 gene and in the SGCE gene has been performed previously. No mutations have been identified. This information might be helpful for the appropriate application of DNA diagnostic testing and for genetic counseling.

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 pathogenic PARKIN mutation 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; IVS3-207T>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 (Arg204Cys and Thr204Met, 2.1%) in heterozygous state were found in the German cohort. None of these 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.


P185
SPINOCERECELLAR ATAXIA TYPE 4: ANALYSIS OF CANDIDATE GENES AND POLYMORPHIC MICROSATELLITES
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The spinocerebellar ataxies (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 chromosomes 3p13.1-q21 (16q22.1) has been described in a Utah family of Scandinavian origin with cerebellar ataxia and axonal neuropathy and further delineated in a German kindred with a similar phenotypic variant. Furthermore, Japanese families with a pure cerebellar ataxia show linkage to the same region. The corresponding SCA4 mutation was narrowed down to 7.9 Mb for the two European kindreds 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 genetic defect or not. We screened 34 candidate genes in the German SCA4 family for point mutations. With the exception 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 SCA4 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, OMIM 257220), a rare, progressive autosomal recessive disorder, is characterized by a late-onset neurovisceral disorder due to mutations in either the NPC1 gene or the HE1 gene, respectively, and is a rare cause of neonatal and childhood death. The frequency of NPC1 mutations in NPC patients and healthy controls is unknown. NPC was diagnosed in all patients by molecular genetic analysis. 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 a retrospective study of 21 patients the development and clinical course of NPC were examined. 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 before the 3rd percentile was found in 9 patients with the neonatal and infantile form of NPC compared to an age-matched 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. Integrating the evidence 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 POLYMORPHIC STRAIN IN PMX2B IN PATIENTS WITH ATAXIA OR HUNTINGTON DISEASE
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Detection of new variations of the polymorphic strain in PMX2B in patients with ataxia or Huntington disease (HD)
Late onset ataxia caused by FMR1 premutations

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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 families harboring 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 50.9 ± 13.7 (range 15-73 y.) and 51 (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, bradykinnesia, and rigidity (Hoehn & Yahr stage 3). After 20 years of disease duration and five years of L-dopa therapy, she had motor fluctuations and dyskinasias. 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 Dardarin 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.

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 inclusion 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 + Casp8+/− mice double-mutant 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 microglia-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 a conditional knockout mouse for Rs1h-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 towards a therapeutic approach to prevent neurodegenerative progression of this retinal disease.
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 homozygous 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.

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 heterogeneous peripheral neuropathies. According to electro-physiological 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. Over all we found six novel mutations in MFN2 (515T, R275W, G298R, S378P, R707W, E744L). This represents 20 % of these CMT2 patients and indicates a first chance for 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 extremities. 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 wheelchair 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 tzo_mitofusin domain, two are clinically sporadic novel mutations. The S378P mutation is located in an evolutionarily highly conserved interdomain region. The here presented patients and MFN2 mutations give deeper insight in the genotype-phenotype correlation in CMT type 2.

Expression analysis of mutant seipin in AIT20 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 AIT20 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 coverted 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 syndrome / dHMN-V.

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

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Distinct hemizygous mutations in the aristless-related homeobox gene (ARX; Xp22.13), the human orthologue of the Drosophila gene aristless, 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 aristless 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, 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.
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 Krt1 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 Krt1 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 3q25.2-2q7. 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 neurological interventions. MRI 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 neurological 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) and 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 WSF1 gene at 4p16.1. Patients develop very early onset type 1 diabetes in association with progressive optic atrophy. The WSF1 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 non-consanguineous parents. Diabetes mellitus was diagnosed with 6 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 WSF1 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 where as the missense mutation in exon 4 was not described yet. Calculation of splice site scores using different programs predicted a clear reduction of score and 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 splicing 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 WSF1 gene mutations but also shows current limits of splice score calculation programs. Recent reports suggest a clear association of WSF1 gene mutations with psychiatric illness but MK up to now does not show any anomaly of that kind.

P199

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

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We recently showed that mutations in the gene for a Ras-like 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 (small/GTPase 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, 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 cell-subcellular 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 as 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.

**P201**

The OSLT 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 STIL in a childhood B-ALL cell line. OSLT shares the first exon with STIL but is transcribed in the opposite direction (OSTL = Opposite STIL). Since the ETV6/OSTL 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 OSLT protein is highly conserved: the human and mouse proteins are 99% homologous and between the human and puffer fish (Fugu rubripes) the protein homology is 83%. A green fluorescent protein OSLT fusion protein expressed in mouse fibroblast cells (NIH3T3) localized to the cytoplasm. Northern blot analysis showed high expression of human OSTL in tests, 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 OSLT-interacting proteins that are important in B-cell receptor signaling and B-cell survival (SIVA, HAX-1). The OSLT - SIVA and OSTL - HAX-1 interactions were confirmed by co-transformation assays in yeast, colony assays in mouse fibroblast cells, and co-immunoprecipitation (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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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 syndromes) 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 chromosome-specific primers and fluorescence in situ hybridisation using cosmid clones. Here we show that the identified deletions vary in size, yet the vast majority of the 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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Léri-Weill dyschondrosteosis and idiopathic short stature is caused by a paracentric inversion in one of her X chromosomes with breakpoints p11.4 and p22.33. Analysis of DNA methylation at the AR locus and acridine-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 inactivated. 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, which have enzymatic activities. 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 coactivator.
P204

