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Abstracts

VORTRÄGE

Symposia

S1-01

Phenotypes of a mutated SWI/SNF complex

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De novo germline variants in several components of the SWI/SNF or BAF complex have been described as causing several entities such as Coffin-Siris syndrome and Nicolaides-Baraitser syndrome, and non-syndromic intellectual disability. Syndromes which can show significant overlap in phenotype such as Borjeson-Forssman-Lehmann syndrome and DOOR syndrome can be caused by mutations in genes with apparently completely different functions and also teratogens like alcohol can cause overlapping phenotypes..
Here the resemblances and differences in the various phenotypes described in literature are discussed, to define each of the phenotypes in more detail, and to establish more detailed genotype-phenotype correlations, if present. In addition, a comparison is made with other entities which show overlap in signs and symptoms, and hypotheses explaining the origin of this overlap are suggested.

S1-02

The Role of microRNAs in Controlling SWI/SNF (BAF) Complexes During Neural Development and Cell Fate-reprogramming

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Complex genetic networks involving transcriptional and epigenetic programs govern the acquisition of a cell fate. Mounting evidence suggests that microRNAs, small RNA molecules that typically repress the expression of their target genes, are important regulators of cellular differentiation. We previously discovered that a set of brain-enriched microRNAs, miR-9/9* and miR-124 (miR-9/9*-124), controls the subunit composition of ATP-dependent BAF chromatin remodeling complexes, promoting the switch of their activities during neural development. In addition, ectopic expression of these microRNAs in human dermal fibroblasts promoted an assembly of neuron-specific BAF subunit composition, leading to conversion of fibroblasts into post-mitotic neurons. Because miR-9/9* and miR-124 are pan-neuronally expressed microRNAs, we hypothesized that transcription factors enriched in specific brain regions would synergize with the generic neurogenic activity of miR-9/9*-124 and guide the neuronal conversion into specific neuronal subtypes. We screened transcription factors highly expressed in GABAergic projection, medium spiny neurons (MSNs), a major neuronal subtype in the striatum, and identified MSN factors that produced a neuronal population highly enriched with GABAergic neurons with miR-9/9*-124. Furthermore, these GABAergic neurons expressed markers of MSNs including DARPP-32 and displayed electrophysiological properties of mature neurons. Further analysis using multiplex single cell gene expression profiling demonstrated that the converted cells expressed transcription factors enriched in MSNs, suggesting an establishment of transcriptional network specific for MSNs. Our results so far highlight the potential of miR-9/9*-124 to be combined with transcription factors to guide the neuronal conversion towards a specific neuronal subtype. We are currently investigating the role of BAF complexes during the neuronal conversion mediated by miR-9/9*-124.

S1-03

SWI/SNF Chromatin Remodelers and Cancer

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Data emerging over the last two years implicate the SWI/SNF (BAF) chromatin remodeling complex as a major tumor suppressor as frequent inactivating mutations in at least seven SWI/SNF subunits have been identified in a variety of cancers. These include inactivating mutations of the gene encoding the ARID1A (BAF250a) subunit in ovarian, endometrioid, bladder, stomach, colorectal and pancreatic cancers; of the PBRM1 (BAF180) subunit in renal carcinomas; of the ARID2 subunit in hepatocellular, lung, and pancreas carcinomas as well as melanomas; of the BRD7 subunit in breast cancers; and of the BRG1 (SMARCA4) subunit in non-small cell lung cancers and medulloblastomas. The SWI/SNF complex includes both core and lineage-specific subunits and utilizes the energy of ATP to modulate chromatin structure and regulate transcription.

My laboratory began studying the SWI/SNF complex when the SNF5 subunit was first identified as a tumor suppressor over a decade ago when it was found to be recurrently and specifically inactivated in a highly aggressive type of pediatric cancer called malignant rhabdoid tumor. We now study the complex using mouse models, cell lines and primary human tumor samples. Our goals are to elucidate the normal function of the complex, identify the mechanisms by which subunit mutations drive cancer formation, and utilize this insight to identify and develop novel therapeutic approaches.

Using mouse models, we have shown that biallelic inactivation of murine Snf5 leads to the rapid onset of cancer formation in 100% of mice with a median latency of only 11 weeks. Heterozygous mice develop rhabdoid tumors that are histologically indistinguishable from their human counterpart and Mx-Cre-mediated conditional inactivation results in 85% of mice developing aggressive mature T cell lymphomas and 15% developing rhabdoid tumors. Intriguingly, the rapid cancer onset arises neither due to defective DNA repair nor due to genome instability, as we have found that the genomes of both the murine and human SNF5-deficient cancers are diploid and indistinguishable from normal cells via high-density SNP arrays. Recently, by sequencing the exomes of 35 human pediatric rhabdoid tumors we have shown that

these cancer genomes contain an extremely low rate of mutations, with loss of SNF5 being essentially the sole recurrent event. Indeed, in two of the cancers there were no other identified mutations. Our results suggest that high mutation rates are dispensable for the genesis of cancers driven by mutation of this chromatin remodeling complex and further that epigenetic dysfunction caused by mutation of the SWI/SNF complex may underlie the broad spectrum of cancers caused by mutation of this complex. Insights into the normal function of SWI/SNF complexes, the mechanisms by which mutation of the complexes drive cancer formation, and potential therapeutic vulnerabilities created by mutation of the complex will be presented.

S2-01

Adventures in Recent Human Evolution Told by Massively Large Catalogs of Human Genetic Variation

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Abstract: Advances in DNA sequencing technology have enabled the collection of massively large data sets of human genetic variation. In this lecture, I will present results from two projects that leverage large-scale sequencing data sets. First, I will describe exome sequencing data from 6,515 individuals, which reveals that most protein-coding variants are rare, arose very recently, and are enriched for deleterious mutations. Second, I will describe a method that we developed to detect Neanderthal lineages that persist in contemporary individuals. Application of this method to whole-genome sequences from over 600 individuals shows that ~20% of the Neanderthal genome survives in present-day humans, there were fitness costs to hybridization, and Neanderthals were a source of adaptive variation for loci involved in skin phenotypes.

S2-03

Genetic and nongenetic inheritance

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Many biologists are calling for an 'extended evolutionary synthesis' that would 'modernize the modern synthesis' of evolution. Biological information is typically considered as being transmitted across generations by the DNA sequence alone, but accumulating evidence indicates that both genetic and non-genetic inheritance, and the interactions between them, have important effects on evolutionary outcomes. I will briefly review the evidence for such effects of epigenetic and cultural inheritance. These issues have major implications for diverse domains, including medicine and human sciences where they may profoundly affect scientific research strategies. For instance, non-genetic inheritance may explain a significant part of one of the major enigma of current molecular biology, namely the case of the missing heritability, which concerns many human supposedly genetic disorders. The missing heritability rather suggests that we should abandon the current genocentric framework of inheritance and adopt a broader view including non-genetic inheritance into an extended theory of evolution.

S3-01

Germline and somatic TERT promoter mutations in melanoma and other cancers

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Human telomerase reverse transcriptase (TERT) encodes a rate limiting catalytic subunit of telomerase that maintains telomere length. Increased telomerase activity is perceived to be one of the hallmarks of human cancers and the transcriptional deregulation of the TERT gene is the major cause of its cancer-specific activation. Though epigenetic regulation and gene amplification had been considered as the possible cause, the precise mechanism behind TERT activation in cancers had mostly remained unknown. We recently described an occurrence of a disease segregating causal mutation at the -57 bp upstream of ATG start site in the promoter region of the telomerase reverse transcriptase (TERT) gene in a large melanoma family with early on-set and severe form of the disease. Screening of the TERT core promoter in tumors from unrelated melanoma patients showed recurrent and mutually exclusive mutations at the -124 and -146 bp from the ATG site with frequency higher than any other mutation reported in melanoma to-date (Horn et al. Science, 2013, 339:959-61). The familial and somatic mutations resulted in de novo creation of CCGGAA/T binding motifs for E-twenty six/ternary complex factors (Ets/TCF) transcription factors, with consequent increase in TERT expression. The TERT promoter mutations have now been shown to be widespread in many cancer types and have been regularly associated with an increased gene expression and adverse forms of the disease. Our recent study on bladder cancer, clearly demonstrated an association of the TERT promoter mutations with a poor patient survival and an increased disease recurrence through a modification by common polymorphism at a pre-existing Ets2 binding site (Rachakonda et al. PNAS 2013, 2013 Oct 7. Epub ahead of print). These findings have resulted in major conceptual advancements and have shown that tumor specific changes in a non-coding region can initiate cancer through change in gene expression and provide in part a definite mechanism for cancer specific TERT over-expression.

S3-02

Telomeres and coronary artery disease

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Coronary artery disease (CAD) has well established life-style, demographic and genetic risk factors. However, at an individual level, both susceptibility to CAD and its age of onset varies considerably, even for subjects with apparently similar risk factor profiles. CAD is an age-associated disease but not an inevitable consequence of ageing. Because of these epidemiological observations, the concept has arisen that CAD may, at least in part, represent a manifestation of premature biological ageing. Using telomere length as a potential marker of biological age, we and others have shown that mean leucocyte as well as vascular wall telomere length is shorter in subjects with CAD compared with controls and that shorter mean telomere length in leucocytes is present before, and independently predicts, the development of clinically overt CAD. These studies have consistently shown that, except in very old subjects, mean leucocyte telomere length in subjects with (or prone) to CAD is similar to normal subjects who are chronologically 8-12 years older. Several studies have shown that risk factors for CAD such as male gender, Type 1 and Type 2 diabetes, obesity, smoking psychological stress and low socio-economic status are associated with shorter leucocyte telomeres. However, adjustment for these risk factors does not attenuate the association between shorter telomere length and CAD, suggesting that the relationship does not simply reflect the effect of these risk factors on telomere attrition. Hitherto, an unresolved question was whether shorter telomere length is simply a marker of biological ageing or whether shorter telomeres play a direct role in the development of CAD. Recently, using genome-wide arrays we identified seven genetic loci associated with telomere length and showed in a Mendelian Randomisation analysis that alleles associated with shorter telomeres were associated with increased risk of CAD. This finding suggests a causal role for shorter telomere length in the aetiology of CAD. The "telomere hypothesis" of CAD therefore has the potential to

integrate different known aspects regarding the aetiology of CAD and explain its variable age of onset and expressivity. This talk will review the evidence for this hypothesis.

S3-03

Prenatal Stress, Telomere Biology, and Fetal Programming of Health and Disease Risk

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Substantial evidence suggests conditions in intrauterine life may play a critical role in subsequent health and disease susceptibility (i.e., the concept of fetal or developmental programming of health and disease). The elucidation of biological mechanisms underlying these effects is an area of active investigation. We suggest that telomere biology may represent a novel mechanism underlying the effects of a disparate set of suboptimal intrauterine exposures on various health and disease risk phenotypes. Telomere biology is known to play a fundamental role in genomic integrity, cellular regeneration, physiology, aging, disease risk and mortality. The initial setting of telomere length (TL) in early life has major implications for telomere maintenance throughout the lifespan. From an evolutionary-developmental perspective, energy substrate availability (i.e., nutrition) and challenges that have the potential to impact the structural or functional integrity and survival of the organism (i.e., stress) likely represent the most important environmental considerations underlying natural selection and developmental plasticity. Maternal stress and nutrition in pregnancy therefore represent attractive candidate processes in the context of fetal programming of telomere biology. Our previous work has established an important role for prenatal stress and stress-related processes in adult telomere biology.

In our recent studies we used data from two longitudinal birth cohorts in which stress- and nutrition-related processes were assessed during pregnancy, and telomere length (TL) was subsequently measured in newborns (cord blood) and infants (buccal cells).

Our results suggest that among the nutrition-related factors maternal lower folate levels (an essential methyl donor) and higher triglyceride concentrations in early pregnancy were significantly and independently associated with shorter newborn TL. Among psychosocial stress-related measures higher maternal pregnancy-specific stress was associated with shorter newborn TL. Maternal estrogen (E3) concentrations during early pregnancy seem to have a protective effect on infant telomere length because they were associated with longer infant TL.

Taken together, our findings provide evidence in humans that maternal nutrition and stress-related processes during pregnancy may exert a programming effect on the newborn and infant telomere biology system. In utero telomere biology represents a potential molecular mechanism whereby different exposures in this critical developmental period before birth could impact subsequent health and disease susceptibility over the life span, including aging and longevity.

S4-01

News from the RNA world or Deciphering the function of RNA modifications or The Epitranscriptome

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Cytosine-5 methylation (m5C) is a widespread modification in both DNA and RNA and the corresponding methyltransferases share many structural features. Whereas the functions of m5C in DNA have been extensively studied, the cellular and molecular functions of the same modified nucleobase in RNA remain unclear. We found that m5C is a common post-transcriptional modification in transfer RNA (tRNA) and other non-coding RNA species. RNA-methylation pathways is an important regulator of stem cell differentiation in various tissues, and loss-of-function mutations in the cytosine-5 RNA methylase NSun2 causes neuro-developmental diseases in humans. Depletion of the m5C modification in tRNAs causes their cleavage by angiogenin and the cleaved tRNA fragments are implicated in the reduction of protein translation rates in response to cellular stress stimuli. Thus, post-transcriptional cytosine-5 methylation is an important and unexpected regulatory pathway to control cellular behaviour.

S4-02

Long noncoding RNAs and enhancers

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Long non-coding RNAs are transcripts functionally expressed in many organisms. Using the ENCODE data covering the human genome, more than 10,000 long non-coding RNAs have been annotated. We have previously found long non-coding RNAs to have enhancer-like activity, and positively regulate genes that are adjacent in the linear genome. To define the precise mode by which such enhancer-like RNAs function, we have depleted factors with known roles in enhancer function and transcriptional activation. Depletion of the components of the co-activator complex, Mediator, specifically and potently diminishes the non-coding RNA-induced activation of transcription. Selected long non-coding RNAs interact with Mediator to regulate its chromatin localization and kinase activity towards histone H3 serine 10. Using chromosome conformation capture we can detect DNA looping between the long non-coding ncRNA loci and their targets, that is dependent on the presence of both the long non-coding RNA and the Mediator complex.

S4-03

Circular RNA

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Usually introns are spliced out of the pre-mRNA in a linear fashion, progressing from the 5 '- towards the 3'-end. However, occasionally splicing back-tracks and joins a 5' splice site to an up-stream 3'-splice site, resulting in a 5'-3' circularized exon. Circularization of exons does occur at low level for many exons and for a significant proportion of transcripts the circularization of one or more exons is more prominent than normal splicing. We have recently characterized two examples where the circular RNA form appear to play an important role in regulating the activity of the microRNAs with profound impact on a number of down stream genes. The most well-characterized example derive from a non-coding antisense transcript to neuronal specific gene CDR1 [1]. Only the circular product is detected in large amounts from this locus in neurons where it acts as a highly efficient microRNA-7 (miR-7) sponge and hence named it ciRS-7 (Circular RNA Sponge for miR-7) [2]. ciRS-7 harbours more than 70 selectively conserved putative miRNA target sites and it is highly and widely associated with Ago-proteins in a miR-7 dependent manner. While the circular RNA is completely resistant towards miRNA-mediated target destabilization, it strongly suppresses miR-7 activity resulting in elevated levels of miR-7 targets. In the mouse brain, we observe overlapping neuronal expression patterns and colocalization of ciRS-7 and miR-7 in distinct regions of the brain suggesting a high degree of endogenous interaction. We also show that ciRS-7 can induce established miR-7 targets including

SNCA, EGFR and IRS2, implicated in Parkinson disease, cancer and diabetes, respectively [3]. The ciRS-7 is itself under the control of miR-671 that cleaves and destroys the ciRS-7. Hence, miRNA with near complementary match to circular RNA may constitute an essential pathway to remove both functional and undesired circular RNA products from the cell. The function of circular RNAs as miRNA sponges appears to be a more general phenomenon based on the observation that circular testis specific RNA, SRY, serves as a miR-138 sponge and that circular SRY expression can increase miR-138 targeted mRNAs. Furthermore, analysis of NGS data have revealed the existence of thousands of or circular RNAs often differentially expressed in a developmental and tissue specific fashion.
We have also studied the mechanism of circular RNA biogenesis and based on this established optimized vectors to express ciRSs as generic platform that enables sponging any miRNA of choice by reprogramming the seed sequences.

[1] Hansen et al. (2011) EMBO J. 30:4414-22
[2] Hansen T.B. et al. (2013) Nature 495:384-8
[3] Hansen et al. (2013) Cancer Res. 73:5609-12

S5-01

Calling Somatic Mutations in Cancer

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Contrary to common belief analysing sequencing data is not trivial. There are many reasons for this, - sequencing reads are short compared to the previous sequencing generation, the output of 2nd generation sequencers makes producing enough sequence for a whole human genome tractable, but dealing with this amount of data is not trivial, reference sequences are not of consistently high quality, certain genomic structures are by their nature difficult to analyse and many more. The analysis of sequencing data is made up of a chain of different analyses; - first analogue data is converted into sequences with base-by-base quality scores. This is usually done using manufacturer's software. Next, reads are aligned to a reference. Different software tools have been developed to do this. However, exhaustive, deterministic alignment is computationally very expensive as differences relative to the reference need to be allowed. The third element is calling of a variant. Depending on the nature of the variant and kind of experiment that is carried out different approaches need to be applied. Calling SNVs, indels and structural variants pose different challenges. Comparing a tumour genome and the matching normal genome from a patient can be fraught by issues such as the tumour having low cellularity or subclones being at low abundance, or that the normal sample is contaminated with the tumour genome due to circulating tumour cells or free, circulating tumour DNA. The fourth part is predicting the functional effect of a difference in a genome. Many tools exist for this; however, there is not much consensus between different tools. Predictions focus on changes in aminoacid sequences while variants outside genes are largely neglected. There are currently no guidelines for the deployment of 2nd generation sequencing in diagnostics. Many issues remain to be resolved.

S5-02

Mutational signatures in chronic lymphocytic leukemia: subclonal evolution and clinical relevance

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Chronic lymphocytic leukemia (CLL), the most frequent leukemia in adults, is amongst the first human neoplasias whose study has benefited from the recent introduction of high-throughput sequencing technologies of outstanding efficiency. The aim of the CLL-ICGC Spanish Consortium is to generate a catalogue of genetic, epigenetic and transcriptomic alterations relevant to the pathogenesis and clinical evolution of this heterogeneous disease. These studies have allowed us the identification of a series of oncogenes and tumor suppressors, such as NOTCH1, SF3B1, POT1, XPO1 and MYD88, which are recurrently mutated in CLL. Functional analysis of these mutated genes together with clinical studies in a large number of CLL patients have led us to define specific genes and molecular pathways that drive the development and progression of this disease. Parallel studies have also shown that CLL development is associated with an impressive reprogramming of the methylation profile of lymphoid cells, which has a profound impact in the transcriptional landscape of CLL as well as in the clinical evolution of patients. Additionally, these genome sequencing analyses have contributed to define the highly dynamic subclonal evolution of this malignancy and its relevance for the clinical outcome of CLL patients. Hopefully, the integration of all this molecular information may be soon useful for the better clinical management of CLL patients and for the development of new treatments for a disease with such a complex and dynamic biography.

S5-03

Mutational signatures in human cancers: detection and clinical relevance

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Cancer is the ultimate disorder of the genome, characterised by not one or two substitutions, indels or copy number aberrations, but hundreds to thousands of acquired mutations that have been accrued through the development of a tumour. The set of mutations observed in a cancer genome is not simply a random accumulation of variants. It is the aggregate outcome of several biological mutational processes comprising an underlying mechanism of DNA damage mitigated by the DNA repair pathways that exist in human cells. Each mutational process will leave its distinctive mark or mutational signature on the cancer genome.
The recent increase in the speed of sequencing, offered by modern sequencing technologies permits unprecedented access to the entire tumour genome of a cancer patient. Utilising this surge in scale, we set out to extract the mutational signatures that bave been operative in 21 whole-genome sequenced breast cancers in a pilot experiment. Our mathematical methods reveal known signatures of mutagenesis as well as novel signatures. We find from further interrogation of nearly 5 million mutations sourced from over 7000 cancers sequenced worldwide, that more than 20 different signatures exist; imprints determined by the underlying source of endogenous and exogenous DNA damage and repair. Delving deep into the architecture of these signatures, we uncover hidden biological insights. Early forays into clinical applications will also be discussed.

S6-01

Twinning: Causes and Consequences

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Historically, twins have fascinated all societies and human geneticists have used twins to assess heritability for complex disorders. Monozygotic (MZ) twinning is essentially unique to humans and may provide insights into early human development. With the advent of molecular studies, it has become clear that discordant MZ twins are common and help to elucidate several pathogenic mechanisms. Maternal physiology during a twin pregnancy is quite different from that of singletons and

may lead to differences in fetal programming and epigenetic stability. Vanishing co-twins are frequent and may also lead to residual maternal physiologic alterations. Maternal fetal microchimerism is increased in twin pregnancies and between dizygotic (DZ) twins if the pregnancy is complicated. ARTs leads to an increase of both MZ and DZ twinning and strains the health care system because of the prematurity associated with twinning. There is also an increase of monochorionic, diamniotic DZ twins with ARTs. Finally, MZ twins are not identical (albeit they do have many more identical DNA sequences than sibs) and they probably represent a fascinating congenital anomaly.

S6-02

Modern Twin Studies in Human Genetics

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The classical twin study has been a powerful heuristic in biomedical, psychiatric and behavioral research for decades. Twin registries worldwide have collected biological material and longitudinal phenotypic data on tens of thousands of twins, providing a valuable resource for studying complex phenotypes and their underlying biology. We will illustrate the continuing value of twin studies in the current era of molecular genetic studies. We show how classical twin methods combined with novel technologies represent a powerful approach towards identifying and understanding the molecular pathways that underlie complex traits. In particular, technological advances allow us to assess the extent to which twins resemble each other at the level of molecular processes that contribute to their phenotypic similarity. We will show how the comparison of discordant MZ twins can lead us into novel pathways associated with disease, and emphasize that a unique advantage of the MZ twin design is the ability to study biological discordance.

S6-03

Twins and integrated omics

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Twins are ideal in epigenetic research, because they are natural controls for genetic background, as well as many parental and environmental effects. Epigenetic studies of twins can provide insights into epigenetic heritability, for example, using DNA methylation patterns as a dynamic quantitative trait.

Current research in monozygotic twins with high discordance rates for common diseases suggests that underlying environmental or epigenetic factors may be involved. Epigenetic studies in disease-discordant monozygotic twins demonstrate the power of this design to successfully identify epigenetic changes associated with common complex disease traits.

The Department of Twin Research (DTR) in conjunction with the Beijing Genomics Institute has embarked on a large-scale epigenetic study of 5,000 adult twins (EpiTWIN). The aim of this study is to use whole genome methylation sequencing to improve our understanding of how genetic and environmental factors can impact on epigenetics and how DNA methylation pattern variation can affect a wide range of complex traits.

This study first explores the heritability and genomic biology of DNA methylation patterns within a large scale family design. Secondly, the function of the changes can be explored using multiple datasets on the same individuals using an integrated o

Selected Presentations (SEL)

sel-01

Identification of a novel susceptibility locus for nonsyndromic cleft lip and palate at chromosome 15q13

Ludwig K.U.[1,2], Böhmer A.C.[1,2], Peters H.[3], Graf D.[4], Gültepe P.[1,2], Al-Chawa T.[1,2], Fier H.[5], Mossey P.A.[6], Steegers-Theunissen R.P.[7,8], Rubini M.[9], Lange C.[5,10], Nöthen M.M.[1,2], Knapp M.[11], Mangold E.[2]

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Cleft lip with or without cleft palate is one of the most common congenital malformations worldwide. It may either occur as part of a complex malformation syndrome, or as an isolated, nonsyndromic anomaly. The latter represent about 70% of all cases. Nonsyndromic cleft lip with or without cleft palate is considered to be of multifactorial etiology, with both genetic and environmental factors contributing to cleft susceptibility. It is a variable phenotype and, based on epidemiological evidence, can be subdivided into nonsyndromic cleft lip only (nsCLO) and nonsyndromic cleft lip and palate (nsCLP).

Genome-wide studies (GWAS, linkage and meta-analyses) and replication approaches have recently led to the identification of 15 susceptibility loci for nonsyndromic cleft lip with or without cleft palate. However, a number of additional genetic risk factors still await elucidation. In the present study we used data from a recent genome-wide meta-analysis integrating data from European and Asian populations (Ludwig et al. 2012, Nature Genetics) and combined these with results from a replication study performed in an independent European trio cohort (n=793; Mangold et al. 2010, Nature Genetics). Integration of subgroup-information on nsCLO or nsCLP revealed rs1258763 on chr. 15q13 as a novel genome-wide significant locus associated with nsCLP in the European population ($P = 2.61\times10\text{-}08$), and in the combined dataset of European and Asian populations ($P = 1.04\times10\text{-}08$). The associated region maps intergenically, between the Gremlin-1 (GREM1) and Formin-1 (FMN1) genes. GREM1 is involved as antagonist in BMP pathways which have been implicated in facial genesis. Sequencing of the entire coding region of GREM1 in 196 patients and 196 controls did not reveal a causal variant. However, statistical burden analysis demonstrated a significant overrepresentation of rare variants within patients ($P = 0.02$), and analyses of Grem1 expression during embryonic craniofacial development in mice might suggest a functional role of Grem1 in lip and secondary palate formation. Of note, the top variant rs1258763 at the 15q13 locus has previously been shown to influence normal variation in facial morphology, namely nose width (Boehringer et al. 2011, EJHG, and Liu et al. 2012, PLoS Genetics).

Our study identified the 15q13 locus as new susceptibility region for nsCLP, a subtype of nonsyndromic cleft lip with or without cleft palate. Our results demonstrate that the combination of increasing sample sizes and precise phenotypic information might help to identify further risk loci for genetically complex traits.

sel-02

A de novo gain-of-function mutation in SCN11A causes loss of pain perception

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The ability to perceive and react to pain is essential to protect the body from injury. Pain also helps to define the limits to which we can stress the body before causing damage. In the condition of "congenital inability to experience pain" self-mutilations, slow-healing wounds and painless bone fractures are the consequence of the lack to sense pain. We show that loss of pain perception results from a specific missense mutation in SCN11A, which encodes the NaV1.9 voltage-gated sodium channel. This de novo mutation (p.Leu811Pro) was identified in independent individuals with the condition using whole-exome sequencing. SCN11A / NaV1.9 is expressed in nociceptors, specialized sensory neurons that transmit pain signals from the body periphery to the central nervous system. We engineered mice to carry the missense mutation in Scn11a and found that these animals have reduced sensitivity to pain. Knockin-mice showed self-inflicted tissue lesions, likewise recapitulating aspects of the human phenotype. Mutant NaV1.9 channels in sensory neurons of knockin-mice are functional, but display excessive activity at resting voltages and cause sustained depolarization of pain-sensing neurons. This gain-of-function in the basal activity of NaV1.9 leads to progressive inactivation of other sodium and calcium channels, the principal components of the action potential in pain-sensing neurons. A resultant conduction block prevents signal transmission to the brain as supported by aberrant synaptic transmission in the spinal cord of knockin-mice. The findings raise the possibility that manipulating NaV1.9 activity could be a new pathway for treating pain.

sel-03

A Novel Method for ChIP-seq Reveals Mutation-Specific Pathomechanisms of HOXD13 and MSX2 Missense Mutations

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Mutations in transcription factors (TF) are frequently involved in the pathogenesis of developmental disorders. However, elucidation of the mutational pathomechanisms has proven to be difficult, especially for missense mutations with a suspected gain-of-function. ChIP-seq, which couples chromatin immunoprecipitation with high-throughput sequencing, is a powerful tool to investigate TF binding sites on a genome-wide scale but a number of technical hurdles have limited its application for the functional characterization of TF mutations. Here, we present a novel methodology using ChIP-seq to explore the effect of missense mutations in TFs. Our method is based on low level expression of the tagged target-TF in chicken mesenchymal stem cells via a retroviral expression system. We elucidated the mechanism underlying a novel missense mutation in HOXD13 (Q317K) associated with a complex hand and foot malformation phenotype. ChIP-seq results for HOXD13wt and HOXD13Q317K showed the wildtype to bind to the published HOXD13 binding site and show that the mutation changes the recognition sequence to that of PITX1, another TF expressed in the limb. A similar shift was not observed with another mutation, Q317R, which is associated with brachysyndactyly, suggesting that the bicoid/PITX1-shift observed for HOXD13Q317K is likely to be related to the severe clinical phenotype. Expression analysis in vitro and viral overexpression in developing chicken limb buds provided functional evidence that the mutation partially converts HOXD13Q317K into a TF with PITX1-like properties.

In a next step, we used our method to investigate loss- and gain-of-function mutations in MSX2, R172H and P148H, which cause enlarged parietal foramina and Boston-type craniosynostosis, respectively. Investigation of wildtype and mutant binding sites confirmed that the MSX2R172H mutant loses most binding sites, whereas the GOF MSX2P148H binding sites largely overlap with those of MSX2wt. Intriguingly, our ChIP-seq results revealed a difference between the MSX2wt and MSX2P148H peaks in the frequency of cofactor binding sites. Recognition sequences for RUNX2, an antagonistically acting cofactor of MSX2, were identified in MSX2wt and MSX2P148H peaks, however to a different degree. Moreover, an extension of our ChIP-seq procedure allowed investigation of MSX2-RUNX2 interaction in vivo. Importantly, this difference in cofactor binding sites provides a molecular basis for the adverse effects of RUNX2 and MSX2 during development of the cranial skeleton that has been suggested to underlie the MSX2P148H craniosynostosis phenotype.Collectively, applying ChIP-seq to functionally characterize the pathophysiology of TF mutations provides a robust platform to identify distinct molecular pathomechanisms without prior knowledge, which can then be verified in custom-designed functional experiments.

sel-04

Mutational Profiling of Germinal-Center Derived B-Cell Lymphomas by Whole Genome Sequence Analysis

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Germinal-center derived B-cell lymphomas (GCB-lymphomas) are the most common B-cell lymphomas. They include follicular (FL), diffuse large B-cell (DLBCL) and Burkitt (BL) lymphomas. In the framework of the ICGC (International Cancer Genome Consortium)-MMML-Seq (Molecular Mechanisms in Malignant Lymphoma by Sequencing)-Project funded by the German Federal Ministry of Education and Research (01KU1002A-J) we have finished whole genome sequencing of currently 60 GCB-lymphomas and matched normal controls; additional samples are ongoing. To control for the physiologic somatic hypermutation active in normal GCB-cells, three sorted lymphocyte populations from two non-tumorous tonsils were sequenced. Genomic analysis of the samples is complemented by transcriptome and miRNAome sequencing. DNA methylation is being determined in all samples by 450K arrays as well as by whole genome bisulfite sequencing in a subset of 29 GCB-lymphomas and two controls.

We observed between 1914 and 5325 somatic single nucleotide variants (SNVs) and small insertions / deletions (indels) in BL (median 2632), between 3027 and 40922 (median 10615.5) in DLBCL, and between 1692 and 12621 (median 4334.5) in FL. Several recurrently mutated genes have been identified, which include known lymphoma genes as well as some genes not previously associated with B-cell lymphomas. On the whole genome level, regional differences in the density of single nucleotide variants were observed. Integration of replication timing data enabled the differentiation into clusters of SNVs in early replicating regions, which affect loci undergoing somatic hypermutation in B-cells or B-cell lymphomas, and broader regions of generally higher mutation density which are late replicating regions of the genome. Examination of the SNV types in their sequence context revealed the activity of different mutational signatures in the different lymphoma subtypes. Finally, the analysis of copy-number balanced and unbalanced rearrangement events (structural variations, SVs, and copy-number alterations, CNAs) identified significant differences in the number of those events between BL, DLBCL and FL, with DLBCL genomes being in many cases much more complex than the genomes of BL and FL. Even complex DNA rearrangements that may have been generated in catastrophic one-off events (chromothripsis) have been observed in DLBCL cases.
In summary, our ongoing whole genome sequencing analysis provides novel insights into the different mutational mechanisms active in the various subtypes of GCB-lymphomas.

W1 Monogenic Diseases I

W1-01

Compound inheritance of a low-frequency promoter deletion and a null mutation in a new gene causes Burn-McKeown syndrome (BMKS)

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Burn-McKeown syndrome (BMKS, OMIM %608572) was first described by Burn et al., in 1992, and is characterized by cardiac defects, choanal atresia, sensorineural deafness and craniofacial dysmorphisms including short palpebral fissures, high nasal bridge and large ears. This distinct condition seems to be rare as only three papers describing three brother pairs and two sporadic patients have been published so far. The presence of similarly affected brothers in three families suggests X-linked or autosomal recessive inheritance. All three brothers were born to non-consanguineous parents.
We performed exome sequencing in the brother pair published by Wieczorek et al., 2003, and in three sporadic patients with the tentative diagnosis of BMKS. We identified two heterozygous nonsense mutations in an autosomal gene, (p.Glu117*) in the brother pair and (p.Glu13*), as well as one frameshift mutation (p.Val44Alafs*48). Screening for deletions (MLPA/array CGH) and mutations (Sanger sequencing) in seven additional families with BMKS revealed three large deletions (0.484, 1.164 and 4.7 MB), containing the entire candidate gene and adjacent genes. Further three patients are currently being analysed, two of them have at least partial gene deletions. In two patients, no mutation was identified. All mutations and one deletion were inherited from a healthy parent, but in no case a second mutation was found in the coding region of the gene. Final results are pending.
Thus, we postulated that a regulatory variant on the other allele might influence the phenotype. Whole genome sequencing was performed in five patients, including one brother pair, to address this question. In all patients, a 34 bp promoter deletion on the other allele of the same gene was identified. Segregation analyses for the promoter deletion by PCR in the families revealed that all patients with a null mutation on one allele had the promoter deletion on the other allele. As the promoter region was not covered in the control genomes which were available, we screened a control cohort of 200 healthy German and 178 South Asian individuals. Four of the German controls carried this promoter deletion, which suggests a minor allele frequency of 1% for this population.
Preliminary results of primer extension analyses of RNA from peripheral blood suggest a negative effect of the 34 bp-deletion on the expression level of this allele. Further functional analyses, reporter gene assays and RNA seq, are currently being performed.
In conclusion, we show that unrelated patients with an inactivating mutation of one allele have a low-frequency promoter deletion on the other allele. No patient had two inactivating mutations or was homozygous for the promoter deletion. Thus, our results indicate that BMKS is an autosomal recessive condition caused by an unusual mode of inheritance and highlight the importance of analyzing regulatory regions of causative genes.

W1-02

Homozygous mutation in the mRNA decapping enhancer EDC3 involved in the nonsense-mediated decay pathway causes autosomal-recessive intellectual disability

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Nonsense-mediated decay (NMD) is a mechanism for degradation and regulation of mRNAs in eukaryotic cells. As a first step, the monomethyl-guanosine cap of the mRNA is removed by a decapping complex involving the decapping factor DCP2. The mRNA is then degraded from 5' to 3' by NMD factors like UPF1 and UPF2. The NMD pathway has already been related to human phenotypes presenting intellectual disability as for instance the deletion of UPF2. The Homo Sapiens Enhancer of mRNA Decapping 3 (EDC3), amongst others, enhances DCP2. Data from yeast and drosophila show an accumulation of mRNAs if EDC3 is impaired.
Here we report a mutation in EDC3 in a consanguineous family with two children presented with mild intellectual disability. Assuming autosomal-recessive inheritance, we undertook autozygosity mapping which revealed two candidate regions with a total length of 33 Mb. Exome sequencing using exome enrichment with Agilent SureSelect All Exon 50Mb kit and a SOLID 5500xl platform revealed one candi-

date mutation; EDC3 NM_025083:c.T161C:p.Phe54Ser. This position is evolutionary highly conserved and was predicted to be disease causing by four in silico programs. It was neither observed in public databases nor in over 300 in house exome. In 280 ethnically matched controls there was one heterozygous person from the same village as the family. Biochemical modeling predicted that serin at position 54 crucially alters the hydrophobic core of the LSm domain of EDC3. This LSm domain has been shown to interact with the decapping factor DCP2 and is therefore very likely essential for the functioning of EDC3. To examine whether the regulation of mRNAs is altered in patient cells, we conducted transcriptome profiling by sequencing (RNA-Seq) on both patients and two controls using RNA from lymphoblastoid cell lines. We identified 14 genes which were significantly altered in their expression (corrected $P < 0.05$). Eleven genes were chosen to be validated with quantitative PCR and we could confirm the RNA-Seq data in 9 of those including the upregulation of GABRA4 and KCNA6. Since the decapping enzyme DCP2 has been reported to prefer longer mRNAs as substrates, we also compared expression levels of long and short mRNAs; we observed that the longest 50% mRNAs showed a significantly higher expression than the shortest 50% mRNAs ($P = 2.20E-16$). This indicates an accumulation of long mRNAs due to less activation of DCP2 by the altered EDC3. Since brain specific proteins tend to be generally longer, this may point towards the isolated neurological phenotype of our patients. Taken together, these data suggest that the variant in EDC3 has a pathogenic effect and is causative for the ARID in the examined family. Further gene expression experiments after siRNA knockdown of EDC3 are ongoing to gain better understanding of the functions of EDC3 and the pathomechanisms leading to intellectual disability.

W1-03

A homozygous mutation in the complex IV assembly factor COX20 (FAM36A) as a novel cause of a dystonia-ataxia syndrome

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A combination of predominant dystonia and mild cerebellar ataxia is referred to as DYTCA syndrome. We examined two affected siblings with healthy, consanguineous, Turkish parents. Both patients presented with a combination of childhood-onset cerebellar ataxia, dystonia, and sensory axonal neuropathy. Routine diagnostic investigations excluded known genetic causes such as mtDNA deletions/depletion or pathogenic mutations in POLG1, CABC1, APTX, COQ2, COQ9, PDSS1, and PDSS2. Biochemical analyses of a muscle biopsy revealed few atrophic fibres, mildly enlarged and proliferated mitochondria, an isolated mitochondrial respiratory chain complex IV deficiency (26.5U/gNCP, normal: 112-351), and coenzyme Q10 deficiency (42.2 nmol/gNCP, normal: 160-1200). By exome sequencing, we identified a homozygous missense mutation (c.154A>C; p.Thr52Pro) in both patients in exon 2 of the COX20 (FAM36A) gene which encodes a complex IV assembly factor. This variant was confirmed by Sanger sequencing, heterozygous in both parents and absent from 427 healthy controls. The exact same mutation was recently reported in a patient with ataxia and muscle hypotonia. Among 128 early-onset dystonia and/or ataxia patients, we did not detect any other patient with a COX20 mutation. cDNA sequencing and semi-quantitative analysis were performed in fibroblasts from one of our homozygous mutation carriers and six controls. In addition to the exchange of an amino acid, the mutation led to a shift in splicing towards the expression of a presumably non-coding transcript that lacks Exon 2. Finally, we measured the form factor in fibroblast cultures from the index patient and a healthy control to assess the mitochondrial network. There was a trend towards increased fusion in COX20 mutant cells compared to control fibroblasts. In conclusion, we report a novel genetic cause of a DYTCA syndrome which is characterized by reduced complex IV activity.

W1-04

Mutations in POGLUT1, encoding protein O-glucosyltransferase 1, cause autosomal dominant Dowling-Degos disease

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Dowling-Degos disease (DDD) is an autosomal dominant genodermatosis which is characterized by progressive and disfiguring reticulate hyperpigmentation affecting the flexures, large skin folds, trunk, face and extremities. We previously identified loss-of-function mutations in KRT5 in fewer than half of the individuals of our DDD cohort.
In this study, we undertook an exome sequencing approach to identify additional genetic causes of DDD by initially focusing on five unrelated affected individuals with similar DDD phenotypes and without KRT5 mutations. Analysis of the exome data revealed three different heterozygous mutations in these five individuals, all of which are in the same gene. These mutations, namely c.11G>A (p.Trp4*), c.652C>T (p.Arg218*), and c.798-2A>C, are within POGLUT1, which encodes protein O-glucosyltransferase 1. By further screening of POGLUT1 in all our unexplained cases of DDD, we identified six additional mutations as well as two of the above described mutations. Immunohistochemistry of skin biopsies of affected individuals with POGLUT1 mutations showed significantly weaker POGLUT1 staining in the upper parts of the epidermis in comparison to healthy controls. We characterized the wild type POGLUT1 and various abberant forms by immunobloting, immunofluorescence studies, protein modeling and transcript analysis. Immunoblot analysis revealed that translation of either wild type POGLUT1 or a mutated form with an amino acid substitution led to the expected size of about 50 kDa, while a nonsense mutation led to translation of a truncated protein of about 30 kDa. Immunofluorescence analysis identified a co-localization of the wild type protein with the endoplasmic reticulum and a notable aggregating pattern for the truncated protein. Protein modeling and transcript analysis further supported the pathogenicity of the identified mutations. Recently, mutations in POFUT1, encoding protein O-fucosyltransferase, were also reported to be responsible for DDD. Interestingly, both POGLUT1 and POFUT1 are essential regulators of Notch activity. Our results fur-

thermore emphasize the important role of the Notch pathway in pigmentation and keratinocyte morphology.

W1-05

Null mutation in PGAP1 impairs GPI-anchor maturation and causes intellectual disability

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Many eukaryotic cell-surface proteins are anchored to the membrane via glycosylphosphatidylinositol (GPI). There are at least 26 genes involved in biosynthesis and remodeling of GPI anchors. We recently reported on two hypomorphic mutations in PGAP2 in two independent consanguineous families with non-specific severe intellectual disability. Further hypomorphic coding mutations in six of these genes have been reported to cause decreased expression of GPI anchored proteins (GPI-APs) on the cell surface and to cause autosomal-recessive forms of intellectual disability (ARID). Now we report for the first time, to our knowledge, on a homozygous mutation in PGAP1, a further gene of this pathway.

We performed homozygosity mapping and exome sequencing in a family with two children with non-specific ARID and identified a homozygous 3 bp deletion (p.Leu197del) in the GPI remodeling gene PGAP1. PGAP1 is a deacylase that removes an acyl-chain from the inositol of GPI anchors in the endoplasmic reticulum immediately after attachment of GPI to proteins. In silico prediction and molecular modeling strongly suggested a pathogenic effect of the identified variant. The expression levels of GPI-APs on lymphoblastoid cells derived from an affected person were normal. However, when those cells were incubated with phosphatidylinositol-specific phospholipase C (PI-PLC), GPI-APs were cleaved and released from lymphoblastoid cells from healthy individuals whereas GPI-APs on the cells from affected person were totally resistant. Transfection with wild type PGAP1 cDNA rescued the PI-PLC sensitivity. These results indicate that GPI-APs were expressed with abnormal GPI structure due to a null mutation in the remodeling gene PGAP1. Our results add PGAP1 to the growing list of GPI abnormalities and indicate that not only the cell surface expression levels of GPI-APs but also fine structure of GPI-anchors is important for normal development. Mutations in GPI synthesis pathway genes seem to have a strong impact on development of the central nervous system and further mutations in this pathway will probably be identified soon.

W1-06

Mutations in PGAP3 impair GPI-anchor maturation causing a subtype of hyperphosphatasia with mental retardation

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GPI-anchored proteins play important roles in many biological processes and mutations affecting proteins involved in the synthesis of the GPI-anchor are reported to cause a wide spectrum of intellectual disabilities (ID) with characteristic additional phenotypic features. Here we describe in total five individuals from three unrelated families in whom we identified mutations in PGAP3, encoding a protein that is involved in GPI-anchor maturation. Three siblings in a consanguineous Pakistani family presented with profound developmental delay, severe ID, no speech, psychomotor delay, and postnatal microcephaly. A combination of autozygosity mapping and exome sequencing identified a 13.8 Mb region on 17q11.2-q21.32 harbouring a novel homozygous c.275G>A variant in PGAP3. Subsequent testing showed elevated serum alkaline phosphatase (ALP, a GPI-anchored enzyme) in all three affected children. In two unrelated individuals in a cohort with developmental delay, ID and elevated ALP we identified compound heterozygous variants c.439dupC and c.914A>G and a homozygous variant c.314C>G. The 1bp duplication causes a frameshift and nonsense mediated decay. Further evidence supporting pathogenicity of the missense mutations p.(Gly92Asp), p.(Pro105Arg) and p.(Asp305Gly) was provided by the absence of the variants from ethnically matched controls, phylogenetic conservation and functional studies on CHO cell lines. Taken together with recent data on PGAP2, these results confirm the importance of the later GPI-anchor remodelling steps for normal neuronal development. Impairment of PGAP3 causes a new subtype of hyperphosphatasia with ID, a congenital disorder of glycosylation that is also referred to as Mabry syndrome.

W2 Cancer Genetics

W2-01

Systematic screening of eight polymerase genes identified germline POLE mutations as relevant cause of unexplained familial colorectal adenomas and carcinomas

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Background: Familial colorectal cancer is a genetically heterogeneous condition, including various gastrointestinal polyposis syndromes and hereditary non-polyposis colorectal cancer (Lynch syndrome, HNPCC). However, in a number of families in whom the clinical criteria of a colorectal polyposis are met or Lynch syndrome is suspected, no germline mutation in the known genes can be found. Missense mutations in polymerase genes (POLD1 and POLE) have recently been identified as a rare cause of multiple colorectal adenomas/carcinomas (Palles et al. 2012).