Htra2-ß1 - a splicing modulator of SMN2 transcripts: functional studies in transgenic and knock out mice
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We have already identified the SR-like splicing factor Htra2-ß1 to restore the correct splicing of SMN2 transcripts: functional studies in transgenic and knock out mice. Since reduced SMN protein levels also lead 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 in which the gene associated with chromosome condensation defect is known. This disorder is characterized by reduced SMN protein levels due to alternative splicing of the SMN2 gene giving rise to SMN-ß proteins which are unable to restore the correct splicing of the 5'UTR of the SMN1 gene. Reduced SMN protein levels are also associated with the phenotype of SMA patients. Since reduced SMN protein levels also lead to reduced Htra2-ß1 protein level, the construction of Tra2-ß1 knock out mice is of particular interest.

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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Triplet 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 CCG repeat in the ZNF9 gene of myotonic dystrophy type 2, but has never been tried to detect expanded FMR1 CGG 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 noninterrupted full mutation alleles.

P207

Screening of hot spot mutations in the Ryndogene 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 anaesthetics and muscle relaxants. If not treated immediately MH episodes might have lethal consequences. A form of myopathy closely associated with MH is associated in many patients with the RYR1 gene, which is associated with a chromosome condensation disorder. So far, we have already described the first congenital disorder in man in which the gene associated with chromosome condensation defect is known. This disorder is characterized by reduced SMN protein levels due to alternative splicing of the SMN2 gene giving rise to SMN-ß proteins which are unable to restore the correct splicing of the 5'UTR of the SMN1 gene. Since reduced SMN protein levels also lead to reduced Htra2-ß1 protein level, the construction of Tra2-ß1 knock out mice is of particular interest.
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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 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 duplication clusters 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 JAA1 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 a third group of candidates were those, which are expressed specifically in heart and skeletal muscle – the preferentially affected tissues in EDM (FLNC, SMPX, POP 1-3, AKAP 7, Nesprin1α; Delta2). Until now two genes, STA and LMNA, have been associated to Emery-Dreifuss muscular dystrophy (EDMD). Further genes are likely to be included in the EDMD gene cluster region, in addition to the breakpoint clusters of STA and LMNA. Our results demonstrated that a considerable number of large NF1 deletions were detected. For 47 of the 77 patients, mRNA quality 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 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 expression was confirmed by FISH which revealed a novel 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. In 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 methods, 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 number of large 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.

P210

MLPA for screening of large alterations affecting the NF2 Gene

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We studied a patient with the acampomelic form of CD with the karyotype 46.X;#(1;7)(q11.2;p23). 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 (DY33) on the derivative Y chromosome. BAC probes up to 800 kb upstream of SOX9 always gave signals on the der(Y) only. BAC probe RP11-878D6, 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 cases which add 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.

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 translocation involving CD with the karyotype 46.X;#(1;7)(q11.2;p23). 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 (DY33) on the derivative Y chromosome. BAC probes up to 800 kb upstream of SOX9 always gave signals on the der(Y) only. BAC probe RP11-878D6, 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 cases which add 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.
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 high-ly characterized craniofacial findings including a thin nose with hypoplastic alae nasi, prominent columella, microcornea, enamel hypoplasia, oligodonty, and microcephaly. Neurologic symp-toms are mainly spastic paraparesis and bladder disturbances. We observed a boy of Turkish-Austrian origin, who was diagnosed with ODDD.

Methods: The complete coding region of the GJA1(connexin-43) gene was investigated by PCR and bidirectional sequencing and avoid-ance of amplification of the pseudogene GJA1P1.

Results: A heterozygous missense mutation c.146 A>C was found, causing an exchange of up to three nucleotides in the first two exchanges counted from the 5' side. Second, asymmetric modification 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 3' side than from the 5' side. Second, asymmetric mod-ified oligonucleotides also showed differences in total exchange rates in the correction of the point mutation.

Conclusions: So far, all ODDD patients who un-derwent molecular studies revealed mutations in the coding region of the GJA1(Cx43) gene. The glutamine residue in codon 49 is highly con-served among species and a mutation at this codon has already been described in ODDD. However, no obvious genotype-phenotype cor-relation can be derived.

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 en-vironmental as well as by genetic factors. In-deed, the genetic basis for specific metabolic disorders that lead to urolithiasis, such as cystinuria and oxaluria is well established; how-ever only little is known about the genetic basis of hypocitraturia as the major stone forming fac-tors in calcium-oxalate stone disease. Urinary citrate concentration is primarily determined by its rate of reabsorption in the proximal tube. 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. Ge-nomic DNA of 13 patients with hypocitraturia less than 1 mmol/d were included in the study, in addition, 5 patients from hypocitraturia fami-lies were analysed. The coding sequence (12 ex-ons) as well as the intron/exon boundaries of NaDC1 was screened by single strand confor-mation analysis (SSCP). To demonstrate the sen-sitivity of our SSCP approach, 10 of the frag-ments were additionally analysed by denaturing high-performance liquid chromatography (DH-PLC). By SSCP and DHPLC analysis, we detect-ed unusual patterns in the fragments of exons 3, 5, 6, and 12 of the NaDC1 coding sequence. Direct sequencing of these variations confirmed base-pair substitutions which correspond to known SNPs (rs11568446 in exon 3, rs11568443 in in-tron 7, rs11568454 in exon 12). 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 hypocitr-aturia.

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 9 exons of the NaDC1 gene. That is unlikely because we found in the same experiment aside the clones with the exchange of all three nucleotides also clones with the ex-change of just one or two nucleotides. Accord-ing to this result we could also suggest that the exchange of the three nucleotides is an inde-pendent exchange of one nucleotide after anoth-er. 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 calculat-ed. So the independent exchange of single nu-cleotides is not very likely. In our experiments we found exchanges of all three nucleotides in a maximum distance of 15 nucleotides. So we cal-culate 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 hy-potheses didn’t fit our experimental data. In ad-dition, we found evidences of a preferred orien-tation 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 3' side than from the 5' side. Second, asymmetric mod-ified oligonucleotides also showed differences in total exchange rates in the correction of the point mutation.

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 asso-ciated 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 defi-ciency, in the majority point mutations.

Patients: We report about 2 patients with bio-chemically confirmed protein C deficiency. A 65-year-old patient from Germany with throm-bosis of the right eye, 8 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 coronary embolism when he was 67 years old.

Methods: Genomic DNA was extracted from pe-ripheral blood leukocytes, followed by PCR im-plication of the 9 exons of the PROC gene, in-cluding 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 was detected in the second patient who is located in exon 9 of the PROG gene. The T-to-C change at nucleotide number 8743 leads to the substitution of methionine (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 PROG 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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Germine 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. Cell suspension 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, characterized by immunofluorescence or RT-PCR. Expression analyses were performed with Atlas 3.6 Arrays (Clontech) and data were verified by comparing them to the GeneChip Human 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 cytokoskeletal 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

MP44 is a scaffolding protein in the connecting cilia of photoreceptors
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Objectives: MP44 (membrane protein, palmito- lated) is a member of the large family of mem- 
brane-associated guanylate kinases (MAGUKs), MAGUKs act as scaffolding proteins at various cell-cell junctions by mediating the assembly and anchoring of protein complexes through var-
tious conserved protein interaction domains. We have demonstrated that MP44 is preferentially transcribed in the retina. The current study aims at the elucidation of MP44 expression in the cili- ary organelles of photoreceptors in various mammalian species.

Materials and Methods: We have generated a panel of highly specific mono- and polyclonal anti-
tibodies raised against various epitopes of the MP44 protein. Immunofluorescence labelling with antibodies against MP44 and known molec- 
ular markers for connecting cilia was per-
formed on fixed and unfixed frozen retinal sec-
tions, on isolated individual photoreceptor inner and outer segments and on intact photorecep-
tor microtubule-based axonemes. Furthermore, we have prepared cytoskeleton-enriched frac-
tions from photoreceptor extracts by sucrose density gradient centrifugation to investigate an association of MP44 with ciliary axonemes.

Results: Cellular localization studies consistent-
ly revealed that MP44 is abundantly present in the synaptic terminals of rod and cone photore-
ceptors. In addition, we provide evidence that MP44 is a component of the non-motile primary cilia (connecting cilia) of photoreceptor cells. Outgrowing primary cells were positive with finnish nephrosis (CNF) and a NPHS1 mutation in one adult kidney and one kidney of a patient with biochemically confirmed protein C deficiency, to confirm the diagnosis and define presymptomatic gene carriers.

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 rearrange-
ments. Therefore it can be concluded that these inversions occurred and were fixed in the ho-
moygous state between 6 and 2 MYA, in the time interval when the human and chimpanzee lineages have separated. This supports the com-
mon 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 ex-
plain the divergence of human and chimpanzee lineages, which took place in East Africa without local separation (sympathy). According to this model in interbreeding populations inversions lead to the suppression of recombination which facilitates the evolution of species-specific hap-
lotypes in the rearranged regions. Since a pos-
tive correlation exists between recombination frequency and interspecies nucleotide substitu-
tion rates, differences in DNA divergence are in-
dicative of a substantial period of heterozygosi-
y with suppression of recombination. We have determined the human/chimpanzee DNA diver-
gence rate at 2.15 loci (total length 2921 bp, aver-
age length 19956 bp) in inverted chromosome fragments and at 21 loci (total length 446939 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 encou-
passed by the inversions and 1.37 % in the co-
linear ones (P < 0.0137, Mann-Whitney-U-Test). Our findings are in agreement with the chromo-
osomal speciation model of restricted recombin-
a within the rearranged regions. New experimen-
tations 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 Mbd4 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 re-
combination and reduction of the diploid chro-
mosome set to a haploid one. To date, about 100 genes have been found, mainly in knockout mouse models, to be implicated in spermatoge-
nesis. Many of the genes that play a role in mei-
otic 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 par-
allel. To this end, we developed a cdNA chip with approximately 500 genes which are in-
volved 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 hu-
man testes, approximately 50 genes were ex-
pressed differentially in testicular and fibroblast cells. Microarray results were validated with re-
verse Northern blots. From the subset of genes that showed at least twofold elevated mRNA lev-
els 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 pro-
teins in the nucleus of spermatogonia, whereas no testicular cell types showed only very weak staining. In particular co-localization was observed in a percentage of nuclei from an exponentially growing fibrob-
last culture. We hypothesize that Dnmt3a and Mbd4 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 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 III)**

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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 α1 (V) and one α2 (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 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 preceded molecular genetic analysis in 15 cases.Mutation screening of all coding exons of the COL5A1 and the COL5A2 gene by direct sequencing of leucocyte DNA has been performed 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 glycine residue and a conserved arginine residue in the triple-helical domain of the proα1 (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 α1 (V), one α2 (V) and one α3 (V) chain prompted us to extend mutation analysis on the COL5A3 gene in COL5A1/COL5A2 negative patients.
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 apparatus 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 a known phenotype. After exclusion of genetic linkage to all known DCM loci we performed a genomewide 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 9.73 Mb 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.