Methods: The mutation screening of the polymerase genes POLD1, POLD2, POLD3, POLD4, POLE, POLE2, POLE3, and POLE4 was performed by a targeted next-generation sequencing approach (Truseq Custom Enrichment Kit, Illumina) on an Illumina HiSeq2000 sequencer using a sample of 192 apparently unrelated patients (145 polyposis patients without APC or MUTYH mutations and 47 familial colorectal carcinoma cases with microsatellite stable tumours meeting the Amsterdam I or II criteria). Data analysis was done by standard protocols using the VARBANK pipeline (CCG, Cologne).

Results: The previously described pathogenic POLE missense mutation c.1270C>G;p.Leu424Val was found in 4 index patients, all of them with a positive family history. We could demonstrate that the mutation segregates with the phenotype in all 8 affected members of 3 families from whom DNA was available. Haplotype analysis showed that 2 of the families are distantly related (confirmed by extended pedigree information), the other 2 families exhibit different haplotypes. Thus, around 2% (3/191) of unrelated families in the whole study cohort and around 5% (3/66) of families with known positive family history are affected by this mutation. The phenotype of the patients ranges from adenomatous polyposis with a maximum of about 100 colorectal polyps in three families to one family meeting Amsterdam I criteria. Interestingly, in some patients duodenal adenomas are described and one patient had duodenal carcinoma. Another patient shows retroperitoneal fibrosis. Moreover, we could identify the POLE mutation c.1306C>T;p.Pro436Ser (also located in the exonuclease domain) in another familial case, segregation analysis is ongoing. Six further probably pathogenic heterozygous mutations could be identified (1 frameshift mutation in POLE2 and 5 rare missense mutations in POLD1, POLD3, POLE and POLE2).
Conclusions: In conclusion, we could identify the previously described POLE mutation in a substantial number of our well characterized sample of polyposis and familial colorectal cancer patients. Screening for that mutation should be considered especially in unexplained familial cases. Furthermore we could extend the previously described phenotypic spectrum to duodenal adenomas and carcinomas and identified new potentially pathogenic variants in POLE and some other polymerase genes.
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W2-02

Combined High-resolution Analysis of Genome and Transcriptome of a Single Cell

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During the last years, a constant increase in technologies that allow detection and even isolation of single cells were developed and are even incorporated in clinical diagnostic settings for the detection of circulating tumor cells (CTCs) and disseminated cancer cells (DCCs) in cancer patients. However, for a deeper understanding of their biological properties, reliable molecular methods for single cell analysis are indispensable, especially as recent studies indicate the clinical utility of CTCs as a "liquid biopsy" to circumvent tissue biopsies of metastatic tumors. Here, we present for the first time combined high-resolution molecular analysis following parallel whole genome (WGA) and whole transcriptome amplification (WTA) of the same single cell. Our approach provides the unique possibility to carry out multiple analyses in parallel on mRNA and DNA from a single cell. Thus, the complex landscape of somatic alterations can be cross-validated using independent nucleic acid amplification methods, and thereby minimizing the risk of detecting artificial sequence errors. Parallel RNAseq analysis of two single cells of the VCaP prostate cancer cell line presenting markedly different gene expression levels of the prostate cancer fusion transcript marker TMPRSS:ERG was performed using Roche 454 GS FLX+ and Illumina HiSeq 1000 platform. Additionally, we developed a protocol for experimental normalization of single cell cDNA libraries to increase the sequence depth of the Roche 454 GS FLX+ system. Consequently, it was possible to detect TMPRSS:ERG fusion transcripts at low levels of gene expression. Furthermore, by using high-resolution aCGH analysis, we demonstrate that high-throughput analysis of both transcriptome and genome of the same single cell is feasible. We used our protocols to comprehensively analyze for the first time genome and transcriptome of a disseminated cancer cell from bone marrow of a metastatic prostate cancer patient. Besides detection of homozygous PTEN loss and AR high level amplification on the genomic level we obtained sequences for 7692 mRNA transcripts. Bioinformatic evaluation and further validation experiments (e.g. AR qPCR, validation of detected gene fusions) allow the qualitative and quantitative assessment of our combined genome-wide single cell analyses. In conclusion, our assays provide powerful molecular tools to analyze tumor cells on a single cell level and therefore are able to characterize the full molecular profile and heterogeneity of systemic cancer.

W2-03

Divergence between high metastatic tumor burden and low circulating tumor DNA concentration in metastasized breast cancer

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Circulating tumor DNA (ctDNA) was reported to represent a highly sensitive biomarker of metastatic cancer disease directly reflecting tumor burden and dynamics. Here we investigated the role of ctDNA in patients with metastatic breast cancer. In an index patient with more than 100,000 circulating tumor cells (CTCs) in serial blood analyses, whole genome, exome, or targeted deep sequencing of the primary tumor, metastases, and 551 CTCs were consistent with a genetically homogeneous cancer. However, the allele fractions (AFs) of ctDNA were only 2-3% in each analysis, which neither reflected the tumor burden nor the dynamics of this progressive disease by far. Indeed, plasma analyses of 71 further patients demonstrated highly variable AFs of mutant fragments, which frequently did not correspond to the tumor burden. Our data show that although ctDNA is a promising new biomarker in patients with cancer, the dynamics of the release of ctDNA into the circulation may not reflect tumor burden and requires further investigation. This observation has important implications for the use of ctDNA as liquid biopsy, as it indicates that tumor cells in some cancer patients with progressive disease may release only small amounts of ctDNA into the circulation, probably because of their low apoptotic rate.

W2-04

Qualification of Lung Cancer DNA methylation markers for liquid biopsy testing

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Elucidation of genome wide epigenetic changes has become a routine application, and is of utmost interest for the development of biomarkers for diagnostics. Along the biomarker developmental chain upon discovery of candidate markers, confirmation and validation are indispensable steps. For nucleic acids based markers the method of choice is qPCR. Focusing on DNA methylation based biomarker development, we have established methylation sensitive restriction enzyme (MSRE)

based qPCR testing for DNA methylation analyses. Efficient assay design tools have been developed enabling high throughput sequence manipulation and qPCR design. Several hundred assays for human DNA methylation targets have been designed and setup. Analytical validation according MIQE guidelines has been conducted using a standard qPCR and Fluidigm's Biomark system. These high throughput MSRE-qPCR assays have been used for confirmation of DNA-methylation biomarkers of cancerous and non cancerous disease. The strategy combining MSRE-digestion and multiplexed preamplification has been optimized for paralleled analyses of candidate methylation markers from spurious amounts of cell free DNA in plasma and serum. Here we will present data on the performance of DNA methylation markers for minimal invasive diagnostics of lung cancer. When only 400µl of plasma of archived samples (n=200) stored for up to 15y were available, at least 10 ng DNA could be isolated and 48 MSRE-qPCR assays conducted confirmed the performance of gene-panels for diagnostic testing. By a methylation-test panel of 5 genes WT1, SALL3, TERT, ACTB, CPEB4 adeno, squamous cell, small cell and large cell -carcinoma of the lung were detected with an AUC of 0.8-0.9. Several combinations including additional 2-5genes let us increase AUCs to 0.85-0.95. Performance of markers was on DNA from sputum (n=100) AUC=0.76.
Although limited by the amount of sample and input DNA, MSRE-qPCR is very efficient and useful to transfer candidate markers derived from tissue based screening to blood-based or minimal invasive tests.

W2-05

Association of the type of 5q loss with complex karyotype, TP53 mutation status and prognosis in AML and MDS

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Background: Deletions of the long arm of chromosome 5 are frequent abnormalities in myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). Their size varies considerably. Jerez et al. (JCO 2012) reported on commonly retained regions using SNP arrays and observed that deletions involving the centromeric and telomeric extremes of 5q are associated with a more aggressive clinical course. From a cytogenetic view loss of 5q either occurs due to interstitial deletions, unbalanced translocations or monosomy 5. While in interstitial deletions the telomeric region of 5q is retained, it is lost in cases with unbalanced 5q translocations and monosomy 5.
Aim: Analyze whether the type of 5q loss (interstitial deletion vs unbalanced translocation/ monosomy) is associated with other biological markers and prognosis in AML and MDS.
Patients and Methods: 1200 patients (pts) with loss of 5q were studied including 627 AML pts (de novo: 454, s-AML: 101, t-AML: 72) and 573 MDS pts (de novo: 511, t-MDS: 62) with a median age of 71.7 yrs (range: 30-90) and 73.2 yrs (range: 34-93). Interphase FISH had been performed in all pts with a probe for EGR1 (5q31) and all showed a heterozygous EGR1 deletion. All cases had been studied by chromosome banding analysis and in addition by 24-color FISH whenever necessary to resolve complex karyotypes. Data on TP53 mutation (TP53mut) status was available in 420 pts (AML: 233, MDS: 187).
Results: AML and MDS cases were separated into 2 groups according to type of 5q loss: 1) interstitial 5q deletion (idel5q): AML: 341/627 (54.4%), MDS: 385/573 (67.2%), and 2) 5q loss due to an unbalanced translocation (ut5q): AML: 286/627 (45.6%), MDS: 188/573 (32.8%). Cases with monosomy 5 (AML: 12, MDS: 1) were assigned to the second group. 530/627 (84.5%) AML pts and 303/573 (52.9%) MDS pts with 5q loss showed a complex karyotype (defined as >3 abnormalities). 195/233 (83.7%) AML and 104/187 (55.6%) MDS pts exhibited a TP53mut. In AML and MDS, patients with ut5q showed complex karyotypes more frequently (MDS: 179/188 (95.2%) vs 124/385 (32.2%); p<0.001; AML: 274/286 (95.8%) vs 256/341 (75.1%); p<0.001). Moreover, in MDS ut5q was associated with TP53mut (64/67 (95.5%) vs 40/120 (40.0%); p<0.001) and shorter survival (15.3 mo vs not reached; p<0.001). In MDS with loss of 5q complex karyotype was an independent adverse prognostic factor (HR=5.34; p=0.032). In AML with loss of 5q TP53mut was the strongest adverse prognostic factor (HR=2.21; p=0.026).
Conclusions: Loss of 5q due to unbalanced translocations encompassing the telomeric 5q region is more frequent in AML than in MDS and in both entities associated with a complex karyotype. MDS pts with ut5q show compared to pts with idel5q a shorter survival. This data suggests that in MDS ut5q reflect chromosomal instability which most probably is associated with progression to AML and adverse prognosis. In AML with loss of 5q the presence of TP53 mutations is the strongest adverse prognostic factor.

W2-06

Submicroscopic Copy Number Changes were identified by Array CGH in 10% of 520 MDS Patients with Normal Karyotype: Deletions Encompass the Genes TET2, DNMT3A, ETV6, NF1, RUNX1, and STAG2 and Are Associated with Shorter Survival

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Background: In MDS, cytogenetic aberrations play an important role for classification and prognostication. The vast majority of chromosome aberrations in MDS are gains or losses of chromosomal material while balanced rearrangements are rare. However, more than 50% of MDS and even more in low risk MDS harbor a normal karyotype. Chromosome banding analysis (CBA) can only detect gains and losses of more than 10 Mb size due to its limited resolution and is dependent on proliferation of the MDS clone in vitro. Array CGH has a considerably higher resolution and does not rely on proliferating cells.
Aims: We addressed the question whether MDS with normal karyotype harbor cytogenetically cryptic gains and losses.
Patients and Methods: 520 MDS patients with normal karyotype were analyzed by array CGH (Human CGH 12x270K Whole-Genome Tiling Array, Roche NimbleGen, Madison, WI). For all patients CBA had been performed. The cohort comprised the following MDS subtypes: RA (n=22), RARS (n=43), RARS-T (n=27), RCMD (n=124), RCMD-RS (n=111), RAEB-1 (n=104), and RAEB-2 (n=89). Recurrently deleted regions detected by array CGH were validated using interphase-FISH (IP-FISH).
Results: In 52/520 (10%) patients copy number changes were identified by array CGH. Only 8 cases (1.5%) harbored large copy number alterations (CNA) >10 Mb in size, as such generally detectable by CBA. These CNA were confirmed by IP-FISH. They were missed by CBA due to small clone size (n=2), insufficient in vitro proliferation (n=3) or poor chromosome morphology (n=3). In the other 44 patients with submicroscopic CNA 18 gains and 32 losses were detected. The sizes ranged from 193,879 bp to 1,690,880 bp (median: 960,176 bp) in gained regions and 135,309 bp to 3,468,165 bp (median: 850,803 bp) in lost regions. Recurrently deleted regions as confirmed by IP-FISH encompassed the genes TET2 (4q24; n=9), DNMT3A (2p23; n=3), ETV6 (12p13; n=2), NF1 (17q11; n=2), RUNX1 (21q22; n=2), and STAG2 (Xq25, deleted in 2 female patients). No recurrent submicroscopic gain was detected. In addition, we performed survival analysis and compared the outcome of patients with normal karyotype also proven by array CGH (n=462) to patients with aberrant karyotype as demonstrated by array CGH (n=52). No differences in overall survival (OS) were observed. However, OS in 35 patients harboring deletions detected solely by array CGH was significantly shorter compared to all others (median OS: 62.1 vs 42.4 months, p=0.023).
Conclusions: 1. Array CGH detected copy number changes in 10% of MDS patients with normal karyotype by chromosome banding analysis. 2. Most of these alterations were submicroscopic deletions encompassing the genes TET2, ETV6, DNMT3A, NF1, RUNX1, and STAG2.

3. Interphase-FISH for these loci can reliably pick up these alterations and is an option to be easily performed in routine diagnostics in MDS with normal karyotype. 4. Patients harboring deletions detected solely by array-CGH showed worse prognosis.

W3 Complex Diseases

W3-01

Mutations causing complex disease may under certain circumstances be protective in an epidemiological sense

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Guided by the practice of classical epidemiology, research into the genetic basis of complex disease usually takes for granted the dictum that causative mutations are invariably over-represented among affected as compared to unaffected individuals. However, employing various models of population history and penetrance, we show that this supposition is not true and that a mutation involved in the etiology of a complex disease can under certain circumstances be depleted rather than enriched in the affected portion of the population. Such mutations are 'protective' in an epidemiological sense and would normally tend to be erroneously excluded from further studies. Our apparently paradoxical finding is due to the possibility of an anti-correlation between complementary causative mutations that may arise as a consequence of the specifics of the population genealogy. This phenomenon also has the potential to hamper efforts to identify rare causative mutations through whole-genome sequencing.

W3-02

Systematic association analysis of human microRNAs with schizophrenia

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Schizophrenia (SCZ) is a common and severe neuropsychiatric disorder. SCZ genome-wide association studies (GWAS) have identified common single nucleotide polymorphisms (SNPs), rare copy number variants (CNVs) and a large polygenic contribution to illness risk, but biological mechanisms remain unclear. Among the strongest GWAS finding is mir-137, a post-transcriptional mRNA regulator involved in neuronal development [Ripke et al., 2011]. The known role of microRNAs (miRNAs) as potent disease modifiers in neuropsychiatric disorders raises the question of whether genetic variation in miRNAs plays a critical role in SCZ etiology.
Results from the largest SCZ meta-analyses with 9,898,078 imputed SNPs from a Sweden national sample and data from the Psychiatric Genetic Consortium (PGC) including a total of 13,833 patients and 18,310 controls [Ripke et al., 2013] provided the basis for this analysis.
Therefore, we implemented a systematic set-based testing for all miRNAs (n=718) defined in the miRBase release 13.0 based on test statistics from GWAS data. To capture regulatory regions, ± 20kb were defined as miRNA boundaries. Alike the popular gene set testing tool VEGAS [Liu et al., 2010], SNPs in within these boundaries were grouped together, corrected for linkage disequilibrium and controlled for the number of SNPs within each miRNA. In this study, we summarized the 10% of most significant SNPs) for each miRNA. Additionally, all miRNA hostgenes (n=340) were analyzed with the same regional boundaries.
From all analyzed miRNAs, 2.76% were significantly associated with SCZ after correction for multiple testing; further 18.90% were nominal significant. As expected from the GWAS results, the strongest association was found for mir-137. Moreover, miRNAs involved in neural and synapse development such as mir-9, let-7, mir-17 and mir-34a as well as in miRNAs with yet unknown function were identified. Further evaluation of targets from significantly associated miRNAs as well as their presence in brain eQTLs is ongoing and will be presented.
Overall, our results give the first unbiased screening of miRNA association based on large SCZ GWAS data and might lead to the discovery of key players suitable for further functional studies.

W3-03

Follow up of loci identified by the International Genomics of Alzheimer's Disease Project confirms TRIP4 as a novel risk locus for AD.

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Alzheimer's disease (AD) is a complex multifactorial disorder that manifests with a progressive loss of cognitive functions which results in the loss of autonomy. The etiology of AD is driven by genetic an environmental factors and the apolipoprotein E4 (APOE4) allele constitutes the strongest genetic risk factor. In the past years, genome-wide association studies (GWAS) have been successful in identifying nine contributing genetic risk loci. Very recently, a breakthrough in the understanding of the underlying genetic causes of AD was achieved by the International Genomics of Alzheimer's Project (IGAP). IGAP performed a large-scale two-step meta-analysis of four GWAS samples for AD which resulted in the identification of 11 additional AD risk loci. Noteworthy, genome-wide significance for seven of these 11 loci was only established in the IGAP replication stage in an independent sample of ~20,000 AD cases and controls (IGAP stage II). In addition to these genome-wide significant loci, the IGAP effort provided a list containing 13 loci that were ranked as suggestive AD risk loci. Follow up of these IGAP suggestive loci is a promising strategy to identify novel AD risk genes. We therefore analyzed a total of 19 SNPs including novel and suggestive AD loci from IGAP analysis in an independent Spanish sample from the Fundació ACE, comprising 1,808 patients and 2,564 controls. Our data confirmed the association of four SNPs from the IGAP step II analysis with nominal significance (rs35349669 at INPP5D, rs190982 at MEF2C, rs1476679 at ZCWPW1, and rs17125944 at FERMT2). In addition, we found a trend towards association for three loci detected during IGAP stage I (rs11218343 at SORL1; rs10498633 at SLC24A4/RIN3; rs8093731 at DSG1). Out of the 9 suggestive loci included in our analysis, only rs74615166 located intronically in TRIP4 (thyroid receptor interacting protein) could be replicated with nominal significance (P=0.0032). A subsequent meta-analysis of the Fundació ACE replication sample and the IGAP sample confirmed this association between rs74615166 and AD with genome-wide significance (P=9.74x10-9; OR=1.31; 95%CI [1.19-1.44]). Hence, our results added a novel Alzheimer risk gene, TRIP4, to the growing list of Alzheimer susceptibility genes. TRIP4 is involved in transcriptional activation following thyroid hormone interaction. Excitingly, the activity of the thyroid hormone system has been associated with an increased risk of Alzheimer's disease. Furthermore, the TRIP proteins also show similar ligand-dependent interaction with the retinoid X receptor (RXR), a particularly exciting finding in light of the recent finding that administration of an RXR agonist, bexarotene, to a mouse model of AD resulted in enhanced clearance of soluble Aβ within hours. Thus, TRIP4 may represent a good target for AD therapies. Further genotyping and re-sequencing efforts to investigate the IGAP loci are underway in order to corroborate further genuine signals.

W3-04

Functional Characterization of Long-QT Syndrome (LQT) and Sudden Infant Death (SIDS) Associated OLFML2B Mutations

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By GWAS we have mapped the strongest human QTL modifying cardiac repolarization (QT interval) to OLFML2B and NOS1AP in 1q23.3. OLFML2B encodes a secreted extracellular matrix (ECM) protein. By mutation screening we have identified an overrepresentation of rare (MAF≤1%) nonsynonymous heterozygous mutations in 125 patients with long-QT Syndrome (LQT; OR=3.62 (1,46-8,93) p=2,9x10-3) and in 93 with sudden infant death syndrome (SIDS; OR=3,01 (1,05-8,65) p=3,2x10-2) but not in 94 adults with sudden cardiac death (SCD; OR=0,57 (0,07-4,41) p=5,9x10-1) compared to 702 population controls. Of 35 missense variants identified, we have selected 14 variants in-silico predicted to negatively affect protein structure (PolyPhen2, SIFT, Mutation taster) for heterologous expression in HEK293 cells and functional analysis. Combining our 702 controls with in-silico data from 6503 WES sequenced individuals from the ESP 4 out of the 14 variants occurred in 2,3,7 and 9 heterozygotes among 7205 persons while the other 10 variants were absent in the expanded control sample.
All OLFML2B protein variants were equally expressed intracellularly. Their secretion into the extracellular space was impaired depending on the mutation ranging from mild reductions to nonsecretion. Co-expression of wildtype and mutant demonstrated dominant negative secretion impairment. Secretion experiments were performed in triplicate at three temperatures and parametrized for statistical analysis (LI-COR Image Studio) using wt-OLFML2B at 37°C as a reference. As expected protein secretion was temperature dependent (30°C>37°C>41°C; p<0,001). In addition secretion was significantly correlated with disease severity (wt>LQT>SIDS; p<0,05) and with allele frequency in the controls (p<0.01). Four out of the 14 variants were investigated by cellular electrophysiology in Xenopus oocytes. They showed significant reduction of the voltage gated KCNH2/Kv11.1 channel (IKr) but no other main cardiac ion channels. The degree of impairmant ranged from −7% to −49% and was also correlated with mutation secretion status.
Taken together the functional proteomic investigation suggests a significant influence of OLFML2B and the ECM on myocardial repolarization. This assumption is supported by nonsecretion being associated with both disease severity and population allele frequency acting in an autosomal dominant manner. Our data support the hypothesis that rare nonsynonymous OLFML2B variants impair repolarization, most likely by failing to assume the correct topological position in the ECM, and confer genetic predisposition to long QT-Syndrome (LQT) and sudden infant death (SIDS).

W3-05

High-density genotyping in alopecia areata identifies TNFSF4 and HLA-C as two new susceptibility loci with genome-wide significance

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Alopecia areata (AA) is a common hair loss disorder which occurs in both sexes and all age groups. The disorder is characterized by a typically sudden onset of patchy areas of hair loss which can occur on the scalp or on the body hair. The course of disease is unpredictable. Individuals with AA may experience complete remission, a chronic course, or progression towards a complete loss of scalp hair (AA totalis) or of scalp and body hair (AA universalis). The precise etiopathogenesis of AA is still unknown. However, immunological and association studies generate accumulating support for the hypothesis that innate and acquired immunity is implicated in the etiopathogenesis of AA. Up to now, ten loci with genome-wide significance have been identified for AA. The association findings include among the HLA region various genes which are assumed to be involved in immune modulating as well as inflammatory processes. To further reveal the immune nature of AA, we analyzed a sample of 778 AA patients and 1,488 controls of Central European origin on a custom-based Illumina BeadChip array, the Immunochip.

To follow up our initial results, in a first step, a targeted analysis of the HLA-region was performed. Stepwise logistic regression analysis established HLA-C as a new susceptibility locus with genome-wide significance (rs9264531; P = 4.41*10-7) and confirmed the well-established HLA alleles HLA-DQA1 and HLA-DQA2 (P = 6.93*10-7; P = 8.55*10-21).

To follow up the most strongly associated susceptibility variants outside the HLA-region, a total of 50 variants were genotyped in an independent Central European AA sample of 1,016 cases and 1,060 controls. To obtain robust evidence for association, we performed a meta-analysis of the data from the discovery and follow-up cohorts, designating TNFSF4 (tumor necrosis factor (ligand) superfamily, member 4) as a new AA susceptibility locus with genome-wide significance (rs4916209, Pcomb. = 6.89*10-08). Interestingly, TNFSF4 encodes the cytokine OX40L, binding with OX40 to a complex. The complex is implicated in wide-ranging immune modulating processes and has a key role in the regulation or maintenance of CD4 T cell response, regulatory T cells, memory CD8 T cells, cytokine production and cytokine receptor signaling. An increase of OX40-OX40L is described in chronic inflammatory diseases. Blockade of the OX40-OX40L complex is thought to suppress the development of autoimmune and chronic inflammatory disorders. Nominal significant association was observed for an additional three loci – FASLG (Fas ligand (TNF superfamily, member 6), THADA (thyroid adenoma associated) and C11orf30 (chromosome 11 open reading frame 30). Interestingly, all three loci were reported to be associated with Crohn`s disease (CD), pointing to a yet widely unknown genetic overlap between these CD and AA. In summary, our results shed additional light on the immune-related pathways of AA and bring forward the blockade of the OX40-OX40L complex as a potential new therapeutical approach for AA.

W3-06

Increased methylation and expression of the IL17REL gene in patients with ulcerative colitis

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Ulcerative colitis (UC) is characterized by severe inflammation and ulceration of the colon and rectum, leading to recurrent diarrhea and abdominal pain. The cause of disease is described as an interaction between different environmental, immunological, microbiological and genetic factors. The IL17REL gene encodes a protein belonging to a newly discovered subfamily of IL17 receptors and harbors a SNP (rs5771069, A/G) that was recently identified as a risk locus for UC (Franke et al.; 2010, Nat Genet 42:292-4). We used bisulphite pyrosequencing and quantitative Real time RT-PCR to investigate promoter methylation and mRNA expression of the IL17REL gene in inflammatory (I-UC) and non-inflammatory (NI-UC) colon biopsies of UC patients as well as colon biopsies of non-IBD patients (controls) and correlated our findings with the IL17REL rs5771069 genotypes. Mean methylation levels of the IL17REL gene were significantly higher in I-UC samples (n =27) compared to NI samples (n =32; p=4.6*10-5) and controls (n =38; p=1.8*10-8). Unexpectedly, relative mRNA expression levels of the IL17REL gene were not lower, but also significantly elevated in the I-UC samples (n =11) compared to the NI-UC samples (n=19; p=0.011) and controls (n =13; p=0.005). Interestingly, increased IL17REL methylation and expression in the I-UC samples appeared to be correlated with the progression of UC. Based on preliminary data, occurrence of the rs5771069 risk allele (G) was not associated with increased IL17REL methylation and expression levels. To analyse if, according to our methylation and expression data, increased promoter methylation of the IL17REL gene coincides with increased promoter activity, we are currently establishing a luciferase assay with the IL17REL promoter cloned in a CpG free luciferase reporter vector, pCpGL. pCpGL plasmids with the IL17REL promoter will then be in vitro methylated using CpG methyltransferases and transient transfection assays will be performed to monitor IL17REL promoter activity with the promoter in either an unmethylated or an in vitro methylated state. Promoter methylation analysis of IL17REL gene might be a useful marker for disease progression in UC and open new avenues to improve diagnostics and targeted therapy.

W4 Clinical Genetics

W4-01

Whole exome sequencing identifies mutations in two cilia-related genes as a probable cause of the new syndromic form of intellectual disability (Tyshchenko syndrome, OMIM 615102).

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Background: Recently, we presented clinical data of three patients from two unrelated families afflicted with a newly defined syndrome (Tyshchenko syndrome, OMIM 615102), a unique combination of minor facial anomalies including prominent eyes, bilateral ptosis and intellectual disability. Other features include cleft palate, hearing loss, heart defects and short stature. Molecular karyotyping in both families did not reveal any significant genomic imbalances [1].

Method: Four members of family 1 (affected mother, her healthy parents and her affected daughter) as well as four members of family 2 (affected son, his healthy parents and his healthy sister) were analyzed by whole exome sequencing (Agilent 50 MB exome) on a SOLiD4 or a HiSeq2000 system, respectively.

Results: In family 1, we identified a heterozygous missense mutation in SEPT2 (OMIM 601506). The mutation is present in the affected mother and her affected daughter and absent in the healthy family members as well as in a large cohort of healthy, unrelated control individuals. According to both polyphen-2 and mutation taster, the mutation is damaging with a probability score of >0.999. SEPT2 is part of a dif-

fusion barrier at the base of the ciliary membrane and is essential for retaining receptor-signaling pathways in the primary cilium [2]. In the second family exome sequencing revealed a novel homozygous mutation in another cilia gene, ALMS1 (OMIM 606844), in the affected patient. Both parents are heterozygous carriers of this mutation; the healthy sister showed only wild type alleles. Like SEPT2, ALMS1 is also located at the base of cilia and contributes therefore to cilial functions [3].
Conclusion: The Tyshchenko syndrome may be caused by mutations in genes coding for proteins that are localized and functioning at the cilial basis. Whether SEPT2 and ALMS1 are directly interacting is not known at present. Further experiments are ongoing to address this question. Our findings support that SEPT2 and ALMS1 mutations are causative for Tyshchenko syndrome and that this syndrome is a ciliopathy.
Acknowledgement: The work was supported by the Bundesministerium für Bildung und Forschung (BMBF) network grant MR-NET 01GS08166.
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W4-02

Investigating the molecular basis of Jeune Syndrome and other non-motile Ciliopathies (174 cases)

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Background: Mutations in genes encoding for cilia associated proteins lead to complex developmental defects in human and although individual ciliopathy diseases are very rare, together they represent a significant human disease burden. Genetic heterogeneity and phenotypic as well as genetic overlap has hampered molecular diagnosis in the past but Next Generation Sequencing (NGS) techniques offer new tools. Jeune Asphyxiating Thoracic Dysplasia (JATD) is a rare ciliopathy characterised by short ribs and life-threatening pulmonary hypoplasia, rare polydactyly and some patients additionally presenting with renal, retinal or liver involvement. Like JATD, Bardet-Biedl-Syndrome (BBS) and Joubert Syndrome are rare autosomal-recessively inherited conditions sharing renal and retinal symptoms and polydactyly occurs in BBS. In contrast to JATD, these conditions also result in a brain phenotype.
Results: We investigated 61 JATD, 37 BBS, 7 Joubert and 9 other ciliopathy cases (total of 114 cases) using Whole Exome Sequencing and further 60 JATD cases using a gene panel. We identified the disease causing gene in > 70% of cases revealing new phenotype-genotype associations in JATD with mutations in DYNC2H1 causing a severe and predominant skeletal phenotype while mutations in IFT140 result in mild thoracic involvement but frequent renal and retinal involvement. In several instances we had to revise the initial clinical diagnosis based on the genetic findings and Copy Number Variations calculated from exome data revealed causative alleles in 10% of all cases.
For JATD, we identified mutations in several new genes encoding previously uncharacterised human cytoplasmic dynein-2 complex components, WDR60 and WDR34 among which mutations in WDR34 represent the second most common cause of JATD overall. Protein-protein interaction between human WDR34 and dynein-light chain LC8 as well as proteomics data suggest this is a new intermediate chain of the mammalian cytoplasmic dynein-2 complex but could also represent a novel link to cytoplasmic dynein-1. WDR34 might further influence NF-kappa B signalling via inhibition of TAK-1, a pathway not previously associated with cilia. We also found mutations in a potential novel mammalian cytoplasmic dynein-2 light chain for which a mouse model is currently under investigation. Last, we identified mutations in IFT172 as a new cause of JATD with severe liver involvement.
Summary: Our findings in this large non-motile ciliopathy cohort demonstrate that WES is a very efficient tool in genetics diagnosis of heterogenous recessive disorders and shows that this is facilitated by deep phenotyping. Compared to NGS gene panel sequencing, WES offers additional opportunities to identify new genes previously not associated with the condition investigated.

W4-03

Characterization of the total ciliopathy variant load dissolves the enigma of oligogenic inheritance in Bardet-Biedl syndrome

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Bardet-Biedl syndrome (BBS) is a clinically and genetically heterogeneous ciliopathy. Primary features are retinal dystrophy, obesity, polydactyly, renal abnormalities, hypogonadism and learning difficulties. Further phenotypic traits out of the ciliopathy spectrum (e.g. cardiac abnormalities, ataxia, hearing defects) are common. Mutations in 18 BBS genes have been described so far without convincing genotype-phenotype correlations. Clinical and genetic overlap exists with other cilia-related disorders, especially Alström syndrome, nephronophthisis, and Joubert syndrome.
More than ten years ago, BBS was among the first Mendelian genetic disorders for which triallelic inheritance as a bridge between Mendelian and multifactorial traits has been proposed (Katsanis et al., Science 2001). As a consequence, BBS was postulated to be not a single-gene recessive disease but a complex trait requiring three mutant alleles at more than one locus to manifest the phenotype. These data still causes some uncertainty for genetic counselling, clinical management and prenatal diagnostics.
By means of NGS (next-generation sequencing) targeting all BBS genes and other genes known or hypothesized to cause ciliopathies at that time (currently in total 306 genes), we clinically and genetically examined in detail more than 100 families suspected to have BBS, the largest cohort analysed so far. To uncover "hidden mutations" such as copy number variations (CNVs) we extended the use of NGS data by quantitative readout of the in total 5546 targeted exons most recently and were able to detect causative CNVs which were key to the diagnosis in hitherto unsolved constellations. In all but three families who fulfilled the diagnostic criteria for BBS, we were able to identify homozygous or compound heterozygous mutations in a single BBS gene or ALMS1, what we call the major disease locus. In most patients, we detected additional mutations at other loci that may well exert a modifying effect on the disease phenotype. However, in contrast to published data, our findings are in accordance with a recessive disease model for BBS and do not support a model in which an additional allele is urgently needed for disease manifestation. Our study widely resolves

the long-standing enigma of triallelic or oligogenic inheritance in Bardet-Biedl syndrome. We further conclude that genetic heterogeneity in BBS is limited. More than 95% of typical BBS patients harbour pathogenic mutations in one of the known disease genes. Our data is of major importance for genetic counselling, prenatal diagnostic testing, and the clinical management of patients and their families.

W4-04

Defining new genes and disease mechanisms for cystic kidney disease and related disorders

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Polycystic kidney disease (PKD) is the most common potentially life-threatening human genetic disorder and paved the way for the growing number of cilia-related disorders (ciliopathies) of which most show cystic kidneys. In contrast to the more heterogeneous field of cystic kidneys, PKD is thought to be restricted to mutations in only three genes: PKD1 and PKD2 in the dominant form ADPKD, and PKHD1 for the recessive counterpart ARPKD. Notably, both ADPKD genes (PKD1 and PKD2) can also be inherited in a recessive way. We present unpublished data and demonstrate that in a subgroup of patients PKD can be mimicked by mutations in a number of other genes (e.g. BBS10, NPHP3, TMEM67, ANKS6). Some of our cases clearly indicate that caution is required when only novel or rare changes are found, especially when only data from single gene sequencing is available. The increasing number of genes that have to be considered in patients with cystic kidney disease is challenging to address by conventional techniques and largely benefits from Next-Generation Sequencing (NGS) based approaches. Parallel analysis of targeted genes by NGS considerably increases the detection rate, allows for better interpretation of identified variants and avoids genetic misdiagnoses. We could recently demonstrate that there is increasing evidence for molecular networks in and next to the cilium (Hoff et al., Nat Genet 2013) explaining overlapping disease phenotypes among members of the same module. Here we present new data which is in line with digenic inheritance between different module members. We also show that some patients harbour mutations in more than just one single gene supporting the idea of a dosage-sensitive network especially in cases with early disease manifestation. We used zebrafish for validation of some of our findings and as a model for vertebrate development to finally gain a better understanding of disease processes and variable expressivity. An accurate genetic diagnosis is crucial for genetic counselling, prenatal diagnostics and the clinical management of patients and their families.

W4-05

Hidden mutations in CdLS - Limitations of Sanger sequencing in diagnostics

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Cornelia de Lange syndrome (CdLS) is a clinically and genetically heterogeneous developmental disorder. Patients are characterized by distinct facial features, growth retardation as well as a cognitive delay. Whereas half of the patients show mutations in the NIPBL gene, mutations in SMC1A, SMC3, RAD21 or HDAC8 can be identified in around 10 % of the patients. These patients tend to be more mildly affected compared to patients with NIPBL mutations which often represent the characteristic CdLS phenotype. Here we report two unrelated patients with characteristic CdLS phenotype. Interestingly, conventional molecular diagnostic (Sanger sequencing) of the five CdLS genes could not detect any disease causing mutation using DNA isolated from blood samples. Very recent data using DNA extracted from buccal mucosa (BM) tissue could identify a high proportion of mosaic mutations in NIPBL which were not detected by investigating DNA from blood samples. Therefore we used Sanger sequencing analysis of the NIPBL gene on DNA from BM of our two patients but also failed to identify a mutation.

Because of the characteristic CdLS phenotype of both patients, DNAs from BM were transferred to our well established Ion Torrent CdLS panel sequencing platform. This custom made gene panel does include the five known CdLS genes beside eleven functionally associated candidate genes and allows very deep sequencing analysis with high coverage. By this we could detect a mosaic nonsense (17%) and a mosaic missense mutation (13%) in NIPBL. Because both mutations were not detected by Sanger sequencing neither using DNA derived from blood nor from BM, we used SNaPshot fragment analysis to screen for the mutant allele. Although SNaPshot analysis could not detect the mutation in blood, distinct signals were detected in DNA isolated from BM, urine sediments and fibroblast of the patients. Subsequent Sanger sequencing approaches using DNA from all four tissues available could only detect clear heterozygous mutation using the fibroblast DNA samples.

Our data further supports recent findings that indicate a high frequency of mosaic NIPBL mutations in patients with CdLS. In addition we could clearly show limitations of classical Sanger sequencing approaches even when using DNA from BM as suggested suitable tissue for molecular diagnostics by recent publications. While both mutations could be identified by Sanger sequencing using fibroblast DNA, the availability of fibroblasts is mostly a highly limiting factor in molecular diagnostics. Regarding to our findings we recommend the use of high coverage sequencing techniques on DNA from BM especially when analyzing patients with characteristic CdLS phenotypes and negative Sanger sequencing results. We hope that our findings clearly highlight the advantages and sensitivity of next generation panel sequencing approaches compared to the much more time, patient material and money consuming Sanger sequencing in molecular diagnostics.

W4-06

In- house cMRI reevaluation leads to a significantly higher mutation detection rate in neuronal migration disorders

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Neuronal migration disorders are an important cause of early and severe psychomotor retardation in combination with a seizure disorder, which is frequently resistant to common schemes of antiepileptic medication. Based on their appearance in cerebral MR imaging (cMRI) neuronal migration disorders (NMD) can be subdivided into classic lissencephaly, subcortical band heterotopia, cobblestone lissencephaly, polymicrogyria and periventricular nodular heterotopia. Here we report our results over 13 years for a cohort of more than 1030 independent patients.

Genetic testing was performed for 1034 index patients either (1) individually after careful consideration of clinical information as well as in-house reevaluation of cerebral MR imaging (n=214) or (2) as assigned by the referring medical doctor (n=820).The individual testing strate-

gies included linkage analysis for suitable families, CNV analysis by MLPA, Sanger sequencing and most recently the introduction of massive parallel sequencing. Overall, pathogenic mutations were identified in the following genes: LIS1 (34), DCX (47), ARX (10), TUBA1A (4), TUBB2B (4), GPR56 (10), FLNA (30), POMT1 (22), POMGnT1 (15), FKTN (3), FKRP (9), ISPD (1), LARGE (2) and DAG (1).
After in-house MRI reevaluation 44% of the patients were classified as "other" meaning that a specific pattern characteristic for one of the currently known monogenic forms of neuronal migration disorders could not be confirmed. 119 NMD patients of study arm (1) were then genetically analyzed leading to the identification of the underlying genetic alterations in more than 35% of the analyzed samples. The mutation detection rate in the radiological subgroups was as follows: classic lissencephaly 65.4%, subcortical band heterotopia 83.3%, polymicrogyria 10.0%, cobblestone lissencephaly 14.3%, periventricular nodular heterotopia 37.5% and complex cortical malformations 20.0%.
In comparison, the mutation detection rate in study arm (2) without in-house cMRI reevaluation was significantly lower (overall mutation detection rate: 18.5%; $p<0.0005$): classic lissencephaly/SBH 18.6%, polymicrogyria 5.8%, cobblestone lissencephaly 25.2%, periventricular nodular heterotopia 19.4%.
Due to improved imaging technologies today severe forms of neuronal migration disorders are more often diagnosed prenatally. In our cohort 49 prenatal samples were tested for neuronal migration disorders. Suspected Walker-Warburg Syndrome showed the highest mutation detection rate (46.6%), whereas pinpointing the mutation in the more heterogeneous group of other complex cortical malformations was more challenging. In addition 37 prenatal samples were tested in subsequent pregnancies.
As genome wide testing strategies will further improve the number of identified genes and genetic alterations, critical interpretation in the context of clinical findings and cerebral imaging will become more important than ever.

W5 Intellectual Disability

W5-01

Molecular Inversion Probe based Resequencing Identifies Recurrently Mutated Genes in Intellectual Disability

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Exome sequencing studies of individuals with intellectual disability (ID) and related neurodevelopmental disorders have identified many de novo mutations but few recurrently mutated genes1,2. We therefore applied a molecular inversion probe (MIP) based resequencing method to screen 42 ID candidate genes - mainly from a previous study2 - in ~2,500 patients with the clinical diagnosis of ID and 1,000 healthy controls. This approach allowed targeted multiplex enrichment of 768 DNAs per week per fte, and barcoding allowed simultaneous sequencing of up to 3,072 samples for the 42 gene panel per HiSeq2000 sequencing run.
We discovered predicted loss-of-function (LoF) events for more than half of the candidate genes, including multiple recurrent de novo nonsense and frameshift mutations in the genes CHD2, PIK3C3 and MYT1L. This together with ongoing 'reverse-phenotyping' of the affected individuals further supports the pathophysiological role of these genes in intellectual disability. For some de novo mutations we identified a weaker minor allele ratio suggestive for post-zygotic de novo events. We were also able to analyze copy number status for these candidate genes, and discovered de novo CNVs, deletions and duplications, that implicate new dosage sensitive genes. Despite the challenging task to prove causality, latest technological improvements allowed entering a golden age of 'neurodevelopmental-gene' discovery which promises to improve not only our understanding of disease but provide fundamental insight into the biology of human brain development.
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W5-02

ARID1B mutations in intellectual disability link chromatin remodelling to neuronal differentiation by derepressing Wnt/β-catenin signaling

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Intellectual disability (ID) and Coffin-Siris syndrome (CSS) are frequently associated with loss-of-function mutations in various components of the BAF chromatin remodeling complex, such as ARID1B. The human BAF complex (SWI/SNF-A complex) repositions and alters the structure of nucleosomes, facilitating the activation or repression of gene transcription. ARID1B is expressed in human brain and ARID1B-associated BAF complexes are important in early stages of murine brain development.
To understand how mutated ARID1B affects human brain development and leads to developmental delay phenotypes, we performed whole transcriptome analysis (RNA-Seq) of peripheral blood lymphocytes from ID patients harboring ARID1B mutations and controls. Prominent transcriptional changes were observed in 452 genes and results were validated by qRT-PCR in a randomly selected subset of genes. Pathway analysis showed upregulation of Wnt/β-catenin target genes as the most pronounced change indicating a general increase of Wnt/β-catenin signaling in patients. In luciferase reporter assays in various human cell lines, overexpression of ARID1B suppressed Wnt/β-catenin-mediated transcription. Reciprocally, siRNA-mediated knockdown of ARID1B increased Wnt/β-catenin signaling. Immunofluorescence microscopy in cells transiently overexpressing ARID1B and β-catenin showed full co-localization in well-defined nuclear puncta not seen when transfected alone. PRMT5 (protein arginine N-methyltransferase 5), which promotes a closed chromatin structure, was enriched in these nuclear puncta, whereas acetylH3K9 (histone 3 acetylated on lysine residue 9), an activator of transcription, was excluded, suggesting that ARID1B promotes the accumulation of negative regulators of transcription to β-catenin sites. Both ARID1B and β-catenin bind directly to the ATP-dependent helicase BRG1 (SMARCA4), an enzymatic subunit of the BAF complex. Co-expression of BRG1 led to significantly larger nuclear puncta while knockdown of BRG1 abolished them, lessening the inhibitory effect of ARID1B on Wnt/β-catenin signaling activation. This suggests that BRG1 mediates the association between β-catenin and an ARID1B-based BAF complex to repress β-catenin-mediated transcription. ARID1B mutations as seen in ID and CSS patients reduced

the ability of ARID1B to localize β-catenin to nuclear puncta and compromised its suppressive effect on β-catenin-driven transcription. In mouse neuroblastoma cells, knockdown of ARID1B induced neurite outgrowth and shifted cell morphology to a neuronal differentiation phenotype and expression of neuronal marker TUJ1. This process is β-catenin-dependent since concurrent knockdown of β-catenin inhibited signaling activation and blocked induction of differentiation.
Altogether, our results show that derepression of Wnt/β-catenin pathway upsets the balance between differentiation and pluripotency in neuronal cells explaining some of the features observed in ARID1B-associated ID syndromes.

W5-03

Biallelic BRF1 mutations alter RNA polymerase III-dependent transcription and cause a neurodevelopmental syndrome with cerebellar, dental and skeletal anomalies

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RNA polymerase III (Pol III) synthesizes tRNAs and other small non-coding RNAs such as 7SK RNA, 5S rRNA, and U6 snRNA and thereby regulates protein synthesis at different levels. Dysregulation of Pol III transcription has been linked to cancer, and mutations in Pol III subunit genes and in genes encoding tRNA processing factors cause different forms of neurogenetic diseases, such as hypomyelinating leukodystrophies and pontocerebellar hypoplasia. Here we describe an autosomal-recessive disease characterized by facial dysmorphic features, short stature, mild-to-moderate intellectual disability, cerebellar hypoplasia, dental anomalies, and scoliosis. Whole exome and Sanger sequencing revealed biallelic missense alterations of BRF1 as the cause of the cerebellar-dental-skeletal syndrome. BRF1 associates with BDP1 and TBP to form the transcription factor IIIB (TFIIIB), which recruits Pol III to target genes. We show that the identified mutations reduce BRF1 occupancy at tRNA target genes in the model organism S. cerevisiae and impair cell growth. BRF1 mutations also reduce Pol III-related transcription activity in vitro. These results show that hypomorphic BRF1 mutations cause a novel neurodevelopmental syndrome and that BRF1-mediated Pol III transcription is required for normal cerebellar and cognitive development.

W5-04

GPM6A is duplicated in a patient with learning disability and influences cholesterol response as well as stress response and long-term memory in Drosophila melanogaster

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In a patient with learning disability and behavioral anomalies we identified a de novo copy number gain of the GPM6A gene by routine SNP-array testing. Glycoprotein M6A (GPM6A) is a transmembrane protein of the PLP/DM20 protein family whose expression is restricted to neurons. GPM6A variants have not yet been implicated in cognitive impairment. Expression analysis in blood lymphocytes showed increased GPM6A expression in our patient compared to healthy controls. We observed an increase in membrane protrusions in patient lymphoblastoid cell lines, thereby supporting a functional effect of this dosage alteration. To further study the function of GPM6A/m6 and the effects of m6 overexpression and knockdown, we employed Drosophila melanogaster as a model organism. We could show that, as described for other animal models before, also expression of Drosophila m6 is stress responsive. Using the courtship conditioning paradigm, we demonstrated that correct m6 levels are necessary for proper long term memory function, which indicates dosage sensitivity of m6 and supports a causative role of the GPM6A duplication for the cognitive impairment found in our patient.
Defects in the close homolog PLP1 are causative for Pelizaeus-Merzbacher disease (PMD), a severe demyelinating neurodevelopmental disorder. Prompted by recent results on successful therapy of the phenotypes in PMD mice by the administration of a cholesterol-enriched diet, we investigated if the phenotypes of GPM6A/m6 dosage alterations could also be improved by cholesterol supplementation. We indeed found that the phenotypes observed in patient cells with GPM6A overexpression as well as in flies with m6 knockdown could be partially improved by a cholesterol-enriched diet. Together with other recent findings, these data point to an increasing role of cholesterol metabolism for some ID genes.

W5-05

Homozygous mutation in fatty acyl CoA reductase 1 (FAR1) in autosomal recessive intellectual disability with early epilepsy

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We examined a consanguineous family with two children presenting with severe intellectual disability, muscular hypotonia, epileptic seizures beginning at the age of 13 months, pronounced constipation, and bruxism. In addition, one individual suffered from congenital cataracts which made surgical correction necessary. After excluding submicroscopic copy number aberrations we performed autozygosity mapping under the assumption of autosomal recessive inheritance and identified 4 candidate regions. Using exome sequencing we identified a single candidate mutation; FAR1 NM_032228.5; c.639-651delAGTAGTCTATCCAinsT, p.EVVYP166-169D. The variant was excluded in 280 ethnically matched control individuals. Molecular modelling predicted a distortion of the active site in altered FAR1 and, thus, a strong impairment in protein function.
FAR1 (fatty acyl CoA reductase 1) catalyzes the reduction of fatty acids to their corresponding alcohols (e.g. the c16 palmitic acid to hexadecanol and the c18 stearic acid to octadecanol) and it is crucial for plasmalogen synthesis that takes place in peroxisomes and the endoplasmic reticulum. Plasmalogens are essential membrane components and protect cells from damage through reactive oxygen species. Previous studies associated reduced brain levels of plasmalogen with Alzheimer's disease, X-linked adrenoleukodystrophy, and Down syndrome. Two enzymes of the plasmalogen biosynthesis pathway, GNPAT and AGPS, were reported to be altered in patients with rhizomelic chondrodysplasia punctata 2 and 3, respectively. Patients with this syndrome exhibit severely reduced plasmalogen levels and their phenotype overlaps with our patients' phenotype.
To further validate the effect of our candidate mutation on protein function we transfected HEK293 cells with wild type and mutant FAR1 and measured hexadecanol and octadecanol concentration in lipid extracts using gas-chromatography. We found that cells transfected with wild type FAR1 have significantly higher levels of hexadecanol and octadecanol than cells transfected with mutant FAR1. This indi-

cates that the activity of altered FAR1 is probably abolished, which is in line with the autosomal recessive inheritance model.
Altogether, our results indicate that FAR1 is associated with autosomal recessive intellectual disability. Further, our study underlines the importance of plasmalogen synthesis and fatty acid metabolism in brain development and function, and extends the number of enzymes affected in peroxisomal disorders.

W5-06

Mutations and deletions of SETD5 are associated with intellectual disability and characteristic facial features and contribute significantly to the microdeletion 3p25.3 phenotype

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Whole exome sequencing (WES) has proven to be a valuable tool to identify causative genes e.g. in several Mendelian disorders. Intellectual disability (ID) has an estimated prevalence of 1.5-2% and its genetic basis remains unclear in most affected persons. However, recently WES studies have shown that a large proportion of sporadic cases is caused by de novo point mutations and small insertions or deletions (indels). Our study aims to detect causative variants by exome sequencing in a cohort of 250 patients with unexplained ID and their unaffected parents and to identify new candidate ID genes.
Here, we present two patients with de novo intragenic mutations of SETD5 (SET domain containing 5, a predicted SET domain-containing histone methyl-transferase) detected by WES as well as four patients with de novo microdeletions in 3p25.3 encompassing SETD5. All six patients presented with common clinical features.
Patient 1 carried an 81 bp deletion which deletes 14 codons and an exon-intron boundary / splice site (chr3:g.9477565_9477645del); RNA studies are in progress. The nonsense mutation detected in patient 2 (chr3:g.9490270C>T, published previously in Rauch, Wieczorek, Graf et al., Lancet. 2012;380:1674-82)) is predicted to cause nonsense-mediated mRNA decay. The microdeletions of patients 3 - 6 were non-recurrent and comprised 148 kb (affecting 4 RefSeq genes, chr3:9,394,944-9,542,885), 371 kb (10 genes, chr3:9,422,487-9,793,524), 2.45 Mb (46 genes, chr3:8,856,000-11,305,600) and 11.16 Mb (71 genes, chr3:61,891-11,220,006), respectively.
All six patients shared some core symptoms including ID and several facial dysmorphisms (especially a long philtrum, anteverted nares, and downturned corners of the mouth). Developmental delay became obvious in all six individuals within the first year of life; the intellectual disability was in the range of mild to moderate (mutations and small deletions) to severe (large deletions). Facial dysmorphisms were more pronounced in microdeletion carriers than in carriers of intragenic mutations. In addition to this core phenotype, all four deletion carriers had a muscular hypotonia and developed a short stature postnatally. The carriers of larger deletions also developed microcephaly postnatally.
The core phenotype common to both groups, i.e. carriers of a microdeletion or of an intragenic mutation, strongly indicates a causative role for SETD5 for certain facial dysmorphisms and, most importantly, for intellectual disability. In addition to this, the additional genes involved in the 3p25.3 microdeletions points to their contribution to the etiology of muscular hypotonia as well as postnatal growth retardation and microcephaly, possibly defining a smallest region of deletion overlap of only four genes for growth retardation and hypotonia.

W6 Technology and Bioinformatics

W6-01

A circulating microRNA profile is associated with age-related macular degeneration

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Age-related macular degeneration (AMD) is the leading cause of severe vision impairment among people aged 55 years and older. Although a plethora of novel candidate genes are continuously indicated to be involved in AMD by large genome wide association studies (GWAS), only few studies so far have actually identified a functional relationship between a gene variant and disease pathology. In contrast, a biomarker which is dysregulated in disease might point towards processes involved in the underlying pathology and thus could help to point to novel therapeutic targets. Recently, circulating microRNAs were found in blood serum/blood plasma as potential novel biomarkers for various diseases.
Here, we aimed to elucidate the role of circulating microRNAs in AMD by genome-wide microRNA expression profiling. We performed sensitivity analyses and found three microRNAs to be associated with AMD (P-adjusted < 0.05). A combined profile of those three microRNA had an area under the curve (AUC) value of 0.718 and was highly associated with AMD (P = 2.61*10^-4). By performing pathway enrichment analysis on genes which are predicted to be regulated by these microRNAs, we were able to identify novel pathways involved in AMD pathology. We found the strongest enrichment of genes in the canonical TGFβ, mTOR, VEGFA as well as the canonical neutrophin pathway. By combining the genetic risk score (GRS) and expression data of the strongest associated microRNA, we were able to fit logistic regression models with a bootstrapped AUC value of 0.887 (95% CI: 0.882-0.893), which significantly improves upon a classification scheme based on genetic factors alone. Taken together, our results strongly implicate specific microRNAs as novel biomarkers involved in AMD disease.

W6-02

Genome-wide analysis of microRNA coding genes in bipolar disorder

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Bipolar disorder is a severe disorder of mood with a lifetime prevalence of about 1%. The disease is characterized by recurrent episodes of mania and depression and shows a high heritability of about 70%. Molecular genetic candidate and lately genome-wide association studies (GWAS) have identified a number of susceptibility genes contributing to the etiology of bipolar disorder. However, the disease relevant pathways and regulatory networks are still largely unknown.

microRNAs are a class of 21-25-nucleotide small non-coding RNAs. They control the expression of their target genes by binding to target sites in messenger RNAs (mRNAs). Each microRNA usually controls up to several hundred target mRNAs, while one mRNA target can be synergistically regulated by multiple microRNAs. This allows microRNAs to integrate different intracellular signals and to regulate various signalling pathways. Accumulating evidence suggests that microRNAs contribute to the basic mechanisms underlying brain development and synaptic plasticity. This in turn suggests their possible involvement in the pathogenesis of various psychiatric disorders, including bipolar disorder.

The aim of the present study was to systematically investigate whether common variants at all known microRNA loci listed in the miRBase database (release 13.0) contribute to the development of bipolar disorder. For this purpose we performed gene-based analyses for all microRNAs and +/- 20kb flanking sequences using VEGAS on the largest existing GWAS dataset of bipolar disorder comprising of 9,747 patients and 14,278 controls (Mühleisen et al., 2013). In this dataset we combined our data obtained from four European countries, Canada, and Australia with the results of the large bipolar disorder GWAS by the multinational Psychiatric Genomics Consortium (Sklar et al., 2011). Our analysis revealed that 98 of the 609 microRNAs showed nominally significant p values. The observed number of microRNAs with a p value of < 0.05 was significantly higher than expected (i.e. n=30, p=0.006), indicating that bipolar disorder-associated microRNAs are enriched within the known microRNA loci. After correction for multiple testing, nine microRNAs showed a significant association with bipolar disorder (let-7g, miR-135a, miR-499, miR-581, miR-611, miR-640, miR-644, miR-708, miR-1908). These included microRNAs known to be involved in neural development, neuronal differentiation and synaptic plasticity.

The investigation of the affected target genes and the underlying regulatory networks is currently underway and will be presented. Preliminary data provide evidence for an involvement of at least two microRNA-regulated networks in the development of bipolar disorder.

AJ. Forstner and A. Hofmann contributed equally to this work.

W6-03

Pathway-based enrichment analysis of genome-wide association results suggests an involvement of NCAM signaling in the etiology of bipolar disorder

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Bipolar disorder (BD) is a severe and highly heritable mental illness that affects more than 1% of a population. Recent genome-wide association studies (GWAS) have identified the first common risk variants with robust statistical support (genome-wide significance and replication), notably for the genes ANK3, CACNA1C, NCAN, and ODZ4. These GWAS also provide strong statistical evidence for the presence of multiple additional risk loci, each contributing a relatively small genetic effect to BD susceptibility. Because larger sample sizes (more power) and denser marker sets (better fine-mapping capability) are necessary to detect these additional loci, we have analyzed 2,3 million autosomal single-nucleotide polymorphisms (SNPs) in the so far largest BD GWAS (9,747 patients, 14,278 controls) and detected 56 SNPs with genome-wide significance (P<5E-8) in five chromosomal regions, comprising three known risk genes (ANK3, ODZ4, TRANK) and two new risk loci (ADCY2, MIR2113-POU3F2). Results were presented at GfH2013.

To further exploit our data regarding possible biological relationships among the top genes and/or supportive evidence for sub-genome-wide significant loci, we applied an approach (INRICH) that tests if association signals in predefined gene sets (pathways) are enriched across independent gene loci (non-overlapping intervals). Test intervals were constructed in two steps. First, GWAS results were filtered for strong to moderate signals (P<5E-4), resulting in 5,312 SNPs. Mapping of these SNPs to the largest gene isoform yielded a basic set of 386 genomic intervals. Secondly, overlapping intervals were merged to avoid multi-counting of clustered genes. Finally, 359 intervals covering 496 genes were tested for enrichment in 430 sets with 3,881 genes (Reactome pathway map). We found that a subset of 10 intervals, each covering a single gene, was significantly enriched in a set of 67 genes that form a pathway for NCAM signaling (P=3.4E-5). The result withstood correction for the total number of sets tested. Of note, among the 10 interval genes were the voltage-dependent calcium channel gene CACNA1C, the sulfate proteoglycan gene NCAN, and the transcription factor gene CREB1.

The present study demonstrates that re-investigation of GWAS data by a systematic enrichment analysis can elucidate relationships between known risk loci and genes that would have not been implicated with BD if one only focuses on ‚top hits'. The NCAM pathway plays an important role in cellular processes for the development and maintenance of the brain, for instance, axonal growth and synaptic plasticity. However, follow-up of the whole gene set as well as of single genes in independent SNP association data are warranted to further support our finding.

W6-04

A Multiplex Cap-Seq Analysis Strategy to Detect Mosaic RB1 Mutations in Blood

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Retinoblastoma is a rare malignant intraocular childhood tumor caused by two mutations, each affecting one of the RB1 alleles. In most children with sporadic unilateral retinoblastoma (>90%), both RB1 mutations occurred de novo in somatic cells. These heterozygous mutations are readily detectable in blood samples with conventional methods. In a few sporadic unilateral retinoblastoma cases, however, the first mutation occurred during the embryonic phase resulting in mutational mosaicism. These mutational mosaics can only be detected if the mutant allele proportion is large enough to give a clear signal in sequencing. This makes it impossible to determine the causative mutation in blood samples of more than 85% of sporadic isolated retinoblastoma patients using conventional techniques. Improving the detection of low-dose mutational mosaics in blood would increase the proportion of patients with sporadic unilateral retinoblastoma for whom the causative mutation can be determined without the need of a tumor sample.

We previously developed a PCR-based deep sequencing protocol (>10,000x coverage) on the Roche 454 Junior platform to determine the causative mutations in DNA from blood of unilateral retinoblastoma patients. We tested the method on 100 blood samples from unilateral retinoblastoma patients with known mosaic RB1 mutations as determined by Sanger sequencing of the tumor. We identified heterozygous and mosaic RB1 mutations in blood samples from 13 patients, including one mutation that was not identified using Sanger sequencing and three mutations that were inconsistent to those determined in Sanger sequencing. The method showed a minimal noise level of 2%, but in certain regions this increased up to 15%, seemingly dependent on the sequence context. The intended use of this method is compromised by two mutations that were not found although they were previously identified by Sanger sequencing. This prompted us to change the sequencing platform to an Illumina HiSeq 2000. As read lengths are shorter on this system, we also changed our PCR-based approach to a capture-based approach, avoiding the break-up of single exons into multiple amplicons.

We used a custom DNA capture of a 200 kb region containing RB1 to perform 24-sample multiplex captures of blood DNA from patients with known mosaic RB1 mutations followed by sequencing of the sample pool on an Illumina HiSeq 2000. First analyses indicate an overall average coverage of ~3700x (range 2635–5245x), an average of 85.2% (range 72.9-87.8%) of the ROI with 1000x coverage. The technology-dependent noise level was below 1% with sequence-dependent rises up to 2%, but not above. However, a CpG island at the 5'-end of RB1 was one of the regions not successfully captured in any of the samples. Here, we present the results of our first analyses to test the robustness of this method with improved baseline noise and the potential capability to detect gross deletions in addition to small mutations.

W6-05

Exome Sequencing and DNA Methylation Analysis of Cardiac Tissue from Patients with Hypoplastic Left Heart Syndrome

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Hypoplastic left heart syndrome (HLHS) is a congenital heart defect (CHD) characterized by severe underdevelopment of the left side of the heart, interfering with the ability to support the systemic circulation after birth. Despite good progress in surgical treatment, the etiology of HLHS remains unknown. Up to now identified genetic causes explain only less than 10% of all CHDs. Nevertheless, somatic genetic, epigenetic or transcriptional alterations potentially associated with CHD in general and HLHS in particular have yet been hardly investigated. Thus, we aimed here at analysing whether somatic cardiac tissue from HLHS shows sequence variants, DNA methylation patterns or altered gene expression.

For DNA methylation analysis genomic DNA extracted from heart tissue, specifically interatrial septum (IAS) samples of 26 HLHS patients and 9 patients with tricuspid valve atresia and underdeveloped right ventricle as control, was assessed using Illumina's HumanMethylation450K BeadChips. To discover genes aberrantly methylated in HLHS, we compared the DNA methylation values of HLHS samples to control samples using the Qlucore Omics Explorer 2.3 software. Additionally, RNA of these tissue samples was subjected to transcriptome profiling using RNA-seq. The same IAS samples of 9 HLHS patients were subjected to exome analysis using targeted enrichment via NimbleGen SeqCap EZ Human Exome Library v2.0 followed by sequencing with Illumina's HiSeq 2000 system.

No significant DNA methylation differences in the IAS samples of HLHS as compared to control samples were detected. Overall, the DNA methylation patterns of both groups HLHS and controls showed strong heterogeneity. After filtering for known SNPs, exome sequencing identified protein changing variants in a total of 21 genes known to be involved in heart development. RNA-seq analyses revealed some of these genes to be also significantly deregulated on the transcriptional level in HLHS samples. Though various validation analyses are still in progress our integrated genomic, transcriptomic and epigenomic approach provides preliminary evidence for somatic changes to be potentially involved in the pathogenesis of HLHS. (Supported by DZHK (German Centre for Cardiovascular Research) partner site Hamburg/Kiel/Lübeck, in the WP NCCR3.3 Heart Failure)

W6-06

Improved exome prioritization of disease genes through cross species phenotype comparison

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Numerous new disease-gene associations have been identified by whole-exome sequencing studies in the last few years. However, many cases remain unsolved due to the sheer number of candidate variants remaining after common filtering strategies such as removing low quality and common variants and those deemed unlikely to be pathogenic (non-coding, not affecting splicing, synonymous or missense mutations annotated as non-pathogenic by prediction algorithms). The observation that each of our genomes contains about 100 genuine loss of function variants with ~20 genes completely inactivated makes identification of the causative mutation problematic when using these strategies alone. In some cases it may be possible to use multiple affected individuals, linkage data, identity-by-descent inference, de novo heterozygous mutations from trio analysis, or prior knowledge of affected pathways to narrow down to the causative variant. In cases where this is not possible or has proven unsuccessful we propose using the wealth of genotype to phenotype data that already exists from model organism studies to assess the potential impact of these exome variants. Here, we introduce PHenotypic Interpretation of Variants in Exomes (PHIVE), an algorithm that integrates the calculation of phenotype similarity between human diseases and genetically modified mouse models with evaluation of the variants according to allele frequency, pathogenicity and mode of inheritance approaches in our Exomiser tool. Large-scale validation of PHIVE analysis using 100,000 exomes containing known mutations demonstrated a substantial improvement (up to 54.1 fold) over purely variant-based (frequency and pathogenicity) methods with the correct gene recalled as the top hit in up to 83% of samples, corresponding to an area under the ROC curve of over 95%. We conclude that incorporation of phenotype data can play a vital role in translational bioinformatics and propose that exome sequencing projects should systematically capture clinical phenotypes to take advantage of the strategy presented here. The Exomiser is freely available at http://www.sanger.ac.uk/resources/databases/exomiser/.

W7 Monogenic Diseases II

W7-01

Deciphering Mitochondrial Disorders by Exome Sequencing

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Mitochondrial disorders are a genetically and clinically highly heterogeneous group of diseases characterized by faulty oxidative phosphorylation. Despite good progress in the field, most disease causing mutations still have to be identified. During the course of three years, we applied whole exome sequencing in 300 unrelated individuals with juvenile-onset mitochondrial disorder. In a quarter of patients, we detected mutations in known disease genes. In another quarter of patients, we identified mutations in genes previously not associated with mitochondrial disorders. Mutations in the majority of genes are rare and could be identified due to loss-of-function alleles in evolutionary conserved genes such as MGME1, the first exonuclease involved in mitochondrial replication. Mutations in other genes are more frequent, with ACAD9 being the most common finding with 15 cases association with isolated respiratory chain complex I-deficiency. More difficult to identify are missense mutations in genes coding orphan proteins such as FBXL4, a protein with yet unknown function associated with reduced mitochondrial protein content. Additional diagnostic chal-

lenges are patients with recessive mutations in more than one gene (MTO1 and LYRM7) resulting in a compound clinical phenotype.
Evolving topics are tRNA modifying enzymes (ELAC2, MTO1 and GTPBP3) and tRNA synthetases, as well as cofactor metabolism defects. The later offers rational therapeutic options as for example riboflavin supplementation in the case of mutations in the riboflavin transporter SLC52A2.
In summary, the genetically heterogeneous group of mitochondrial disorders is an example par excellence for the application of genome wide sequencing, which allows for comprehensive detection of disease causing mutations and rapid identification of novel disease genes. Further improvement of the sequencing technology holds promise for a further increase in diagnostic yield by optimizing coverage and the detection of indels and copy number variants. However, several issues remain to be considered including how to tackle diseases caused by di- or oligogenic mutations or how to identify mutations with dominant effect. Nevertheless, in addition to shedding light on mitochondrial physiology, newly identified genes promise options for new treatments.

W7-02

Constitutive Activation of PRKACA in Adrenal Cushing's Syndrome

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Background:
Endogenous hypercortisolism, referred to as Cushing's syndrome, is associated with excess morbidity and mortality. When Cushing's syndrome is severe, patients suffer from catabolic symptoms such as muscle weakness, skin fragility, osteoporosis and severe metabolic sequelae. Corticotropin-independent Cushing's syndrome is caused by tumors or hyperplasia of the adrenal cortex.
Methods:
We performed exome sequencing of ten cortisol-producing adenomas and matched control tissue to identify somatic mutations and evaluated recurrent mutations in candidate genes in adenomas of additional 171 patients. We further performed genome-wide copy number analysis in 35 patients with cortisol-secreting bilateral hyperplasias. We studied the effects of these genetic defects both clinically and in vitro.
Results:
Exome sequencing revealed somatic mutations in the PRKACA gene, which encodes the catalytic subunit of cyclic AMP-dependent protein kinase (PKA), in 8 of 10 adenomas (c.617A>C, p.Leu206Arg in seven and c.595_596insCAC, Leu199_Cys200insTrp in one patient). Overall, PRKACA somatic mutations were identified in a total of 22 of 59 adenomas (37%) from patients with overt Cushing's syndrome; these mutations were not detectable in patients with subclinical hypercortisolism (n=40) or in other adrenal tumors (n=82). Among 35 patients with bilateral cortisol-producing hyperplasias, 5 (two of whom were first-degree relatives) carried a germline copy number gain of the chromosome 19 region, including the PRKACA gene. In vitro studies demonstrated impaired inhibition of both PKA catalytic subunit mutants by the PKA regulatory subunit, while cells from patients with germline chromosomal gains showed increased protein levels of the PKA catalytic subunit; in both instances, basal PKA activity was increased.
Conclusions:
This study links genetic alterations of the catalytic subunit of PKA to human disease. Germline duplications of this gene result in bilateral adrenal hyperplasias, whereas somatic PRKACA gain-of-function mutations lead to unilateral cortisol-producing adrenal adenomas.

W7-03

Extending the molecular basis of isolated and syndromic microcephaly

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Microcephaly is a neurodevelopmental disorder characterized by reduced head circumference at birth and varying degrees of intellectual disability. Microcephaly can occur isolated, with no other obvious abnormalities, or it may be part of a syndrome of congenital anomalies, and therefore associated with other abnormalities. To extend our knowledge about genes associated with isolated and syndromic forms of microcephaly, we initially performed whole-exome sequencing (WES) in different patients born to consanguineous and non-consanguineous parents, presenting with phenotypical features of primary microcephaly/syndromic microcephaly, including growth retardation, developmental delay, mental retardation and facial dysmorphism. Combining WES strategy with determination of homozygous stretches of identified variants, we found homozygous mutations in different new genes and additionally were able to prove causality of very recently identified genes associated with different forms of microcephaly.
We identified a novel splice-site mutation in CDK5RAP2, c.4005-9A>G, in a consanguineous family from Turkey with Seckel syndrome leading to a frame-shift and premature truncation of the protein (p.Arg1335Serfs*3). Functional analysis revealed that the identified mutation leads to loss of CDK5RAP2 function inducing severe defects in mitosis and spindle organization and resulting in cells with abnormal nuclei and centrosomal pattern. These data provide first evidence that mutations in CDK5RAP2 are associated with both primary microcephaly and Seckel syndrome.
Furthermore, we identified the first homozygous mutation, c.2524G>A, in RAD50 predicted to result in the change p.Val842Ile. RT-PCR analysis of mRNA derived from primary patient fibroblasts showed that the c.2524G>A mutation induces skipping of exon 15 of RAD50 causing a frameshift and premature protein truncation (p.Met800Phefs*7), thereby leading to complete loss of protein function.

Additionally, we identified a German family with three children all presenting with severe neonatal, persistent, therapy-resistant seizures, and severe postnatal microcephaly who were compound heterozygous for the mutations c.228dupA and c.638dupA in the BRAT1 gene proving further evidence that mutations in BRAT1 underlie this new clinical entity referred to as lethal neonatal rigidity and multifocal seizure syndrome.
Finally, in a consanguineous family with two affected children presenting with primary microcephaly we were able to identify a homozygous frameshift mutation in a novel, non-characterized centrosomal protein of the CCDC family. The c.813_814delGA mutation is predicted to lead to premature protein truncation, p.M271Ifs*2, indicating further heterogeneity in primary microcephaly.
In summary, we were able to expand the spectrum of genes and mutations associated with primary or syndromic microcephaly and we could prove causality and identify new genes involved in the pathogenesis of microcephaly.

W7-04

Loss of CRIM1 causes colobomatous macrophtalmia with microcornea in human and mouse

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Macrophtalmia, colobomatous, with microcornea (MACOM, OMIM 602499), is a rare, autosomal dominantly inherited malformation of the eye, which is characterized by microcornea with increased axial length, coloboma usually involving the optic disc, and severe myopia. The causative gene has been mapped to the 2p23-p16 region, but it has not been identified to date. We performed whole-exome sequencing (WES) in two affected individuals from the largest reported pedigree of MACOM, which includes 13 affected individuals from three generations. Since no shared novel variation was found in the WES data in the linked region from both individuals, we performed CNV analysis by comparing the coverage of all exons in the WES data of the patients with the coverage of 26 controls. We detected a total of 110 and 89 statistically significant CNVs ($p<0.05$), but only one of them was present in both patients within the linked interval ($p=1.2*10^{-27}$ and $p=6.4*10^{-21}$). This heterozygous deletion was predicted to span 22 kb and to include exons 14 to 17 of CRIM1 and most of the 3'UTR of FEZ2. We performed a quantitative PCR using primers for exon 6 of CRIM1. Genomic DNA from 11 affected individuals from this family showed a reduction in the amount of CRIM1 when compared to healthy family members. To identify the breakpoints, we analyzed the WES data looking for split-reads, which align to both ends of the deletion. Even though our WES protocol did not include enrichment of the UTRs, one of the breakpoints was close enough to the last exon of FEZ2 to be covered by 4 reads in one patient and 5 reads in the other. We could thus precisely identify the breakpoints at chr2:36757668 and chr2:36780274 and interestingly, we found a 4-bp microhomology (CTTG) flanking the deletion. We confirmed this result with a breakpoint-PCR, which was positive in all affected family members and in none of the healthy family members. Crim1 is a transmembrane protein containing six cysteine-rich von Willenbrand factor type C repeat domains (VWFC) and an insulin-like growth factor-binding domain, with pleiotropic roles in the development of different organs, including eye, central nervous system, kidney, vasculature and placenta. The deletion is predicted to produce a truncated protein, missing the last VWFC domain, the transmembrane domain and the cytoplasmic domain. To investigate the mouse ocular phenotype caused by loss of Crim1, we crossed a Crim1-flox mouse line with the Ap2α-cre mouse line, which expresses Cre in the head surface ectoderm. Strikingly, we observed developmental alterations of eye development in these mice leading to severe anatomical and morphological changes, which are overlapping with the abnormalities observed in human. Taken together, these findings identify CRIM1 as the causative gene for MACOM syndrome, and provide an example of how WES can be used for identifying CNVs and determining the exact location of breakpoints.

W7-05

Mutations in CERS3 cause autosomal recessive congenital ichthyosis in humans.

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Autosomal recessive congenital ichthyosis (ARCI) is a rare genetic disorder of the skin characterized by abnormal desquamation over the whole body. In this study we report four patients from three consanguineous Tunisian families with skin, eye, heart, and skeletal anomalies, who harbor a homozygous contiguous gene deletion syndrome on chromosome 15q26.3. Genome-wide SNP-genotyping revealed a homozygous region in all affected individuals, including the same microdeletion that partially affects two coding genes (ADAMTS17, CERS3) and abolishes a sequence for a long non-coding RNA (FLJ42289). Whereas mutations in ADAMTS17 have recently been identified in autosomal recessive Weill-Marchesani-like syndrome in humans and dogs presenting with ophthalmologic, cardiac, and skeletal abnormalities, no disease associations have been described for CERS3 (ceramide synthase 3) and FLJ42289 so far. However, analysis of additional patients with non-syndromic ARCI revealed a splice site mutation in CERS3 indicating that a defect in ceramide synthesis is causative for the present skin phenotype of our patients. Functional analysis of patient skin and in vitro differentiated keratinocytes demonstrated that mutations in CERS3 lead to a disturbed sphingolipid profile with reduced levels of epidermis-specific very long-chain ceramides that interferes with epidermal differentiation. Taken together, these data present a novel pathway involved in ARCI development and, moreover, provide the first evidence that CERS3 plays an essential role in human sphingolipid metabolism for the maintenance of epidermal lipid homeostasis.

W7-06

Sensory neuropathy with bone destruction due to a mutation in the membrane-shaping atlastin GTPase 3

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A hallmark of hereditary sensory and autonomic neuropathies (HSAN) is loss of nociception, which can have deleterious consequences. We analysed a family with therapy-refractory plantar ulcerations and progressive destruction of the pedal skeleton necessitating amputation in one member. The inheritance pattern was autosomal dominant. Subsequently sensory neuropathy with loss of pain perception and mildly impaired touch sensation without muscle atrophy was noted. By whole-exome sequencing in the two most distant affected family members we detected a missense mutation in a highly conserved amino acid residue of atlastin GTPase 3 (ATL3), an endoplasmic reticulum shaping GTPase. The same mutation (p.Tyr192Cys) was identified in a second family with similar clinical outcome by screening a large cohort of 115 patients with hereditary sensory and autonomic neuropathies. In both families the mutation segregated with the disorder and showed complete penetrance. ATL3 is a paralog of ATL1, a membrane-curvature generating molecule involved in spastic paraplegia and hereditary sensory neuropathy. ATL3 proteins are enriched in three-way junctions, branch points of the endoplasmic reticulum that connect membranous tubules to a continuous network. Mutant ATL3-p.Tyr192Cys failed to localize to branch points, but instead disrupted the structure of the tubular endoplasmic reticulum, suggesting that the mutation exerts a dominant-negative effect. Identification of ATL3 as novel disease-associated gene exemplifies that alterations in membrane shaping-proteins are a major emerging pathway in axonal degeneration. Furthermore, this case illustrates the central role of nociception for the preservation of the weight-bearing parts of the skeleton.

W8 Epigenetics

W8-01

Automated methylation analysis of amplicons from bisulfite flowgram sequencing

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The Roche 454 GS Junior sequencing platform allows locus-specific DNA methylation analysis using deep bisulfite amplicon sequencing. However, bisulfite-converted DNA reads may contain long T homopolymers, and the main sources of errors on pyrosequencing platforms are homopolymer over- and undercalls. Furthermore, existing tools do not always meet the analysis requirements for complex assay designs with multiple regions of interest (ROIs) from multiple samples. We have developed the amplikyzer software package to address the above challenges. It directly aligns the intensity sequences from standard flowgram files (SFF format) to given amplicon reference sequences, without converting to nucleotide FASTA format first, avoiding information loss by rounding flow intensities, and taking special measures to correctly process long homopolymers. It offers a variety of options to analyze complex multiplexed samples with several regions of interest and outputs useful statistics and publication-quality analysis plots without mandatory manual interaction. This allows our software to be used as part of automated pipelines as well as interactively. The underlying analysis algorithms, using a novel hybrid flowgram-DNA sequence representation are described. We also discuss configuration options and use cases of our open source amplikyzer software and present exemplary results. The software, including required libraries, is available at https://bitbucket.org/svenrahmann/amplikyzer/downloads.

W8-02

A mouse model for human RB1 imprinting

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The human retinoblastoma gene, RB1, is imprinted. Parent-of-origin specific DNA methylation is acquired on CpG85, a CpG island within the pseudogene PPP1R26P1, which got integrated into intron 2 of the RB1 gene. The unmethylated CpG85 on the paternal allele serves as promoter for an alternative RB1 transcript, transcript 2B. Transcription of transcript 2B interferes with transcription of the regular RB1 transcript on the same allele. PPP1R26P1 contains a second CpG island (CpG42), which is biallelically methylated.
The mouse Rb1 does not contain PPP1R26P1 and is not imprinted. To determine if the integration of PPP1R26P1 is sufficient to result in skewed expression of Rb1, we generated a knock-in of human PPP1R26P1 in intron 2 of the murine Rb1 gene, using homologous recombination in mouse embryonic stem (ES) cells.
To be able to distinguish expression of the two Rb1 alleles in ES cells, a single nucleotide variant in exon 3 of Rb1 was introduced first. In vitro methylation and expression analyses were performed. Next generation bisulfite sequencing of CpG85 and CpG42 revealed differences in their susceptibility to DNA methylation, gaining methylation at a median level of 4% and 18%, respectively. We showed enrichment of RNA polymerase II at and transcription from the unmethylated CpG85 in PPP1R26P1. However, these transcripts could not be connected to downstream exons of Rb1 so far. Using the single nucleotide variant in exon 3 of Rb1, quantitative primer extension analyses (SNaPshot) revealed reduced expression of full-length Rb1 from the targeted allele. These results identify human PPP1R26P1 as a cis-repressive element in vitro.
To determine if CpG85 acquires imprinted differential DNA methylation in the mouse germ line, we used targeted ES cells to generate mice carrying human PPP1R26P1 in the Rb1 gene. Chimeras were obtained from two independent ES cell clones, but germ line transmission of the targeted allele was achieved with one clone only. The selection cassette was already removed by breeding with a Cre expressing mouse strain. Currently, the mice are backcrossed to obtain them on a pure strain background for DNA methylation and allelic expression analyses. First results will be presented.

W8-03

Epigenetic Changes during Male Germ Cell Development – The Role of SPOC1 (PHF13) in Meiosis

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Germ cells are responsible for the transmission of an individual´s genome to the next generation. Correct and sustained germ cell development is therefore of fundamental importance for genome integrity. In addition, transmission of epigenetic information to the next generation via the male germ line has been demonstrated recently. Spermatogenesis is controlled by unique gene expression programs involving epigenetic reprogramming of histone modifications and DNA methylation. The main event of this reprogramming takes place at the prophase I of meiosis during which homologous chromosomes pair and recombine. At unpaired regions, chromatin is repressed at the pachytene stage by meiotic silencing, a tightly epigenetically regulated process known as Meiotic Sex Chromosome Inactivation (MSCI).
We have recently identified the gene SPOC1 (PHF13) which encodes a protein involved in protein-chromatin interaction binding to the

histone modification H3K4me3. We could show that deletion of Spoc1 leads to severe hypoplasia of the testis and infertility in male mice. These mice showed progressive loss of germ cells due to apoptosis in the pachytene stage. Functional analyses of the Spoc1-knockout mice using whole genome expression arrays revealed a defect in the transcriptional silencing of the sex chromosomes (MSCI) during meiosis.
Here we report on the molecular mechanisms responsible for altered MSCI and infertility in the Spoc1-/- mice. We were able to demonstrate that the induction of the XY-body, the subnuclear compartment where MSCI takes place, appeared normal in the knockout mice, indicating that the increased level of transcription on the sex chromosomes represents the result of an epigenetic effect. Extensive immunofluorescence experiments with meiocyte spreads showed specific changes of the histone modifications H3K27me2, H3K36me2/3, H3K9ac, H3K27ac, and H2AK119ub within the XY-body of knockout cells. Interestingly, some of these modifications are also important in epigenetic regulation of developmental genes by bivalent chromatin. Furthermore we detected a clonal apoptosis pattern of the pachynemas in the knockout testes. Together with the fact that expression of Spoc1 in the testis is restricted to undifferentiated spermatogonial stem cells (SSCs) in the germinal epithelium, this strongly suggests that epigenetic misregulation takes place during SSC differentiation but becomes deleterious later in differentiation, during meiosis and MSCI.

W8-04

DNA methylation profiling of pediatric acute lymphoblastic leukemia with MLL translocations

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Acute lymphoblastic leukemias (ALL) with rearrangements of the Mixed-Lineage Leukemia (MLL) gene account for the majority of infant ALL cases and are associated with an unfavorable prognosis. The MLL gene is highly promiscuous and undergoes fusion with more than 70 translocation partners. Pathomechanisms and prognosis might vary depending on the respective partner genes. Besides the chromosomal translocation involving the MLL gene, which is supposed to be the primary genetic event in these ALLs, the genome of MLL rearranged leukemias seem to be very stable. Only very few recurrent somatic mutations (mainly affecting the FLT3 and RAS genes) and chromosomal imbalances have been described and the mutation rate is comparably low (~0,23 per Mb). The MLL (alias: KMT2A) gene is located in chromosomal region 11q23 and encodes a lysine (K)-specific methyltransferase. Besides others the protein contains a SET domain, which is responsible for its histone H3 lysine 4 (H3K4) methyltransferase activity which mediates chromatin modifications associated with epigenetic transcriptional activation. Thus, deregulation of the epigenome seems to be the major pathogenetic mechanism driving leukemogenesis in MLL-rearranged ALL.
As part of the International Human Epigenome Consortium project BLUEPRINT (funded by EU under 282510) we aim at providing full reference epigenomes of MLL rearranged pediatric ALL in order to better characterize and understand the epigenetic mechanisms underlying the pathogenesis of these leukemias. As an initial step to this goal, we here performed array-based DNA methylation profiling on genomic DNA from leukemic cells of 26 pediatric patients with MLL+ ALL of the BFM-ALL pediatric study group. These data were compared to 22 samples from different non-neoplastic B-cell precursor populations. The patient cohort included 21 infants (<1 year) and 5 children aged 2, 5,7,17 and 18 years. The majority of the patients carried the translocations t(4;11) (15 cases) or t(11;19) (6 cases). The aberration t(9;11) was present in two cases, t(1;11) was present in one case.
The methylation patterns of the analyzed ALL samples were strikingly homogenous. The few methylation differences detected between e.g. ALLs with t(4;11) and t(11;19) are confounded by the different age at diagnosis associated with the both translocations. We identified 774 CpG loci hypermethylated and 201 CpG loci hypomethylated in MLL rearranged leukemias compared to the benign B-cell precursors. Whereas hypermethylated regions were enriched for CpG islands and polycomb repressed regions, hypomethylation seems to preferentially target enhancer regions. The differentially methylated loci were enriched mainly for 5 different transcription factor binding sites.
In summary, the DNA methylation patterns of MLL rearranged ALLs significantly differs from that of normal B-cell precursor cells but is quite homogenous within the leukemias with some variation in dependence of translocation and age.

W8-05

(Epi-)genomic analyses of lung cancer patients for prediction of chemotherapy resistance

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Lung cancer is the most frequent cause of cancer-related death worldwide. This is partly due to high incidence rates, but also due to the fact that they are diagnosed at advanced stages and that they are frequently resistant against chemotherapies. To identify chemotherapy resistance mechanisms we collected primary lung tissue and xenograft tumor material from 25 lung cancer patients and performed next generation sequencing to identify copy number variations, mutations and epigenetic alterations. Chemotherapy response values were generated for each of these mice for five different chemotherapeutic drugs and used to group the tumors in responders and non-responders. Here we will present comprehensive data on genomic and epigenomic differences likely underlying chemotherapy resistance. In addition we used a mathematical computer model (PyBios) for the simulation of cancer-related cellular processes and the prediction of the effectiveness of chemotherapies. The approach we present may function as a prototype for the identification of therapy resistance mechanisms and will result in biomarkers for the stratification of tumor patients.

W8-06

Structure / function relationships between the inactive X chromosome territory (CT) and active CTs studied by super-resolution microscopy

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Transcriptional silencing of most of the genes in one X chromosome in female mammals has evolved as a dosage compensation mechanism between males and females. X chromosome inactivation (XCI) starts at the blastocyst stage when Xist RNA is transcribed from the committed inactive X (Xi). Maintenance of inactivation is further accomplished by epigenetic factors corporately mediating the chromosome-wide silenc-

ing of gene activity. XCI is associated with a distinct chromatin condensation resulting in Xi territories that are highlighted as structural hallmarks in female nuclei by a prominent DNA staining intensity, the so-called "Barr body". Thus, the Xi territory (Barr body) represents a unique system to analyze structure-function relationships of repressed versus transcriptionally active chromosome territories (CTs).
Using DNA probe sets, which target physically or functionally defined sub-chromosomal segments, we compared the higher order folding structure in Xi-and Xa-territories at the subchromosomal level by 3D multicolor FISH and quantitative 3D image analysis. Using super-resolution 3D-structured illumination microscopy (3D-SIM), we compared the 3D ultrastructure of the Xi with active CTs in somatic cells. We demonstrate that chromatin condensation in the Xi does not result from a uniformly increased compaction at the nucleosome level, but rather from a collapse of a specific nuclear subcompartment. All CTs form a network of structurally linked chromatin domain clusters (CDCs). CDCs of active CTs are lined by a spatially confined perichromatin region (PR), which harbors decondensed chromatin enriched with transcriptionally competent markers. The PR network is co-aligned with a contiguous channel system, the interchromatin compartment (IC), which starts at nuclear pores and pervades CTs. PR and IC channels together form the intricate active nuclear compartment (ANC) where transcription occurs at the periphery of compact CDCs. The Xi differs from active CTs by a significant collapse of the ANC and a closing up of CDCs clusters. The mainatenance of rudimentary ANC channels may be essential for the structural integrity of Xi and for providing accessibility of factors required for (sparse) transcription and replication. Xist RNA foci localize within the rudimentary ANC channels suggesting their dynamic interaction with silenced genes / regulatory sequences. Exploring the spatio-temporal process of Barr body formation in early differentiating embryonic stem cells (ESCs) we found that initial Xist RNA spreading precedes Barr body formation which occurs together with subsequent RNA polymerase II (RNAP II) exclusion. Autosomal transgenic Xist induction triggers an "autosomal Barr body" with less compacted chromatin and incomplete RNAP II exclusion demonstrating the X-chromosomal context required for a typical Barr body.

W9 Disease Mechanisms and Treatment

W9-01

Mapping the genetic architecture of gene regulation in whole blood

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Background: We aimed to assess whether whole blood expression quantitative trait loci (eQTLs) with effects in cis and trans are robust and can be used to identify regulatory pathways affecting disease susceptibility.
Materials and Methods: We performed whole-genome eQTL analyses in 890 participants of the KORA F4 study and in two independent replication samples (SHIP-TREND, N=976 and EGCUT, N=842) using linear regression models and Bonferroni correction.
Results: In the KORA F4 study, 4,116 cis-eQTLs (defined as SNP-probe pairs where the SNP is located within a 500 kb window around the transcription unit) and 94 trans-eQTLs reached genome-wide significance and overall 91% of all eQTLs (92% of cis-, 84% of trans-eQTLs) were confirmed in at least one of the two replication studies. Different study designs including distinct laboratory reagents (PAXgeneTM vs. TempusTM tubes) did not affect reproducibility (separate overall replication overlap: 78% and 82%). Immune response pathways were enriched in cis- and trans-eQTLs and significant cis-eQTLs were partly coexistent with published results from other tissues (cross-tissue similarity 40-70%). Furthermore, four chromosomal regions displayed simultaneous impact on multiple gene expression levels in trans, and 746 eQTL-SNPs have previously reported clinical relevance. We demonstrated cross-associations between eQTL-SNPs, gene expression levels in trans, and clinical phenotypes as well as a link between eQTLs and human metabolic traits via modification of gene regulation in cis.
Conclusions: Our data suggest that whole blood is a robust and informative tissue for eQTL analysis and may be used both for biomarker studies and to enhance our understanding of molecular mechanisms underlying gene-disease associations.