P227
Frequencies of mutations in the GJB2 gene in Egyptian and Romanian patients with autosomal-recessive non-syndromic hearing loss
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2) Otolaryngology, Assiut
3) Human Genetics, Tübingen

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 (72.1%) 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.

P226
Searching for mutations in the OTOA gene in a family with deafness
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3) Human Genetics, Tübingen

We used an array of 6101 human bacterial artifical 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 site of these 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.

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 cutaneous hyperpigmentation, somatic and 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. 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 disease occurred due to non-allelic homologous recombination 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. Deletion 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. We detected translocation breakpoints that map in or near the CACNA2D3 gene suggests that this chromosomal region might be prone to genomic instability and thus these heterozygous microdeletions encompassing various parts of CACNA2D3 might be present in sporadic patients with ZLS.

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 the mp28 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 function. To study the function of mp28, we generated several transgenic mouse lines that either mp28 is transported by 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 dynein complex, 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.

Identification 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 share eight cysteines in the extracellular loops throughout evolution. Moreover, amino acid substitutions of one of these cysteines in human TMEM16E causes gnathodiaphyseal dysplasia, an autosomal dominant skeletal disorder. 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 and in situ 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 immobilize antibodies 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 heterologously 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.

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 resistance (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 PPR than in PGR. Two of the proteins (P231 and G5) were identified as protein kinase C beta I 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 (0.1 - 0.59) 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 NFB- 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 proteins

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Spinal muscular atrophy (SMA) is an autosomal recessively inherited motor neuron disease. The disease determining survival of motor neuron gene 1 (SMN1) is homozygously lost in 95% of SMA patients, while intragenic SMN1 mutations 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 anti-convulsant VPA possess histone deacetylase (HDAC) inhibitor properties. We experimentally 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 deacetylation pathways, 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 hallmark 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 (156) came from Austria, three were from Bulgaria and four from Slovenia. We identified 14 different mutations in 41 genes (25%) that were already previously described in the literature. These consisted of missense and protein-truncating mutations as well as single nucleotide deletions, which clustered in the two functional MeCP2 regions, the methyl-cysoine-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 (ESB, 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 arise 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.

The role of Pelo (Pelo) during the cell cycle

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The Fas system is a receptor-ligand signaling 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 signaling 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 murined 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 characterized a gene trap mutant mouse line (line 40) and found that the gene trap vector was inserted in intron 8 of the Faf1 gene. Heterozygous animals appeared normal, however 15% of heterozygous males are infertile. All seminiferous tubules in testes of infertile heterozygous mutants were replaced by Sertoli cells and spermatogonia. Genotyping of the progeny of heterozygous intercrosses shows the absence of Faf1 homozygous mutant and suggests an embryonal lethal phenotype. These results suggest that the Faf1 is essential for germ cell and embryonic development.

P236
Mutation in either the Drosophila pelota (Pelo) or the S. cerevisiae homologue, dom34 cause defects of spermatogenesis and oogenesis in Drosophila, and delay of growth and failure of sporation in S. cerevisiae. The findings of these mutants are suggestive of a requirement for 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 number 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 embryo that the lethality of Pelo null embryos is due to defect in cell proliferation. Increase of percentage of cells exhibiting polyplody 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. Approach to establish Pelo-/- cells by culture of Pelo-/- ES cells in medium containing high concentrations of G418 failed to deliver a Pelo deficient cell lines. 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 localized at the stress actin filaments. To overcome the early embryonic lethality of the Pelo deficient mice, generation of conditional knock-out mice is underway.

P237
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 signaling 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 signaling 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 murined 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 was inserted in intron 8 of the Faf1 gene. Heterozygous animals appeared normal, however 15% of heterozygous males are infertile. All seminiferous tubules in testes of infertile heterozygous mutants were replaced by Sertoli cells and spermatogonia. Genotyping of the progeny of heterozygous intercrosses shows the absence of Faf1 homozygous mutant and suggests an embryonic lethal phenotype. These results suggest that the Faf1 is essential for germ cell and embryonic development.

P238
Novel mutations in BCOR in three patients with oculo-facio-cardio-dental syndrome, but none in Lenz microphthalmia syndrome

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seinChip technology just like protein A/agarose, for a sufficient precipitation we tested several (surface enhanced laser desorption/ionisation). The cell lines U-2 OS and MCF-7 were used to detect endogenous E2F and possible binding partners by the ProteinChip technology SELDI. The cell lines U-2 OS and MCF-7 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 regulation of E2F family. Pathways affecting the influence on DNA replication or cell cycle regulation.

This work was supported by BMWF and IFKF.

P240 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 allelic 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 prerequisite 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 unambiguously be deduced from the genotypes at all together 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 ancient immigration to Europe by two genetically homogeneous but well separated groups of immigrants. Two waves of migrations 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 population samples in the analysis.
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 replying 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 isochore 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 intra-species 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 pericentric 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 pericentric 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 origins, 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 unmasked transcribed 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 unmasked transcribed

P244

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

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Leri-Weil syndrome (LWS) is a well characterised inherited short stature syndrome associated with mesomelic shortening of the lower limbs and the forearms. Patients are often affected by Madelung deformity of the forearms. Phentotypic inter- and intrafamilial heterogeneity 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 10-20 % 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 simultaneously 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 an 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-Weil syndrome.