W9-02

Deletions of Chromosomal Regulatory Boundaries are Associated with Congenital Disease

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Recent data from chromosome confirmation capture analysis (Hi-C) indicate that the human genome is divided into conserved megabase-sized local chromatin self-interacting regions called topological domains. These topological domains form the regulatory backbone of the genome and are separated by regulatory boundary elements or barriers. Copy-number variations (CNVs) can potentially alter the topological domains architecture by deleting or duplicating the barriers and thereby allowing enhancers from neighboring domains to ectopically activate genes causing misexpression and disease, a mutational mechanism that has recently been termed "enhancer adoption". The Human Phenotype Ontology (HPO) database provides information for over 2,800 genes associated with the known Mendelian diseases, and was used in this study to relate the phenotypes of the CNVs to those of monogenic diseases of genes in or adjacent to 922 deletion cases recorded in the DECIPHER database.
We identified human enhancers specific to ten different tissue types by analysis of DNAse hypersensitivity data, and used these results and the HPO information to categorize the DECIPHER deletions. Using this computational approach most deletions can best be explained by dosage effects affecting phenotypically relevant genes located within the deletions themselves. However, up to 12% of the deletions can be best explained by the potential effects of tissue-specific enhancers that are brought by the deletion into the genomic vicinity of phenotypically relevant genes that lie adjacent to the breakpoints, or by a combination of the two mechanisms. Our results suggest that enhancer adoption caused by deletions of regulatory boundaries may contribute to a substantial minority of CNV phenotypes and should thus be taken into account for their medical interpretation.

W9-03

Functional analysis of SHANK2 mutations identified in schizophrenia patients

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Mutations in the SHANK3 gene and common variants associated with the SHANK1 gene were previously described in schizophrenia patients suggesting a role for these genes in pathological processes. In our study we focused on the last remaining member of the SHANK gene family – SHANK2. We sequenced all exons and exon-intron boundaries of the ProSAP1A_AB208026 isoform of SHANK2 by Sanger sequencing using genomic DNA isolated from 481 schizophrenia patients (177 trios and 304 singleton patients) and 374 unaffected individuals. We found ten variants that affect protein structure which are only present in the patient group. We used mutation prediction tools that are based on evolutionary protein conservation and chemical properties of amino acids to select the four most promising variants. Such in silico investigation is helpful to estimate a functional relevance of mutations, but cannot substitute the functional analysis itself. To analyze the functional impact of the four selected mutations, we conducted overexpression and knockdown-rescue experiments in primary hippocampal neurons from rat with a major focus on morphological changes of the neurons. Another major point of our study was to investigate the effect of different SHANK2 isoforms and schizophrenia mutations on the actin structures. We used COS-7 cells as a model system and live cell TIRF (Total Internal Reflection Fluorescence) microscopy for imaging of actin structures. Additionally, we also performed actin polymerization assays to measure in vivo F-actin/G-actin ratio in our mutants compared to SHANK2 wild type. With these functional tests we were able to show for the first time a functional effect of SHANK2 variants which were identified in schizophrenia patients.

W9-04

Impaired miRNA regulation as molecular cause of altered 5-HT4 receptor signalling in irritable bowel syndrome

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Irritable bowel syndrome (IBS) is a complex gastrointestinal (GI) disorder in which disturbed motility is thought to play a role. 5-HT4 receptors regulate motor function and are targeted therapeutically in the treatments of IBS with constipation (IBS-C) and functional constipation. We hypothesize that disturbed 5-HT4 receptor regulation or functional defects in the 5-HT4 receptor gene (HTR4) may be involved in the motor dysfunction seen in these patients.
As 3' untranslated regions (3'UTRs) are a major site of posttranscriptional regulation, we screened different HTR4 isoforms in a small IBS pilot cohort for variants in their 3'UTRs. In this cohort, we identified the rare SNP (rs201253747) HTR4b c.*61T>C to be exclusively present in IBS with diarrhoea (IBS-D) patients (2/98 in IBS-D, 0/100 in IBS-C and 0/92 in controls). In a subsequent replication study, we confirmed the polymorphism as significantly enriched in IBS-D patients compared with healthy controls (p=0.033; OR=3.09; 2185 healthy controls, 613 IBS-C and 829 IBS-D patients were tested in total).
The SNP locates in a putative miR-16 family binding site. We show that HTR4 is specifically downregulated by this miRNA family via multiple target sites. Furthermore we describe a novel isoform of HTR4b with an alternatively spliced 3'UTR. This isoform escapes miR-16 regulation in reporter assays when the HTR4b c.*61C allele is present, suggesting an increase of 5-HT4 receptor expression in HTR4b c.*61C carriers .
In conclusion, we have shown for the first time that HTR4 is susceptible to miRNA regulation and that the c.*61T>C polymorphism impairs the regulation of HTR4 via the miR-16 family. Thus we postulate that people carrying this SNP may have a higher 5-HT4 receptor activity and therefore higher risk of developing IBS-D.

W9-05

Osteoporosis: Filamentous-actin bundling, a novel mechanism underlying bone development

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Osteoporosis affects a large proportion of the human population, particularly women after menopause. Recently we reported that pathogenic loss-of-function variants in the plastin 3 (PLS3) gene, localized on Xq23 are causative of familial osteoporosis and osteoporotic fractures at a young age in males and can lead to osteoporosis at a variable age in females. Furthermore a rare variant in PLS3 (rs140121121) associated with a 2-fold increased risk for fractures among elderly heterozygous women in two large cohorts from Rotterdam. This data indicates that PLS3 is also a novel etiologic factor involved in common, multifactorial osteoporosis (Van Dijk et al., N Engl J Med, Oct 2013).

PLS3 is an ubiquitously expressed actin bundling protein that largely influences the dynamics of the actin cytoskeleton. We have demonstrated that PLS3 mRNA co-injection dose dependently rescued malformations of the craniofacial muscular-skeletal system induced upon pls3 morpholino injection in 3 dpf and 5 dpf col1a1:eGFP transgenic zebrafish. Additionally, body axis and tail phenotype were also rescued upon PLS3 mRNA co-injection. Remarkably, affected patients with PLS3 loss of function mutations only presented a bone phenotype. The absence of systemic manifestations has led us to hypothesise that other F-actin bundling proteins may compensate for the loss of PLS3 in other tissues. Interestingly, α-Actinin (ACTN) was found to be overexpressed in fibroblasts of affected individuals, possibly preventing more severe disease manifestations. Indeed co-injection of zebrafish with pls3 morpholinos and ACTN1 or ACTN4 mRNA, rescued the muscular-skeletal phenotype induced by pls3 knock-down. Moreover, analysis of femora by micro computed tomography (μCT) in 3 months old transgenic mice overexpressing human PLS3 showed significant differences between male and female cortical and trabecular bone structures depicting increased cortical thickness as well as increased trabecular thickness and altered shape when compared to control mice. These results strongly indicate an essential function of PLS3 and other F-actin bundling proteins as new regulators of bone development and maintenance. From these observations we further speculate that women overexpressing PLS3, a situation found in 5% of the population may show decreased risk for osteoporosis.

W9-06

iPS-cell derived basal keratinocytes for autosomal recessive congenital ichthyosis

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The generation of induced pluripotent stem (iPS) cells from a somatic source like dermal fibroblasts by transient or permanent introduction of the four stemness factors OCT4, cMYC, SOX2, and KLF4 is today a widely accepted technique to preserve cells from patients with rare conditions and to study underlying pathomechanisms. We are on the way to establish patient-specific 3D full-thickness skin models with iPS-cell derived keratinocytes to enhance our studies on protein-replacement therapies, especially for patients with autosomal recessive congenital ichthyosis (ARCI). Up to date treatment for most keratinisation disorders is only symptomatic and clearly insufficient, especially owing to the critical lack of suitable disease models. Therefore we have generated iPS cells from patients with ARCI and other genodermatoses. To investigate the disease of our patients in differentiated cells, we have focused on ectodermal differentiation of iPS cells using stringent culture methods and selected feeder techniques. Recently we were able to generate highly pure keratinocytes expressing markers of the basal epidermis, including keratin 18, keratin 14, p63, as well as integrins of the basement membrane zone in feeder and defined culture systems. Thus we were successful to suppress neuronal outgrowth at the neuro-ectodermal switch and to enhance ectodermal commitment repressing noggin expression with human recombinant BMP4 supplementation during early differentiation. Our strategy omits cell sorting to minimize cell loss and costs and produces iPS-derived keratinocytes within 40 days of differentiation. The ectodermal marker keratin 18 is expressed in most cells from day 12. In contrast, from day 12-18 few single cells show keratin 14 expression; only from day 29-35 all cells show a clear and structured keratin 14 expression signal indicative of epidermal development. At this time point cells also express integrin alpha 6 and integrin beta 4 as markers for basal keratinocytes, but no keratin 10. Currently, we are using our iPS cell-derived keratinocytes for first 3D tissue model studies. Only iPS-cell derived keratinocytes allow us to generate patient-specific 3D tissue models in larger quantities, which will be essential to assess drugs for these orphan diseases and pave the way for individualized therapies.

EDUCATIONAL SESSIONS

EDU 1

Panel- und Exomdiagnostik

Peter Bauer (Tübingen), Tim M Strom (Neuherberg, München)

Die Einführung von Next-Generation Sequenziertechnologien in die klinische Diagnostik erfordert neue Strukturen für die Datenproduktion und Datenauswertung. Dabei geht es einerseits darum, das dieser Technik inhärente Potential zur Automatisation zu nutzen. Andererseits müssen Elemente der Qualitätssicherung für diese Technologie definiert werden, damit nicht nur eine leistungsfähige, sondern auch eine sichere Diagnostik angeboten werden kann. Im Prinzip erfordert die Datenauswertung lediglich einen Vergleich einer vollständigen Liste der sequenzierten Varianten mit einer umfassenden Liste von krankheitsverursachenden Varianten. In der Praxis stellen weniger die technischen Limitationen der Sequenziertechnik als die fehlende Annotation der meisten seltenen Varianten ein Problem dar, das in Zukunft nur durch eine umfassende Sammlung der Allelefrequenzen von bevölkerungsweiten Sequenzierprojekten und durch hochwertige Mutationsdatenbanken gelöst werden kann.

Für die Diagnostik werden derzeit vor allem die Multi-Gen Panel Sequenzierung (MGPS) und die klinische Whole Exome Sequenzierung (WES) verwendet. Während die technische Datenproduktion in entsprechend ausgestatteten Diagnostiklaboren zentralisiert werden kann, verlangt die diagnostische Auswertung eine enge Kommunikation zwischen Labor und den Einsendern, oft vermittelt über spezialisierte klinische Genetiker. Dabei zeichnet sich jetzt schon ab, dass diese Kommunikation, die Vernetzung von Diagnostiklaboren insgesamt und der Datenaustausch zwischen diesen, einem fundamentalen Wandel unterworfen werden. Darüber hinaus erfordert die Anwendung im klinischen Umfeld spezifische Vorbereitungen im Umgang mit der Mitteilung von sekundären Befunden, den Vorgaben des Gendiagnostik-Gesetzes, der Datenspeicherung, des Datenschutzes und den Normen in akkreditierten Laboren.

In dieser EDU-Session werden wir an Beispielen aus der MGPS und WES diese Aspekte vorstellen und diskutieren. Insbesondere sollen neue Elemente für die Indikationsstellung, Qualitätssicherung und Datenverarbeitung bei der diagnostischen Anwendung von NGS vorgestellt werden, die das außerordentliche technologische Potential für Patient und Arzt zu einer sicheren Diagnostikanwendung machen können.

EDU 2

Mikrozephalie

Moderation: Bernd Wollnik (Köln) und Ute Hehr (Regensburg)

Mikrozephalie ist ein häufig vorkommendes klinisches Zeichen unterschiedlichster Entwicklungsstörungen des Gehirns. Die weltweite Prävalenz wird mit etwa 2 % angegeben. Eine Mikrozephalie ist durch ein verringertes Volumen des Gehirns gekennzeichnet und häufig mit geistiger Behinderung und dem Auftreten einer Epilepsie assoziiert. Die Identifizierung und funktionelle Charakterisierung ursächlicher Gene für die primären, isolierten Formen (MCPH) als auch für syndromale Mikrozephalien (z. B. Seckel-Syndrom und MOPDII) haben uns faszinierende Einblicke in die molekulare Pathogenese von Mikrozephalien verschafft. Es konnte gezeigt werden, dass Veränderungen fundamentaler zellulärer Mechanismen zu neuronalen Differenzierungsstörungen und Mikrozephalie führen. Bekannte Gene und zugrunde liegende Mechanismen werden vorgestellt.

Im klinischen Alltag ist die genetische Abklärung isolierter und auch syndromaler Mikrozephalien eine große, nicht selten frustrane Herausforderung. Für den Großteil der derzeit publizierten, mit einer primären Mikrozephalie assoziierten Gene wurden ursächliche Mutationen überwiegend in arabischen Populationen in häufig konsanguinen Familien beschrieben. Systematische, Genotyp-basierte Daten zur Mutationshäufigkeit, dem genspezifischen klinischen Spektrum und der Häufigkeit assoziierter extrazerebraler und Hirnfehlbildungen u.a. auch in mitteleuropäischen Populationen liegen bisher nicht vor. Jedoch erscheint auch für sporadische Patienten mit primärer Mikrozephalie und ansonsten altersgerechter Entwicklung eine molekulargenetische Untersuchung zumindest von ASPM und WDR62 gerechtfertigt. Umgekehrt finden sich Mikrozephalien nicht selten auch bei Patienten mit angeborenen Hirnfehlbildungen und erfordern erweiterte individuelle differentialdiagnostische Erwägungen. Als Faustregel kann dabei gelten, dass Patienten mit angeborenen strukturellen Hirnfehlbildungen mit wenigen Ausnahmen tendenziell eher erst postnatal eine Mikrozephalie entwickeln und eine altersgerechte psychomotorische Entwicklung eher die Ausnahme ist. Obwohl zukünftig die Anwendung von Hochdurchsatz-Sequenzierverfahren hilfreich für eine molekulare Diagnosestellung sein wird, kann sie auch in Zukunft den klinischen Sachverstand bei der differentialdiagnostischen Abklärung der Mikrozephalie nicht ersetzen. Unverändert ist die klinische Expertise zwingende Voraussetzung sowohl für die Erarbeitung zielführender diagnostischer Strategien unter Berücksichtigung der Familienanamnese und des individuellen klinischen Bildes incl. assoziierter (Hirn)fehlbildungen als insbesondere auch für die Interpretation genetischer Varianten.

EDU3

DNA-Methylierung: Vom Nachweis bis zur klinischen Relevanz

Moderation: Bernhard Horsthemke (Essen), Reiner Siebert (Kiel)

Die Identifizierung und der Nachweis epigenetischer Veränderungen gewinnen zunehmend an Bedeutung nicht nur in der humangenetischen Forschung sondern auch in der klinischen Diagnostik. Unter den epigenetischen Modifikationen ist die DNA-Methylierung die bislang am besten untersuchte. Da sie zudem vergleichsweise stabil ist, eignet sie sich auch gut für den diagnostischen Einsatz. Dieser Workshop hat deshalb zum Ziel, über den aktuellen Stand der Bedeutung von Veränderungen der DNA-Methylierung im klinischen Kontext zu informieren und die verschiedenen Methoden für deren Nachweis vorzustellen.

Im ersten Teil wird T. Haaf (Würzburg) die Grundlagen der DNA-Methylierung vorstellen. Neben den verschiedenen Formen der DNA-Methylierung (Methyl-Cytosin und Hydroxymethyl-Cytosin) wird auf die Mechanismen der Methylierung und Demethylierung eingegangen. In die Relevanz der DNA-Methylierung für Entwicklung, Zelldifferenzierung und Krankheitsentstehung wird eingeführt.

K. Buiting und J. Beygo (Essen) werden über konstitutionelle Veränderungen der DNA-Methylierung berichten. Insbesondere wird dabei auf die Diagnostik von Imprintingerkrankungen und auf die Untersuchung der X-Inaktivierung bei X-chromosomal-rezessiven Erkrankungen eingegangen. In die Methoden zum Nachweis Locus-spezifischer DNA-Methylierung wird eingeführt. Die Prinzipien sowie die Vor- und Nachteile von z.B. Methylierungsspezifischer MLPA und „Targeted Next-Generation Bisulfite-Resequencing“ werden vorgestellt. Diagnostische Algorithmen, wie sie u.a. im Rahmen des BMBF-geförderten Netzwerkes „Imprinting-Erkrankungen“ erarbeitet werden, sollen erörtert werden.

Im dritten Teil wird die Bedeutung somatischer Veränderungen der DNA-Methylierung diskutiert (R. Siebert, Kiel). Dabei wird auf die Bedeutung der DNA-Methylierung als Biomarker z.B. bei der chronisch lymphatischen Leukämie (CLL) und bei Hirntumoren eingegangen. Array-basierte Methoden zum Nachweis von Veränderungen der DNA-Methylierung und ihr Einsatz zur Identifizierung von Biomarkern für häufige Erkrankungen z.B. im Rahmen des Internationalen Humanen Epigenom-Consortiums (IHEC) werden vorgestellt.

C. Bock (Wien) gibt abschließend eine Übersicht über zukünftige Entwicklungen in der Analyse der DNA-Methylierung einschließlich des „Whole Genome Bisulfite Sequencing", wobei insbesondere die bioinformatischen Herausforderungen beleuchtet werden. Darüber hinaus werden internationale Vergleichsstudien zum Benchmarking der verschiedenen Technologien zum Nachweis von DNA-Methylierung vorgestellt.

EDU 4

Der ungelöste Fall

Moderation: Dagmar Wieczorek (Essen), Anita Rauch (Zürich)

Diese EDU-Session soll dazu dienen, ungeklärte Fälle aus dem Auditorium zu besprechen, ein diagnostisches Procedere zu entwickeln und optimalerweise auch Diagnosen zu finden. Schön wäre es aber auch, wenn neben ungelösten Fällen auch ungewöhnliche und/oder seltene gelöste Fälle aus dem Auditorium vorgestellt würden. Es können auch Fälle vorgestellt werden, die durch „Next Generation Sequencing" gelöst wurden entweder als ‚call for patients' oder zur Diskussion fraglich kausaler Befunde.

Optimalerweise sollten die (un)gelösten Fälle (max. 6 Folien) bis zum 15.03.2013 per e-mail an anita.rauch@medgen.uzh.ch und dagmar.wieczorek@uni-due.de geschickt werden. Zumindest aber sollte ein Beitrag mit Angabe des Titels angemeldet werden, damit wir einen Überblick über die Anzahl der Präsentationen haben. Ein Vorschlag für eine Powerpoint-Präsentation für die gelösten und ungelösten Fälle ist unter http://www.gfhev.de/de/kongress/wissenschaftl_programm.htm abrufbar.

POSTER

P-Basic Molecular Mechanisms

P-Basic-001

Characterization of a neuronal phenotype in a mouse model for Noonan Syndrome

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Constitutional dysregulation of the Ras-mitogen activated protein kinase (MAPK) signaling pathway can lead to Noonan Syndrome (NS), Neurofibromatosis type 1 (NF1), Costello Syndrome or similar disorders, collectively called "RASopathies". These disorders are characterized by an overlapping pattern of physical abnormalities and mild to severe cognitive impairment. Their common molecular basis is an overactive Ras-MAPK signaling pathway due to mutations in the genes of components or modulators of this signaling cascade. In animal models for NF1 and NF1-like Syndrome, it has been shown that mutations in the homologous genes can lead to impaired cognitive function and reduced synaptic plasticity (Costa et al., 2002, Denayer et al., 2008). However, the molecular pathogenesis for the cognitive impairment still remains to be elucidated. This study was aimed at further investigating the consequences of dysregulated Ras-MAPK signaling in neuronal cells of a mouse model for NS.

We used a conditional knock-in mouse model expressing the oncogenic allele Ptpn11D61Y (Chan et al., 2009) bred with an EMX1IREScre strain that starts cre expression from embryonic day 10.5 (Gorski et al., 2002). In this way we circumvent embryonic lethality and receive heterozygous offspring with an activated mutation restricted to the excitatory cells of forebrain. Primary cultures of dissociated hippocampal neurons from newborn Ptpn11D61Y animals and control littermates were prepared to characterize the neuronal phenotype.

ERK is downstream kinase of the Ras-MAPK signaling pathway, which translocates to the nucleus upon phosphorylation and is involved in the regulation of cellular gene expression. In the brain, reconfiguration of expression of neuronal genes represents an important mechanism that underlies functional adaptations of neuronal function during experience-induced and homeostatic plasticity. While the Ptpn11D61Y mutation is usually found to lead to a higher level of phosphorylated ERK (pERK) in overexpression studies, we found no differences in the nuclear level of pERK comparing wild type and heterozygous cells under basal conditions. However, a neuronal activity-driven induction of nuclear translocation of pERK was severely affected in the heterozygous Ptpn11D61Y neurons, suggesting dysregulation of the activity-induced neuronal signaling. In line with this finding, we found differences in the size of total recycling synaptic vesicle pools, which is subject of regulation during homeostatic adaptation in neurons.

In summary the results show no changes under basal conditions, but upon stimulation we found a lack of response in the heterozygous mutant cells, which has not been described so far. This suggests aberrant activity-induced nuclear signaling and deficits in homeostatic plasticity in Ptpn11D61Y neurons, which might underlie the intellectual disability found in patients with NS.

P-Basic-002

Autistic-like behaviour and neurodevelopmental disruption in mice lacking Foxp1

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Neurodevelopmental disorders are multi-faceted diseases that can lead to intellectual disability, autism spectrum disorder and language impairment. Mutations in the Forkhead box FOXP1 gene in humans have been linked to all these disorders, suggesting that it may play a central role in various cognitive and social processes. To address these questions, we have generated and characterised a mouse where Foxp1 is deleted specifically in the brain. Foxp1 KO mice exhibit a distinct neurodevelopmental phenotype, including disruption of the developing striatum. Foxp1 mutant mice also show an affected neuronal morphogenesis, a reduced excitability and an imbalance of excitatory to inhibitory input in CA1 hippocampal neurons. Loss of Foxp1 also results in hyperactivity and cognitive and social deficits. Together, these findings provide first insights into how defects in the FOXP1 gene in the brain may lead to cognitive and social disorders in affected patients.

P-Basic-003

Deep sequencing unravels the molecular signatures in two different mouse models for Alzheimer disease

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Alzheimer disease (AD) is a progressive neurodegenerative disorder characterized by the development of extracellular amyloid plaques, which are composed of amyloid-β (Ab). It has been demonstrated that soluble oligomeric Ab, but not plaque-associated Ab, correlates best with cognitive dysfunction in AD patients. In the present work we used deep sequencing to identify genes that are differentially expressed two different AD mouse models. We intended to compare the molecular profile comparing the 5XFAD model having a robust plaque pathology and at 12 months of age deficits in the spatial reference memory, with the novel Tg4-42 model expressing only Abeta4-42. The Tg4-42 model also develops spatial reference memory at the same age without any evidence of plaque formation. We identified ~100 genes differentially expressed in the 5XFAD compared to wildtype mouse brain. In the Tg4-42 we were able to detect 40% genes that were also found in the 5XFAD mouse. 60% of the genes in the Tg4-42 mouse are novel and not found in the 5XFAD mouse. Taking into account that the 5XFAD mouse is a model for the rare familial variant of AD and that the Tg4-42 model mimics the sporadic AD variant, the novel genes might be of special interest to better understand the molecular cascade of pathological events leading to neuronal death and eventually memory loss.

P-Basic-004

The role of MEST in the etiology of Silver-Russell syndrome: Expression analysis in upd(7) fibroblasts

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Silver-Russell syndrome (SRS) is a congenital imprinting disorder characterized by pre- and postnatal growth restriction. SRS patients show a typical triangular face with a prominent forehead; body asymmetry, a clinodactyly V and feeding difficulties are also reported. Molecular defects of two chromosomes are known to be associated with SRS: Up to 50% of patients show an epigenetic alteration in the imprinting control region 1 (ICR1) on chromosome 11p15, and 7-10% are carriers of a maternal uniparental disomy of chromosome 7 (upd(7)mat). On chromosome 7, several genes are consistently discussed to contribute to the pathophysiology of SRS: GRB10 in 7p13 as well as COPG2 and MEST in 7q32 have been suggested as candidates because of their imprinting status, their expression patterns and their function in embryonic development delineated from mouse models. In 11p15, in particular the genes H19, IGF2, KCNQ1OT1 and CDKN1C are promising candidates as they exhibit similar properties like the chromosome 7 factors. To elucidate the pathophysiological influence of the upd(7)mat we analyzed the expression patterns of the chromosome 7 encoded imprinted genes as well as of other genes that are associated with imprinting disorders. Expression analysis was performed in fibroblasts from patients with an upd(7)mat and a carrier of a segmental upd(7q)mat with an additional MEG3-DMR hypomethylation. As expected, we could confirm the down regulation of MEST by qPCR. Furthermore, we observed a reduced expression for CDKN1C, KCNQ1OT1 as well as for IGF2. These patterns were compatible with those from studies in upd(11p)mat fibroblasts reported recently and therefore indicate that the pathoetiology is similar for chromosome 7 and 11 associated SRS. The patient with the additional MEG3-DMR epimutation also presented an increased expression of MEG3 and DLK1. To further characterize the molecular basis of SRS in upd(7)mat patients and to elucidate the role of MEST in the so called imprinted gene network (IGN) we performed a siRNA knockdown of MEST in a placenta derived trophoblastic cell line: first findings correspond to that obtained from upd(7)mat fibroblasts.

P-Basic-005

FTO levels affect the miRNAome

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A block of single nucleotide polymorphisms (SNPs) within intron 1 of the FTO (fat mass and obesity associated) gene is associated with variation in body weight. The gene encodes a 2-oxoglutarate-dependent RNA demethylase, which has been shown to demethylate 3meU, 3meC, 1meA in vitro and 6meA in vitro and in vivo. Our previous work suggests that increased expression of FTO leads to increased body weight. Overexpression of FTO in HEK293 cells affected the transcript levels of genes related to RNA splicing and metabolism, whereas knockdown of FTO was followed by changes in the transcripts levels of genes involved in the response to starvation. Interestingly, in FTO deficient cells the mRNA level of LIN28B - a master regulator of the biogenesis of let-7 miRNAs - was reduced by 50%.

To investigate whether FTO levels affect the levels of let-7 and other miRNAs, we performed high-throughput sequencing of small RNA preparations from HEK293 cells overexpressing and lacking FTO. In cells with increased expression of FTO, five miRNAs – let-7e, miR-

3945, miR-3622b, miR-372 and miR-566 showed significantly increased steady-state levels. Decreased levels (>20%) were revealed for 12 miRNAs. In case of FTO deficiency, 40 miRNAs showed increased levels >20%, with the following top ten scorers (from ≈50 to 1330 fold changes): miR-651, miR-152, miR-5704, miR-210, miR-6739, miR-6514, miR-6506, miR-6787, miR-5583, miR-7158. Decreased levels (>20%) were observed for 217 miRNAs. The following miRNAs – miR-137, miR-548ay, miR-656, miR-1908, miR-548h, miR-22, miR-196a, miR-769, miR-4755 and miR-335 were those with most decreased levels. Five members of let-7 family –let-7a, let-7e, let-7f, let-7g and let-7c showed a moderate reduction (20-50%), however, these differences could not be detected by qPCR. Interestingly, qPCR revealed a reduction of the mature miR-7 miRNA in FTO deficient cells (confirming the RNA-seq data), but not of the primary transcripts of miR-7 (pri-miRNA), indicating that FTO affects the processing of pri-miRNAs to pre- and mature miRNAs, possibly by modulating the degree and/or pattern of RNA methylation.
We suggest that altered levels of FTO do not only affect mRNA levels, but also miRNA levels. Currently we investigate whether miRNAs (or pri- and pre-miRNAs) are methylated at adenosine residues. Further investigations will help to find a possible link between FTO function, miRNA biogenesis and obesit

P-Basic-006

Functional analysis of CBL germline mutations: aberrant EGFR trafficking underlies a Noonan syndrome-like phenotype

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Noonan syndrome is a congenital disorder comprising a characteristic face, short stature, various heart defects, learning difficulties and a predisposition to malignancies. Heterozygous germline mutations in genes encoding components of RAS-MAPK signaling pathways cause Noonan syndrome. In addition, mutations in the CBL tumor suppressor gene have been reported in patients with a Noonan syndrome-like phenotype. CBL encodes a multivalent adaptor protein with ubiquitin ligase activity, which promotes vesicle-mediated internalization and degradation of the epidermal growth factor (EGF) receptor (EGFR). Here, we investigated the functional consequences of disease-associated CBL amino acid changes p.K382E, p.D390Y and p.R420Q on ligand-induced EGFR trafficking. Expression of CBL^{K382E}, CBL^{D390Y} or CBL^{R420Q} in EGF-stimulated COS-7 cells resulted in increased levels of surface EGFR and significantly reduced amounts of intracellular EGFR; both consequences indicate ineffective EGFR internalization. Accordingly, receptor-mediated uptake of EGF was decreased. Moreover, p.K382E, p.D390Y and p.R420Q lesions abrogated the promoting effect of CBL on EGFR degradation. Together these data suggest that pathogenic CBL mutations severely affect vesicle-based EGFR trafficking. Since we detected stronger ERK phosphorylation in cells expressing mutant CBL than in those expressing wild-type CBL, we concluded that aberrant EGFR trafficking results in augmented RAS-MAPK signaling, the common trait of Noonan syndrome and related RASopathies. Thus, our data establish EGFR trafficking as a novel disease-relevant regulatory level in the RASopathy-network.

P-Basic-007

In-depth characterisation of retinal pigment epithelium (RPE) cells derived from human induced pluripotent stem cells (iPSC)

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Purpose: To establish and comprehensively characterise retinal pigment epithelium (RPE) cells derived from adult human dermal fibroblasts via induced pluripotent stem cell (iPSC) technology.
Materials and Methods: Adult human dermal fibroblast cultures were established from skin biopsy material and subsequently reprogrammed following polycistronic lentiviral transduction with OCT3/4, Sox2, Klf4 and l-Myc. Chromosomal integrity was assessed by karyotyping. RPE cell differentiation was achieved by induction with RPE medium enriched for nicotinamide and Activin A. After 8 weeks, pigmented clusters of RPE cells were manually excised and subcultured. Human iPSCs were characterised by RT-PCR expression of specific stem cell markers and immunofluorescence. Induced RPE cells were characterised by confocal microscopy, scanning electron microscopy (SEM) and functional analysis, the latter including feeding experiments with porcine photoreceptor outer segments (POS) and measurements of transepithelial resistance (TER).
Results: Fibroblast-derived human iPSCs showed typical morphology and regular karyograms. Furthermore, they revealed distinctive stem cell marker properties based on RNA- and protein-expression profiling. Subsequently, human iPSCs were differentiated into pigmented clusters reminiscent of RPE cells. These cells maintained typical hexagonal RPE-morphology during subcultivation. Starting at passage 6 replicative senescence increased. RNA expression of mature PRE markers RPE65, RLBP and BEST1 were found in comparison to human iPSCs. Confocal microscopy demonstrated localisation of BEST1 at the basolateral plasma membrane while SEM demonstrated typical microvilli at the apical side of RPE cell. With regard to functional aspects, iPSC-derived RPE cells phagocytosed and shredded POS. Finally, TER measurements showed a significant increase and maintained high levels of TER indicating functional formation of tight junctions.
Conclusion: Our data demonstrate the successful reprogramming of human adult skin biopsy-derived fibroblasts to iPSCs and their differentiation to RPE-cells histologically and functionally indistinct from native RPE cells.

P-Basic-008

Overexpression of synphilin-1 promotes clearance of soluble and misfolded alpha-synuclein without restoring the motor phenotype in aged A30P transgenic mice.

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Lewy bodies and neurites are the pathological hallmark of Parkinson's disease. These structures are composed of fibrillized and ubiquitinated alpha-synuclein suggesting that impaired protein clearance is an important event in aggregate formation. The A30P mutation is known for its fast oligomerization, but slow fibrillization rate. Despite its toxicity to neurons, mechanisms involved in either clearance or conversion of A30P alpha-synuclein from its soluble state into insoluble fibrils and their effects in vivo are poorly understood. Synphilin-1 is present in Lewy bodies, interacting with alpha-synuclein in vivo and in vitro and promotes its sequestration into aggresomes, which are thought to act as cytoprotective agents facilitating protein degradation. We therefore crossed animals overexpressing A30P alpha-synuclein with synphilin-1 transgenic mice to analyze its impact on aggregation, protein clear-

ance and phenotype progression. We observed that co-expression of synphilin-1 mildly delayed the motor phenotype caused by A30P alpha-synuclein. Additionally, the presence of N- and C-terminal truncated alpha-synuclein species and fibrils were strongly reduced in double-transgenic mice when compared with single-transgenic A30P mice. Insolubility of mutant A30P and formation of aggresomes was still detectable in aged double-transgenic mice, paralleled by an increase of ubiquitinated proteins and high autophagic activity. Hence, this study supports the notion that co-expression of synphilin-1 promotes formation of autophagic-susceptible aggresomes and consecutively the degradation of human A30P alpha-synuclein. Notably, although synphilin-1 overexpression significantly reduced formation of fibrils and astrogliosis in aged animals, a similar phenotype is present in single- and double-transgenic mice suggesting additional neurotoxic processes in disease progression.

P-Basic-009

A comparative transcriptome analysis identifies FGF23-regulated genes in HEK293 cells stably expressing KLOTHO

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Phosphate is the most abundant anion in the human body and is crucial for various biological functions like cellular activity and bone mineralization. Phosphate homeostasis is regulated in a complex process that involves the interplay of different organs, tissues and systems. A key regulator of phosphate homeostasis is fibroblast growth factor 23 (FGF23). It is mainly secreted from osteocytes and osteoblasts, circulates in the blood and binds to a specific receptor complex composed of FGF receptor 1 (FGFR1) and KLOTHO in the kidney. As elevated FGF23 levels are the main cause of hypophosphatemia in monogenic disorders of phosphate homeostasis (XLH (MIM 307800), ADHR (MIM 193100), ARHR1 (MIM 241520) and ARHR2 (MIM 613312)), further studies on the regulation of phosphate metabolism are necessary to identify possible therapeutic targets. FGF23 activates FGFR1/KLOTHO to inhibit renal phosphate reabsorption and to suppress 1,25-dihydroxyvitamin D3 synthesis. However, little is known about FGF23/FGFR1/KLOTHO signaling and downstream targets of FGF23 contributing to its phosphaturic action. For this purpose, we established an in vitro cell system of FGF23-inducible HEK293 cells that stably express KLOTHO (HEK293-KL). To find differentially expressed FGF23-induced transcripts, we performed whole transcriptome analysis. We used the technology of RNA-Seq, which is a massively parallel sequencing approach to allow genome-wide analysis of gene expression profiles at a far higher resolution than is available with microarray-based methods. Genome-wide transcriptional changes in HEK293-KL cells specifically caused by FGF23 were defined by comparing the transcriptome of FGF23-induced HEK293-KL cells with the transcriptome of not induced HEK293-KL cells. We tried to identify new FGF23-responsive genes that might belong to a network of factors involved in the regulation of phosphate homeostasis.

P-Basic-010

Upstream open reading frames regulate cannabinoid receptor 1 expression under baseline conditions and during stress

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The endocannabinoid system (ECS) plays a crucial role in the regulation of a variety of physiological functions, such as learning and memory processing, vegetative control, energy homeostasis, immunity and stress response. It acts through different endogenous endocannabinoids which are able to bind to the cannabinoid receptor subtypes 1 and 2 (CNR1 and CNR2). The CNR1 is not only associated with phenotypes such as cognitive performance, addiction and anxiety, but is also known to be crucially involved in cellular responses to acute and chronic stress conditions. The molecular mechanisms leading to altered CNR1 expression under acute or chronic stress exposure are not completely understood so far. It is known that the 5'- and 3'untranslated regions (UTRs) of genes can harbor regulatory elements, such as upstream open reading frames (uORFs) that are capable of influencing the expression pattern of the main protein coding region.
In our study, we investigated the influence of putatively functional uORFs present in the five known mRNA variants of the human CNR1 gene on transcription and translation under baseline conditions and various stress conditions in vitro. The functional analysis performed with reporter gene assay and quantitative realtime PCR revealed that two of these variants contain upstream open reading frames that modulate gene expression both under baseline condition and conditions of cellular stress. Thus our findings suggest that the functionally relevant uORFs found in the 5'UTR variants of CNR1 are part of the stress response mechanisms that protect cells from unfortunate conditions.

P-Basic-011

Respiratory Distress and Early Neonatal Lethality in Hspa4l/Hspa4 Double Mutant Mice.

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Heat shock protein HSPA4L and HSPA4 are closely related members of the HSP110 family that acts as co-chaperone. We generated Hspa4l-/-Hspa4-/- mice to investigate a functional complementarity between HSPA4L and HSPA4 during embryonic development. Hspa4l-/-Hspa4-/- embryos exhibited marked pulmonary hypoplasia, and neonatal death. Compared to lungs of wild-type, Hspa4l-/- and Hspa4-/- embryos, Hspa4l-/-Hspa4-/- lungs were characterized by diminished saccular spaces and increased mesenchymal septa. Mesenchymal hypercellularity was determined to be due to an increased cell proliferation index and decreased cell death. A significant increase in expression levels of pro-survival protein BCL-2 may be the cause for inhibition of apoptotic process in lungs of Hspa4-/-Hspa4l-/- embryos. Accumulation of glycogen and diminished expression of surfactant protein B, prosurfactant protein C and aquaporin 5 in saccular epithelium suggested impaired maturation of type II and type I pneumocytes in the Hspa4l-/-Hspa4-/- lungs. Further experiments showed a significant accumulation of ubiquitinated proteins in the lungs of Hspa4l-/-Hspa4-/- embryos, indicating an impaired chaperone activity. Our study demonstrates that HSPA4L and HSPA4 collaborate in embryonic lung maturation, which is necessary for adaptation to air breathing at birth.

P-Basic-012

RAD51B: A crucial player in Homologous Recombination

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The five RAD51 paralogs RAD51B (B), RAD51C (C), RAD51D (D), XRCC2 (X2) and XRCC3 (X3) are known to play a key role in DNA maintenance via Homologous Recombination Repair (HRR). Two different structures are formed by the paralogs: the RAD51C-XRCC3

(CX3) complex and the RAD51B-RAD51C-RAD51D-XRCC2 (BCDX2) complex. The latter was found to consist of two subcomplexes, RAD51B-RAD51C (BC) and RAD51D-XRCC2 (DX2). The interplay between these proteins was shown by Yeast Two/Three-Hybrid (2/3H) studies in vivo and purified proteins derived from baculovirus or human cell systems in vitro. In our study we investigated the interactions between the paralogs and RAD51 in the Mammalian 2/3H system. Our experiments confirm the disposition of RAD51B to engage in a dimeric subcomplex with RAD51C. In addition, they show a crucial role of RAD51B for the formation of the BCDX2 complex by activating RAD51C for interaction with RAD51D or by stabilizing this interaction. Moreover, we observed an interaction between RAD51B and XRCC2 which was assisted by RAD51D. This finding lends support to a likely ring structure of the BCDX2 complex, previously only observed by electron microscopy. Such ring formation may require the presence of certain DNA configurations as we could not demonstrate an interaction between RAD51B and XRCC2 by co-immunoprecipitation using DNA-free HEK 293 protein extracts. XRCC3 inhibited the interaction within the BC and DX2 complexes, in varying degrees. On the other hand, CX3 complex formation was influenced by other paralogs and RAD51. These findings suggest regulatory effects of different complex formation among one another which function up- (BCDX2) or downstream (CX3) of RAD51 recruitment to DNA damage sites. Damage induction by the DNA interstrand crosslinking agent mitomycin C increased the interaction strength between C and B, that of C and D with additional B and that of C and X3 to form the CX3 complex in M2H and in M3H analyses using B and D as additional proteins. These findings lend further support to regulatory influences between BCDX2 and the CX3 complex formation. The Fanconi anemia mutation R258H of RAD51C did not to diminish or even abolish the interactions of the protein with B, D or XRCC3 suggesting other pathogenic effects.

P-Basic-013

Single-nucleotide profiling of 5-hydroxymethylcytosine on a genome-wide scale in TET1-overexpressing HEK293 cells

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In mammals, 5-methylcytosine (5mC) predominantly exists in the context of CpG dinucleotides. This DNA modification is often associated with gene repression and essential for normal development. To date, little is known about the mechanisms regulating DNA methylation dynamics. Recently, it has been shown that enzymes of the TET family - consisting of TET1, 2 and 3 - can convert 5mC into 5-hydroxymethylcytosine (5hmC). 5hmC is found at low levels in the genome and several biological functions of 5hmC have been proposed. On the one hand, it could represent an intermediate product in active or passive DNA demethylation, on the other hand, it might also have direct effects by displacing 5mC-binding proteins or recruiting 5hmC-specific effectors. Apart from the conversion of 5mC into 5hmC it has been shown that the TET enzymes can also modulate transcription by recruiting other protein complexes to their target sites.

To further elucidate the function of TET1, we generated a stable HEK293 cell line that inducibly overexpressed TET1. Overexpression was induced in three biological replicates and verified by qRT-PCR and western blotting. DNA dot blot analysis revealed a global increase of 5hmC in TET1-overexpressing cells. Reduced Representation Hydroxymethylation Profiling (RRHP, Zymo Research) was performed to map 5hmC modifications at single-nucleotide resolution on a genome-wide scale. We observed a 3.7-fold increase in total 5hmC levels. More detailed analyses showed a moderate increase of 5hmC at most sites, whereas a small fraction of sites showed a very strong increase of 5hmC. Interestingly, this fraction consisted mostly of sites that had a basal level of 5hmC before TET1 overexpression. The sites showing a strong increase of 5hmC were present in all genomic regions analyzed, including promoters, exons, introns, CpG islands and intergenic regions.

Our data suggests that many 5mC sites in the genome can be oxidized by increased levels of TET1, that they differ in their susceptibility to oxidation by TET1, and that this differential susceptibility is not related to structural elements of a gene but probably to the DNA sequence or chromatin context.

P-Basic-014

MicroRNA 497-195 cluster miRNAs antagonize Bmp-signaling in bone

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Background: Treatment with recombinant BMP2 has become a therapeutic option for poor fracture healing in conditions such as large size defects, spinal fusion, and oral surgery. However, in these procedures supraphysiological doses of BMP2 are necessary. Extracellular factors such as follistatin, gremlin, noggin, and sclerostin play a major role in the antagonism of Bmp-signaling as they sequester BMPs to an inactive complex and prevent their interaction with BMP receptors.

Methods/Results: In this study we identified the miR-497-195 cluster as the most prominent member of the miR-15 family in bone formation in vivo and during osteoblast differentiation in vitro. Using alkaline phosphatase assays and alizarin red staining we showed an inhibitory effect of early miR-195-5p overexpression on differentiation and mineralization of primary calvaria osteoblasts. Microarray experiments and quantitative PCR were used to identify BMP2 responsive genes and to confirm differential expression of known BMP2 target genes upon miR-195-5p mimic treatment. Western blots were used to characterize the impact of miR-195-5p on proteins of the BMP signaling cascade in the context of BMP2 treatment. Using human mesenchymal stem cells we analyzed the impact of BMP2 treatment on miR-497-195 cluster microRNA expression in osteoblast precursor cells. Finally, in a rat large-size defect model we were able to show that high dose BMP2 treatment is associated with increased miR-195-5p and miR-497-5p expression levels.

Conclusion: Here, we present evidence that the miR-497-195 cluster miRNAs act as an intracellular antagonist of Bmp-signaling in bone cells. Interestingly, these miRNAs were upregulated in response to treatment of bone defects with recombinant BMP2. We suggest that the inhibition of Bmp-signaling by miR-497-195 cluster miRNAs might be a possible explanation for the need of supraphysiological concentrations to promote healing of non-unions and large-size defects.