P245

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

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2) Ibsen Medizinische Universität Halle-Wittenberg, Institut fuer Humangenetik und Medizinische Biologie, Halle

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 filaments (NM-IF) and is being down regulated by the intrinsic Dicer enzyme. Telomerase inhibition and shRNA-mediated 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 is a protein which is involved in the biological function of the genetic instability as well as MSI in cells. Scapinin has been found to bind to cytoplasmic actin and further being a putative regulatory subunit of protein phosphatase-1 (PP1). It is attached to the nuclear matrix-intermediate filaments (NM-IF) and is being down regulated by the intrinsic Dicer enzyme. Telomerase inhibition and shRNA-mediated 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.

Abstracts
P247

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 domains. Mutations in the TRPS1 gene lead to the tricho-rhino-phalangeal syndrome types I and III (TRPS/III), which present with a broad spectrum of facial abnormalities, brain dysmorphologies, 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 mimicking 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.

P249

Stable siRNA mediated conditional knock-down in mammalian cell lines.

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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. Nevertheless, siRNA technology has some important caveats. Firstly, the depletion of gene activity. 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 Lysyl 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.

Stable siRNA constructs showing a down-regulation of more than 40% in the luciferase reporter gene assay were integrat-ed 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 (bHLH) 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, 37KRRK4 and NLS2, 73KRGK77), a highly conserved 5 aa region, called NSEEED-domain, and a WR-domain in the C-terminus of the protein.

To understand the role of the second nucleo-
The Glaucoma risk associated Gln368X
Myocilin variation impairs the mitochondrial 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 localization; 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 aggregates in vacuoles 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.

Establishment and characterization of a primary testicular cell culture from a patient with Frasier Syndrome
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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 similar- ly 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, a protein that contains 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-activating factors, predicted binding sites within this region will be modified by mutation.

Comparison of the human, mouse and fugu gentic 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 tested 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.

Cohen syndrome: Mutational and transcriptional analysis of COH1
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10) Charité, University Medicine of Berlin, Inst. of Medical Genetics, Berlin, Germany
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Cohen syndrome is a rare autosomal recessive disorder, clinically highly variable and mainly characterized by developmental retardation, craniofacial dysmorphism, renal 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 consislent 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 frameshift or nonsense sequence alterations. The lack...
of a second pathogenic mutation in some pa-
tients points to the existence of further alterna-
tive 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 proteins, which
is similar to VPS13p from yeast and therefore
studying the expression of
and Northern hybridization. Furthermore, we are
Therefore, we have embarked on a detailed

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The pericentric inversion of chimpanzee chromosome 11 homologous to human chromosome 9 is associated with repositioning of the centromere Kehrer-Sawatzki H.(1), Szamalek J.(1), Tänzer S.(2), Penberthy L.(1), Mørk trous J.(1), Schwarz-Herzke B.(1), Schoenen F.(1), Karg er-Sawatzki H.

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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 char-
terized the breakpoints of the pericentric inver-
sion which distinguishes chimpanzee chro-
mosome 11 (PTR 11) and the homologous chro-
mosome 9 in humans (HSA 9). The break of ho-
mo-logy between PTR 11 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 ortho-
logous to HSA 9q22.1. Thus the chimpanzee specific inversion of PTR 11 was associated with neoacentromere close to the inversion breakpoint and the inactivation of the ancient centromere. Over evolutionary time, this neoacentromere 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, GTF2H2 and the TFNR gene. We hypothesized that haploinsufficiency of TFNR may cause brain atrophy and neuronal dysfunction. 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 nucle-
us, 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 zinc finger protein. It was shown that ZNF297B is mainly expressed in the nucleus in many tis-

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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 tran-
scription factors (previously called HNF-3/-fork-
head transcription factors). The 2.7-kb transcript of the murine Foxq1 gene is expressed promi-

nently in stomach and kidney. Expression of Foxq1 was detected in stomach and kidney dur-
ing pre- and postnatal development. Immunohis-

tologic analysis revealed that the expression of
Foxq1 is located to parietal cells in the gastric

unital. Parietal cells are responsible for the output

1-hormon. Foxq1 is located to parietal cells in the gastric

neural regulation of gastrin secretion. To de-
termines the function of Foxq1, we have generat-
ed knock out mice by deleting the whole coding region of Foxq1. Foxq1 deficient mice are viable and fertile. No apparent histological abnor-
malities can be observed in stomach and kidney. Us-
ing RT-PCR assays and Northern-Blot analysis we found altered expression of genes which are involved in regulation of gastric acid secretion.

Expression of hormone, 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 re-

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 in-
tragenic 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, un-
explained hyperthermic episodes, bowing of the long bones, and distal arthroplasia. The clin-
ic, radiological, and histological similarities be-
tween 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 muta-
tions in the leukemia inhibitory factor receptor (LIFR) gene. The LIFR gene maps to chromo-
some 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 report-
ed so far occurred in consanguineous families and were homozgyous. Thus, information con-

cerning the spectrum of mutations within the LIFR gene is very limited and phenotype-geno-
type correlations are not yet evident.

In order to provide additional data, we have se-

quenced the LIFR coding exons from six pa-
tients with congenital bowing of the long bones

including three that were diagnosed with SWS.
One patient was homozygous for the 1798C>T
(R500W) mutation which has been found before
mainly in families with consanguineous parents.
Another patient was compound heterozygote and both mutations (the paternal H116Y muta-
tion as well as the maternal S133T mutation)
have not yet been described. Furthermore, these mutations do not a priori lead to premature ter-
mination of translation, which is in contrast to most of the mutations reported so far. These are either nonsense mutations or frame shift muta-
tions. 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 nRNA 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

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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. Comparison 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 Lmxtf1 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 promoter in order to perform detailed expression studies.

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. NBS1 is indeed involved in CSR. Here we show, by conditioned immortalisation of the murine homologue (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.

Infanteil Hypophosphatasia due to a new compound heterozygous mutation in the ALPL gene - functional evidence for a hydrophobic side-chain
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Abstracts

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, 657delE5, which is hypomorphic and leads to the expression of amino- and carboxy-terminal nibrin fragment 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, lysesates were simultaneously precipitated with anti-Me11 antisera. We found the NBSp70 protein in all 657delE5 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 compound heterozygous 657delE5 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.

Infanteil Hypophosphatasia due to a new compound heterozygous mutation in the ALPL gene - functional evidence for a hydrophobic side-chain
Uttsch E.(1), Brun-Head G(2), Gravouil Aplatolou C(1), Karle S(1), Jacobs U(1), Ludwig M(3), Dörn H-G(1), Rascher W(1), Mornet E(2), Dötsch J(1)
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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 ALP 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 craniofacial 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/24 h creatinine and renal ultrasonography confirmed nephrocalcinosis. Vitamin D was withdrawn and the patient was started on calcitonin, hydrochlorothiazide and feeding by nasogastric tube. Nonetheless, the girl’s overall clinical condition continued to deteriorate and she died at the age of 5 months from respiratory failure. Sequence analysis of all regions of functional significance of the ALP 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 I201T change would affect its so far unknown functional properties. Residue R374 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.

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 contains repetitive elements. We aimed to establish the mutation spectrum for FANCD2, 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 pyrimidine-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 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 Yb8. 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 the FANCD2 gene is frequent and in aberrant splicing causing exon skipping, exonisation of intrinsic sequence, activation of cryptic and creation of new 3’ splice sites.

Mutations in the MITF-gene in Waardenburg syndrome type 1 and type 2

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Hereditary haemorrhagic telangiectasia (HHT, or Osler-Weber-Rendu syndrome) is an autosomal dominant disease characterized by recurrent 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 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.

P265

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 SOCS1 is known to dephosphorylate 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 promoter/nucleroid plasmid together with mutant SHP2 plasmid results in a downregulation of SOCS1 promoter activity. This downregulation of SOCS1 activity by mutant SHP2 can be re-dosed 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 promot er 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.

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 SOCS1 is known to dephosphorylate 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 promoter/nucleroid plasmid together with mutant SHP2 plasmid results in a downregulation of SOCS1 promoter activity. This downregulation of SOCS1 activity by mutant SHP2 can be re-dosed 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.

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

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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 FAX2 and MATF 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 information, 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.

P268
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 cultures of mammalian cells 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 cystine 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.

P269
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 dependend 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 activity completely. Mutations at Tyr 139 which were detected in patients with the VKCFD2 phenotype) abolished VKOR activity completely. Mutations at Tyr 139 which were detect- ed 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 analogs 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.

P270
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 telangiectatic tumors with a hemispheric shape were identified histologically as cylindromas and trichodiscomas. The occurrence of these special skin lesions is known as Brooke-Spiegler syndrome. To date there are reported about 40 families with this disease condition. The patient’s mother and her younger sister were affected. The patient and her younger sister were tested 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 of the CYLD gene for a debiquitinating 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.
pleies 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 assay further the nature of the alternatively spliced transcript found here, functional experiments are in progress.

P273

Organization and expression of murine TSPY
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TSPY (testis-specific protein, 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 TSTN-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, arildactyl, 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 TSPY. 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 fate of a unique 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 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, gTSPY, 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.

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 have been discovered during the last decade, causes for hypogammaglobulinemia with B cells present in peripheral blood, were observed. 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 activation status of B cells. The phenotype mimics 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 naive 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 are not affected to suffer from lymphoproliferation and autoimmunity. Interestingly, also heterozygous mutations display a phenotype. 4. The homozygous loss of the inducible co-stimulator (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.

P275

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 dysmorphism. 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 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 from 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 in PHF6. Sequence variants 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 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.

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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 mis-
sense and nonsense mutations in the common
part of isoforms I and II, whereas mild pheno-
types are generally caused by mutations in the
unique sequence part of isoform I. In addition,
there is evidence that mild forms show no pref-enevent inactivation of any X chromosome.

Gapo syndrome, there is only a single report of
a non-syndromic, autosomal dominant inheri-
tance of this condition (Shokeir, Clin. Genet. 5:
322-326, 1974; OMIM 123530). We have now
ascertained and clinically characterized a three
generation family of German descent segregat-
ing an autosomal dominant form of primary fail-
ure of eruption and aim at identifying the genet-
ic basis of this disease.