P-Basic-015

Spectrum of NEK1, DYNC2H1, and IFT80 mutations in short rib-polydactyly syndrome / asphyxiating thoracic dystrophy

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Several disorders have been associated with defects of a variety of proteins involved in cilia formation, maintenance and function. These ciliopathies affect the intraflagellar transport, components of the cilia, the basal body, or the centrosome. Many of the associated phenotypes include brain malformations, polydactyly, kidney cysts, and skeletal abnormalities. In particular, this phenotypic spectrum is present among patients with short rib-polydactyly syndromes (SRPS). This group constitutes the most frequent lethal autosomal recessive osteochondrodysplasias. According to their phenotype the SRPS are classified into four distinct types: Saldino-Noonan (I), Majewski (II), Verma-Naumoff (III) and Beemer (IV) and include the phenotypically related asphyxiating thoracic dystrophy (ATD) and Ellis-van Crefeld syndromes (EVC). From this group of disorders, the underlying cause has been identified in NEK1, DYNC2H1, IFT80, TTC21B, WDR19/34/35/60 and EVC1/EVC2 for patients with SRPS or overlapping phenotypes.
Characterizing the underlying cause of SRPS II, we identified NEK1 mutations and postulated a digenic diallelic inheritance in the NEK1 and DYNC2H1 genes. Screening of additional 16 patients referred with the diagnosis of SRPS/ATD we identified further NEK1 and DYNC2H1 mutations in 8 patients. No mutations in NEK1, DYNC2H1 or IFT80 were present in 8 of 16 patients. All of the identified DYNC2H1 mutations were missense or nonsense, whereas the NEK1 mutations include splice-site, nonsense, missense mutations or complex rearrangements. Overall we identified 6 different NEK1 mutations in 4 patients. Only one patient was homozygous for a missense mutation. Surprisingly, two non-related patients showed the same splice-site mutation in the NEK1 gene on one allele indicating a possible founder mutation. Until now, all known NEK1 mutations were localized in the kinase, basic, coiled-coil domain or in the c-terminal region of the protein. 4 different DYNC2H1 mutations were present compound heterozygous in 2 patients according to an autosomal recessive inheritance. No further patients with mutations in both genes, NEK1 and DYNC2H1, were identified, but in 2 additional patients we only found one heterozygous DYNC2H1 mutation, each. Here, we neither found a mutation in the second allele of DYNC2H1 nor an additional mutation in NEK1 or IFT80. Considering a possible digenic diallelic inheritance, this might indicate a mutation in another, yet to be identified, gene.
In summary, even though we were able to identify the underlying cause in 50% of the patients, our results confirm the genetic heterogeneity and the broad clinical spectrum of ciliary disorders. Based on our estimation of more than 400 cilia-associated genes we expect to identify mutations in other yet unknown genes in the remaining patients using further exome sequencing.

P-Basic-016

Unraveling The Genetic Basis Of Innate Immune Response In TLR4-Activated Human Monocytes

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Toll-like receptors (TLRs) play a key role in innate immunity. Apart from their function in host defense, dysregulation in TLR-signaling can confer risk to autoimmune diseases, septic shock or cancer. Despite major advancements in our understanding of how the innate immune system recognizes pathogens, the genetic basis for differences in innate immune responses is only poorly defined. This study was aimed to characterize the genetic basis of variation in gene expression in TLR4-stimulated human monocytes. For this purpose we isolated monocytes of 136 individuals and stimulated them with lipopolysaccharide (LPS) to activate Toll-like receptor 4 (TLR4). From these donors, we performed transcriptome profiling at three time points (0 min/90 min/6 h) and genome-wide SNP-genotyping. Using the differential expression upon LPS treatment revealed 222 genes that are regulated by expression quantitative trait loci (eQTLs) at 90 min. and 213 genes at 6 hours. Among these, we show that SNPs conferring risk to primary biliary cirrhosis (PBC), inflammatory bowel disease (IBD) and celiac disease are immune response eQTLs for novel candidate genes, bringing new insights into the pathophysiology of these disorders in the context of TLR4-activation. Altogether this study significantly enhances our knowledge on the innate immune system and its genetic determinants.

P-Basic-017

Profiling the Methylomes of Neurons and Glia

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The epigenome exerts an essential influence on the regulation of cellular proliferation and differentiation. One of the most emerging fields in epigenome research is the analysis of post-DNA synthesis base modifications, e.g. the methylation of cytosine (5mC).
It is known that the DNA-methyltransferases (DNMTs) add a 5-methylation to cytosine residues preferentially within so called CpG-islands in the promoter region of genes. This modification can lead to a subsequent inactivation of transcription. This way the gene transcription can be regulated on DNA level. DNA-methylations then contribute to the cellular diversity but they also contribute to the development of diseases that are not based on mutations within the DNA-sequence. Important examples are neurodegenerative diseases like Alzheimer's disease or Parkinson's disease as most of these cases do not show mutations within the DNA-sequence.
The human brain consists of a mixture of different cell types. The most important ones are neurons and glia. The latter ones can be divided into astrocytes, oligodendrocytes and microglia. This enormous diversity of cells in the human brain leads to numerous problems for researchers that exert to investigate mechanisms that occur only in distinct cellular sub-populations of the human brain.
Till date, it is almost impossible to investigate distinct cell types of the brain. Even a precise isolation of cortical regions leads to a mixture of numerous different cell types as there are up to 50 % glial cells within the human cortex.
Here, we describe a novel, highly efficient method that allows us to isolate and investigate highly pure neurons and glia from human brain samples.
We evaluated the efficiency and purity of our technique and performed genome-wide methylation-analysis on human neurons and glia using highly pure isolated neurons and glia.
Analysis of genome-wide methylation data reveal, that human neurons and glia show distinct epigenetic methylation profiles that are very homogenious within one cell type but that show highly significant differences between different cell types.

In summary, we were able to use our newly developed highly efficient method of isolating neurons and glia in order to generate high-resolution methylation profiles in isolated human neurons and glia.

P-Basic-018

Deletion of the Epigenetic Factor SPOC1 Leads to Highly Variable Abnormalities during Murine Development

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SPOC1 (PHF13) was identified as a novel epigenetic factor whose expression is negatively correlated with the survival time in patients with ovarian cancer. The gene is located in chromosome region 1p36 and is deleted in a number of patients with 1p36 deletion syndrome (MIM #607872). Associating dynamically with chromatin in cell lines, the protein could be shown to play a role in chromosome condensation and cell division. SPOC1 binds to H3K4me3 and is indispensable for spermatogonial stem cell differentiation in the testis and sustained spermatogenesis in mice due to its role in epigenetic regulation of meiosis. Spoc1-/- mice show a wide range of defects. The homozygous mice surviving until weaning age are viable and adult animals do not show any obvious malformation. However, offspring genotype distribution is strongly shifted towards wildtype due to high perinatal lethality in the Spoc1-/- mice.

Here we report on an additional developmental phenotype in prenatal and newborn Spoc1 knockout mice in a congenic C57Bl/6 background. These mice show highly variable CNS defects ranging from apparently healthy animals to mice with severe embryonic defects in head development, marked by exencephaly, anencephaly, malformations of the facial skeleton, and anophthalmia. The severe abnormalities are perinatal lethal and may, at least in part, explain the reduced number of Spoc1-/- offspring. Spoc1 is ubiquitously expressed in all proliferating cells but shows exceptionally high expression in undifferentiated spermatogonia and stem cells of the hair follicle. Comparative genome wide expression analyses performed on E15.5 Spoc1-/- and wild type embryos revealed a number of developmental factors and pathways to be dysregulated. Among the differentially regulated genes, a significantly enriched portion shows trimethylation of both lysines 4 and 27 of histone H3 in the E14.5 mouse brain. This bivalent chromatin state, marked by both activating and repressive histone modifications, is a hallmark of a large number of developmental genes. This combination of histone modifications is known for its ability to exert temporal control of transcription, allowing for rapid activation and inactivation of genes. Bivalent chromatin is associated with the activity of trithorax and polycomb complexes. Components of the latter have been demonstrated to interact with SPOC1, suggesting a similar role in development.

With respect to its general role in maintenance and differentiation of stem cells the present data indicate a role in the epigenetic control of differentiation processes during development as well. The histone modification patterns of its target genes suggest that it may perform its function through an effect on bivalent chromatin marks and via an interaction with polycomb repressive complexes.

P-Basic-019

Population-specific differences in gene conversion patterns between human SUZ12 and SUZ12P indicate the dynamic nature of interparalog gene conversion

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Nonallelic homologous gene conversion (NAHGC) resulting from interparalog recombination without crossover represents an important influence on the evolution of duplicated sequences in the human genome. In 17q11.2, different paralogous sequences mediate large NF1 deletions by nonallelic homologous recombination with crossover (NAHR). Among these paralogs are SUZ12 and its pseudogene SUZ12P which harbour the breakpoints of type-2 (1.2-Mb) NF1 deletions. Such deletions are caused predominantly by mitotic NAHR since somatic mosaicism with normal cells is evident in most patients. Investigating whether SUZ12 and SUZ12P have also been involved in NAHGC, we observed gene conversion tracts between these paralogs in both Africans (AFR) and Europeans (EUR). Since germline type-2 NF1 deletions resulting from meiotic NAHR are very rare, the vast majority of the gene conversion tracts in SUZ12 and SUZ12P are likely to have resulted from mitotic recombination during premeiotic cell divisions of germ cells. A higher number of gene conversion tracts was noted within SUZ12 and SUZ12P in AFR as compared to EUR. Further, the distinctive signature of NAHGC (high number of SNPs per paralog, high number of shared SNPs between paralogs), a characteristic of many actively recombining paralogs, was observed in both SUZ12 and SUZ12P but only in AFR and not in EUR. A novel polymorphic 2.3-kb deletion in SUZ12P was identified which exhibited a high allele frequency in EUR. We postulate that this interparalog structural difference, together with low allelic recombination rates, caused a reduction in NAHGC between SUZ12 and SUZ12P during human evolution.

P-Basic-020

Pelota regulates the development of extraembryonic endoderm through activation of bone morphogenetic protein (BMP) Signaling

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Abstract

Pelota (Pelo) is ubiquitously expressed, and its genetic deletion in mice leads to embryonic lethality at an early post-implantation stage. In the present study, we conditionally deleted Pelo and showed that PELO deficiency did not markedly affect the self-renewal of embryonic stem cells (ESCs) or their capacity to differentiate in teratoma assays. However, their differentiation into extraembryonic endoderm (ExEn) in the embryoid bodies (EBs) was severely compromised. Conversely, forced expression of Pelo in ESCs resulted in

spontaneous differentiation towards the ExEn lineage. Failure of Pelo-deficient ESCs to differentiate into ExEn was accompanied by the retained expression of pluripotency-related genes and alterations in expression of components of the bone morphogenetic protein (BMP) signaling pathway. Further experiments have also revealed that attenuated activity of BMP signaling is responsible for the impaired development of ExEn. The recovery of ExEn and down-regulation of pluripotent genes in BMP4-treated Pelo-null EBs indicate that the failure of mutant cells to down-regulate pluripotency-related genes in EBs is not a result of autonomous effect, but rather to failed signals from surrounding ExEn that induce the differentiation program. Moreover, Pelo-null fibroblasts failed to reprogram towards induced

pluripotent stem cells (iPSCs) due to inactivation of BMP signaling and impaired mesenchymal-to-epithelial transition. Thus, our results indicate that PELO plays an important role in the differentiation of ESCs into ExEn through activation of BMP signaling.

P-Basic-021

Dppa3 binds to the IG-DMR of the Dlk1-Dio3 imprinting cluster and prevents its imprinting loss during iPS cell generation.

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Reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) often results in defects at the genetic and epigenetic level. Of particular interest is the loss of imprinting at the paternally imprinted Dlk1-Dio3 cluster in a majority of the iPSC clones, which ultimately fail to show full pluripotent cell characteristics. In the present study, we analyzed the expression of germ cell (GC) marker genes (Blimp1, Fragilis, and Dppa3), that have been described to become activated early during somatic cell reprogramming. We found that Dppa3, the guardian of the maternal genome and imprinted loci that protects them against DNA demethylation during early embryonic development, was present only in iPSC lines showing expression of Gtl2 (Gtl2on), an indicator of normal imprinting at Dlk1-Dio3 cluster, but not in iPSCs with loss of imprinting (Gtl2off). Subsequently, we found that exogenous Dppa3 together with classical reprogramming factors can efficiently reprogram somatic cells into iPSC clones that all display normal imprinting at Dlk1-Dio3 region. Next, we performed chromatin immunoprecipitation assays with a Dppa3 antibody on embryonic stem cell (ESC) chromatin and found Dppa3 to be associated with a specific region within the intergenic differentially methylated region (IG-DMR) of Dlk1-Dio3. Further studies indicated that the forced expression of Dppa3 during reprogramming leads to the maintenance of imprinting at this cluster by counteracting and reducing the binding of Dnmt3a, a de novo DNA methyltransferase that establishes DNA methylation. Collectively, our results show that Dppa3 is a genetic factor necessary for preventing abnormal imprinting at the Dlk1-Dio3 region during somatic cell reprogramming.

P-Basic-022

Molecular genetic analysis of Autosomal Recessive Retinitis Pigmentosa & Leber congenital amaurosis in Pakistani Population

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Retinitis pigmentosa (RP) and Leber congenital amaurosis (LCA) are devastating eye diseases that cause degeneration of Rod and Cone photoreceptors leading to severe vision impairment and blindness. LCA is known to be the most severe type of retinal dystrophy causing blindness or severe visual impairments in early infancy whereas in RP disease onset is later and progression can be rather variable. LCA is usually inherited as an autosomal recessive trait while RP has different patterns of inheritance such as autosomal dominant, autosomal recessive, or X-linked. LCA and RP have in common an immense genetic heterogeneity with 18 and 50 known genes causing LCA and RP, respectively. Little is known about the prevalence of these different disease genes in different populations.

In this study we aimed to identify the genetic defects associated with retinal dystrophies in the Pakistani population focusing on selection of consanguineous families with multiple affects. We applied homozygosity mapping based on genotyping data from genome-wide SNP arrays followed by candidate gene analysis (direct sequencing of coding exons as well as deletion mapping) in homozygous regions. Using this approach we were able to identify a novel homozygous 110 kb deletion encompassing exons 1-4 of the LCA5 gene and further upstream sequence in all three affected members of family MA23.

Direct sequencing of all exons and flanking introns of the NMNAT1 gene detected a missense mutation of c.25G>A, p.Val9Met in MA31 family. Segregation analysis further revealed the mutation in the remaining three affected members.

In family MA48 with three affected members suffering from autosomal recessive retinitis pigmentosa we observed larger homozygous regions on chromosomes 8 and 10 including several known RD genes. Sanger sequencing of all exons and flanking intron sequences revealed a novel putative splice site mutation at c.256-2 A>G in the C8ORF37 gene. As it was previously known that splice site mutations could result in exon skipping, activation of cryptic splice sites, creation of a pseudo-exon within an intron, or intron retention [Nakai and Sakamoto, 1994], we are currently performing a Minigene assay (which is currently ongoing) to clarify the functional consequence of this acceptor site mutation.

Our findings reveal a novel gene 'C8ORF37' among the Pakistani population to be the cause of autosomal recessive Retinitis Pigmentosa in our family and a large homozygous deletion in LCA5 to be the reason behind the recognizable phenotype cause of MA23, and a missense mutation to be responsible for Leber congenital amaurosis in MA31 family.

In conclusion our results suggest that Homozygosity mapping is an efficient strategy for mapping rare recessive disorders affecting members of inbred populations.

P-Basic-023

Interaction of the Cohen Syndrome-associated protein Coh1 with Rab6 and Ric1/Rgp1 emphasizes its role for Golgi function

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Postnatal microcephaly, mental retardation, and progressive retinal dystrophy are major features of the autosomal recessive Cohen syndrome, which is caused by mutations in COH1 (VPS13B). COH1 is a protein of 3997 amino acids, which harbors two short regions homologous to yeast vacuolar protein sorting-associated protein 13 (Vps13p), and was therefore classified as one of four mammalian VPS13 family members. Recently, our cell biological characterization identified COH1 as Golgi-enriched scaffold protein important for Golgi structure maintenance. Similarly to previous results for Vps13p in yeast, we show that the function of mammalian COH1 as a membrane-associated scaf-

fold at the Golgi is linked to its interaction with the Golgi-associated small GTPase RAB6 and its guanine nucleotide exchange factor (GEF) complex RIC1/RGP1. We observed strong co-immunoprecipitation of COH1 with the constitutive active mutant of RAB6. Further analysis using RNAi-mediated depletion of RAB6A/A′ results in dispersal of COH1 into the cytoplasm, suggesting that Golgi-association of COH1 is dependent on RAB6 activity. Confirming results were found by overexpression of wild-type and mutant RAB6B constructs showing decreased membrane association of COH1 upon constitutive inactive RAB6B co-expression. However, RAB6 localization is not affected by RNAi-mediated depletion of COH1. Recently, inactivity of RAB6 was found to interfere with neurite extension in primary hippocampal neurons. To analyze a potential impact of COH1 on neuron development, we depleted Coh1 in primary rat hippocampal neurons (E18) using RNAi. Down-regulation of the Coh1 mRNA level to ~50% significantly reduced the length of the longest forming neurite by ~40% compared to control-transfected neurons, establishing a critical role for RAB6-dependent COH1 function in neuritogenesis. Our collective data suggest that neurological manifestations in Cohen syndrome may be due to deficient Golgi-associated membrane traffic and signalling during neuronal differentiation.

P-Basic-024

One third of cerebral cavernous malformation probands are minors: predictive genetic testing becomes an issue

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Cerebral cavernous malformations (CCM) are prevalent vascular malformations occurring in familial autosomal dominantly inherited or isolated forms. Once CCM are diagnosed by magnetic resonance imaging, the indication for genetic testing requires either a positive family history of cavernous lesions or clinical symptoms such as chronic headaches, epilepsy, neurological deficits, and hemorrhagic stroke or the occurrence of multiple lesions in an isolated case. Following these stringent inclusion criteria, the mutation detection rates are extraordinarily high: in a consecutive series of 105 probands, mutations were found in 87% of familial and 57% of isolated cases. 31 novel mutations were identified with a slight shift towards proportionally more CCM3 mutations carriers than previously published (CCM1: 60%, CCM2: 18%, CCM3: 22%). In-frame deletions and exonic missense variants requiring functional analyses to establish their pathogenicity were rare: An in-frame deletion within the C-terminal FERM domain of CCM1 resulted in decreased protein expression and impaired binding to the transmembrane protein heart of glass (HEG1). Notably, 20% of index cases carrying a CCM mutation were below age 10 and 33% below age 18 when referred for genetic testing. Since fulminant disease courses during the first years of life were observed in CCM1 and CCM3 mutation carriers, predictive testing of minor siblings became an issue.

P-Basic-025

Developmental defects and premature ageing in lamin B receptor deficient mice

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The lamin B receptor (LBR) is a multifunctional inner nuclear membrane protein with structural impact on nuclear shape and chromatin organisation. Further, LBR belongs to the C14 sterol reductase family and has enzymatic activity in sterol metabolism. LBR mutations have been shown previously to cause dose-dependent hyposegmentation of granulocyte nuclei in heterozygous or homozygous Pelger-Huët-Anomaly. Heterozygous LBR mutations change blood morphology without causing any associated pathology. In contrast, homozygous mutations in LBR cause a spectrum of systemic malformations ranging from heart defects, brachydactyly and mental retardation, as occurs in Pelger-Anomaly, to severe cutaneous derangements, as seen in the recessive ichthyotic mice (ic), and finally to prenatal lethality, which is found in Greenberg dysplasia.

To elucidate the mechanisms responsible for the variety of disease manifestations in individuals with lamin B receptor deficiency, we studied prenatal and postnatal development in ic/ic mice (Spontaneous mutation Lbr icJ 1088insCC, which is a functional null mutation). We found first but modest differences in some of the homozygous embryos around mouse embryonic day E10. However, we noticed the highest mortality perinatally and around weaning. The survival varied between a few hours and several weeks or months. Generally, all homozygous mice display growth retardation and severe ic/ic skin defects. In addition to the previously described phenotypes ichthyosis, alopecia, nuclear hyposegmentation and occasional soft tissue syndactyly, we observed other manifestations as increased frequency of hydrocephalus, abnormal histology of heart and muscle cells and an abnormal fat distribution. The latter manifestations of Lbr deficiency overlap with those of Lamin A diseases, especially with Progeria, and with processes in physiological ageing. We therefore studied protein glycation as a biomarker of ageing. Compared to liver and heart of controls, ic/ic mice showed a significantly increased accumulation of intracellular glycated proteins Arg-pyrimidine, carboxyethyllysine and pentosidine. In contrast, we did not see changes in the accumulation of carboxymethyllysine as well as in advanced glycosylated end product (AGE) modification of the extracellular matrix.

Summarizing, manifestation in ic/ic mice start prenatally and are life threatening perinatally and around weaning.If homozygous mice survive these critical intervals, symptoms overlap with premature ageing. We conclude that the lamin B receptor is essential both for development and healthy ageing.

P-Basic-026

Deciphering the genetic basis of idiopathic short stature

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Human growth is a highly complex and multifactorial trait, with an estimated heritability of about 80 - 90 %. Since 3 % of the general population present with shortness of stature, growth retardation in an individual is one of the common medical concerns in childhood. After excluding common defects like Turner Syndrome, SHOX defects and known syndromic forms, the underlying cause remains unknown in approximately 80 % of patients (idiopathic short stature).

Uncovering the genetic basis of short stature is therefore not only important for clinical diagnosis, prognosis and genetic counseling of affected individuals and their families, but is also a prerequisite for future development of therapeutic approaches.

While Genome-wide association studies identified hundreds of common single nucleotide polymorphisms and copy number variants (CNVs) contributing to the height variation in the healthy population, we confirmed a frequent disease – rare variant hypothesis by the identification of pathogenic CNVs in patients with severe short stature.

Our results implied a heterogeneity with more than 200 genes involved in idiopathic short stature by power analysis. Furthermore, we found evidence for both autosomal recessive and autosomal dominant inheritance in several families. To attribute this problem in the identification of monogenic causes of short stature we established the SHORT-NET project. By identification of novel genes and functional characterization of their impact on a cellular level we aim to unravel the underlying mechanisms involved in idiopathic short stature.

The project now includes more than 400 clinically characterized families with idiopathic short stature. As part of this project we already identified and functionally characterized mutations in a histonacetyltransferase, KAT6B, and a novel microtubuli associated protein, MAP4, as novel rare causes of short stature based. In individuals where positional data is not available we expect to find disease causing mutations in 2 or more patients within the same gene by exome sequencing of 50 patients with a probability 0.84. The SHORT-NET project will address the problem of heterogeneity in short stature, and the results will help to demonstrate the complex genetic mechanisms involved in both cellular and individual growth.

P-Basic-027

Mechanisms underlying non-recurrent microdeletions causing neurofibromatosis type-1 (NF1)

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About 5% of patients with NF1 have large deletions within the NF1 gene region located on chromosome 17q11.2. These NF1 microdeletions do not only encompass the NF1 gene but also its flanking genes. The mechanisms underlying recurrent NF1 microdeletions have been investigated in great detail, whereas those underlying non-recurrent (atypical) NF1 microdeletions are not well characterized. NF1 microdeletions with non-recurrent breakpoints are highly variable in terms of their size, breakpoint position and number of deleted genes. In this study, we investigated 20 atypical NF1 deletions using high-resolution custom aCGH and performed breakpoint-spanning PCRs. Sequence analysis of breakpoint-spanning PCR products indicated that 15 deletions exhibit simple breakpoint junctions without further complexities and hence were probably mediated by NHEJ/MMEJ. These findings suggest that microhomology-dependent mechanisms are frequently underlying atypical NF1 deletions. However, two of the 20 deletions investigated exhibit insertions of truncated SVA elements at their breakpoints causing considerable breakpoint complexity, as determined by inverse PCR, semi-specific PCR and Genome Walker analysis. Large genomic deletions associated with SVA element insertions at the breakpoints have not been previously reported. We postulate that SVA insertions, occurring via retrotransposition, that trigger large genomic rearrangements may represent an as yet uncharacterized mechanism responsible for causing CNVs with non-recurrent breakpoints.

We further noted that 14 (70%) of the 20 atypical NF1 microdeletions had their proximal breakpoints located within a 38-kb region of SUZ12P. This enrichment of atypical NF1 deletion breakpoints in SUZ12P is remarkable since the breakpoints of recurrent type-2 NF1 deletions are also located within the pseudogene. The accumulation of breakpoints associated with recurrent and non-recurrent NF1 deletions within SUZ12P is indicative of its genomic instability. The analysis of FISH, microsatellite markers and insertion/deletion polymorphisms indicated somatic mosaicism in 12 of the 20 (60%) atypical NF1 deletions. Thus, not only recurrent type-2 NF1 deletions, but also a considerable proportion of atypical NF1 deletions, are of postzygotic origin.

P-Cancer Genetics

P-CancG-028

Genomic background of neuroblastomas with intra-tumor heterogeneity of MYCN amplification

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Background: MYCN amplification (MNA) is the most powerful therapy-stratifying marker in neuroblastoma (NB). With recent technological advances and the analysis of different pieces of individual tumors it became evident that approximately 20% of MYCN amplified NBs carry the amplification in only a fraction of tumor cells ranging from a few amplified cells to up to the majority (~70%) of all tumor cells. Although the existence of intratumor heterogeneity of MNA (hetMNA) is well known today, its clinical meaning is still unclear, compromising the patients' assignment to specific treatment strategies. To learn whether the genomic background of hetMNA tumors differs to the genomic background of homogenously MNA (homMNA) tumors and non-amplified NBs, we looked for common segmental and numerical chromosome aberrations, allelic imbalances and the expression of the favorable NB marker (CD44).

Material and methods: Ultra-high resolution SNParray analyses (2.6 million copy number markers) and interphase FISH on various tumor and bone marrow samples (BM) obtained from 20 hetMNA, 22 homMNA and 110 nonMNA NB patients were performed. Median patient age of the hetMNA patients group was 13.5 months (range 6-168), 13

patients were below and 7 above 18 months of age. CD44 staining was done by fluorescence labeled antibodies on cryo-sections.
Results: Besides hetMNA, seven tumors showed no segmental chromosomal aberrations (noSCA), another four were heterogeneous concerning both, MNA and SCA (hetSCA), one with two SCAs and eight exhibited a high number (>7) of SCAs (highSCA) from these eight one with chromothripsis and two with deletions within the ATRX1 gene. Acquired whole chromosome uniparental disomy (wcUPD) occurred in 15/20 (75%) of hetMNA tumors, in 4/22 (18.2%) homMNA tumors and in 38/110 (34.5%) of the nonMNA NBs. wcUPD of chromosome 11 was predominately found in the hetMNA group (10/15), three out of four in the homMNA group and 7/38 in the nonMNA group also showed wcUPD11. The increase of SCAs correlated with age (no/hetSCA: 11/13 infants and highSCA: 7/7 patients >18m). By contrast, UPD11 decreased with age (UPD11: 9/13 infants and 1/7 patients >18m). Furthermore, hetMNA tumors were frequently CD44+, which is not the case in homMNA tumors.
Conclusions: The high frequency of wcUPDs, especially UPD11, in hetMNA tumors has not been described so far and could represent a hallmark of hetMNA NBs. Moreover, it was unexpected that hetMNA tumors in contrast to homMNA tumors can either totally lack SCAs or also bear a multitude of them. Taken together, hetMNA tumors differ in their genomic make up from fully amplified and non-amplified tumors.

P-CancG-029

Analysis of Aberrant DNA Methylation in Lung Cancer

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Lung cancer is the most common cause of cancer related death in males and the third common cause in females in Germany. Identification of molecular mechanisms by which environmental factors contribute to the development of lung cancer might improve not only lung cancer prevention but also prognosis and therapy of this cancer entity. DNA methylation, an enzymatically catalyzed and reversible covalent modification of the DNA, belongs to the family of epigenetic modifications allowing the cell to adapt its genetic activity according to the environmental conditions. In the German Center for Lung Diseases (DZL, supported by the BMBF) we investigate alterations in the DNA methylation pattern in lung cancer tissue samples and corresponding normal controls using an array-based BeadChip approach.
It is known that fixation of the sample material with formalin prior to BeadChip analysis can affect the results of BeadChip analyses. Consequently, we first compared the influence of fixation on the outcome of BeadChip analysis. From 6 patients each a lung cancer tissue sample and a corresponding tumor free lung tissue sample were collected. The samples were separated into three pieces. One piece of each sample was fixed with formalin, another one by the non-crosslinking HOPE-technique (Hepes-glutamic acid buffer mediated Organic solvent Protection Effect). Subsequently, both became paraffin embedded. As a reference, the remaining third piece was cryo-preserved. We showed that using the HOPE-technique instead of formalin largely prevents the introduction of formalin-fixation related artifacts.
Subsequently, we investigated the DNA methylation pattern of 90 lung tumor samples of selected entities (e.g. squamous cell carcinoma, adenocarcinoma, large cell carcinoma, PECA) and compared the methylation status between entities and the corresponding normal controls. Altogether, we identified >620 loci aberrantly methylated in lung cancer as compared to controls (FDR<1.62x10-24, s/smax> 0.415).
In this study we compared suitability of formalin and HOPE fixation for array based DNA methylation analysis and subsequently analyzed alterations in the DNA methylome of 90 lung tumor samples as compared to the corresponding controls.

P-CancG-030

Whole-genome sequencing of plasma DNA reveals frequently occurring copy number changes in patients with metastatic breast cancer

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With the increasing number of available predictive biomarkers, clinical management of cancer is becoming more and more reliant on the accurate serial monitoring of tumor genotypes. Circulating cell-free DNA (cf-DNA) collected from plasma of patients offers a unique opportunity for monitoring tumor genomes in a non-invasive manner. It is a potential surrogate for the tumor itself, and often referred to as "liquid biopsy". The amount of cf-DNA that derives from tumor cells changes owing to the size and state of the tumor. The proportion is also conditioned by clearance, degradation and other physiological filtering events of the blood and lymphatic circulation.
We addressed whether complex tumor genomes may be identified non-invasively from peripheral blood of metastatic breast cancer patients. Plasma DNA concentration and size distribution were determined and followed by qualitative and quantitative analysis for subsequent whole-genome sequencing at a shallow sequencing depth using the Illumina MiSeq® platform to establish genome-wide copy number profiles. In order to identify highly significant somatic copy-number changes and genes affected by such copy-number alterations 25 plasma samples were enrolled in a GISTIC2.0 (Genomic Identification of Significant Targets in Cancer) analysis. Furthermore, to test whether the observed copy-number changes are specific for breast cancer, we conducted a comparison with data of 913 breast tumor samples that were generated from the Broad Institute.
A subset of metastatic patients had a biphasic size distribution of plasma DNA fragments with elevated concentrations of plasma DNA, and a high percentage of mutated DNA fragments. Using GISTIC2.0 analysis 15 significant gains and 13 significant losses were detected in the plasma DNA samples. Regions on chromosome 11q13.3 (including the CCND1 or FGF3/4/19 gene) and 12q15 were significantly gained, whereas chromosomal regions on 8p23.1 and 6q21 were frequently deleted. In comparison with 913 breast tumor samples from the Broad Institute 10 gains and 8 losses were shared implying that the detected copy-number changes were breast cancer specific.
The techniques used in this study allow serial monitoring of tumor genomes with a simple blood test. They could revolutionize the management of cancer patients through the detection of mutations leading to resistance to targeted therapies, personalized therapeutic monitoring and non-invasive follow-up of the disease.

P-CancG-031

Sequential Cytogenetic Evolution of Burkitt lymphoma/leukemia

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Burkitt lymphoma/leukemia (BL) is a highly aggressive B-cell lymphoma. The biological hallmark of BL is the overexpression of the oncogene MYC on chromosome 8 due to its juxtaposition to the immunoglobulin heavy chain (IGH) locus on chromosome 14 in the translocation t(8;14)(q24;q32) or, in its variants, to the IG light chain loci on chromosomes 2 and 22 in the translocations t(2;8)(p12;q24) and t(8;22)(q24;q11). Typical BL is characterized by an overall low chromosomal complexity and favorable prognosis with only few relapses. However, in addition to MYC rearrangements, secondary chromosomal rearrangements may be present contributing to disease evolution and progression. We have investigated the karyotypic evolution of 5 pediatric and one adult BL sequential samples using conventional cytogenetic techniques. For validation, we performed a review of karyotypes of (primary) BL listed in the Mitelman database.
In total 14 samples from 6 BL patients comprising 6 samples at primary diagnosis, 7 samples at relapse at different times, and 2 samples during monitoring of front-line chemotherapy were investigated. The presence of the t(8;14) or its variants was confirmed by fluorescence in situ hybridization (FISH) with a specific break-apart probe for the MYC gene and a MYC-IGH tri-color-dual-fusion probe (all from Abbott Vysis). Five out of 6 samples (83%) showed the t(8;14) and one sample (17%) the t(8;22). Complex karyotypes with >3 alterations were seen in 2 cases at diagnosis and in all 6 cases at relapse or during monitoring. The mean number of aberrations (including MYC rearrangement) at diagnosis was 2. Sequential specimens showed significantly higher karyotypic complexity with a mean number of alterations of 8 ($p<0.05$). Third samples presented more aberrations than second samples (12 and 6, respectively). Analysis of the Mitelman database delivered similar results. Recurrent secondary cytogenetic numerical and structural alterations were +7, +12, +21, duplication of 1q, and losses of 6q, 13q22 and 17p11-13.
In conclusion, we present here a longitudinal study of BL with sequential cytogenetics available showing clonal evolution and an increasing load of karyotypic changes. Moreover, in addition to increasing karyotype complexity we also observed strong evidence for a direct (sub) clonal evolution in BL. These findings point to an important role for karyotype complexity and clonal evolution in disease progression and relapse of BL and therefore might contribute to the dismal prognosis of patients with relapsing disease.
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P-CancG-032

HOPX a putative tumour suppressor gene involved in cervical carcinogenesis

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Background:
Persistent infection with a high risk (HR) human papillomavirus (HPV) type is a prerequisite for the development of cervical cancer (CxCa). This process is accompanied by numerous genetic alterations. In previous investigations we observed a frequent under-representation of chromosome 4 and different parts of chromosome 4. These chromosomal regions are thus of particular interest, since loss of genetic information is indicative for the location of putative tumour suppressor genes. In microarray analyses we found the homeobox gene HOPX (4q12) to be consistently down-regulated in cervical cancers.
Objectives:
The aims of the present study were to validate the differential expression of HOPX in cervical tissues and to analyze its properties in different functional assays.
Methods:
Validation of the candidate gene expression was done by qRT-PCR and by immunohistochemical staining in normal cervical tissues, high-grade precancers (CIN2/3) and CxCa. To assess the promoter methylation status of HOPX, methylation specific qRT-PCR was performed. Moreover, HOPX was expressed ectopically in various cell lines to analyze its effect on cell proliferation, colony formation, anchorage-independent growth, cell migration, apoptosis and cellular senescence.
Results:
The mRNA expression level of HOPX was significantly ($p<0.01$) lower in CxCa as compared to CIN2/3 or normal tissue. Immunohistochemical analyses revealed that protein expression of HOPX is in line with the RNA data. Methylation data showed that promoter methylation of HOPX was observed in the majority of CxCa. Reconstitution of HOPX expression resulted in a significant reduction in cell proliferation and colony formation in CaSki and HPKII cells. These effects are not due to an increase in apoptosis or senescence.
Conclusions:
In conclusion, our results suggest that HOPX has characteristic tumour suppressor gene properties and loss of HOPX function may contribute to the transformation process in cervical carcinogenesis. On-going gene knock-down experiments may provide further support for this hypothesis.

P-CancG-033

Functional Characterisation of the Isocitrate Dehydrogenase 1 (IDH 1) R132H Mutation in Gliomas

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Glioblastoma multiforme (GBM) is the most prevalent and aggressive brain tumor in adults. A patient diagnosed with GBM and receiving therapy is left with a median survival time of one year. This poor prognosis emphasizes the importance of understanding the genetic processes leading to tumor development in the human central nervous system in order to find new therapeutic strategies.
There are two types of GBMs: primary GBMs, which arise de novo, and secondary GBMs, which progress from lower grade astrocytoma. It was shown that both types differ in their genetic and epigenetic profiles as well as in their clinical outcome. Most notable is the prevalence of Isocitrate Dehydrogenase 1 and 2 (IDH1/2) mutations, which are more frequently found in secondary GBM (~80% of cases as opposed to ~5% of primary GBM). The enzyme IDH1 converts isocitrate to alpha-ketoglutarate (αKG) while reducing nicotinamide dinucleotide phosphate (NADP+). Due to the mutation at arginine 132 of IDH1, the enzyme has a reduced ability to produce αKG while its production of 2-hydroxyglutarate (2HG) is increased. Mutations in IDH1/2 are thought to be an early, if not initiating, event in gliomagenesis, but their mechanism is not entirely elucidated. The accumulation of 2HG, which is considered to be an oncometabolite, cannot explain all the observed phenomena of the mutation.
We have stably transduced three cell lines (one established cell line of GBM origin, one primary patient-derived GBM cell line, and one

of immortalized astrocytes) with IDH1 R132H using viral vectors and checked the presence of the mutation on the genomic, transcriptomic, and protein level using Sanger sequencing and Western blot. Using liquid chromatography-tandem mass spectrometry (LC-MS/MS), we could detect a significant increase of 2HG in cells with the IDH1 R132H as compared to cells transduced with IDH1 wt. Interestingly, there was a decrease in proliferation of the cells with IDH1 R132H and an increase in cell size in 2D cell cultures. This effect was confirmed in 3D spheroid cell cultures.
In summary, we have developed an in vitro model to further study the effect of the IDH1 R132H mutation on the energy balance and the transcriptome of the cells. We are currently running experiments to check the metabolic and transcriptomic profiles of the transduced cells. These results will also be presented.
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P-CancG-034

Rapid and efficient method for molecular diagnostics of patients with Peutz-Jeghers syndrome

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Peutz-Jeghers syndrome [PJS, MIM # 175200] is a rare, hereditary predisposition characterized by the presence of hamartomatous polyps in the gastrointestinal tract, occurrence of lentigines on mucous membranes (mainly on lips, oral cavity and nostrils) as well as by increased risk of malignant tumours of various internal organs. The incidence of the syndrome, depending on the examined population, is estimate to fluctuate from 1:300 000 even up to 1:25 000 of births.
PJS is an autosomal dominant disease caused by mutations in the STK11 gene [MIM # 602216] located on the small arm of chromosome 19 at position 19p13.3. This gene comprising 22 637 base pairs consists of 10 exons, nine of which contain a coding sequence of 48.6 kDa protein made up of 433 amino acids which fulfils the function of serine-threonine kinase. Small mutations of the STK11 gene constitute the highest percentage of changes causing the disease, therefore, the elaboration of a rapid and cost-effective method of their detection is a key aspect in the advancement of genetic diagnostics of patients suffering from PJS.
Aim of our study was to develop such methodology based on high resolution melting (HRM) analysis, one of the more novel techniques employed in screening investigations which allows identification of small, unknown sequence changes within the examined fragments. The tests were performed on DNA obtained from 41 probands with diagnosed Peutz-Jeghers syndrome. The small mutations of the STK11 gene were detected in 22 families (54%). No additional small mutations were found in the remaining patients subjected to sequencing of the entire coding sequence of the gene. The employed HRM method exhibited 100% effectiveness with respect to the group of our patients.
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P-CancG-035

Reliable single cell array CGH for clinical samples

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Comprehensive analysis of minute quantities of genomic DNA has become relevant in a variety of forensic, diagnostic and biological studies. In cancer research, single-cell technologies become increasingly important to study the course of metastatic spread of cancer cells. Disseminated cancer cells (DCCs) and circulating tumor cells (CTCs) that comprise the precursor cells of distant metastases or therapy resistant cells are extremely rare. The detailed molecular analysis of these cells may help to identify key events of cancer cell dissemination, metastatic colony formation and development of therapy resistance. Using the Ampli1™ whole genome amplification (WGA) technology and high-resolution oligonucleotide aCGH microarrays we optimized conditions for the analysis of structural copy number changes in single cells. The resulting protocol enables reliable detection of numerical genomic alterations as small as 0.1 Mb. Moreover, analysis of single cells subjected to fixation and staining procedures used to detect DCCs showed no significant impact on the outcome of the analysis, proving the usability of our method for clinical cancer research. In a proof-of-principle study we tracked the chromosomal changes of single DCCs over a full course of high-dose chemotherapy treatment by isolating and analyzing DCCs of an individual breast cancer patient at four different time points. The developed protocol enables detailed genome analysis of single-cell genomes. The results from an exemplary case report of a breast cancer patient provide evidence that DCCs surviving selective therapeutic conditions may be recruited from a pool of genomically less advanced cells, which display a stable subset of specific genomic alterations. Preliminary results have shown that the analysis can be completed within 48 hours, indicating its applicability to pre-implantation genetic diagnosis.

P-CancG-036

Calcium and CaSR dependent bone metastasis in renal cell carcinoma

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Renal cell carcinoma (RCC) is the most frequent type of kidney cancer. It is associated with poor prognosis for affected patients due to its high metastatic potential. The process of metastasis begins with the dissociation of single tumour cells through the extracellular matrix into the blood circuit. Once in the blood system the tumour cells can get stuck in the capillary system and finally invade the corresponding organs. The high incidence of bone metastases that occur in approximately 30% of patients with RCC suggests, that the microenvironment of the bone-tissue especially attracts renal carcinoma cells to form bone metastases. To date it is not known why some renal cell carcinomas tend to form metastasis in bones, while others of the same type do not. We therefore aimed to determine if Ca2+ originating from bone (e.g. due to the bone remodelling processes) is a chemotaxin for circulating tumour cells that could facilitate bones as a target organ. One way of perceiving extracellular Calcium is via the Calcium sensing receptor (CaSR).

Since expression of the CaSR is either reduced in different cancers, or CaSR signalling may differ dependent on the type of cancer, stage and grade, we aimed to correlate CaSR expression and signalling with the fate of primary RCCs in respect of their metastatic behaviour. Based on our study with 11 matched specimens and 3 primary RCC cells for each of the 3 categories non-metastasized RCC, RCC metastasized into the lung and RCC metastasized into bones during a five-year period after nephrectomy, we can show that a higher expression of CaSR in primary RCCs correlates with the appearance of bone metastases. Our data also argues for a Calcium dependent physiological switch as soon as tumour cells with high CaSR levels reach the bones by upregulating the Akt pathway and downregulating the gate keeper of apoptosis PTEN.

P-CancG-037

Somatic neurofibromatosis type 1 (NF1) inactivation events in cutaneous neurofibromas of a single NF1 patient

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Background NF1 (MIM#162200) is a relatively frequent genetic condition which predisposes to tumor formation. Main types of tumors occurring in NF1 patients are cutaneous and subcutaneous neurofibromas, plexiform neurofibromas, optic pathway gliomas (OPG), and malignant peripheral nerve sheath tumors (MPNST).
Methods To search for somatic mutations in cutaneous (dermal) neurofibromas, whole exome sequencing (WES) was performed on seven spatially separated tumors and two reference tissues (blood and unaffected skin) from a single NF1 patient. Validation of WES findings were done using routine Sanger sequencing or Sequenom IPlex SNP genotyping.
Results Exome sequencing confirmed existence of the known familial splice site mutation NM_000267:c.3113+1G>A in exon 23 of NF1 gene (HGMD ID CS951480) in blood, unaffected skin, and all tumor samples. In five out of seven analyzed tumors we additionally detected second hit mutations in the NF1 gene. Four of them were novel and one was previously observed. Each mutation was distinct demonstrating the independent origin of each tumor. Only in two of seven tumors we detected an additional somatic mutation not associated with NF1.
Conclusion Our study demonstrated that somatic mutations of NF1 are likely the main driver of cutaneous tumor formation. The study provides evidence for the rareness of single base pair level alterations in the exomes of benign NF1 cutaneous tumors.

P-CancG-038

Inhibition of Tumorigenic Properties of Metastatic Breast Cancer by miR-122-5p Targeting ADAM10

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MicroRNAs are negative regulators of protein coding genes. Recently, microRNA-122 was proved to play an important role in hepatocellular carcinoma, but its functions in breast cancer (BC) remain unknown. Overexpression and activating mutations of ErbB family members were implicated in the development and progression of most of tumor types. In most of metastatic breast cancer cells, HER2 receptors are known cleaved by an ectodomain sheddase, ADAM10, to liberate HER2 extracellular domain (ECD). This provides ligand-independent growth to breast cancer cells and Trastuzumab, anti-HER2 agent, cannot inactivate HER2 by binding ECD portion of it. This process is clinically crucial since high levels of serum HER2 ECD in metastatic breast cancer patients are associated with a poorer prognosis. Also, HER2-amplified patients with low-HER2 ECD levels may get more benefit from Trastuzumab-based scheme. Thus, our aim is to provide a novel therapeutic approach for breast cancer by inhibiting HER2 sheddase activity with miR-122-5p and increasing activity of Trastuzumab. Real-time PCR was used to identify use of transcriptional profiling of miR-122-5p in vitro approaches to identify major source of HER2 sheddase activity in breast cancer cell lines (CRL-2329,MDA-MB-231,CRL-1500,MCF-7,SK-BR-3) and in CRL-4010 (control). First four breast cancer cell lines are Her2-, but SK-BR-3 is Her2+. We found down-regulation in expression of miR-122-5p that selectively inhibited ADAM10 expression in mRNA level and so increased HER2 shedding in our cell lines, except SK-BR-3. miR-122-5p expression level was significantly higher in SK-BR-3 cell line than other cell lines and control. Moreover, SK-BR-3 cell line showed highest expression level among the other four and control. This result is very consistent with our hypothesis because our results indicates that ADAM10 expression level increases in Her2+ cell line and miR-122-5p expression level also increases to suppress the sheddase activity of ADAM10 however increase in miR-122-5p expression is not high as increase in ADAM10 expression in Her2+ cell line so sheddase activity of ADAM10 cannot be inhibited totally in Her2+ cell line. So, we proved a positive correlation observed only between ADAM10 inhibition and reduction of HER2 ECD shedding in a cell based assay. In brief, we suggest that the inhibition of ADAM10 by miR-122-5p specifically may provide a novel therapeutic approach by sensitizing breast cancer cells to Trastuzumab targeting ECD of HER2 in these cells with active HER2 signaling.