Material and Methods: Genomewide 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 has
now been completed including the en-
tire chromosomes 1 to 6. Thus far, there is no
evidence for genetic linkage. Best fit modell-
ing predicts a maximum lod score of 2.4 with
the given family constellation. Additional affected
family members are being ascertained at pres-
ent in an effort to reach lod scores over 3.0.

Conclusions: The full genome will be com-
ponently inactivated.

P278
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 het-
erogeneous. In 20% of cases, family history in-
dicates autosomal dominant inheritance (ADRP).
Twelve ADRP pedigrees have been mapped to date,
with genes identified in all cases. However, mu-
tations in these genes only account for half of all
Acanthus disease cases. 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 mem-
bers noticed visual impairment in the early 3rd
decade, others not before early 4th decade. Pro-
gression of visual impairment ranges from rapid
to moderate. Linkage studies using microsatel-
itez markers for all known ADRP loci (NRL (RP27),
CRX, RP1, PIM1K (RP9), IMPDH1 (RP10),
CA4 (RP17), and FSCN2 as causative genes in this
family. Where markers were not in-
formatice (loci for PRPF31, RDS, and RHO, re-
espectively), the entire coding regions of the cor-
responding genes were sequenced. In case of
PRPF8 and PRPF13 mutations have only been
described in restricted parts. We found no mu-
tations in either gene by sequencing these re-
gions. As there is no male-to-male transmission
in this family, and since mutations in PRPGR (un-
derlying X-linked RP3) have been described in
families with apparent ADRP, markers flanking
the RP3 locus were typed and showed no
linkage. Genomewide linkage analysis will be
performed in order to identify the ADRP locus in
this family.

Primary failure of eruption of permanent
teeth – a genome-wide linkage analysis
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3) University of Regensburg, Institute of
Human Genetics, Regensburg, Germany

Objectives: Tooth eruption is a complex, local-
ized, bilateral symmetric and timed process.
Eruption disturbances are due to various rea-
sons and include ectopic position of the tooth
gem, mechanical interferences and failure in the
eruption mechanism caused by systemic factors
or local eruption disturbances. Although distur-
bances of tooth eruption are associated with
many systemic disorders (e.g. ecotodermal dys-
plasia with adrenal cyst, Rutherfurd syndrome or

P276
No evidence for a role of SLC1A5 in 19q13 in
the aetiology of cystinuria
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Cystinuria is an inherited metabolic disorder
d 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 identi-
fied: 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 cystin-
uric patients does not reach more than 80% and
is influenced by several factors (screening tech-
iques, ethnic origin, classification of patients).
Therefore, the role of further genes in the aetiol-
y of cystinuria has been postulated. Interest-
ingly, 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 cystin-
uric patients with linkage with 19q13 but without
detectable mutations in SLCT9A7. 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 possi-
ibility that other genes are involved in cystinuria.
Further molecular studies will clarify the complex-
nature of this disorder.

P279
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 (>80%) asso-
ciated with the known intragenic SNP haplotype
5'-CCGGA-3'. HapMap data indicate that the
CYP1B1 gene and the 5'-CCGGA-3' SNP hap-
lotype is located within one linkage disequilib-
rium (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 homozgyous
or compound heterozygous form and are further-
more homozygous for the intragenic 5'-CCGGA-
3' haplotype. One PCG patient belongs to a
Costa Rican PCG family, one to a German PCG
family and 7 are clinically sporadic PCG pa-
tients. The microsatellites flanking CYP1B1 vari-
ed much more as the intragenic 5'-CCGGA-3'
SNP haplotype. Since this observation hampers
the hypothesis of a founder effect based on the
intragenic SNP haplotype we have analysed 7
newly assigned high frequency SNPs within or
close to the CYP1B1 gene. Although the above
mentioned mutations are within the identical in-
tragenic 5'-CCGGA-3' SNP haplotype, the ad-
ditional SNPs allowed us to order the mutations
in two subgroups. The Arg355Stop, Arg368His,
7901del13 and 622delC mutations are all with-
in one SNP haplotype (CAGCCCGTGAATT),
while the Gln398X, His437Asn and 1208insC and
8037dup10 are embedded in a second SNP
haplotype (TAGCCGAGTACGA). It is highly prob-
able that almost all of the CYP1B1 mutations asso-
ciated with the intragenic 5'-CCGGA-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 com-
pared to homozgyous PCG patients.

P280
Autosomal Recessive Mesoxial Synostotic
Syndactyly with Phalangeal Reduction Maps
to Chromosome 17p13.3
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Ankara, Turkey
3) Quaid-I-Azam University, Department of
Biological Sciences, Islamabad, Pakistan
4) Cumhuriyet University, Faculty of
Medicine, Department of Orthopaedics and
Traumatology, Sivas, Turkey
Abstracts

We have cloned the DNA fragment containing

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 characteristic cranial suture formation. Candidate genes have been excluded in a region without any known or predicted regulatory function of candidate genes on chromosome 9 or 11 involved in craniosynostosis. No causative mutation was described DNA. Assuming a regulatory function of the chromosome 9 and 11 breakpoints. The breakpoint on chromosome 9p15 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 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 lead to a dysregulation of flanking genes on chromosome 9 or 11 involved in cranial suture formation. Candidate genes have been evaluated.

P282

Autosomal dominant periniosis maps to chromosome 3p


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Familial periniosis is a novel autosomal-dominant genodermatos. 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 erythematos. Extensive investigation of 3 affected individuals of a multigenerational nonconsanguineous German kindred could exclude the presence of an nuclease deficiencies or anomalies in each of them cause deficiencies or anomalies in each of them cause

P284

Interactions of proteins associated with Bardet-Biedl syndrome

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Bardet-Biedl syndrome (BBS; MIM #209900) is a genetically heterogeneous, pleiotropic 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-RCI) 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 multissubunit 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

Cytogenetic discrepancies in prenatal diagnosis: Tissue specific mosaicism in extraembryonic and embryonic cells

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Abstracts

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 homoygous 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.

The QF-PCR in routine prenatal diagnosis of the common human trisomies (1/2 years of practice)

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A rapid prenatal test for the common human tri somies 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 (sem)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 recommended 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 (min imum 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 diplodipcy at molar pregnancies. Material and Methods: 15 different highly polymorphic STR markers (Ampestr Identifier 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 diplodipcy was confirmed if all 15 STR polymorphisms of product of conception were monoallelic 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 micrognathia, 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, micrognathia, 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 micrognathia and short femurs. If micrognathia 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 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.


P292
Familial arhinia, choanal atresia and microphthalmia
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We describe three females (aunt and nieces) with variable manifestations of arhinia, choanal atresia, microphthalmia and hypertelorism with normal karyotype 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.

P293
Protein biosynthesis and infection – is the elongation factor 2 the possible link?
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2) Inst. of Human Genetics, Halle
3) Inst. of Immunol., Halle
4) Internal Med. III, Halle

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 metabolite 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 TNFα (20ng/ml), Ps. pyocyanea (10ng/ml), endotoxin (1µg/ml), inflammatory markers including TNFα (20ng/ml), Ps. pyocyanea (10ng/ml), endotoxin (1µg/ml), IL-1α (200ng/ml), Ps. exotoxin A (10ng/ml), endotoxin (1µg/ml), IL-1β (100ng/ml), on mRNA expression of the cardiac EF2. For this investigation neonatal rat heart myocytes (approx. 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-1β or TNFα. However, the two exogenous toxins, Ps. pyocyanea 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 atherosclerotic (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 ApoA1, 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 European populations, SNPs, except within ApoE, showed different association signals and did not allow a prediction of atherosclerotic 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.
A repeat polymorphism in the CLCN7 gene influences bone density in patients with autosomal dominant osteopetrosis (ADO) type II and in postmenopausal women. Konnak U (1), Branger S (2), Ostertag A (2), Benichou O (2), de Vernejoul M (2). 1) Charité University Hospital, Institute for Medical Genetics, Berlin 2) Hôpital Lariboisière, INSERM U806, Paris

Higher concentrations of the CIC-7 chloride channel cause autosomal dominant osteopetrosis (ADO) type 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 normal 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 penetration. 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.

A whole-genome scan in 155 German sib pairs with attention-deficit/hyperactivity disorder substratifies linkage to chromosome 5p. Heidebrand J (1), Dempfle A (2), Friedel S (1), Saar K (3), Herpertz-Dahlmann B (4), Kieß F (5), Warnke A (6), Heiser P (2), Hinney A (1), Nürnberg P (3), Konrad K (4). 1) University of Duisburg-Essen, Essen 2) University of Marburg, Marburg 3) MDC, Berlin 4) University of Aachen, Aachen 5) Bezirksklinik Regensburg, Regensburg 6) University of Würzburg, Würzburg

Objective: 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 ascertainment 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 initial- ly 404 autosomal and X-chromosomal microsatellite markers (average distance 10cM). In total, 425 individuals were genotyped. Additional markers on chromosome 5p at the DAT1 (SCL6A3) locus were subsequently genotyped: DSS2005, the DAT1 VNTR, rs8347 (in exon 9) and rs11546774 (in exon 15).

Results: The highest multipoint LOD score in the initial analysis was obtained on chromosome 5p at the marker DSS807 (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 and one VNTR 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 (rs11546774) 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 0.098).

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.


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The aim of this study was to evaluate the influence of genomic variants in the angiotensinogen gene (AGT) point mutations, 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 ≥140/90 mmHg after 20 gestational weeks; proteinuria ≥1 g/l or superimposed preeclampsia (blood pressure ≥140/90 mmHg before 20 gestational weeks; proteinuria ≥1 g/l) and 100 controls without 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 L34F [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.


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Sarcoidosis is a complex inflammatory disease of unknown cause that primarily affects the lungs 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 significant 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.
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. The aim of the Cited2 project is to identify disease-causing mutations. For this purpose, we sequenced all coding exons of a total of 15 candidate genes that are expressed in a wide range of fetal and adult tissues. We identified significant polymorphisms in the Cited2 gene for CHD and found an association with CHD.

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 miRNA satellite markers, linked to 7 different autosomal loci implicated in the etiology of ARMD with and without drusen formation, have been mapped to the Xq 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 sequenced for mutation using PCR, SSCP (Single Strand Conformational Polymorphism) and sequencing.

Results: An association between two alleles at hepatic 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 (chi-square=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.
nous blood samples (mothers) and umbilical cord blood samples (fetuses). The coding sequences of PIgf (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 polymorphism in exon 1 of the non-coding sequence of PIgf 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 PIgf gene nor in the FLT1 gene, thereby providing no evidence for a relevant role of both genes in the etiology 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 period. 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-occurrence 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 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-occurrence of the T2DM accelerate the occurrence of CAD by the mechanisms differing from those operating in the nonsmoking and the non T2DM patients.

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