P-CancG-039

A new missense germline mutation affecting the proofreading domain of polymerase δ (POLD1) apparently predisposes to colorectal adenomas and carcinomas

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The lifetime risk of colorectal cancer (CRC) is increased by several tumour predisposition syndromes such as Lynch syndrome (caused by Mismatch Repair Gene mutations), Familial Adenomatous Polyposis (APC mutations) or Familial Adenomatous Polyposis type 2 (MUTYH mutations). Recently, germline mutations in the exonuclease domain of the genes POLE (p.Leu424Val) and POLD1 (p.Ser478Asn) have been identified conferring a high risk of colorectal adenomas and carcinomas. These mutations exhibit high penetrance and autosomal dominant inheritance. The phenotype includes early onset development of multiple colorectal adenomas and carcinomas. In addition, the mutation in POLD1 was also associated with endometrial carcinomas and brain tumours. Both mutations map within the proofreading (exonuclease) domains of the resulting polymerases (residues 268-471 and 304-517 of POLE and of POLD1, respectively). Therefore the term Polymerase Proofreading-Associated Polyposis (PPAP) was suggested. Furthermore, a probably pathogenic mutation in POLD1 (p.Pro327Leu) was detected in a patient with multiple adenomas. Authors concluded the loss of proofreading activity in a still working polymerase as the

pathogenic mechanism (Palles et al. Nat Genet 2013; 45:136-44; Briggs and Tomlinson J Pathol 2013; 230:148-53).
Here we report on a 63 year old patient who presented with approximately 40 colorectal polyps developed throughout the last six years. Firstly, this raised the suspicion of attenuated familial adenomatous polyposis. His brother died of colorectal cancer at the age of 63. The paternal grandfather also had polyps and died of colorectal cancer at 70 years of age. The father of the patient died an accidental death at the age of 36. At the age of 19 the patient's daughter died of a tumour located in the spine. Two sons of one of the patient's sisters also suffered from colorectal polyps. The sister herself refused a colonoscopy.
Molecular genetic analysis revealed no mutation in the genes APC, MUTYH and POLE. However, the heterozygous sequence alteration c.1379T>G, p.Leu460Arg was detected in POLD1 (NM_002691.2). The affected nucleotide is highly conserved (phyloP 4.4; phastCons 1.0) as well as the coded amino acid residue up to Saccharomyces cerevisiae (considering 11 species). All four in silico prediction programs applied (SIFT, PolyPhen-2, AGVGD, MutationTaster) categorised the mutation as pathogenic. Especially the location of the alteration within the exonuclease domain of POLD1 is indicative of an apparently pathogenic mutation.
The family resembles the recently reported two POLD1 mutation (p.Ser478Asn) carrying families, with a family history of polyps and CRC and missense mutation located within the exonuclease domain of POLD1.
In conclusion, molecular analysis of POLE and POLD1 should be considered in families with clustering of colorectal adenomas, CRC or other cancers, in which no mutations in APC, MUTYH or Mismatch Repair Genes could be identified.

P-CancG-040

Increasing genomic and epigenomic complexity is a hallmark of the evolution from in situ to clinically manifest follicular lymphoma

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In situ Follicular Lymphoma (FLIS) is characterized by the presence of a clonal B-cell population with immunophenotypic features of classical follicular lymphoma but restricted to one or few germinal centers. Like classical follicular lymphoma, the cells carry the t(14;18)(q32;q21) translocation which juxtaposes the BCL2 gene to the immunoglobulin heavy chain (IGH) locus, causing constitutive expression of the anti-apoptotic protein BCL2. FLIS is considered to be a potential precursor lesion of clinically manifest Follicular Lymphoma (mFL). However, the events associated with progression of FLIS to mFL are largely unknown. Thus, we here studied the presence of chromosomal imbalances and changes of DNA methylation in selected genes in 6 paired samples of microdissected FLIS and mFL from the same patient using DNA extracted from formalin-fixed, paraffin-embedded (FFPE) tissue. Bisulfite pyrosequencing was performed on a selected set of genes (DAPK1, EYA4, GRB10, TWIST1, GAS7, TUSC3, ETV1) known to be de novo hypermethlyated in mFL (Martin-Subero et al., Blood. 2009, PlosOne. 2009). Chromosomal imbalances were studied by array CGH using the Human Genome CGH Microarray 244K platform (Agilent Technlogies, Santa Clara USA). Five out of six patients showed copy number alterations (CNA) only in the manifest FL whereas no alterations were observed in the in situ counterpart (FLIS 0.8 CNA/case; mFL 6.3 CNA/case). In a comparison of methylation status between FLIS and classical FL, 2 out of 4 cases presented a higher levels of DNA-methylation in the manifest FL as compared to FLIS (p=0.012 and p=0.012). These data support the hypothesis that FLIS represents a FL precursor lesion of long-lived clonal B-cells carrying the t(14;18) with no or few secondary genetic changes. Moreover, we provide evidence that there may be more than one distinct lesion driving the progression from FLIS to manifest lymphoma.
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P-CancG-041

Functional analysis of mutations in polymerase epsilon gene that predispose to polymerase proofreading associated polyposis (PPAP)

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Germline mutations in the exonuclease domain of genes encoding the catalytic subunits Polymerase δ and ε, i.e. POLD1 and POLE, predispose to "polymerase proofreading associated polyposis" (PPAP) resulting in multiple colorectal adenomas and carcinoma with high penetrance and dominant inheritance. Moreover, somatic mutations in the ED of POLE have been frequently found in sporadic CRCs and endometrial carcinoma. Tumors, with both inherited and somatic EDMs, were microsatellite stable and showed a mutator phenotype with a dramatic increase of base substitutions indicating impaired proofreading. To assess the functional consequences of POLE germline and somatic mutations, we characterized exonuclease domain mutant alleles in S.pombe. We therefore generated constructs encoding the equivalent changes in the fission yeast protein and determined the effect of this change on reversion of the ade6-485 allele, 5-fluoroorotic acid and canavanine resistance. The somatic mutations including P286R, S297F, and S459F showed a dramatically increased mutation rate (up to more than 100-fold) compared to the wild type strain. V411L was only slightly increased (4-7 fold). Also the germline variant L424V showed increased mutation rates (2-11-fold) compared the wild type strain. As expected whole genome sequencing of POLE mutated strains revealed an elevated rate of base substitutions with a similar mutation spectrum as observed in human tumors. This data indicate that EDMs in POLE indeed lead to proofreading deficiency and can induce replication errors during synthesis of oncogenes and tumor suppressors resulting in tumor formation.

P-CancG-042

No association between biallelic MSH6 germline mutations and MUTYH-associated polyposis phenotype

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Background: Biallelic mutations in mismatch repair (MMR) genes (in heterozygous state causative for hereditary non-polyposis colorectal cancer (HNPCC/Lynch syndrome)) are known to cause an early onset tumor predisposition syndrome (constitutional mismatch repair deficiency, CMMR-D) including colorectal cancer, hematologic cancer, brain tumors, and café-au-lait macules. One patient of our HNPCC cohort was found to carry a biallelic MSH6 missense mutation (c.3226C>T;p.Arg1076Cys) that is likely to be pathogenic. The patient's phenotype however is not typical for a biallelic MMR gene muta-

tion, but rather dissembles a MUTYH-associated polyposis (MAP). MUTYH analysis in this patient revealed no mutation.
Gu et al. (2002) reported on a functional interaction between the base excision repair (BER) protein MUTYH and the MSH6 protein. They showed that the MSH2/MSH6 complex enhances the binding affinity to the mismatched DNA substrate and the glycosylase activities of MUTYH. Hence we hypothesized that biallelic mutations in the MSH6 gene (especially the MSH6 missense mutation c.3226C>T;p.Arg1076Cys) might impair MUTYH function and therefore lead to an MAP-like phenotype.
Methods: To investigate, if there are more patients with a polyposis phenotype and biallelic MSH6 mutations, we examined 145 apparently unrelated polyposis patients without APC or MUTYH mutations (most of these patients show an attenuated phenotype and are sporadic cases). MSH6 mutation screening was performed by a targeted next-generation sequencing approach (Truseq Custom Enrichment Kit, Illumina) on an Illumina HiSeq2000 sequencer. Data analysis was done by standard protocols using the VARBANK pipeline (CCG, Cologne).
Results: We identified nine different heterozygous missense mutations in MSH6, one of those in four, another one in three different patients. Four of these mutations are known variants presumed to be benign. For the other five rare mutations no data regarding pathogenicity were found in databases or literature. Based on in-silico tools (Mutation Taster, PolyPhen-2, SIFT) no definite prediction regarding pathogenicity could be made either. We did not find the mutation c.3226C>T;p.Arg1076Cys in any further patient. Only one patient harboured two MSH6 variants, both probably benign. Therefore no further patient was found to carry two presumably pathogenic MSH6 mutations.
Conclusions: We could not find further evidence that biallelic mutations in the MSH6 can cause a MAP-like phenotype. A minor effect of MSH6 variants on MUTYH function can still not be excluded. Functional analyses are needed to elucidate the impact of MSH6 variants on MSH6/MUTYH-interaction.

P-CancG-043

OncomiRs Variation in Cancer Tissues, Act as a Tumor Suppressor and Oncogene

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Background: Over than 530 types of microRNA-noncoding RNA molecules, about 22 nucleotides- are encoded by human genome that interference with mRNA and by destruction or repression of translation adjusts their expression. Each mi RNA regulates the expression of about 200 genes and their mutation would increases the probability of malignancies dramatically. 10% of mi RNAs have shown lack of expression balance in several tumors so they act as tumor suppressors (TS) and also as oncogenes. The micro RNAs that are involved in inducing neoplasm are named as oncogenic micro RNAs (OncmiR).
Objective and Design: This is to scrutinize OncomiRs express variation in tumors by reviewing articles published at NCBI database from 1995 to 2012. The headings "OncomiRs variation in cancer", "miRNA irregular expression", "OncomiR and cancer" were searched.
Result: miRNAs regulate cell-cycle control, cell differentiation and development, cell proliferation and apoptosis and also play considerable role in tumor initiation and progression. . These molecules are interfering with some crucial oncogenes and TSs such as MYC, RAS, p53, etc and modify their expression. Studies have shown widespread dysregulation of these molecules at an early stage in various cancers. Specific oncomirs loss- or gain-of-function contributes to specific tumors. E.g. MicroRNA-21 (miR-21) which functions as an oncogene is overexpressed in breast cancer. MiR-196a2 variant boosts colorectal cancer probability. MiR-15 and miR-16 induces apoptosis and deletion of their gene in B-lymphocyte cells causes chronic lymphocytic leukemia (CLL). MiR-34a acts as neuroblastoma tumorigenesis repression and loss of function causes neuroblastoma. Amplification and of the miR-17-92 cluster played a role in development of lymphoma and lung cancer. Overexpression of miR-221 and miR-222 are contributed to papillary thyroid carcinoma. MiR-31, miR-96, miR-133b, miR-135b, miR-145 and miR-183 are correlated with colorectal cancer and etc.
Conclusion: one of the marked characteristic of cancer tissues is the oncomiRs irregular expression; the dysregulation of miRNAs disrupt tissues critical pathway, act as a tumorgenesis and develops cancer subsequently. Expression profiling of tumors has identified prognostic, diagnostic and therapeutic horizons in deal with diverse cancers. Diagnosing the type of mutation in oncomiRs would be efficacious for choosing the appropriate therapeutic approaches by inducing the expression of tumor suppressor or repressing the expression of oncogene.

P-CancG-044

Variant c.*126G>A in NFKBIA gene as a risk allele in Polish patients with differentiated thyroid cancer

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Thyroid carcinomas belong to tumors with well prognosis, slow progress and low benignity but with tendency to recurrences and regional or remote metastasis. Papillary and follicular thyroid cancer are the most frequent in endocrine system with unidentified genetic background.
In this focus, very important seems to be searching for molecular markers of disease course, good or poor prognosis and response on medical treatment as well. It is expected that SNP polymorphisms research in genes demonstrating association with neoplastic diseases will be helpful in understanding of molecular mechanisms of thyroid gland tumors development and allow to better diagnosing.
We analyzed c.*126G>A polymorphism (rs696) in NFKBIA gene. Groups of 548 patients with differentiated thyroid cancer and 535 individuals from population group were examined. Sequence variants were determined by pyrosequencing.
There were observed differences in allele and genotype frequencies. In patients with thyroid cancer allele G was present with frequency 0,581and allele A with frequency 0,418 compared with 0,521 and 0,478 in population group respectively. The differences were more significant when considerate men and women separately. Allele G in males with DTC was observed with frequency 0,651 comparing with males population control 0,533; allele A with frequency 0,349 in patient males and 0,467 in males population.
Regarding lower frequency of the disease in males, detected differences may indicate on association of allele G with thyroid cancer risk.

P-CancG-045

Detection of presumably low penetrance germ line mutations associated with colorectal cancer – results of a prospective comparative study of germ line mutation analysis by next generation sequencing and classical molecular pathology testing

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Colorectal adenocarcinoma (CRC) is one of the most commonly diagnosed malignancies worldwide and ranks fourth and third for cancer-related deaths among males and females, respectively. Current studies estimate that up to 25% of CRCs involve inherited susceptibility. However, germ line mutation testing is typically offered only to a subset of patients fulfilling defined diagnostic criteria. The aim of our study was to determine whether unbiased screening of colorectal cancer cases increased the overall detection rate of germ line mutations.
We tested 18 CRC associated genes for germ line mutations in 152 consecutive cases from the university hospital Erlangen-Nuremberg, who underwent CRC surgery using a customized NGS gene panel (AmpliSeq) on the Ion Torrent PGM (LifeTech). In parallel, all patients were evaluated for Bethesda criteria with a standardized clinical procedure and all tumors were investigated for microsatellite instability (MSI), immunohistochemistry (IHC) for the classical MMR genes and for the BRAF*V600E somatic mutation. We identified 11 cases with bona fide germ line mutations, 10 in known MMR genes and one in APC, all predicted to be causative by several in silico prediction programs suggesting a diagnosis of HNPCC or FAP. Of the 10 cases with mutations in MMR genes only one with a MLH1 mutation showed MSI and loss of MLH1 according to IHC in the tumor.
To further characterize tumor status we sequenced the same gene panel in tumor DNA from the 11 patients with predictive causative mutations, from 12 patients with unclassified variants and from 5 BRAF*V600E positive tumors. All BRAF*V600E positive tumors and the tumor from the MSI positive case showed a hypermutated phenotype indicative of defective MMR. All other tumors investigated showed a non-hypermutated phenotype despite the predisposing germ line mutation indicative of a sporadic cancer. In 2 cases, one with the germ line APC missense mutation and one with an unclassified variant, we found a somatic nonsense second hit mutation in the tumor suggestive of an attenuated FAP. Interestingly, these cases did not show polyposis, compatible with the extreme spectrum of attenuated FAP with older age of onset and fewer to no polyps.
In conclusion somatic mutation pattern was in agreement with tumor IHC and MSI testing. Only one of the 10 MMR mutation cases was confirmed as a HNPCC case based on germline and tumor analyses, while in the other 9 cases the tumor was suggestive of a sporadic origin, indicating non-penetrance of the germline mutation. Overall, in 3 cases (2%) a fully penetrant germ line mutation was identified by unbiased sequencing of which only one would have been identified using established diagnostic protocols. Thus by the use of NGS screening in CRC cases we were able to identify low penetrant germline variants in MMR and APC genes in cases who did not fulfill current diagnostic criteria. In mutation positive cases, confirmatory analysis in the tumor is warranted.

P-CancG-046

Retrospective analysis of genomic and transcriptional changes in a case of Ewing sarcoma tumor progression determined by whole transcriptome and exome semiconductor-based sequencing

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Ewing sarcoma is a cancer that often presents in the second decade of life and is usually associated with a chromosomal translocation t(11;22)(q24;q12) that results in a EWS/FLI1 gene fusion. Long term survival rates for subjects with metastases can be less than 10%. Four independent cell-lines have been established from a subject who succumbed to metastatic disease following relapse after myeloablative chemotherapy. Whole-transcriptome and exome sequencing of normal primary bone marrow-derived stromal fibroblasts (cell-line COG-FB-425), Epstein-Barr Virus (EBV) transformed normal lymphoblasts (cell-line COG-V-455), a pre-therapy primary tumor-derived cell-line (CHLA-9), and a post-chemotherapy metastatic tumor-derived cell-line (CHLA-10), was conducted on an Ion Torrent Proton™ system to profile the differences in gene expression and differences in exonic DNA sequence to characterize the molecular changes associated with primary tumorigenesis and disease persistence after treatment. All cell lines matched by short-tandem repeat analysis. The presence of the EWS/FLI1 fusion gene in the tumor cells was confirmed and the breakpoint determined from both observation of chimeric reads in the RNA-seq data and exome sequence analysis. Exome datasets, collected to >140X average depth of coverage, indicate apparent loss of heterozygosity genome-wide in CHLA10 consistent with cytogenetic analysis that shows tetraploidy in this cell-line. Results from RNA-seq also indicate numerous instances, genome-wide, of differing transcript isoform expression and exon usage between normal, primary tumor, and metastatic tumor cells suggesting an increasing genomic mutational burden in the evolution of the disease, and pointing in particular toward aberrant regulation of RNA-splicing components. One co-expressed, first exon-sharing pair of sense/antisense transcripts corresponding to the gene FEZF1 and transcript FEZF1-AS1 that is unique to the tumor lineage is the subject of further investigation. Taken together, the combination of RNA-seq and exome-sequencing on normal cells and primary vs. post-chemotherapy tumor is providing a broad and deep view of molecular signatures in tumor progression and indicating that a significant role is played by changes in non-coding RNA expression.

P-CancG-047

Acute Lymphoblastic Leukemia (ALL) with Low-Hypodiploid/Near-Triploid Karyotype is a Specific Clinical Entity Characterized by a High TP53 Mutation Frequency

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Background: B lymphoblastic leukemia/lymphoma by the WHO classification 2008 (ALL) is subdivided into 5 subgroups defined by specific translocations (t(9;22); t(v;11q23); t(12;21); t(5;14); t(1;19)) and by the number of chromosomes, respectively. The hyperdiploid subset is well defined containing blasts with 51 to 65 chromosomes. In contrast, the hypodiploid subgroup is heterogeneous and comprises ALL with <46 chromosomes.
Aim: To characterize ALL with low hypodiploidy with respect to cytogenetic abnormalities, molecular mutations and clinical outcome.
Patients and Methods: Out of 878 ALL patients we selected 29 cases harboring a hypodiploid clone with 1) a chromosome number of ≤40 or 2) a near triploid clone with 56-78 chromosomes that had arisen from a low hypodiploid clone by duplication of the chromosome set. Data on karyotype, array CGH and mutation status of NRAS, KRAS, TP53, NOTCH1, FBXW7 and intragenic deletions of IKZF1 was available in all pts.
Results: All cases were negative for t(12;21)/ETV6-RUNX1, t(9;22)/BCR-ABL1 and translocations involving the MLL gene. The pattern of chromosomal losses was non-random: One chromosome (chr.) 3 and 7 was lost in all 29 cases. Also frequently deleted were chr 17 (n=28), 15 (n=27), 16 (n=26), 13 (n=25), 9 (n=20), 4 (n=19), 12 and 20 (n=18, each). Less frequently lost were chr 2 (n=14), 8 (n=12), 14 (n=10), 5 (n=8), 18 (n=7) and 11 (n=6). Rarely lost were chr 6, 22 (n=5, each), 1, 10, 19

(n=4, each), the sex chr X (n=4) and Y (n=3). Chr 21 was retained in all cases. In 14 pts with doubling of the low-hypodiploid karyotype we observed the same typical pattern of gains and losses with two copies of lost chr and four copies of retained chr. The only recurrent structural abnormality was a submicroscopic 9p deletion encompassing the CDKN2A/B locus (heterozygous n=3, homozygous n=4) detected by aCGH. As 16 additional pts showed a monosomy 9, only in 6/29 pts two CDKN2A/B copies were present. Mutational analyses revealed no mutations in IKZF1, FBXW7, NOTCH1 and KRAS and only one mutation in NRAS. However, we discovered a remarkably high frequency of TP53 mutations in 93.1% (27/29) of pts. In 26/27 pts with TP53mut and in both pts with TP53wt chromosome 17 was monosomic. Further, both pts with TP53wt and monosomy 17 showed a remarkably low chr number of 25 and 28 chr compared to TP53mut pts who showed at least 32 chr. The median overall survival was 18.5 months.
Conclusions: ALL with low hypodiploidy is characterized by a typical pattern of chromosome losses, a very high TP53 mutation frequency and poor outcome. A duplication of the low-hypodiploid karyotype occurs frequently and results in a near triploid karyotype based on the definition by merely counting chromosomes but in fact is a very low tetraploid chromosome set. Our data suggests the introduction of a novel WHO entity within the B lymphoblastic leukaemia/lymphoma group showing low-hypodiploid/very low-tetraploid karyotype and concomitant TP53 mutation.

P-CancG-048

Prostate cancer risk regions in 8q24 and 17q24 are differentially associated with somatic TMPRSS2:ERG fusion status

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Deep sequencing of prostate cancer (PrCa) genomes has recently pointed at an early role of ETS gene fusions in tumorigenesis, and revealed a characteristic landscape of structural rearrangements accompanying the oncogene translocation. Since PrCa in general has been proven partially heritable, we considered fusion positive PrCa as a distinct tumor entity that may be traced back to specific sets of germline risk factors. In a collaborative setting involving 1,221 cases with somatic status of the oncogene TMPRSS2:ERG we have investigated 27 common PrCa risk variants, known from previous GWAS studies, for their particular contributions to fusion positive or negative subclasses. Mantel-Haenszel meta-analysis was used to compare frequencies of risk alleles between the two subtypes. In a first set of 552 cases the PrCa risk loci 10q11 (rs10993994: OR = 1.35; p = 0.015), 19q13 (rs2735839: OR = 1.73; p = 0.0035), 17q24 (rs1859962: OR = 1.29; p = 0.038) and 8q24 (rs16901979: OR = 0.53; p = 0.021) appeared associated with TMPRSS2:ERG status at nominal significance. A replication round comprising a further 669 cases verified a selective involvement of 17q24 in fusion positive PrCa (stage 1 and 2 combined: p = 0.0016), and of 8q24 in fusion negative PrCa (stage 1 and 2 combined: p = 0.0007). Both loci, 17q24 and 8q24, are gene deserts, where the causal process for PrCa susceptibility is not fully understood to date. Noteworthy, one additional locus (rs1447295) in 8q24, which represents a risk region independent from rs16901979, also showed association with fusion status in our study (stage 1 and 2 combined: OR = 0.70; p = 0.0025). The concurrence of both 8q24 variants being associated with fusion negative PrCa is not due to linkage disequilibrium, and indicates a common pathogenic mechanism that is unrelated to ETS activity. In contrast, ETS pathway involvement at the 17q24 locus is plausible, since long range interactions have previously been described between risk genotypes and the nearby SOX9 gene, encoding a transcription factor which was observed to be co-expressed with ERG in the androgen driven profile of fusion positive tumors. In brief, our study further supports the distinctness of ETS positive vs. negative PrCa, and demonstrates in principle, that molecular subtypes could represent different entities also on the germline level. Larger sets of PrCa cases with somatic typing will be required in order to identify patterns of corresponding predisposing factors.

P-CancG-049

Functional consequences of epigenetically regulated tumor suppressor miRNA-449 family in hepatocellular carcinoma

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Introduction:
Chromatin remodeling is a common hallmark in human tumor cells and this process strongly alters the transcription of many genes and microRNAs (miRNAs). We could show that histone deacetylases (HDAC1-3) are consistently upregulated in primary hepatocellular carcinoma (HCC). Selected miRNAs have been shown to play important roles in carcinogenesis. Until now, it is largely unknown which miRNA genes are altered due to histone deacetylation in HCC.
Aims:
We hypothesized that the altered expression of miRNAs due to chromatin remodeling may play a fundamental role in hepatocarcinogenesis.
Methods:
Therefore, we induced histone acetylation by HDAC inhibitors trichostatin A (TSA) and by siRNA silencing of HDAC1-3, respectively, to identify deregulated miRNAs and their target genes in four HCC cell lines (HepG2, HLE, HLF, and Huh7) and two immortalized liver cell

lines (THLE-2 and THLE-3). Differentially expressed mRNAs and miRs were identified by expression profiling.
Results:
Upon histone acetylation, the tumor suppressor miRNA-449a was reactivated and its target gene c-MET downregulated, which induced strong effects on proliferation and survival in HCC. MiR-449a together with miR-449b and miR-449c belong to the relatively unknown miR-449 family which is coded in the second intron of the CDC20B gene. So far, only a few direct targets of miR-449a have been validated. MiR-449b and miR-449c have not yet been analyzed in depth. The miR-449 family shares seed sequences and target genes with the p53-inducible miR-34 family. Using qPCR we also found that miR-449b and miR-449c are significantly upregulated after HDAC inhibition, in contrast to the p53-inducible miR-34 family. Interestingly, our first results indicate that miR-449b and miR-449c reduce proliferation and strongly promote apoptosis in HCC cell lines after HDAC inhibition.
Recently, we could show that transfection of miR-449a, b and c into the cell lines HLE led to a significantly reduced expression of BCL9L in RNA. BCL9L increases the expression of a subset of canonical Wnt target genes but also regulates genes that are required for initiation of colon cancer.
Conclusions:
In particular we expect that histone deacetylation and many putative target genes of epigenetically deregulated tumor suppressor miR-449 family can be targeted by new therapeutic agents.

P-CancG-050

Community Driven Development of the Ion AmpliSeq™ AML Genes Research Panel

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Acute myeloid leukemia (AML) is characterized by somatic alterations in a set 30-40 genes. Identification of these aberrations in a cost effective manner has become an important aspect of hematological research. We describe the development, together with four leading European centers in leukemia research, of the Ion AmpliSeq™ AML genes research panel . In a first step, the collaborating centers agreed on the list of relevant targets to go into amplicon design, then the targets were shared with a larger number of centers in various geographies for review in order to yield broad acceptance of the AML research panel in the community. After design we conducted internal performance, specificity and sensitivity testing. Finally, the panel will undergo verification testing across the four initial centers to demonstrate robustness and reproducibility.

P-CancG-051

Recurrent mutation of the JAK3 gene in T-cell prolymphocytic leukemia

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T-lymphocytic leukemia (T-PLL) represents a highly aggressive malignancy in the group of T-cell lymphomas with a median survival time of less than one year. The hallmark chromosomal aberration of T-PLL is the inv(14)(q11;p32)/t(14;14)(q11;q32) or its variant t(X;14)(q28;q11). These aberrations, which lead to activation of the TCL1 or MTCP1 oncogene, respectively, are supposed to be primary genetic events in T-PLL. Nevertheless, they cannot drive leukemogenesis alone and additional aberrations are required for full transformation into T-PLL. Janus Kinase 3 (JAK3) plays an important role in T-cell maturation. Moreover, activating JAK3 mutations have recently been reported in various T-cell malignancies including early T-cell precursor acute lymphoblastic leukemia (ETP ALL), T-cell acute lymphoblastic leukemia (T-ALL), adult T-cell leukemia/lymphoma (ATLL) and natural killer/t-cell lymphoma (NKTCL). Therefore we analyzed the JAK3 gene in T-PLL cells from a cohort of 32 T-PLL.
In all cases, we confirmed the presence of the hallmark changes inv(14)(q11;p32), t(14;14)(q11;q32) or t(X;14)(q28;q11) by interphase FISH using probes for the TCRAD, TCL1 and MTCP genes. By this approach we determined that the tumor cell content in all cases exceeded 31% (median: 67%, range 31-99%). Then, we applied a sequential Sanger sequencing strategy for analysis of JAK3, targeting the regions of the gene recurrently affected in T-cell neoplasms. By this approach we discovered 14 potentially activating mutations in JAK3 in 11 of 32 patients (34%). The most frequently detected mutation in JAK3 was M511I in exon 11 which was present in 8 of the samples with a JAK3 mutation and has previously been described as an activating change in other T-cell malignancies. Furthermore we detected a R657Q mutation in exon 15 in two patients coexisting with M511I. In another case the mutations V674F and V678L in exon 15 could be shown to be located on the same allele in cis.
Protein modelling and comparison to mutation analysis in other JAK family members suggest the mutations likely to activate JAK3, e.g. by disrupting the activation loop and the interface between N and C lobes, increasing the accessibility of the catalytic loop.
In addition to molecular changes of JAK3 we detected alterations affecting chromosome band 19p13 containing the JAK3 locus in 4 of 21 patients lacking a JAK3 point mutation indicating that alterations of JAK3 other than point mutation might also contribute to T-PLL pathogenesis.
In conclusion we show recurrent activating mutations of JAK3 in T-PLL. The inhibition of JAK3 in patients carrying an activating aberration in JAK3 could, thus, provide new therapeutic opportunities to treat this severe malignancy with a currently very poor prognosis.

P-CancG-052

Genome-wide methylation analysis in skin fibroblasts of patients with secondary malignant neoplasms after childhood cancer

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Despite its rarity, childhood cancer is the leading cause of disease-related death in children aged under 15 years. Due to significant advances in treatment over the past 30 years, a cure or long-term remission for a substantial proportion of children (75%) can be nowadays achieved. Nevertheless, a careful long-term follow-up program is essential, as besides endocrine, metabolic and skeletal sequela the development of an unrelated second malignant neoplasm (SMN) is common and occurs in about one of five patients. It is well known that radiation and/or chemotherapy constitute risk factors for second malignancies, but as interindividual variability exists, it is reasonable to assume that inherited or acquired genetic and epigenetic changes are also involved.

To test the hypothesis, that the methylome signature of somatic cells in SMN patients reflects their secondary cancer risk, we analyzed the genome-wide DNA methylation in untreated primary fibroblasts of ten SMN cases compared to ten matched single neoplasm controls, using Infinium HumanMethylation27 BeadChip. Descriptive analysis revealed 84 CpG sites out of a total of 27.578 CpG sites showing methylation differences of 10% or higher in the SMN group in comparison to the control group. The conspicious CpG sites and corresponding genes were prioritized using the degree of methylation differences and literature data on known association with tumor formation and/or cancer susceptibility. Genes that were chosen for further analysis amongst others are CDKN2B, KCNA3, PHACTR3, BCL2, FXYD2 and RIP1. Methylation in these genes is currently validated by bisulfite pyrosequencing in a larger cohort consisting of 22 cases versus matched 22 controls.

To our knowledge, this is the first study whose aim is to establish a methylation signature specific for second neoplasms after a childhood cancer. By this approach we intend to gain knowledge on susceptibility factors that will enable further research on the underlying mechanisms and the development of preventive strategies. On the other hand, the development of a methylation-based SMN risk profile will permit the diagnostic identification of persons at particular high risk to offer them special monitoring programs.

P-CancG-053

Exome sequencing identifies potential new candidate genes for colorectal adenomatous polyposis

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Background: In up to 50% of families with colorectal adenomatous polyposis no germline mutation in the currently known genes APC (causing familial adenomatous polyposis (FAP)) or MUTYH (causing MUTYH-associated polyposis (MAP)) can be identified.

Methods: To uncover new causative genes, the exomes of 11 unrelated APC and MUTYH mutation negative polyposis patients were sequenced (Illumina HiSeq platform). For data analysis and variant filtering the GATK software 2.1-8, ANNOVAR, and in-house tools were applied. The variants were filtered for truncating variants and frequent alterations were excluded. Afterwards a targeted next-generation sequencing approach (Truseq Custom Enrichment Kit, Illumina) of the candidate genes was performed using a validation sample of 192 unrelated patients (145 mutation negative polyposis patients and 47 familial colorectal carcinoma cases with microsatellite stable tumors meeting the Amsterdam I or II criteria).

Results: In one patient, an APC nonsense mutation in mosaic state (10% of reads, coverage 249) was recognized. In the remaining 10 cases, 66 genes were affected by biallelic truncating variants (recessive model) in at least 1 patient and 63 genes were affected by truncating heterozygous variants (dominant model) in at least 2 patients. After detailed visual inspection of the variants in a read browser (Integrative Genomics Viewer) to exclude obvious sequencing artifacts, and data mining according to functions and pathways, 6 genes of high interest remained (2 for the recessive, 4 for the dominant approach), some of which are involved in cell adhesion, proliferation, or recombination repair. By Sanger sequencing 1 of the recessive genes (ZSWIM7) and 3 of the dominant genes could be confirmed (DSC2, HEATR5A and PDE4DIP). The mutation in PIEZO1 presented in heterozygous rather than homozygous state (recessive model; low coverage). In the validation cohort, no additional truncating mutation was found in either of the five genes. Missense variants were filtered for a MAF < 0.001 and a deleterious effect predicted by at least 2 in silico analysis tools. Altogether, 8 different non-recurrent heterozygous rare missense variants were identified in single patients. In PIEZO1, 4 variants could be detected, 2 variants were identified in DSC2 and HEATR5A, respectively. Further workup including segregation analysis in the familial cases is ongoing.

Conclusions: In a pilot exome sequencing study we identified new potentially causative genes for yet unexplained adenomatous polyposis. By targeted sequencing of the affected genes in a validation sample of 192 unrelated polyposis / familial colorectal carcinoma patients several heterozygous missense mutations with a predicted deleterious effect could be detected in 3 of the 5 genes (PIEZO1, DSC2 and HEATR5A). PIEZO1 and DSC2 are interesting candidates due to their involvement in cell adhesion. (Supported by German Cancer Aid and BONFOR programme of the University of Bonn)

P-CancG-054

The Association of Mutations in RUNX1 and CSF3R with the Development of Leukemia in Severe Congenital Neutropenia: A new pathway in leukemogenesis

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Congenital neutropenia (CN) is a rare inherited disorder of hematopoiesis with a 20% risk of evolving into acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS). Using next-generation sequencing in 31 CN patients who developed leukemia, we found that 20 of the 31 patients (64.5%) had mutations in RUNX1 (runt-related transcription factor 1). Of these 20 patients, 19 had inherited mutations associated with CN. Intriguingly, the majority of patients with RUNX1 mutations (80.5%) also had acquired CSF3R (colony stimulating factor 3 receptor) mutations. Other leukemia-associated mutations (EP300, FLT3-ITD, CBL, and SUZ12) were less frequent. In eight patients, we detected two distinct heterozygous RUNX1 mutations. Ten patients with RUNX1 mutations developed monosomy 7 and six patients developed trisomy 21 at diagnosis of leukemia. In contrast to their high frequency in CN evolving into AML, RUNX1 mutations were found in only 9 of 307 (2.9%) patients with de novo pediatric AML. RUNX1 mutations were

mainly found in pediatric AML patients with an adverse prognosis. A sequential analysis at stages prior to overt leukemia in ten CN/AML patients showed that RUNX1 mutation is a late event in leukemogenic transformation.

In 6 of the 10 patients with RUNX1 mutations, a CSF3R mutation occurred prior to RUNX1 mutations (24-192 months prior to CN/AML for CSF3R mutations vs. 1-36 months prior to CN/AML for RUNX1 mutations). Interestingly, monosomy 7 or trisomy 21 appeared after acquisition of RUNX1 mutations and no additional chromosomal aberrations were detected by array-CGH. Single-cell analyses in two patients revealed that RUNX1 and CSF3R mutations were segregated in the same malignant clone. Moreover, functional studies demonstrated elevated G-CSF-induced proliferation with diminished myeloid differentiation of hematopoietic CD34+ cells after co-transduction with mutated RUNX1 and CSF3R, in comparison to cells transduced with mutated RUNX1 or mutated CSF3R only. The importance of RUNX1 mutations in leukemogenic transformation was substantially strengthened by the analysis of a unique family with two siblings suffering from CN that subsequently transformed to AML. In both children, cooperating RUNX1 and CSF3R mutations were detected that were not present in healthy family members. Taken together, the high frequency and the time course of cooperating RUNX1 and CSF3R mutations in CN patients who developed leukemia suggests a unique molecular pathway of leukemogenesis similar to that reported in the Gilliland-Griffin two-hit hypothesis for AML development. The concomitant detection of RUNX1 and CSF3R mutations represents a useful biomarker for identifying CN patients with a high risk of progressing to leukemia or MDS.

P-CancG-055

Unexpectedly high prevalence of Immunoglobulin Light Chain Gene aberrations as revealed by FISH in Multiple Myeloma and MGUS

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Multiple myeloma (MM) is a malignant B-cell neoplasm characterized by an uncontrolled proliferation of aberrant plasma cells in the bone marrow. Chromosome aberrations in multiple myeloma are complex and represent a hallmark of the disease, involving many chromosomes that are altered both numerically and structurally. Nearly half of the patients are non-hyperdiploid and show IGH translocations with the following partner genes: CCND1, FGFR3 and MMSET, MAF, MAFB and CCND3. The remaining 50% of the patients are grouped into a hyperdiploid group that is characterized by multiple trisomies involving chromosomes 3, 5, 7, 9, 11, 15, 19 and 21. In this study we analyzed the immunoglobulin light chain kappa (IGK, 2p12) and lambda (IGL, 22q11) loci in 150 patients, mostly with multiple myeloma and in a few cases monoclonal gammopathy of undetermined significance (MGUS), without IGH translocations. We identified aberrations in 26.6% (= 40 patients) including rearrangements (12%), gains (12%) and deletions (4.6%). In the 6 of 18 patients with IGK or/and IGL rearrangements, we detected a MYC rearrangement which suggests that MYC is the translocation partner.

P-CancG-056

The mutational landscape of primary lymphomas of the central nervous system determined by whole exome sequencing

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Primary lymphomas of the central nervous system (PCNSL) are aggressive B cell lymphomas confined to the central nervous system associated with a poor prognosis. They are classified as diffuse large B cell lymphoma (DLBCL) but there is evidence that they have unique features with respect to their molecular features and pathogenesis as compared to systemic DLBCL. To allow deeper insight into this malignant disease, we performed whole exome sequencing in 9 PCNSL. Excluding single nucleotide polymorphisms (SNP) this analysis detected a median of 728 aberrations per lymphoma (range: 521-958). A total of 1809 of these mutations (median: 203, range: 139-253) were potentially protein-changing. The spectrum of somatic point mutations was conserved, with G>A/C>T transitions being the most frequent events. With regard to mutational processes, we identified three distinct mutational signatures contributing to a significant number of somatic mutations in the PCNSL analyzed. Comparison of previously generated PCNSL and systemic DLBCL gene expression profiles revealed 22 of the mutated genes identified to exhibit a differential mRNA expression. Considering SNP array data of PCNSL, a total of 34 genes mutated in at least two PCNSL are involved in recurrent genomic imbalances and partial uniparental disomies. Thus, novel gene candidates potentially contributing to lymphomagenesis were identified. Otherwise, the genes recurrently targeted by mutations widely resembled those recurrently mutated in extracerebral DLBCL. This study provides novel insights into PCNSL mutational processes, which might be of relevance in the pathogenesis of this lymphoma entity.

P-CancG-057

Mutational landscape of ALK-positive diffuse large B-cell lymphoma

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Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous entity of mature aggressive B-cell malignancies. We have recently described a very rare subtype of DLBCL characterized by activation of the ALK gene through chromosomal translocation which particularly affects children and young adults (Gascoyne et al., Blood, 2003; Gesk et al., Leukemia, 2005). Here we characterized the mutational landscape of an ALK-positive DLBLC cell line with a complex near-tetraploid karyotype using a combination of SNP array analysis and exome sequencing. Custom exome sequencing of the ALK-positive DLBCL cell line identified a total of 456 somatic mutations. Of those, 30% (135/456) were protein changing including 86% missense and 8% stopgain mutations. To identify those mutations which might be driver mutations we applied different filters. As the tetraploid karyotype carried the supposedly initiating ALK-translocation in duplicate, we can conclude that the

malignant transformation occurred before the polyploidization. By using the mutated allele frequency (MAF) we can, thus, distinguish late occurring mutations from early occurring mutations, as e.g. a MAF of ~1 means that all alleles carry the mutations which points to an early mutation event. In contrast, a low MAF indicates a late event likely derived from culturing. Therefore, we eliminated those mutations with a MAF lower than 0.3. Further filter criteria were the occurrence of the mutation within a functional domain, a prediction indicating the mutation to affect protein function, a previously described association of the mutated protein with cancer and/or lymphoma, the detection of mutations in the same gene in other DLBCL and overlap with known copy number changes derived from SNP array analyses including the mutated gene. Applying these criteria we came up with 20 possible candidate genes which might play a role in development of ALK-positive DLBCL. Using Sanger sequencing we were able to validate all these 20 candidate gene mutations as somatic events in the tumor cell line.
These results offer a starting point for the identification of the gene(s) relevant for the development and progression of ALK-positive DLBCL. To unravel the clinical significance of the identified candidate genes further studies on primary tumor tissues have to be performed.

P-CancG-058

RAD51C unclassified variant and a pathogenic MSH2 mutation in a male patient with breast and pancreatic cancer

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The cumulative risk for breast cancer is 10% for females and less than 1‰ for males in the general population. A monogenic cause is suspected in about 5-10% of these cases. An increased risk for breast cancer has been reported for male carriers of mutations in the BRCA1 and BRCA2 genes. However, the contribution of mutations in other cancer related genes to male breast cancer remains unknown.
We report on a 57-year-old man who was diagnosed with pancreatic cancer at the age of 52 years and with breast cancer at the age of 53 years. Family history showed breast and multiple ovarian cancers in female relatives, as well as early-onset colon cancers and further malignancies suggestive of Lynch syndrome (HNPCC).
The pathogenic mutation c.1661+1G>A in the MSH2 gene was detected in our patient, thus confirming the diagnosis of Lynch syndrome. Furthermore, analysis of the BRCA1, BRCA2 and RAD51C genes was performed in our patient as well. BRCA1 and BRCA2 analysis yielded normal results. In the RAD51C gene the previously described possibly damaging missense mutation c.790G>A (p.Gly264Ser) was identified.
Patients with Lynch syndrome are predisposed to pancreatic cancer and the cumulative risk may be increased approximately 8 to 9 fold. The risk for ovarian cancer for carriers of a mutation in the MSH2 gene is estimated at about 8%-11%. However, studies have not consistently demonstrated a higher than expected incidence of breast cancer in Lynch syndrome. It has been shown that mutations in the RAD51C gene are associated with a markedly increased risk for ovarian/breast cancer. Therefore, a possible functional role of the mutation c.790G>A in the RAD51C gene in the pathogenesis of breast cancer in our patient needs to be taken into consideration.

P-CancG-058a

Beyond BRCA1 and BRCA2: results from screening 94 genes in a large cohort of patients with familial breast and ovarian cancer.

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Background. Breast and ovarian cancer (BC/OC) predisposition, often seen in families with a high incidence of BC or young patients, has been associated with a number of high-, moderate-, and low-penetrance susceptibility genes. Prior to the introduction of next generation sequencing (NGS), only a small subset of these susceptibility genes (mostly BRCA1, BRCA2 and RAD51C) have been sequenced on a routine basis. With the advent of NGS-based panel sequencing, a routine screening of all genes currently associated with familial breast cancer became feasible. Here we report the results of panel-based screening of 94 genes that have been associated with hereditary cancer predisposition.
Method. Selection criteria for patients to be included in this study were defined by the German Consortium for Breast and Ovarian Cancer. They include, for instance, the number of cases within the family, the age of onset and the occurrence of ovarian cancer. High risk patients with previously excluded mutations in BRCA1 and BRCA2 were also included in the study. NGS was performed on an Illumina MiSeq sequencer, with 150 bp paired end sequencing chemistry. Target enrichment was performed with the Illumina TruSight cancer panel, which includes 94 genes associated with both common (e.g., breast, colorectal) and rare cancers (http://www.illumina.com/products/trusight_cancer.ilmn).
Results. In 28 % of the patients, BRCA1 or BRCA2 variations have been found. These were either clearly pathogenic protein truncating mutations (12 %) or very rare, unclassified missense variations with high probability of effect (16 %). In 39 % of the patients we found rare, unclassified missense variants in low penetrance susceptibility genes, especially NBN (nibrin) and ATM. In one case with early onset of breast cancer and no familial history, a putative splice relevant mutation in TP53 could be identified, which is currently being investigated on cDNA level. Despite the large set of 94 genes, 33 % of the patients did not reveal any convincing sequence variation. In order to complement the sequence variant detection by a comprehensive copy number analysis, a custom array has been designed that covers the same 94 target genes that are represented on the sequencing panel. Since many of the susceptibility genes are tumor suppressors, it is likely that exon losses or amplifications will also contribute to the mutation spectrum of these genes.
Conclusion. The extension of mutation screening beyond BRCA1 and BRCA2 reveals disease-causing mutations in high-penetrance genes, like TP53, as well as mutations in low-penetrance susceptibility genes. However, the enormous number of unclassified sequence variants and the detection of mutations and of carriers for hereditary diseases other than breast cancer predisposition poses a huge challenge for genetic counselling.

P-Clinical Genetics

P-ClinG-059

Experience of CARID: large scale sequencing and sharing data to decipher the genetics of autosomal recessive intellectual disability

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High-throughput DNA sequencing recently led to progress in understanding the genetics of autosomal recessive intellectual disability (ARID). It also has become evident that intellectual disability is extremely heterogeneous and that the majority of the underlying genes are still unknown. In Erlangen, we performed exome sequencing in 68 index patients, so far. We identified a pathogenic mutation in a known ARID gene in 11 families. We identified a convincing homozygous candidate mutation in novel genes in 33 families and in 5 of those partly proved the pathogenicity on functional level. Hence, we probably clarified ARID etiology in 44 families (65% of the samples). Nevertheless, proving the relevance of a particular gene for ARID is often lacking; therefore additional mutations in independent families are necessary.
For this purpose, we initiated the Consortium of Autosomal Recessive Intellectual Disability (CARID) to join forces and as a platform for exchanging information on candidate ARID genes. CARID includes 10 international groups interested in the genetics of ARID. So far, 174 families and 290 candidate mutations are registered in the CARID database. On average, each family has 1.67 homozygous candidate mutations. 7 genes have mutations in two different families. Based on this, Lincoln-Petersen analysis (mark and capture) predicts that the total number of ARID genes may be as high as 3,553 genes which is in agreement with recent literature. Identifying at least two mutations in each gene would be similar to the coupon collectors' problem. We simulated the number of needed families under the simplified assumption that the prevalence of mutations in different genes is equal and found out that about 39,429 cases are needed to identify 2 mutations in each of 3,500 genes. We also simulated this under the rather realistic assumption of an exponential distribution of the prevalence of mutations in genes; the number of needed families is then much higher; about 2.37 millions. Taken large scale sequencing efforts as a precondition, it becomes obvious that deciphering the genetics of ARID can only be approached via intensive cooperation and sharing of data worldwide.

P-ClinG-060

Prenatal Diagnostics in a Case of Inherited Mitochondrial DNA Mutation (m.13513G>A)

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An index case of infantile mitochondriopathy with hypertrophic cardiomyopathy, lactic acidosis and MRT changes was diagnosed with complex I defect in the muscle biopsy and subsequent mitochondrial DNA sequencing revealing the variant m.13513G>A. This variant results in a missense mutation in complex I subunit ND5: p.D393N and is known to be associated with mitochondriopathy. It was found with a heteroplasmy of 77% in muscle and 74% in blood.
In the healthy mother the sequence variant was found at a lower degree (30% in urine, 10% in saliva and 8% in blood) as compared to the index patient.
To test if the foetus of an ongoing pregnancy would be affected we decided to test chorion tissue material with respect to the m.13513G>A variant. In this tissue the variant was found with a heteroplasmy of 35% and thus in the range of the healthy mother. Pregnancy was continued and showed no abnormalities. In the newborn child testing of the variant revealed a heteroplasmy of 25% in blood. Up to now the child is apparently healthy.
The case report demonstrates that prenatal diagnosis is feasible in cases of mitochondrial DNA sequence variants. Prerequisite for this procedure is the estimation of the threshold for the respective sequence variant which requires diagnostic experience in cases with the same variant and investigation of different tissues in the pedigree.

P-ClinG-061

Severe end of Baraitser-Winter cerebro-fronto-facial syndrome due to a specific ACTB mutation

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Our patient is the second daughter of a healthy, non-consanguineous German couple. Pregnancy was complicated by hygroma colli, pleural effusions and ascites. She was born 38th gestational week with normal birth measurements. Hypertelorism, broad nasal root and tip, high arched eyebrows, long palpebral fissures with ectropion of lower lid, wide mouth, long flat philtrum, grooved chin, large posteriorly rotated ears, pterygium colli and low posterior hairline were described. In addition widely spaced nipples, pectus excavatum, prominent umbilicus, short 5th fingers and preaxial polydactyly of both feet were observed. Bilateral coloboma of iris, retina and chorioidea were diagnosed. MRI scan showed periventricular nodular heterotopias as a sign of a migrational defect. The patient walked with 2 ½ years, spoke with 3 years and seizures started at the age of 3 years. At the age of 10 and 24 years she was small, obese and microcephalic, had severe intellectual disability and was very shy.
Conventional and molecular karyotyping was normal, mutation analysis of ACTB gene showed the same heterozygous missense mutation as described in the first patient by Di Donato et al in 2013 (NM_001101.3:c.359C>T; p.Thr120Ile). Both patients resemble each other and show an identical malformation pattern and were both initially diagnosed with Fryns-Aftimos syndrome. The discovery of the mutations in the non-muscular actin genes as the cause of both Baraitser-Winter and Fryns-Aftimos syndrome led to the lumping of both conditions within the Baraitser-Winter cerebro-fronto-facial syndrome. Although no clear genotype-phenotype correlation could be made so far, the mutations affecting Thr at position 120 in actin beta seem to cause the most severe end of the clinical spectrum. In contrast, the same amino acid change in actin gamma presents with a significantly milder phenotype and is not associated with any malformation additional to pachygyria.
Reference: N. Di Donato et al., Eur. J. Hum. Genet. 2013, 1-5

P-ClinG-062

Whole exome sequencing indentifies CCDC22 missense mutation as a cause of X-linked recessive Ritscher-Schinzel / 3C syndrome

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Background Ritscher-Schinzel (RSS) syndrome / 3C (cranio-cerebro-cardiac) syndrome (OMIM#220210) is a rare and clinically heterogenous developmental disorder characterized by craniofacial abnormalities, congenital heart defects, and cerebellar brain malformations. Affected individuals have severe developmental delay. A recent study of Canadian cohort identified mutations in KIAA0196 gene, which encodes WASH complex subunit strumpellin, being a cause of a form of RSS/3C syndrome. We have searched for genetic causes of the RSS/3C like phenotype in the Austrian family with two affected children.
Methods To search for disease-causing mutation, whole exome sequencing (WES) was performed on samples from parents and two affected male children. Prior to WES, CGH array comparative genomic hybridization was applied. Validations of WES and segregation studies were done using routine Sanger sequencing.
Results Exome sequencing detected revealed a missense mutation (c.1670A>G;) in exon 15 of CCDC22 gene, which maps to chromosome Xp11.23. Western blots of immortalized lymphoblastoid cell lines (LCLs) showed decreased expression of CCDC22 and an increased expression of WASH1 but a normal expression of strumpellin and FAM21 in the patients cells.
Conclusion We identified a mutation in CCDC22 gene as the cause of the form of RSS/3C syndrome characterized in the described family. A hypomorphic mutation in CCDC22 was previously reported in association with the case of syndromic X-linked intellectual disability, which had phenotypic overlap with RSS/3C syndrome. Thus, different inactivating mutations affecting CCDC22 associate with RSS/3C syndrome like phenotypes.

P-ClinG-063

NGS panel for diagnostics of myofibrillar myopathies

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Myofibrillar myopathies (MFMs) have in common a slowly progressive weakness of distal and proximal muscles with usually late onset and typical morphological alterations starting at the Z-disk of muscle fibres. Known causative genes of MFMs are DES, CRYAB, MYOT, LDB3 (ZASP), FLNC and BAG3. Mutations in FHL1, DNAJB6 and TTN have also been reported as causative for MFM, but can also cause other subtypes of myopathies. Inheritance is mostly autosomal dominant except for X-linked FHL1 mutations and rare autosomal recessive mutations in DES or CRYAB.
We developed a NGS panel with eight MFM genes and applied it for 36 clinicopathologically diagnosed MFM patients. The coding exons of all thus far known causative genes of MFM except the large TTN gene were included in the panel. Target enrichment was performed with the Access Array System of Fluidigm followed by sequence analysis on Roche's GS Junior. NGS data were analysed with GenSearch NGS (PhenoSystems) and variants confirmed by Sanger sequencing. Twenty-four patients were analysed by this panel. In 12 additional patients, only FLNC was sequenced by this method since mutations in all the other genes had previously been excluded by Sanger sequencing. Exon 343 of TTN (NM_001267550) comprising a mutation hot spot for MFM was screened separately by Sanger analysis in all 36 patients.
Five of the patients were diagnosed with a causative heterozygous mutation. The known DES mutation c.1049G>C, p.Arg350Pro (rs57965306) was found in two patients. In another patient the known ZASP mutation c.494C>T, p.Ala165Val (rs121908334) was identified. A further patient was diagnosed with the CRYAB mutation c.116C>T, p.Pro39Leu which has not been described before, but was uniformly predicted as damaging by tools like SIFT, PolyPhen-2 and MutationTaster. The fifth patient was diagnosed with the BAG3 variant c.626C>A, p.Pro209Gln. It has not been described before, but prediction tools classify it as disease causing and a C>T (Pro>Leu) exchange at the same position is known as causative for MFM (rs121918312).
In three further patients, the variants could not reliably be classified as pathogenic or polymorphism and were categorised as unclassified variants (UVs). In two patients, different heterozygous variants were found in the TTN gene. The variant c.95297C>T, p.Ser31766Phe is known as rs191484894 with a minor allele frequency of 0.2 % and was found three times in the 1000 Genomes Project. The other variant, c.95351C>T, p.Ala31784Val, has not been described before. Both are predicted as causative. The FLNC variant c.6595G>A, p.Gly2199Arg is predicted inconsistently and not annotated in any database.
In total, five pathogenic mutations and three UVs were found with this panel for MFMs comprising eight genes and a hot spot TTN exon. The genes DNAJB6 and FHL1 provided no variants during this study of 36 patients. The panel has proven to be a fast and practical method for the diagnostic screening of several MFM genes in parallel.

P-ClinG-064

Next-generation sequencing detects an ASXL3 stop mutation (Bainbridge-Ropers syndrome) in a patient with intellectual disability but untypical symptoms

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Bohring-Opitz syndrome (BOS, OMIM 605039) is characterized by distinct craniofacial features and postures, feeding problems, intrauterine growth restriction, failure to thrive and severe intellectual disability (ID) and is associated with mutations in ASXL1. Mutations in its ortholog ASXL3 lead to a Bohring-Opitz syndrome like phenotype (Bainbridge-Ropers syndrome, OMIM 615485). Affected children show a very variable clinical phenotype with BOS-like features.
We report on a 13 year old Turkish boy from non-consanguineous parents with prenatal regular growth. He had postnatal microcephaly, severe ID, hypertrichosis, hypotonia of the trunk, feeding problems, crowded teeth, alternating periods of apnoe and hyperventilation and postnatal short stature (<P3). He did not speak and was unable to walk. Defining features for Bohring-Opitz syndrome like nevus flammeus of the forehead, seizures and intrauterine growth restriction were not present. Karyotyping, SNP array, diagnostics for Smith-Lemli-Opitz syndrome, ATRX syndrome, CDG syndrome and metabolic disorders gave normal results.
We used next-generation sequencing for diagnosis using Kingsmore panel and detected a de novo heterozygous stop mutation in the ASXL3 gene in exon 11 (c.G1369T; p.E457*). The mutation is located in a region with high vertebrate conservation, and where previously reported mutations had severe phenotypic effects.
Considering the highly conserved protein position together with already published pathogenic mutations (Bainbridge et al. Genome

Medicine 2013, 5:11) we consider this de novo stop mutation as disease causing. This example shows clearly that a patient with untypical phenotype can be diagnosed using panel diagnostics.

(3) Mohler PJ et al., 2004, Proc Natl Acad Sci USA 2004
(4) Kapplinger et al, 2009, Heart Rhythm
(5) Kiehne, N., Dissertation FB Biologie der TU Darmstadt 2011

P-ClinG-065

Genotype-phenotype dilemma in a patient with the homozygous SCN5A mutation E1053K

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Background:
Mutations in the cardiac sodium channel gene may cause either Brugada syndrome (BrS, loss of function), long qt-syndrome (LQTS, gain of function), dilated cardiomyopathy or progressive conduction disease. Usually from the electrocardiographic phenotype either a gain or loss of function can be supposed. In most of the inherited cardiac arrhythmias syndromes phenotypical expression can be very variable. In particular this is often the case in cardiac sodium channelopathies, where in some cases SCN5A mutations are not directly causal for the occurrence of BrS (1, 2).
Case: A young female patient without any known cardiac disease suffered from sudden cardiac death while sleeping at the age of 16 years. Autopsy did not reveal any explanation for her sudden death. By sequencing of exon spanning region of the genes KCNQ1, KCNH2, SCN5A, KCNE1 and –E2, KCNJ2, SCN4B, PKP2, DSP, DSG2, DSC2 and most of the exons (3,8,10,12,14,15,37,41,44-47,49,50, 83,88,89,90,93-97,99,100-105) of RYR2 gene, a mutation in the SCN5A gene (p.E1053K, heterozygous) could be detected.
This mutation was previously described in a Patient with BrS (3) as well as in a patient with LQTS (4) and in a patient with syncope and the suspicion of a latent dilated cardiomyopathy in MRI (5).
Calculation of the functional effect with PolyPhen2, Mutation taster and SIFT did not show consistent results (PolyPhen-2 possibly damaging 0,824; Mutation Taster: disease causing 0,9999; SIFT: tolerated 0,07).
Cascade family screening revealed a homozygous carriership of this mutation in the 41year old aunt and a heterozygous carriership in the mother of the index patient.
Suprisingly, a thorough clinical examination including resting ECG, Ajmaline testing, exercise ECG, cardiac MRI did not reveal any pathological phenotype in the homozygous carrier.
Unfortunately, the mother of the deceased girl refuses any clinical examination at present and their parents have already died for non-cardiac causes.
The SCN5A H558R polymorphism, known as a genetic modifier in SCN5A mutation carriers, could be excluded in the deceased as well as in the homozygous mutation carrier.
Conclusion: Until now, genetic information in this case was even more confusing than helpful for risk stratification of the remaining family members. Either the mutation p.E1053K is not the disease causing mutation or it is only disease causing in the context of a specific genetic or other unknown background what remains currently unclear.

(1) Probst et al., Circ Cardiovasc Genet 2009
(2) Postema et al., J Cardiovasc Eletrophysiol 2011

P-ClinG-066

A de novo 2p15 microduplication with global development delay and muscular hypotonia

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Background
Microdeletion syndrome 2p15-16.1 was first described in 2007 and goes along with intellectual disability, autistic features, structural brain anomalies, microcephaly and distinctive craniofacial dysmorphism. Literature search for microduplication 2p15 does not reveal any case reports so far. The Decipher database includes 10 cases of microduplication of the 2p15 region, two of which occurred de novo and are similar in size to the duplication of the propositus.
Case report and results
We saw a 23 month old boy with global development delay and muscular hypotonia. Sleeping and feeding difficulties were reported during the first two years of life. Solid food was not tolerated until the age of 18 month. Physical examination showed normal measurements and several nonspecific minor facial anomalies. The family history was unremarkable except febrile seizures in the father and three paternal aunts. By Molecular Karyotyping we detected a de novo duplication of 0.9 Mb in size on chromosome 2 arr[hg19] 2p15(61,406,990-62,311,815)x3dn. The duplicated region comprises 6 genes: AHSA2, CCT4, COMMD1, FAM161A, USP34, and XPO1.
Conclusions
We found a de novo microduplication 2p15 in a patient with global developmental delay and muscular hypotonia. The patient and two comparable cases reported in the Decipher database share the common feature of developmental delay. We suggest that the microduplication at 2p15 might be the cause for the observed global developmental delay of the patient.

P-ClinG-067

Partial duplication of ZFHX4 causes a recognizable phenotype with congenital ptosis and intellectual disability

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Background: Microdeletions in 8q21.11 have been associated with a recognizable phenotype including intellectual disability, bilateral ptosis, macrocephaly and prominent low set ears. The region of overlap from individuals with very similar phenotypes comprises three genes, MRPL9P1, LOC100192378, and ZFHX4. The loss of the latter gene was assumed to be responsible for the phenotype.
Case report and results:
The patient is the only child of non-consanguineous Caucasian parents. The child presented prenatally with a symmetric ventriculomegaly and white matter cysts detected by fetal MRI. The cysts could not be seen anymore, when the MRI was repeated postnatally at the age of four months. At the age of 1 year the girl showed developmental delay, bilateral ptosis, macrocephaly and prominent low set ears. Her mother also showed bilateral ptosis, but none of the other symptoms.
By molecular karyotyping we found an intragenic duplication of approximately 30 kb affecting two exons of ZFHX4 (arr[hg19] 8q21.11(77.606.817-77.636.835)x3mat). Customized array CGH revealed that the mother was a carrier of the duplication in a mosaic form.
Conclusions:

It seems very probable that the duplicated fragment was inserted into ZFHX4. This should have led to the disruption of this allele and therefore caused the phenotype. Our data therefore support the hypothesis that mutations in ZHFX4 cause a recognizable phenotype.

P-ClinG-068

Unexpected molecular genetic findings by Next-Generation-Sequencing in a young ataxia patient.

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Background: An 18 year-old patient of non-consanguineous parents had experienced ataxic symptoms since age 16. The symptoms in the once excellent student were accompanied by progressive dementia, psychiatric changes, ocular apraxia, vertical ophthalmoparesis and non-specific elevation of liver enzymes, lactate and CK. He has a 14 year old brother with similar symptoms. Her father and half-brother also have psychiatric symptoms, the half-brother suffers from schizophrenia, autism and facial hypotonia.
Material and Methods: The patient was evaluated for Niemann-Pick-type C disease and SCA17 but no mutation could be identified. His DNA was subjected to Next Generation Sequencing of 128 ataxia-associated genes on the next diagnostic level.
Results: The patient was found to carry a published autosomal dominant Emery Dreyfuss Muscular Dystrophy type 4 (EDMD4) mutation in SYNE1. Moreover, he carried heterozygous mutations in WFS1 and PEX7, and a potential splicing variant in VPS13A. Of those, only the WFS1 mutation was also present in his brother. All mutations had been inherited through the mother. VPS13A alone has not been tested in all first-grade relatives, yet.
Conclusion: The unexpected diagnosis of EDMD4 was made in the patient explaining some but not all of his symptoms. Post-hoc, this could be confirmed by further clinical investigations. Moreover, NGS has provided a few more equally surprising differential diagnoses that will allow a targeted in-depth clinical evaluation of the patient and his brother. Afterwards, the most promising candidate genes will be completely evaluated by complementary sequencing and MLPA analysis. There is some evidence that at least two different monogenic disorders segregate in the family. Segregation analysis of all variants is warranted in the rest of the patient's family.

P-ClinG-069

Clinical variability in a family with EFTUD2-associated mandibulofacial dysostosis, Guion-Almeida type

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Mandibulofacial dysostoses (MFD, OMIM 610536) comprise a clinically and etiologically heterogeneous group of conditions which are caused by an improper development of the first and second branchial arches. Distinguishing the different types of MFD can be challenging as many entities are not well characterized and the etiologies of many MFD types remain unknown. Recently, heterozygous mutations in the EFTUD2-gene (elongation factor Tu GTP binding domain containing 2-gene), encoding for a spliceosomal GTPase, were identified as the cause for mandibulofacial dysostosis, Guion-Almeida type (MFDGA) (Lines et al., 2012). MFDGA is a rare craniofacial condition originally characterized by severe and progressive microcephaly, choanal atresia, maxillary and mandibular hypoplasia, microtia, and developmental delay. In addition, Gordon and colleagues found that 8 out of 10 patients presenting with MFDGA had esophageal atresia (Gordon et al., 2012). Further descriptions of patients with EFTUD2-mutations suggested that microcephaly is a variable phenotypic feature of MFDGA (Luquetti et al., 2013; Voigt et al., 2013). One third of the patients present with thumb-anomalies so that it remains to be determined whether MFDGA should be re-classified as an acrofacial dysostosis (Voigt et al., 2013).
We now report three individuals, a mother (patient 1) and her two sons (half-brothers, patients 2 and 3) with heterozygous EFTUD2-mutations. All three family members presented with mandibular hypoplasia. Both boys had developmental delay and microcephaly. Patient 2 also presented with confirmed sensorineural hearing loss, a submucous cleft palate, downslanting palpebral fissures, hypoplastic maxillary and zygomatic bones, microtia, cup-shaped ears and bilateral preauricular skin tags. Patient 3 had suspected hearing loss, abnormally shaped helices, but did not show any of the other features seen in patient 2. Interestingly, the mother presented with a normal head circumference. The clinical features of these patients underscore the phenotypic and intrafamilial variability of EFTUD2-associated mandibulofacial dysostosis and emphasize that microcephaly is not an obligatory feature. The observed variability might suggest that MFD caused by EFTUD2-mutations is an underdiagnosed entity and must be included in the differential diagnosis.

P-ClinG-070

A novel case with mosaic genome wide paternal uniparental disomy: a rare condition which is possibly underdiagnosed?

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Mosaic genome wide paternal uniparental disomy is a rare condition which is viable only in the presence of normal biparental cells (androgenic/biparental). So far, only thirteen cases have been described in the literature. Among others, mosaic genome wide paternal uniparental disomy leads to dysregulation of imprinted genes and can therefore result in complex phenotypes including partial features of various imprinting disorders. Interestingly, in almost all cases reported the initial clinical diagnosis was Beckwith-Wiedemann syndrome. Here we report on a newborn girl presenting prenatally with placentomegaly and polyhydramnion, and postnatally with macrosomia, adrenocortical macrocyst, severe hypoglycemia, hyperinsulinism, hepato- and pancreatomegaly, features typical for BWS. She also showed jejunum atresia, progressive chylothorax and chylous ascites, features not described in BWS so far. The child died at the age of 23 days due to abdominal compartment syndrome and cardio-respiratory insufficiency. Because of the initial clinical diagnosis of BWS, MS-MLPA and microsatellite analysis (MSA) in peripheral blood was performed, and a paternal uniparental disomy (UPD) for chromosome 11 was detected. CGH array analysis revealed in addition a triplication of about 350 kb on 14q32.13 in the patient and a duplication of the identical size in the father. To further clarify this finding, MSA for chromosome 14 was performed and showed paternal UPD for chromosome 14 also. Subsequently, extended MSA for other chromosomes was performed and showed paternal UPD for each chromosome tested, leading to the molecular diagnosis of a genome wide paternal uniparental disomy (GWpUPD), which could be confirmed by SNP array analysis. In the MSA analyses, a very faint peak for a maternal allele was visible for some loci, indicating that the patient is a somatic mosaic for the GWpUPD and has a low degree of biparental cells in blood. To learn more about the degree of mosaicism and its tissue distribution, we performed methylation

analysis at differentially methylated regions of five imprinted loci by high throughput bisulfite sequencing on the Roche 454 GS Junior system in blood, pancreas and heart. By this we found that the patient has 10-20% biparental cells in pancreas, ~20% biparental cells in blood, but ~70% biparental cells in heart, indicating that the degree of mosaicism varied between different tissues and that the amount of GWpUPD cells in pancreas and blood is very high and might explain the severe phenotype of the patient. In conclusion, the BWS phenotype seems to be the predominant phenotype in our patient and in almost all patients with mosaic GWpUPD reported so far. Without the molecular finding of the triplication on chromosome 14 in our patient the GWpUPD would probably not have been recognized. Thus, it is possible that mosaic GWpUPD is underdiagnosed in patients with clinical signs of Beckwith-Wiedemann syndrome plus additional clinical features.

P-ClinG-071

A Homozygous ARL13B Mutation Leads to Joubert Syndrome with Renal Cysts and Retinal Dystrophy

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Introduction: Joubert syndrome is an inherited ciliopathy clinically characterized by muscular hypotonia, cerebellar ataxia, oculomotor apraxia, breathing anomalies, and developmental delay. Due to a distinct cerebellar and brain stem malformation, axial MRI scans typically show the "molar tooth sign". Additional features such as kidney anomalies, retinal dystrophy, polydactyly, and other findings consistent with ciliopathies are frequently observed. So far, biallelic recessive mutations in more than 20 autosomal genes and mutations in 1 X-linked gene have been identified. To date, only four patients from two families have been published with mutations in ARL13B. None of them had renal abnormalities, and only one patient had a "nonspecific pigmentary retinopathy".

Case report: We present a 20-years-old female patient with Joubert syndrome as well as renal cysts and cone-rod dystrophy. Screening for mutations in CEP290, AHI1 and TMEM67 revealed no causative mutation. Based on suspected consanguinity of the parents, an SNP array analysis was performed and showed homozygosity for a chromosomal region on 3q11.2. Subsequent sequencing of the ARL13B gene which is located in the homozygous region revealed a homozygous mutation, c.598C>T (p.Arg200Cys). The mutation results in an amino acid change at a conserved position within the coiled-coil domain and is predicted to alter protein function. Since the same mutation has been identified before in another patient with Joubert syndrome, it is supposed to be pathogenic.

Conclusion: We present a new patient with ARL13B-associated Joubert syndrome. To our knowledge, this is the first ARL13B patient with renal cysts and cone-rod dystrophy, expanding the phenotypic spectrum of patients with ARL13B mutations. Therefore, presence of renal cysts and cone-rod dystrophy should not exclude ARL13B from genetic analyses. Furthermore this case is an example for the need of a carefully recorded family history. In case of consanguinity, homozygosity screening can still be helpful to identify causative mutations in autosomal recessive disorders, also in times of gene panel diagnostics.

P-ClinG-072

Analysis of a novel gene for Noonan syndrome, RIT1 in patients suggestive of Noonan syndrome and negative for other known Noonan syndrome genes

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Noonan syndrome (NS) is among the most common Mendelian genetic diseases (approximately 1/2,000 live births). Most cases (50%-84%) are sporadic. This multisystem disorder showing autosomal dominant inheritance is caused by mutations dysregulating the RAS-MAPK (RAS/mitogen-activated protein kinase) pathway. It is characterized by short stature, heart defects, pectus excavatum, webbed neck, learning problems, cryptorchidism and facial dysmorphism. RASopathies describe a class of disorders, including Noonan syndrome (NS), Costello syndrome (CS), cardio-facio-cutaneous syndrome (CFC), neurofibromatosis type 1 (NF1) and others that share a common pattern of congenital anomalies. Recently a new gene, RIT1, encoding a member of the RAS subfamily was identified. Gain-of-function mutations in RIT1 gene were found in 9% of individuals with Noonan syndrome or a related condition but with no detectable mutations in known Noonan-related genes. Clinical manifestations in the RIT1-mutation-positive individuals were consistent with those of Noonan syndrome.

In order to confirm these findings we examined 11 patients suspicious of Noonan syndrome/RASopaties including one abortion with Hydrops fetalis and one prenatal case showing increased nuchal translucency > 6mm in the 15.th week of gestation and normal karyotype. In an initial study all patients were screened for mutations in well known genes for Noonan syndrome or RASopathies. We designed a RASopathies/Noonan syndrome gene panel comprising 12 chromosomal genes (including PTPN11, SOS1, RAF1, KRAS, BRAF, NRAS, MAP2K1, CBL, SHOC2, MAP2K2, HRAS and NF1). Sequencing was performed on the Illumina MiSeq Next-Generation Sequencing platform. Data analysis was performed using the CLCbio workbench (v6.5) and custom developed Perl scripts. The target regions, in total encompassing 46,485bp, were enriched via in-solution oligonucleotide hybridization and capture (Illumina TSCE). On average, 95-97% of the reads could be mapped to the human genome (build hg19), of which between 63-66% were on target. Target regions with a coverage level of less than 20x were re-analyzed by Sanger sequencing to obtain complete coverage of all coding regions and adjacent splice sites (-20/+10). No causal mutations could be identified in all patients.

In a second step all patients were screened for mutations in RIT1 gene (NM_006912.5 and NM_001256821.1) via Sanger sequencing.

P-ClinG-073

A novel homozygous truncating mutation in the ALS2 gene leading to juvenile primary lateral sclerosis

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Primary lateral sclerosis affects the upper motor neurons. The age of onset of the juvenile form is less than 25 years. It generally progresses over one to two decades of life. Juvenile primary lateral sclerosis is an autosomal recessive form and is caused by truncating mutations in the ALS2 gene presumably leading to a loss of function of the gene product. ALS2 codes for ALSIN which is a guanine nucleotide exchange factor for the small GTPase RAB5 and is involved in intracellular endosomal trafficking.

We report here on a 12-year-old girl with ALS2 due to a novel homozygous truncating mutation in the ALS2 gene. The patient is the only

child of a healthy consanguineous couple. She started to walk without support at the age of 11 months. However, at the age of 17 months, she still could not properly run and felt very insecure in her movements. A computed tomography at this age revealed osteochondrosis with discopathy (L 1/2) and discopathy with small medial protrusion at L5/S1. She began to speak first words at the age of 12 months. She increasingly lost the ability to walk and had to use a rollator at nursery age. At the age of 8 years, her clubfoot and leg muscles were corrected surgically. After that, her muscle power further decreased. Now, at the age of 12 years, she attends a special education school and has increasing difficulties in handwriting. She is also weel chair bond and has difficulties to speak and swallow.
We examined the patient´s DNA for causative mutations using a gene panel that allows parallel sequencing of 1222 genes known to be involved in rare, recessive pediatric genetic diseases, mental retardation and related disorders. It had been developed in the group of Hans-Hilger Ropers at the Institute for Molecular Genetics. We identified a homozygous insertion of guanine at position 3783 (c.3783insG) in exon 24 of the ALS2 gene. This insertion leads to a frameshift (p.1261fs*6X) and a premature stop codon at aminoacid position 1267 (wt. 1658) of the ALSIN protein. The resulting C-terminally truncated ALSIN protein lacks the VPS9 (vacuolar protein sorting 9) domain that is essential for the activation of RAB5 via the guanine-nucleotide exchanging reaction and the endosomal localization of the ALSIN protein. In a previous study, a patient with another homozygous truncating mutation at a nearby position (p.M1206X) also resulting in a loss of the VPS9 domain showed a clinical presentation very similar to that of the patient reported here. Overall, our data strongly support the usage of the gene panel mentioned above for diagnostic purposes and it supports the role of ALS2 mutations in neurodegenerative disorders.

P-ClinG-074

The phenotype of proximal microdeletion 3p25.3: two patients with intellectual disability, epilepsy and stereotypic behaviour

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Background
Interstitial deletions in 3p25.3 ranging in size from 0.64 to 6.3 Mb have been published for 6 patients. These patients show variable features of the 3p- syndrome consisting of intellectual disability (ID), short stature, microcephaly, hypotonia, facial dysmorphism and congenital malformations such as congenital heart disease. Microdeletions of the proximal part of 3p25.3 have not been reported so far.
Clinical reports
Patient 1 is a 3-year-old girl affected by severe developmental delay (DD) accompanied by absence of speech, generalized epilepsy with absence seizures, mild truncal ataxia and stereotypic hand movements. Patient 2 was referred at 5 years of age because of severe DD, exhibiting an inability to speak until the age of 4, myoclonic seizures, impaired social interaction, stereotypic behavior with handwringing and ataxia with a broad-based gait. Both children showed normal growth. Dysmorphism or congenital malformations could not be detected. The respective brain MRIs were unspecific and normal.
Results
Molecular karyotyping showed overlapping de novo interstitial deletions 3p25.3 of 1.125 Mb and 1.5 Mb in the two unrelated patients. The deletions are localized proximal to the published cases.
Discussion
Both patients have severe DD with absence of speech or significant delay in speech development, epilepsy and Rett-like stereotypic hand movements in common. In addition, both patients presented symptoms of ataxia leading to the differential diagnostic consideration of Angelman syndrome. The patients do not show facial features or malformations of the 3p- syndrome supporting that the responsible genes are localized more telomeric. DECIPHER indicates a further patient with a similar phenotype (Pat. 251803) who has an overlapping 1.33 Mb deletion in 3p25.3. The smallest region of overlap of these three patients is approximately 540 kb in size and contains five genes, including SLC6A1 and the 3' end of SLC6A11, two sodium- and chloride-dependent GABA transporters that may contribute to their neurodevelopmental and seizure phenotype.
Conclusion
Two novel patients and one patient reported in the Decipher database with overlapping microdeletions 3p25.3 show a similar phenotype with severe DD, epilepsy, ataxia and stereotypic behaviour in the absence of dysmorphism and major malformations. The deletion is assumed to be causative for the phenotype. The smallest region of overlap contains two sodium- and chloride-dependent GABA transporters.

P-ClinG-075

Germline PTPN11 mutation in a boy with megalencephaly-capillary malformation (MCAP) syndrome

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BACKGROUND: Megalencephaly-capillary malformation (MCAP) syndrome is an overgrowth syndrome which is diagnosed by clinical criteria. Recently, somatic and germline mutations in genes that are involved in the PI3K-AKT-pathway (AKT3, PIK3R2 and PIK3CA) have been described to be associated with MCAP and/or other related megalencephaly syndromes.
METHODS AND RESULTS: We performed trio-exome sequencing in a 6-year-old boy and its parents. Striking features were macrocephaly, cutis marmorata, angiomata, asymmetric overgrowth, developmental delay, midline facial naevus flammeus, toe syndactyly and postaxial polydactyly – thus, clearly an MCAP phenotype. Interestingly, we could deduce only one pathogenic de novo germline mutation in the PTPN11 gene, which has so far been associated with the Noonan syndrome, as well as the related LEOPARD syndrome. In the blood, even a deep sequencing approach did not reveal any alteration in the known megalencephaly genes. Deep sequencing results from saliva are still pending.
CONCLUSIONS: To our knowledge, this report is the first description of a PTPN11 germline mutation in an MCAP patient. Data from basic science suggest that some PTPN11 mutations might lead to the activation of the PI3K-AKT-pathway, which has been associated with megalencephaly syndromes.

P-ClinG-076

NGS based whole X-exome analysis reveals a novel WDR45 missense mutation in a family with 3 males with non-syndromic intellectual disability

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X-exome sequencing in an adult male patient with intellectual disability, epileptic seizures during childhood and otherwise normal somatic

development revealed a novel missense mutation c.698G>A; p.R233H at a highly conserved position in WDR45, a gene located in Xp11.23. Segregation analysis confirmed the presence of the mutation in his two similarly affected monocygotic younger twin brothers and their healthy mother with skewed X-inactivation (15:75). The mutation could be excluded in the maternal grandfather and both healthy maternal uncles of the patients.
De novo mutations located throughout the coding sequence in the autophagy gene WDR45 have been previously described in patients with a recently established subtype of neurodegeneration with brain iron accumulation (NBIA). Mutations in WDR45 have been associated with an X-linked dominant form of NBIA, predominantly affecting females and presumed to be nonviable in males with germline mutations. Most mutations were truncating, with the exception of two missense mutations affecting highly conserved residues. Similar phenotypes in few reported males and females have been attributed to somatic mosaicism in surviving males and skewing of X-inactivation of females (Haack et al. AJHG 91, 1144-1149, 2012; Saitsu et al. Nat Genet 45, 445-450, 2013).
Taking into account the highly conserved position together with the segregation mode in the family we consider the missense mutation c.698G>A; p.R233H to be probably pathogenic. A retrospective analysis of brain MRI in the index patient at the age of 10 years was not suspicious for iron deposition in the globus pallidus. The primary developmental delay and stable course of the disease until young adulthood and transmission of the mutation via a healthy carrier expends the clinical spectrum of the WDR45 associated phenotype in X-linked intellectual disability.

P-ClinG-077

A duplication of the HNF1ß gene as a rare cause of cystic kidney disease?

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Microdeletions in 17q12 including the HNF1ß gene and point mutations of the HNF1ß gene (17q12) are well known to cause MODY type V. Furthermore, a broad spectrum of renal malformations has been associated. The most common kidney manifestation is cystic dysplasia (type POTTER II). Renal agenesis and hypoplasia, hyperuricaemia, as well as other urogenital malformations may also be part of the phenotypic spectrum, which is then denoted as RCAD (Renal Cysts and Diabetes) syndrome. Further features like mild mental retardation and neurological symptoms have rarely been associated with the syndrome. However, so far no reliable genotype-phenotype correlation could be delineated. In about 50% of cases a deletion of the whole HNF1ß gene and the flanking chromosomal material including further genes like the LHX1 gene can be detected. Although break points are rarely determined because of the applied methods (Multiplex ligation-dependent probe amplification), studies including array data narrow down the minimal critical region of the deletion to app. 1.1 Mb or 1.5 Mb respectively. In contrast, patients carrying the reciprocal duplication of the 17q12 region frequently present with global developmental delay, behavioural disturbances, learning difficulties, epilepsy and/or autism but without kidney findings. Oesophageal atresia has been associated in two families. To our knowledge only one family carrying the 17q12 microduplication has so far been described with multicystic kidney dysplasia (Faguer et al. Kidney Int. 2011).
Here, we present the molecular and clinical data of a 21-year-old female with hypertension, diabetes and moderate chronic renal insufficiency with proteinuria. The patient suffered from seizures with onset at the age of 8 years and was afterwards adjusted to anticonvulsant treatment. Enlarged kidneys with multiple cysts were diagnosed in early childhood; renal ultrasound at the age of 20 showed only slight progression of renal impairment. Molecular analysis revealed a 17q12 duplication including the HNF1ß gene. We therefore assume that cystic dysplasia might also be associated to the microduplication in rare instances. To determine the exact break points of the duplication further studies are currently carried out.

P-ClinG-078

11p15-associated Imprinting Disorders: complex molecular findings require multilocus testing

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The chromosomal region 11p15 is a key player in molecular processes regulated by genomic imprinting. Genomic as well as epigenetic disturbances affecting the two imprinting control regions (ICRs) in 11p15 are associated either with Silver-Russell syndrome (SRS) or Beckwith-Wiedemann syndrome (BWS): SRS patients carry ICR1 hypomethylations, maternal 11p15 duplications or maternal uniparental disomies (UPD), whereas the opposite alterations are characteristic for BWS. In the last years, a growing number of patients affected by imprinting disorders (IDs) has been reported to show a hypomethylation at the two ICRs in 11p15 as well as at further imprinted loci on other chromosomes. The molecular basis of these multilocus methylation defects (MLMDs) is widely unknown, however an interaction between trans-localized imprinted genes via a so-called Imprinted Gene Network (IGN) has been suggested. Cases with other types of molecular disturbances than aberrant methylation (e.g. UPD, chromosomal imbalances) support this IGN concept. The complex molecular alterations as well as the overlapping and sometimes ambiguous clinical findings in ID patients often make the decision for a specific ID test difficult. As aforementioned, the 11p15 loci are consistently affected in all ID patients with more complex alterations, but the pattern of affected loci is nearly unpredictable. We therefore suggest to implement molecular tests in routine ID diagnostics which allow the detection of a broad range of (epi)mutation types (epimutations, UPDs, chromosomal imbalances) and cover the currently known ID loci. The need to apply multilocus tests (methylation-specific MLPA, MS-SNuPE) is corroborated by our experience from routine diagnostics in more than 710 patients referred as SRS or BWS: (a) Multilocus tests increase the detection rates as the tests ascertain numerous loci. Thereby the chance to identify even slight mosaic hypomethylation patterns is growing. (b) Patients with unusual phenotypes and unexpected molecular alterations will be detected. (c) The testing of rare imprinting disorders becomes more efficient and quality of molecular diagnosis increases. (d) The tests identify MLMDs.
In future, the detailed characterization of clinical and molecular findings in ID patients will help us to decipher the complex regulation of imprinting and thereby provide the basis of an individualized therapeutic management in IDs.

P-ClinG-079

Targeted and genomewide NGS data disqualify mutations in MYO1A, the „DFNA48 gene", as a cause of deafness

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MYO1A is considered the gene underlying autosomal dominant non-syndromic hearing loss type DFNA48, based on eight heterozygous mutations, including six small in-frame alterations and one nonsense mutation, reported in a single publication. By next-generation sequencing (NGS) targeting 66 deafness genes in 109 hearing-impaired patients, we identified three families that provide strong evidence against a causative role of MYO1A in inherited deafness: Two novel nonsense mutations (p.Tyr740* and p.Arg262*) and a previously described missense mutation were identified not only in the index patients in heterozygous state, but also in unaffected relatives. The hearing deficit in these families was clearly due to mutations in other deafness genes, MYO7A, EYA1 and CIB2, respectively. All but two of the altogether ten MYO1A mutations have been annotated in dbSNP, and population frequencies (dbSNP, 1000 Genomes and Exome Sequencing Project) above 0,1% contradict pathogenicity under a dominant model. Moreover, one healthy individual was even homozygous for the nonsense mutation p.Arg262*, compatible with a previously reported homozygous Myo1a knockout mice lacking any overt pathology. We conclude that MYO1A is dispensable for normal hearing and may even represent a non-essential gene. MYO1A adds to the list of „erroneous disease genes" which will constantly expand with increasing availability of data from large-scale sequencing projects. The potential of NGS to reveal such „false disease genes" is important to avoid pitfalls in diagnostics and genetic counseling.

P-ClinG-080

Renal fibrosis is the common feature of Autosomal Dominant Tubulointerstitial Kidney Diseases (ADTKD) caused by MUC1 or UMOD mutations

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For decades ill-defined autosomal dominant renal diseases have been reported, which originate from tubular cells and lead to tubular atrophy and interstitial fibrosis. These diseases are clinically indistinguishable, but caused by mutations in at least four different genes: UMOD, HNF1B, REN and, as recently described, MUC1. Affected family members show renal fibrosis in the biopsy and gradually declining renal function, with renal failure usually occurring between the 3rd and 6th decade of life.

In this study we describe 10 families and define eligibility criteria to consider this type of inherited disease, as well as propose a practicable approach for diagnosis. In contrast to what the frequently used term "Medullary Cystic Kidney Disease" (MCKD) implies, development of medullary cysts is neither an early, nor a typical feature, as analyzed by MRI.

Having found the putative genetic cause in three of ten families, the other seven families remained unclear. Two families were sufficiently large and we were able to collect blood samples from numerous members consecutively. We performed a genome-wide linkage analysis and subsequently a haplotype analysis narrowing down the location of the disease-linked locus. This analysis confirmed a significant 3.4 Mb locus at a predescribed locus on chromosome 1q21 for the first family. As the results of the second family coincide with this locus we had a shared linkage locus with an overall LOD-score clearly reaching genome-wide significance. Therefore, it is very likely that these families belong to the formerly classed MCKD1 disease. We performed whole exome sequencing for affected and healthy individuals of these two families. In a further attempt, we performed targeted genomic sequencing for the complete linkage locus at 1q21 for these two families and affected individuals of further families without mutation, which showed no segregating variants in any of the genes, including MUC1. However, it needs to be stressed that the VNTR region of the MUC1 gene is masked in both these analyses due to fundamental technological deficiency. Next to Sanger sequencing and semiconductor-based gene panel sequencing of the aforementioned four genes, we established SNaPshot minisequencing for the predescribed insertion mutation in the high GC-containing VNTR region of MUC1. In 3 and 4 families we found mutations in the UMOD and the MUC1 gene, respectively, leaving 3 families unsolved to date.

On the basis of clinical and pathological characteristics we propose the term "Autosomal Dominant Tubulointerstitial Kidney Disease" (ADTKD) as a new name for this entity. We anticipate that using this new terminology will enhance recognition and correct diagnosis of affected individuals, facilitate genetic counseling and stimulate research into the underlying pathomechanisms.

P-ClinG-081

A novel de novo mutation in PIK3CA in a boy with megalencephaly, hemangiomas and multiple anomalies

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We report on a male patient whose macrocephaly was noted prenatally. At birth, his length was 50cm, his weight 3870g and his head circumference 38.7cm (+3 SDS). Postnatally, he was readmitted due to hypoglycemia. He was also diagnosed with hyperbilirubinemia and two small ventricular septum defects which closed spontaneously within the first year of life; furthermore an umbilical granuloma, hypospadias, muscular hypotonia and multiple hemangiomata at several fingers and both ears were noted. His megalencephaly (+5.3 SDS at last visit, 3 y. old) and its resulting particular head formation characterized by dolichocephaly and long face had prompted MRT and CT analyses which excluded premature closure of cranial sutures, but revealed an Arnold-Chiari malformation. Moreover, he had cutis laxa particularly at the hands and feet and a suspected left doubled kidney. Gross motor development was delayed with sitting and crawling at 12-14 months and walking at 23 months. Fine motor activity and speech development were appropriate for his age. Genetic testing for several overgrowth syndromes and conventional and molecular karyotyping revealed no mutation. X-inactivation pattern in the mother was normal.

Recently, de novo germline or postzygotic mutations in three core components of the phosphatidylinositol 3-kinase(PIK)-AKT pathway have

been described in children with megalencephaly, capillary, cortical as well as distal limb malformations and mild connective tissue dysplasia (Rivière et al., Nat Genet. 2012). When screening mutational hot spots of these three genes in our patient, we identified a yet undescribed heterozygous de novo variant c.333G>C; p.Lys111Asn in exon 2 of PIK3CA. This variant is neither annotated in dbSNP (build 138) nor listed in NHLBI Exome Sequencing Project (ESP) or 1000 genomes database. The corresponding amino acid position is highly conserved in vertebrates and is located in a hydrogen-bonded turn between the PIK-ABD and PIK-RBD domains of the protein. For a mutation in the neighboring region (c.353G>A, p.Gly118Asp), significant structural alterations have been described (Orloff et al. Am J Hum Genet 2012). A further variant Arg -> Gln at amino acid position 88 has been described in patients with megalencephaly. It may disrupt the interaction between the PI3K-ABD domain and the N-terminal lobe of PI3K/PI4K kinase domain possibly affecting the conformation of the kinase domain. Indeed, functional data for this variant demonstrated increased lipid kinase activity resulting in constitutive PI3K signaling. Since the novel mutation p.Lys111Asn lies in a hydrogen-bonded turn between the PIK-ABD and PIK-RBD domain, it most probably also affects the conformation of the protein and therefore affects protein function.
We suggest that patients with megalencephaly, capillary malfomations and mild connective tissue dysplasia should be considered for mutation screening of members of the phosphatidylinositol 3-kinase(PIK)-AKT pathway.

P-ClinG-082

Highly variable intrafamilial manifestations of a CCM3 mutation ranging from acute childhood cerebral haemorrhage to late-onset meningiomas

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Familial cerebral cavernous malformations (CCM) are vascular malformations due to loss-of-function mutations in one of three genes, CCM1, CCM2 and CCM3. The disorder follows autosomal-dominant inheritance. Major symptoms are headaches, seizures, cerebral haemorrhages and focal neurological deficits. However, penetrance is reduced and clinical presentation may be highly variable as illustrated by the following three-generation family with a segregating CCM3 mutation.
The index patient presented at the age of 2 years with acute gait disturbance and a right-sided hemiparesis. Magnetic resonance susceptibility weighted imaging (MR-SWI) showed an acute intracerebral haemorrhage of a left-sided central cavernoma as well as multiple smaller cavernomas. The haemorrhagic lesion was treated surgically and the boy recovered from his neurological symptoms.
Family history revealed that the mother of the boy experienced a transient left-sided ptosis and miosis at the age of 22 which led to the detection of multiple supratentorial cavernomas. She is currently 41 years old and didn´t develop any further neurological symptoms. Physical examination showed several pinhead-sized red spots on her palms and bluish subcutaneous nodules on the arm, leg and waist.
The maternal grandfather had a history of intermittent blurred vision, dizziness, and tickling since his fifties. At the age of 55, he was diagnosed to have four extraaxial dural-based meningiomas which were treated with gamma knife radiosurgery. Cerebral cavernomas were not reported in the grandfather.
The tentative diagnosis of familial CCMs was made, and direct sequencing of the CCM3 gene in the index patient showed a typical yet previously unpublished heterozygous one basepair deletion in exon 7 (c.317delA) which is predicted to cause a frameshift and a premature stop codon (p.K106Rfs*20). The same mutation was found in the mother and in the maternal grandfather.
Until recently extra-axial dural-based MRI lesions in patients with CCM3 mutations were assumed to be cavernous angiomas, but so far no histopathological data were available. Surprisingly, Riant et al. recently showed that at least some of these lesions are histologically true meningiomas. This finding is of importance since meningiomas and cavernomas usually show different biological behaviour and require different treatment strategies.
The present case underlines the high clinical variability of CCM3 mutations and should raise awareness that CCM3 mutations are a potential cause of multiple meningiomas.

P-ClinG-083

Classical karyotyping vs molecular karyotyping (arrayCGH) in a case of trisomy 9 mosaicism

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Trisomy 9 mosaicism is considered to be a rare chromosomal abnormality with limited survival and a characteristic pattern of multiple anomalies. The features commonly associated with trisomy 9 include growth retardation, facial dysmorphisms, skeletal abnormalities, congenital heart disease and intellectual disability. More than 50 cases have been reported, most of which were diagnosed after birth.
We report a case of a one month old baby girl with craniofacial abnormalities, hydronephrosis and multiple contractures. ArrayCGH analysis was performed and detected trisomy 9 mosaicism in approximately 40% of the cells. Classical karyotyping of lymphocytes revealed trisomy in about 3% of the metaphases. Previous prenatal analysis of cell cultures from amniotic fluid had not shown the mosaic trisomy 9 constitution. Mosaicisms are expected to be exhibited at different levels in different tissues. In addition, cell culturing leads to a bias in terms of the ratio between trisomic and disomic clones and very likely underestimates the percentage of trisomic cells in conventional karyotyping. Our data show the importance of using uncultured tissue (amniotic fluid and blood) and the value of microarray technology in the assessment of mosaicisms.

P-ClinG-084

Investigation of the association of trinucleotide expansion in intron 2 of the TCF4 gene and Fuchs Dystrophy

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Fuchs endothelial corneal dystrophy (FECD) is a genetic disorder of the corneal endothelium. The late onset form of this disorder affects as much as 4% of the population in the USA over the age of 40 with symptoms such as corneal edema and visual acuity decreases up to complete blindness. Therefore, FECD is a significant reason of the corneal transplantation performed every year. The FECD is genetically heterogeneous. Repeat expansion and variations (e.g. rs613872) in the transcription factor 4 (TCF4) have been identified as a major contributor to the disease (Wieben et al. 2012, Stamler et al. 2013).
Up to now, we tested an association between the intronic TGC trinucletide repeat expansion in TCF4 and FECD in 16 affected patients and 81 control persons. The investigation was done by sequencing, ALF-investigations as well as special bidirectional triplet-primed (TP)-PCR. The TP-PCR is a method to analyze the presence or absence of repeat expansions. TP-PCR is easier and faster than southern blotting. The

TP-PCR is a PCR with three primers. This method is already used for diagnostic analysis of other repeat expansion diseases. Behind the expanded TGC the highly associated single nucleotide polymorphism, rs613872 in intron 3 of the TCF4 is under investigation.
We found in 14 FECD patients (87%) a TGC repeat expansion>50. Two patients (13%) had a normal repeat length <40. In comparison, 9 (11%) of the 81 unaffected controls showed a repeat expansion >50. 72 (89%) of control persons showed normal TGC repeats length < 40.
The single nucleotide polymorphism rs613872 in intron 3 of the TCF4 showed in 14 patient (87,5%) the heterogeneous genotype TG vs. 21/81 (24%) in unaffected controls. One patient (1/16; 6,25%) showed the genotype GG vs. 5 controls (5/81; 6%). One patient showed the genotype TT (1/16, 6,25%) vs. 60 probands in the control group (60/81;70%).
In summary, TP-PCR is a fast and sensitive method for the detection of the presence or absence of repeat expansion. The TGC repeat expansion in the TCF4 is strongly associated with FECD. A repeat length >50 is highly specific for the FECD disease.
Further, the genotype TG for the polymorphism rs613872 was found in 87,5% of patients. The correlation from polymorphism and repeat expansion regarding prediction and syndromes will be analyzed on the end of this study. We assumed that repeat expansion and the polymorphism rs613872 are disease causing for FECD. It is not yet clear whether rs613872 or the repeat expansions are causative for FECD.
Literatur:
Wieben et al. (2012): A Common Trinucleotide Repeat Expansion within the Transcription Factor 4 (TCF4, E2-2) Gene Predicts Fuchs Corneal Dystrophy; Plos One, November 2012, Volume 7, Issue 11
Stamler et al. (2013): Confirmation of the association between the TCF4 risk allele and Fuchs endothelial corneal dystrophy in patients from the Midwestern United States. Ophtalmic Genet. 2013 Mar-Jun; 34(1-2):32-4.

P-ClinG-085

Unique duplication 2p16.3p23.1 in a male newborn with growth retardation, tachypnoea, congenital heart defect and hypothyroidism

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Some 30 cases of partial trisomy 2p have been reported, most of them in the pre-microarray era as unbalanced segregational products of parental translocations. Growth retardation and intellectual disability (ID) were common symptoms but reported phenotypes show considerable variability. We report on a newborn with growth retardation, multiple congenital anomalies (MCA), tachypnoea, and hypothyroidism associated with a unique and pure duplication 2p16.3p23.1.
The patient is the first child of healthy, non-consanguineous, caucasian parents (maternal age 33 years, paternal age 36 years. The family history is unremarkable. The mother was treated with Levothyroxin throughout the pregnancy due to hypothyroidism. At 24 weeks of gestation sonography showed a ventricular septal defect, oligohydramnios and intrauterine growth retardation. Following a cesarean section at 36 weeks, the APGAR score was 5/6/7, birth weight 2310 g (p27), head circumference 32 cm (p25) and length 48 cm (p50). Postnatal respiratory distress required intubation. After extubation at the age of two weeks the child developed tachypnoea without significant impact on the blood gas parameters. Echocardiography confirmed a perimembraneous ventricular septal defect with a significant left-right-shunt, a patent foramen ovale (PFO) and patent ductus arteriosus (PDA). Due to a high TSH level with normal thyroid sonography medication with Levothyroxin was started. Brain MRI showed a hypoplastic vermis cerebelli and a „pale optic nerve". Abdominal ultrasound and EEG were normal. Convergent strabism was diagnosed. Feeding problems required a gastric tube.
First seen as a newborn the baby showed dysmorphic features including a slightly asymmetric head, micrognathia, hypertelorism, a short neck, short fingers, a low frontal and parietal hairline, prominent infraorbital wrinkles and widely spaced nipples.
At the age of one month episodes of tachypnoea, stridor due to laryngomalacia and feeding problems were persistent. All growth parameters were below the third percentile.
Conventional as well as chromosomal microarray analysis (Affymetrix Cytoscan HD Array; hg19) showed an interstitial duplication of approximately 21 Mb on the short arm of chromosome 2 (46,XY,arr2p 16.3p23.1(30,798,825-52,506,229)x3) harbouring at least 16 OMIM disease genes. The karyotypes of both parents were normal.
This is a unique, apparently de novo interstitial duplication of 21 Mb in the chromosomal region 2p16.3-p23.1. The patient shares some common clinical features such as growth retardation, heart defect, facial dysmorphisms with published cases with similar and overlapping duplications. The persisting tachypnoea remains unexplained. Detailed genotype-phenotype correlations will be presented.

P-ClinG-086

A non-classical IFITM5 mutation located in the coding region causes severe Osteogenesis imperfecta with prenatal onset

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Osteogenesis imperfecta (OI) is a hereditary connective tissue disorder characterized by a wide range of skeletal symptoms. Most patients have dominantly inherited or de novo mutations in COL1A1 or COL1A2. Up to 5% of patients have OI type V, characterized by hyperplastic callus formation after fractures, calcification of the membrane interossea of the forearm, and a mesh-like lamellation pattern observed in bone histology. Recently, a heterozygous mutation in the 5´-untranslated region of IFITM5 (c.-14C>T) was identified as the underlying cause of OI type V, and only this specific mutation was subsequently identified in all patient cohorts with this OI subtype. We now present the first case of a heterozygous mutation within the coding region of IFITM5 (c.119C>T; p.S40L). The mutation occurred de novo in the patient and resulted in severe OI with prenatal onset and extreme short stature. At the age of 19 months, the typical clinical hallmarks of OI type V were not present. Our finding has important consequences for the genetic "work-up" of patients suspected to have OI, both in pre- and in postnatal settings: The entire gene – and not only the 5´-UTR harbouring the "classical" OI type V mutation – has to be analyzed to exclude a causal role of IFITM5. We propose that this should be part of the initial diagnostic steps for genetic laboratories performing SANGER sequencing in OI patients.

P-ClinG-087

Panel analysis of 60 lower motor neuron disease genes using the Ion Torrent PGM

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Comprehensive and prompt molecular genetic diagnostics of genetically heterogeneous disorders is one of the key challenges in current DNA diagnostics – especially in neonates. Due to financial and time reasons, genetic testing is most often focussed on the genes with highest detection rates. While this certainly produces the highest diagnostic yield, such an approach self-evidently leaves a certain portion of patients without diagnosis. Among the medical conditions currently rather selectively tested, lower motor neuron disease are one of the toughest nuts to crack: Differential clinical diagnosis, which would allow targeted molecular genetic testing, often requires elaborate or even invasive testing such as muscle biopsies while time is short.
In the frame of NeurOmics, a large European Union-funded FP7 project we compiled a panel of 60 genes known to be associated with medical conditions from phenotypically overlapping spectrum of lower motor neuron diseases (LMNDs) including proximal and distal spinal muscular atrophies, amyotrophic lateral sclerosis and hereditary motor neuropathies. Our panel covers nearly 300 Kb of genomic material and comprises the coding sequences as well as the UTRs of these genes. Following exclusion of the most obvious candidates as the first diagnostic tier, target DNA fragments from the patient's sample were enriched using our custom Haloplex LMND enrichment kit. Subsequent massive parallel sequencing was carried out on an IonTorrent PGM using 200 bp sequencing chemistry. Major challenges in setting up our pipeline were the known pitfalls of semiconductor sequencing such as reliable calling of homopolymeric regions. In addition to the NGS pipeline, an extended questionnaire was developed, which collects detailed clinical information about the patients and thereby allows drawing genotype-phenotype correlations in cases with an uncertain diagnosis. Initial validation using samples with known mutations as well as samples of which exome data was available demonstrated a robust detection rate of previously detected variants. Sequencing of DNA from more than 20 unsolved LMND cases identified detrimental variants in several genes initially not considered as potential candidates for the respective individuals based on their clinical records. However, subsequent segregation analysis and concerted clinical examination verified our molecular genetic report thus demonstrating the diagnostic power of our LMND panel. In parallel, the diagnostic yield of our panel was evaluated by performing whole-exome-sequencing (WES) as well as panel sequencing of one and the same individuals. These first results already illustrate the potential and diagnostic valence of our panel.
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P-ClinG-088

Genetic heterogeneity in Möbius-Kallmann phenotype: reclassification of patients with congenital facial paresis and Kallmann syndrome into CHARGE Syndrome and 'TUBB3 E410K Syndrome'

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Möbius syndrome is a rare entity clinically defined by congenital paresis of sixth and seventh cranial nerve (CN) and associated findings. Kallmann syndrome is defined by disturbance of the first CN with impaired sense of smell and hypogonadotropic hypogonadism. To date, only a few patients with a combination of Möbius syndrome and Kallmann syndrome were described. Not all of them had abducens paresis. One patient showed a mutation in the FGF8 gene and very recently 8 patients with congenital facial paresis and Kallmann syndrome with a specific mutation c.1228G>A (p.E410K) in the TUBB3 gene called the 'TUBB3 E410K syndrome' were described.
We here report on three further patients with congenital facial paresis and Kallmann syndrome, unraveling the genetic cause in two of them.
The first patient is a 36 years old female with congenital facial paresis on the right side, hearing impairment on both sides and impaired sense of smell. She had hypogonadotropic hypogonadism which was treated with estrogen and growth hormone therapy during puberty. She was diagnosed with Kallmann syndrome and Möbius syndrome since childhood. On clinical examination she showed a dysplastic right ear. FGFR1 analysis did not reveal pathogenic mutations. In CHD7 gene analysis a mutation c.7879C>T (p.Arg2627*) was detected, confirming CHARGE syndrome.
The second patient is an 18 years old male patient with bilateral congenital facial paresis, congenital strabismus divergens, ptosis, impaired sense of smell and hypogonadotropic hypogonadism. Analysis of the TUBB3 gene showed the mutation p.E410K confirming the 'TUBB3 E410K syndrome'.
The third patient is a 36 years old female with congenital paresis of the 6th and the 7th CN, involvement of the 1st CN with impaired sense of smell and hypogonadotropic hypogonadism. She was also diagnosed with Möbius syndrome and Kallmann syndrome since childhood. Screening the HOXB1 and TUBB3 genes did not show pathogenic mutations. Screening the genes associated with Kallmann syndrome (PROKR2, CHD7, FGFR1, FGF8, PROK2) also did not show pathogenic changes. Chromosomal analysis in this patient was normal.
We here describe three patients with overlapping phenotype showing congenital facial paresis and Kallmann syndrome resembling the Möbius-Kallmann phenotype described in literature in rare cases. Two of the patients were reclassified into CHARGE syndrome and 'TUBB3 E410K syndrome', respectively. These examples illustrate the genetic heterogeneity in Möbius-Kallmann phenotype. We would like to emphasize the importance of clinical examination, especially regarding facial dysmorphisms and eye movement disorders to classify these patients.

P-ClinG-089

Next-Generation-Sequencing (NGS) diagnostics in X-linked intellectual disability – mutation and spectrum and the role of skewed X-inactivation in carrier mothers

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Background
X-linked intellectual disability (XLID) is a genetically heterogeneous disorder with more than 100 genes known to date. Most genes are responsible for a small proportion of patients only, which has hitherto hampered the systematic screening of large patient cohorts.
Methods
We performed targeted enrichment, next-generation sequencing and analysis of the coding and flanking intronic regions of 107 XLID genes in a cohort of 150 male patients. 100 patients had sporadic intellectual disability, and 50 patients had a family history suggestive of XLID. We

also analyzed two female patients with severe ID and epilepsy because they had strongly skewed X-inactivation.
Results and conclusions
Target enrichment and high parallel sequencing allowed a diagnostic coverage of >10 reads in males and >20 reads in females for ~95% of all coding bases of the XLMR genes. The average depth on target was 64,99x. Systematic gene dosage analysis for low coverage exons detected one pathogenic hemizygous deletion in one patient. Moreover, we found 18 pathogenic mutations in 13 XLID genes (AP1S2, ATRX, CUL4B, DLG3, IQSEC2, KDM5C, MED12, OPHN1, SLC9A6, SMC1A, UBE2A, UPF3B and ZDHHC9). 13 mutations were present in the group of 50 familial patients (26%), and 5 mutations among the 100 sporadic patients (5%). Mutations in IQSEC2 were detected in both female ID patients, suggesting that this gene might play a considerable role in female encephalopathies.
The mutation rate in the cohort of sporadic patients corroborates previous estimates of 5-10% for X-chromosomal defects in male ID patients. This study broadens the mutational and associated clinical spectrum of rare XLID genes for which fewer than 10 mutations had been reported to date. The application of NGS-based approaches on larger patient cohorts will provide the basis for a comprehensive and detailed understanding of the genetics of XLID.

P-ClinG-090

Disease course and mutational spectrum in neurodegeneration with brain iron accumulation caused by mutations in C19orf12

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Background Neurodegeneration with brain iron accumulation (NBIA) encompasses a group of rare neurodegenerative disorders hallmarked by brain iron deposits. A growing list of genetic loci has been subsumed under the NBIA umbrella, including PANK2, PLA2G6, FA2H, ATP13A2, C20orf72, CP, WDR45 and FTL. Absence of the orphan mitochondrial protein C19orf12 leads to mitochondrial membrane protein-associated neurodegeneration (MPAN). It was identified as the second most common cause of NBIA in a Polish cohort, accounting for 19 out of 52 index cases. Preliminary observations indicated a wide clinical spectrum ranging from formerly idiopathic NBIA to Parkinson disease.
Methods and Results In order to complete the clinical picture of this NBIA subtype and to investigate the prevalence of C19orf12 mutations in non-Polish populations, we performed a mutation screen in undiagnosed NBIA cases coupled to standardized assessment of clinical phenotype data. This analysis established a genetic diagnosis in 35 cases. Clinical features initially encompassed gait changes or neuropsychiatric abnormalities followed by progressive spastic paresis, progressive dystonia and cognitive decline. Optic atrophy, axonal neuropathy and absence of the "eye of the tiger" sign in MRI are features to distinguish MPAN from pantothenate kinase-associated neurodegeneration (PKAN) which is the most frequent subtype of NBIA. In contrast to early-onset PKAN, MPAN patients survive well into adulthood. In the end-stage of disease MPAN patients suffer from severe dementia, spasticity, dystonia, and parkinsonism.
Conclusions Our results suggest that mutant C19orf12 is the second most common cause of NBIA not only in Poland but also in other European populations and should be tested in "eye of the tiger" negative NBIA patients especially if the patient is affected by optic atrophy, neuropsychiatric features and neuropathy.

P-ClinG-091

Large deletions in the NSDHL gene in two patients with CHILD syndrome

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CHILD syndrome is a very rare X-linked disorder characterized by congenital hemidysplasia, icthyosiform nevus and ipsilateral limb defects that can range from digital hypoplasia to complete amelia. Mutations in the NSDHL gene (NAD(P)H steroid dehydrogenase-like protein) have been identified in patients suffering from CHILD syndrome. The NSDHL gene encodes for an enzyme involved in cholesterol biosynthesis. In most cases, small mutations in the NSDHL gene, usually being nonsense-mutations, have been reported to date. Also, three patients carrying a large deletion have been identified: a deletion encompassing the complete NSDHL gene, a deletion including exon 6-8 and a microdeletion in the promoter/enhancer region including exon 1 of the NSDHL-gene.
Here, we report on two unrelated Chinese girls diagnosed with CHILD syndrome.. Patient 1 had an inflammatory ichthyosiform nevus on the right side of her body and absence of phalangeal bones of the right foot. Patient 2 showed an inflammatory epidermal nevus and deformed toes and fingers on the right side of her body. Sequencing of the NSDHL gene in both patients did not identify a mutation. A subsequently performed qPCR analysis to detect deletions or duplications revealed a large deletion encompassing exon 4-7 of the NSDHL gene in patient 1 and a deletion including exon 5-8 in patient 2. Our findings indicate that large deletions in the NSDHL locus may be rather common in cases of CHILD syndrome.

P-ClinG-092

Coincidence of reciprocal translocation and COL2A1 mutation in a fetus with severe skeletal dysplasia

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Prenatal sonographic examinations during the first pregnancy of a 28-year-old woman revealed generalized hydrops fetalis and severe micromelia; therefore, fetal achondrogenesis was suspected. Chorion villus sampling was performed during 12th week of gestation. A reciprocal translocation t(4;17) was detected. Subsequent chromosomal analysis of maternal and paternal blood showed that the patient's mother was carrier of the same reciprocal translocation. SNP array analysis of both fetus and mother did not provide evidence for chromosomal imbalances or copy number variations that could be associated with the fetal phenotype. Due to the poor prognosis, the family opted for termination of the pregnancy. Radiography and autopsy revealed small chest, micromelia, cupping of the metaphysis of the tubular bones, absent ossification of vertebral bodies and an underdeveloped and severely disorganized growth plate. As these features suggested a possible diagnosis of achondrogenesis, molecular genetic analyses were carried out which led to the detection of a heterozygote mutation in the COL2A1 gene (c.1529G>A, p.Gly510Asp). This mutation has been described earlier as a cause of achondrogenesis type II, an autosomal-

dominant disease leading to severe micromelic dwarfism. Both parents were proven not to be carriers of this mutation.
The coexistence of a cytogenetic (reciprocal translocation) and a molecular genetic (COL2A1 mutation) abnormality in the fetus carries important implications for genetic counselling. Recurrence risk for achondrogenesis type II (the actual fetal disorder) in the case of a de novo mutation seems to be low, although there are recent reports on recurrence of this disease within the same family, probably due to germline mosaicism. However, the risk of unbalanced gametes as a possible consequence of the maternal reciprocal translocation (which might be considered an incidental finding in this pregnancy) is an important issue to be discussed with the family.

P-ClinG-093

Identification of a new intronic BMPR2 mutation due to an Alu insertion and early diagnosis of heritable pulmonary arterial hypertension in a large family with a mean clinical follow-up of 12 years

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Mutations in the bone morphogenetic protein receptor 2 (BMPR2) gene can lead to hereditary pulmonary arterial hypertension (HPAH) and are detected in more than 80% of cases with familial aggregation of the disease. Factors determining disease penetrance are largely unknown.
A mean clinical follow-up of 12 years was accomplished in 46 family members including echocardiography, stress-Dopplerechocardiography and genetic analysis of TGF-β pathway genes. Right heart catheterization and RNA analysis was performed in members with pathological findings. Manifest HPAH was diagnosed in 8 members, 4 were already deceased, two died during the follow-up, two are still alive. Normal pulmonary artery systolic pressure at rest but hypertensive response to exercise (HR) has been identified in 19 family members. Analysis of BMPR2 transcripts revealed aberrant splicing due to an insertion of an intronic Alu element adjacent to exon 6. All HPAH patients and 12 further asymptomatic family members carried this insertion. During follow-up two family members carrying HR and the Alu insertion developed manifest HPAH.
This is the first report of an intronic BMPR2 mutation due to an Alu element insertion causing HPAH in a large family which has been confirmed on RNA level. Only those members that carried both HR and the mutation developed manifest HPAH during follow-up. Our findings highlight the importance of including further methods such as RNA analysis into the molecular genetic diagnostic of PAH patients. They suggest that at least in some families HR may be an additional risk factor for disease manifestation and penetrance.

P-ClinG-094

Genetic dosage compensation in a family with co-occurrence of PMP22 duplication and PMP22 deletion

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Charcot-Marie-Tooth disease, type 1 A (CMT1A) and Hereditary Neuropathy with liability to Pressure Palsies (HNPP) are two autosomal dominantly inherited neuropathies caused by converse gene dosage alterations of chromosome 17p11.2-p12 including PMP22. CMT1A based on a heterozygous PMP22 duplication is characterized by peripheral neuropathy, distal muscular atrophy, sensory deficits and general reduced nerve conduction velocities. In contrast, a heterozygous PMP22 deletion results in HNPP characterized by recurrent episodes of focal compression neuropathy with weakness and sensory deficits. To date there has been no report of an individual harboring both a PMP22 duplication on one chromosome 17 and a PMP22 deletion on the other chromosome.
We describe a large three generation family with CMT1A or HNPP patients, as well as two sisters with co-occurrence of PMP22 duplication and PMP22 deletion who neither have a characteristic CMT1A nor HNPP phenotype.
MLPA assay was performed on several relatives of a 55-year-old female patient with CMT1A due to PMP22 duplication. Her mother and one of her sisters with typical features of CMT1A carried the PMP22 duplication as expected. Remarkably, one of her brothers carried a PMP22 deletion consistent with his neuropathic symptoms characteristic for HNPP. Furthermore, MLPA analysis of two sisters both with unspecific neurologic symptoms and minimal electrophysiological aberrations neither characteristic for HNPP nor CMT1A presented with normal genotypes. However, analysis of a daughter of one of these sisters displayed a heterozygous PMP22 deletion. We therefore assumed co-occurrence of a maternally inherited PMP22 duplication and a paternally inherited PMP22 deletion in the initially inconspicuous woman. Her father was deceased; further paternal family members were not available. Familial segregation of 3 polymorphic microsatellite markers within the segment in region 17p11.2-p12 confirmed that the two sisters having normal results in MLPA both carried the maternal duplication of PMP22 and the paternal deletion of PMP22.
To our knowledge, this is the first report of co-occurrence of PMP22 duplication and PMP22 deletion. While double trouble of separate neuropathy causing mutations generally results in a more severe phenotype, this is an exceptional situation where an increased protein expression from a duplicated allele seems to be fully compensated by the reduced expression of the deleted allele. The neurological findings of the two sisters are currently under detailed review.
Despite normal MLPA findings in these sisters, their offspring carry either the PMP22 duplication or the PMP22 deletion.
The reported family is a unique and exceptionally rare example of independently segregating and partly compensating gene defects in CMT1A and HNPP and gives exciting insights into PMP22 gene function.

P-ClinG-095

Potential Modifier Effects in Senior-Løken Syndrome

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Nephronophthisis (NPHP), an autosomal recessive cystic kidney disease, is the most frequent genetic cause for end-stage renal disease in the first two decades of life. To date, mutations in more than 15 genes have been identified as causative with a homozygous deletion in the NPHP1 gene being the most common mutation. Ten percent of the affected individuals additionally suffer from retinitis pigmentosa, constituting the renal-retinal Senior-Løken syndrome (SLSN). Some of the SLSN patients carry the mutations in one of the NPHP genes, a genotype-phenotype correlation, however, could not be observed. By positional cloning and exome capture, two additionally genes (IQCB1, SDCCAG8) were identified, which are specifically associated with SLSN.
Here, we present a 24-year old man with SLSN. Visual loss started at the age of 3 years, retinitis pigmentosa was diagnosed at the age of 8 years. At the age of 19 years the patient suffered from renal impairment leading to the diagnosis of a Senior-Løken syndrome. The family history was inconspicuous.

In a first step, multiplex ligation-dependent probe amplification (MLPA) was performed in this patient showing a heterozygous deletion in NPHP1, which is not sufficient to explain SLSN in this patient. Further mutational analysis in the NPHP1 gene did not show an additional point mutation. Using sequencing by synthesis (next generation sequencing) all remaining NPHP genes were analyzed demonstrating two causative heterozygous mutations in IQCB1 (p.Phe142Profs*5, p.Arg489*) and an additional heterozygous mutation in NPHP4 (p.Ala983Thr). Prediction tools like MutationTaster and PolyPhen-2 classified all identified mutations as most likely pathogenic. All identified mutations were confirmed by Sanger sequencing. Segregational analysis was denied from the parents.
In this case the IQCB1 mutations could explain the SLSN phenotype but are potentially modified by the heterozygous mutations in NPHP1 and NPHP4. For a small number of NPHP patients a digenic/oligogenic inheritance has previously been suggested. However, it cannot be ruled out, that both mutations in NPHP1 and NPHP4 in this patient are not pathogenic. This case demonstrates the challenge of interpreting mutations and their clinical significance. Further functional studies will be needed to elucidate the pathogenic mechanisms.

P-ClinG-096

Genetics in disguise - role of pharmacogenetics in the fetal anticonvulsant syndrome?

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There is an increasing discussion on the genetic susceptibility for adverse pregnancy outcomes like drug-induced birth defects. One of the main principles of teratology is the interaction of environmental and genetic factors. This has been confirmed by several animal studies with different strain-specific risks for malformations after exposure to teratogens. In humans, a strong genetic susceptibility for adverse events has been proposed based on the higher recurrence risk for fetal anticonvulsant or valproate syndrome, including neural tube defects, after a first affected child (Moore et al., 2002).
Valproate is frequently used for the treatment of epilepsy, but is also a well-known teratogen leading to an increased risk of malformations and neurodevelopmental delay. In the Berlin Institute of Clinical Teratology outcomes of 415 prospectively ascertained pregnancies with first trimester exposure to valproate were evaluated. Pregnancy outcomes were 324 live births (including two twin pregnancies), 33 spontaneous abortions, 57 elective terminations of pregnancy and one stillborn. The cumulative incidence of spontaneous abortion was within the normal range (21%, 95%-CI 12-36). Congenital anomalies were observed in 16% of prospectively ascertained cases.
Although valproate is considered a drug with a high teratogenic potential, only a minority of women treated during pregnancy have children with congenital anomalies. Pharmacogenetic studies could help to identify risk factors for the development of adverse pregnancy outcomes. For this approach well documented cases, including careful individual clinical and comprehensive genetic evaluation of each case are required. Candidate genes that might protect the embryo/fetus from teratogenicity include a variety of genes involved in metabolism and drug transport as well as specific receptors. In the special situation of pregnancy both the fetal and maternal genome have to be considered.

P-ClinG-097

Excessive mutational fallout of STRC in patients with high frequency hearing loss

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Approximately half of all hearing loss is due to genetic defects, with non-syndromic hearing loss comprising 70% of all genetic cases. Nearly one out of every 1000 newborn children is affected by hearing loss. Molecular genetic analysis of genes responsible for hearing loss is important for diagnosis. Defects in GJB2 (connexin 26; MIM: 121011) and GJB6 (connexin 30; MIM: 604418) constitute many of the non-syndromic cases that are solved. The GJB2 and GJB6 mutation negative cases require further investigation, which calls for the development of new diagnostic assays that can detect causative mutations and solve these otherwise unsolvable cases. Based on a genome-wide SNP array study, we successfully determined STRC (MIM: 606440) as a larger than expected contributor of non-syndromic hearing loss. STRC is on chromosome 15q15.3, has 29 exons and is included in the DFNB16 locus for non-syndromic hearing loss. The coded protein, stereocilin, is associated with the outer hair cells. In knockout mouse experiments, stereociliary crosslinks were absent in the outer hair cell. These crosslinks are responsible for opening transduction channels when outer hair cells are mechanically deflected. In humans, STRC hearing loss is progressive and is characterized by increased thresholds in the higher frequencies with childhood onset. In a SNP array study which contained 93 GJB2/GJB6 mutation negative non-syndromic hearing loss patients, we detected five heterozygous deletions, two homozygous deletions and ten individuals with a copy-neutral loss of heterozygosity (LOH) overlapping with STRC. One additional case was a confirmed heterozygous deletion carrier by quantitative real-time PCR. For molecular clarification of the heterozygous and the LOH cases, a Sanger sequencing method was established, which excludes the STRC pseudogene. This pseudogene is 100 kb away and shows a coding sequence identity of 99.6% and a genomic identity of 98.9% to the STRC gene. Three more cases having a heterozygous mutation could be solved through an additional hemizygous mutation, which are predicted as having negative effect on the protein. Furthermore, twenty patients with characteristic high frequency hearing loss but negative microarray result were Sanger sequenced using our method. One heterozygous and one compound heterozygous mutation could be identified. Altogether, 6% of our patient cohort was diagnosed by homozygous deletion, heterozygous deletion with Sanger sequence hemizygous mutation and compound heterozygous mutation, which is more than what we would normally expect given the genetic heterogeneity of non-syndromic hearing loss. We detected heterozygous carriers in five of 94 patients. This supports the understanding that STRC is an important gene to screen in hearing loss diagnostics.

P-ClinG-098

Identification of a 2q31.1-32.1 Interstitial Deletion in a Female Patient with Developmental Delay, Mild Mental Retardation and Dysmorphic Features

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We report on a patient with an interstitial deletion of the long arm of chromosome 2 at 2q31.12q32.1. She had a mild mental retardation, a high forehead, upslanting palpebral fissures, telecanthus, unusual ear shape with dysmorphic, simple pinnae, a bulbous nasal tip, full cheeks and a short philtrum. She showed delayed dentition and dental crowding, spoon fingers, deep palmar creases, short feet with flat foot arches,

hallux valgus, x-legs, stamping gait and striae rubrae on hips and thigs. The deleted region overlaps with the deletions found in previously reported cases. Array CGH analysis (Agilent 244K) revealed an interstitial deletion of 5.43 Mb leading to hemizygosity for 25 genes. A few of these genes are known disease-causing genes (OMIM) and good candidates to explain the cognitive and behavioral phenotype associated with the 2q31.1q32.1 deletion, namely AGPS, PRKRA, TTN, ITGA4, NEUROD1 and PDE1A. The HOXD Cluster is not deleted in our patient.

P-ClinG-099

Hyperphosphatasia with Mental Retardation syndrome: clinical spectrum and genetic heterogeneity due to defects of the GPI anchor synthesis pathway

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Four different genes of the glycosylphosphatidylinositol anchor synthesis pathway, PIGV, PIGO, PGAP2, and PGAP3 have recently been implicated in hyperphosphatasia-mental retardation syndrome (HPMRS), also known as Mabry syndrome, a rare autosomal recessive form of intellectual disability. The aim of this study was to delineate the mutation spectrum of these genes as well as the associated phenotypic spectrum in a cohort of 18 individuals diagnosed with HPMRS on the basis of intellectual disability and elevated serum alkaline phosphate as minimal diagnostic criteria. Biallelic PIGV mutations were identified in about 50 % of unrelated families with HPMRS. The most frequent mutation detected in about 80 % of affected families is the c.1022C>A PIGV mutation, which was found in both, the homozygous as well as the heterozygous state. Six further mutations found in PIGV and PIGO are novel. Our findings in the largest reported cohort to date significantly extend the range of reported clinical manifestations and demonstrate that the severe end of the clinical spectrum presents as a multiple congenital malformation syndrome with a high frequency of Hirschsprung disease, vesicoureteral and renal anomalies as well as anorectal malformations. At the other end of this spectrum HPMRS could present as apparently non-syndromic form of intellectual disability. PIGV mutations are the major cause of hyperphosphatasia-mental retardation syndrome, whereas mutations of the other involved genes are less frequent.
HPMRS displays a clinical variability regarding associated malformations and growth patterns but the main clinical features are developmental delays often with seizures, hyperphosphatasia, particular facial anomalies, and brachytelephalangy.

P-ClinG-100

Two novel mutations in the LOR gene in two families with loricrin keratoderma

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Loricrin keratoderma (MIM #604117) is a rare autosomal dominant disorder characterized by honeycomb palmoplantar keratoderma and generalized mild ichthyosis, often associated with digital constriction (pseudoainhum). Loricrin keratoderma is caused by heterozygous mutations in the LOR gene (MIM *152445). Loricrin is a small glycine-serine-cysteine-rich basic protein, synthesized in the granular layer. It is deposited beneath the plasma membrane and cross-linked to several cytosolic proteins, which form the cornified cell envelope (CE), a protective barrier against environment. The clinical phenotype of patients with mutations in the LOR gene can be very variable. These patients can be diagnosed with the ichthyotic variant of Vohwinkel's syndrome, progressive symmetric keratoderma or the congenital ichthyosiform erythroderma born as a collodion baby. Until now, 11 families with loricrin keratoderma with 4 different heterozygous single base pair insertions in the LOR gene have been reported. All mutations lead to an elongated loricrin protein because of delayed termination.
Here we report two novel heterozygous insertion mutations in the LOR gene in three patients from two French families with the clinical characteristics of loricrin keratoderma. Patient 1 is a 21 year old woman with the novel heterozygous insertion mutation c.646_647insGCAGCAGGTC, p.Gln216Argfs*123 in exon 2 of the LOR gene. Patient 2 (29 year old man) and patient 3 (his 68 year old mother) carried both the novel heterozygous insertion mutation c.798_799insT, p.Gly267Trpfs*69 in exon 2 of the LOR gene.
Our report confirms the causal association of the LOR gene with loricrin keratoderma and the importance of insertion mutations in loricrin keratoderma.

P-ClinG-101

A 24 bp deletion in ELN causing a Marfan-like phenotype

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We report on a 27 year old male presenting with a Marfan-like phenotype. The patient had a mild myopia, a high palate and broad uvula, skin striae, a plain flat foot, a positive wrist sign as well as long fingers and toes and an increased arm span-to-height ratio. Moreover a threefold patellar luxation was reported. Marfan syndrome was suspected three years ago when funnel chest surgery was performed. A cardiological examination was consecutively recommended but revealed no abnormalities.
The recently revised diagnostic criteria for Marfan syndrome integrate information from multiple sources including personal medical history and physical examination. Those features are weighted and grouped to derive a "systemic score". In the absence of a family history of Marfan syndrome, a systemic score ≥7 in combination with an aortic root enlargement (Z-score ≥2.0) is sufficient for the diagnosis. Our patient achieved a systemic score of 7 but did not show an aortic dilatation.
We used an individual, Multiplex-PCR based Ion AmpliSeq Kit capturing 98,44% of coding regions to analyze the genes FBN1, ACTA2, ELN, CBS, FBN2, MYH11, COL3A1, SLCA10, SMAD3, TGFBR1, TGFBR2 and TGFB2. Surprisingly we identified a small heterozygous in frame deletion of 24 bp (c.1178_1201del24bp) in exon 20 of the ELN gene leading to a loss of 8 amino acids (p.Gly393_Ala401delinsAla). This result was confirmed by Sanger sequencing. We suppose that expression of a mutated protein leads to the disruption of elastic fibre architecture. Up to now mutations in Elastin are only known for autosomal dominant Cutis laxa (ADCL) and as a cause for aortic aneurysms but are not described as causative for other Marfan phenotype features.
For further evaluation physical examination and molecular analysis in the patient´s parents is planned.

P-ClinG-102

A familial case of Cornelia de Lange syndrome caused by NIPBL gene mutation

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Cornelia de Lange syndrome (CdLS) is caused by mutations in the NIPBL gene in 60% of the cases. The most important clinical signs are typical craniofacial features, upper limb defects, mental retardation, short stature and hirsutism.
In the literature NIPBL mutations have been described both in individuals with mild and severe clinical symptoms. Typically individuals with truncating NIPBL mutations have a severe phenotype. The majority of cases are caused by de novo mutations and less than 1% of individuals with NIPBL-related CdLS have an affected parent.
We describe a rare case of familial CdLS with a mild phenotype caused by a NIBPL mutation. The index patient was a two year old Caucasian boy with microcephaly, synophrys and arched eyebrows, ptosis of the left eye, small hands with simian crease, short stature, cryptorchidism and speech delay. Interestingly, his 30 year old mother was found to carry the same mutation. She presented with characteristic facial CdLS features including synophrys with arched eyebrows, short stature, brachydactyly and learning disabilities.
Molecular analyses revealed a previously unknown frameshift mutation c.8307delA (p.Asp2770Thrfs*9) in the last exon (exon 47) of the long isoform of the NIBPL gene (NM_133433.3). This mutation is predicted to affect a functionally noncritical region of Nipped-B-like protein. In addition, this mutation does not alter the short isoform (NM_015384.4) of the NIPBL gene, which lacks the last exon containing the mutation. In conclusion, these findings may explain the milder phenotype observed in our patients.

P-ClinG-103

Bi-allelic PTEN mutation in two patients with extreme macrocephaly and mild intellectual disability

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Heterozygous mutations in PTEN cause a spectrum of disorders including macrocephaly, tumor predisposition, autism and cognition impairment as well as skin lesions. Homozygous or compound heterozygous mutations have not been reported yet. Based on mouse models it was assumed that those would be lethal.
We examined two siblings with an extreme macrocephaly of 63 cm (17 years old female, + 5.9 SD) and 64 cm (15 years old male, + 5.5 SD) and a mild intellectual disability that presented at the institute of human genetics in Lübeck. All further examinations were unremarkable. Especially, the affected persons show no autistic symptoms, no skin lesions, and no tumors. Their heterozygous parents as well as further obligatory carriers are healthy and there is no excess of tumors in the family history.
Since the parents were consanguineous, we assumed autosomal recessive inheritance. We undertook autozygosity mapping and identified one candidate locus on chromosome 10 between 87.99 and 91.02 Mb (3.03 Mb). Exome sequencing revealed one mutation located in the candidate locus that is not reported in public databases and in over 300 in house exomes, and which is highly conserved and predicted to be pathogenic by four in silico programs. This mutation is located in PTEN; NM_000314: g.Chr.10:89711927T>C; c.T545C; p.Lys182Ser and has been recently described in patients with PHTS (PTEN hamartoma tumor syndrome).
The clinical phenotype of intellectual disability and macrocephaly suggests a clear impact of this mutation on the protein function. Since the heterozygous carriers in our family are healthy and this homozygous mutation is obviously not lethal, we assume that this mutation is hypomorphic. Functional analyses to test the residual catalytic activity of PTEN are ongoing using cell lines of our patients as well of healthy persons and of patients with different mutations in PTEN.
We illustrate in this study that a bi-allelic mutation in PTEN is not necessarily lethal and does not always lead to tumor predisposition; we postulate an extension of the phenotype of PTEN mutations to autosomal recessive macrocephaly with mild intellectual disability.

P-ClinG-104

Twenty-one years to the right diagnosis - Clinical overlap of Simpson-Golabi-Behmel syndrome and Beckwith-Wiedemann syndrome

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Simpson-Golabi-Behmel syndrome (SGBS) is an X-linked recessive overgrowth syndrome caused by mutations in the glypican 3 (GPC_3) gene. SGBS is characterized by pre- and postnatal overgrowth, "coarse" facial features and a spectrum of congenital malformations (e.g. macroclossia, organomegaly, Wilms tumor, umbilical hernia) which overlap with Beckwith-Wiedemann syndrome (BWS).
We report on a 21 year old patient who was diagnosed BWS shortly after birth. Polyhydramnios, macrosomia, splenomegaly and large hypoechogenic kidneys were noted at 30 weeks gestation by prenatal ultrasound. The boy was born at 36 weeks of gestation by cesarian section due to fetal macrosomia. Birth weight was 4300 g, length 55 cm and head circumference 38 cm (all >97th centile). Apart from macrosomia examination after birth showed macroglossia, mild prognathism, nephromegaly, peripheral pulmonary stenosis, slightly dilated cerebral ventricles, ear lobe creases and rather small hands and feet. Due to a persistent ductus omphaloentericus with Meckel diverticulum, surgical intervention took place 2 weeks after birth. Tube feeding was necessary during the first weeks of life, and recurrent apnea and bradycardia episodes persisted. At 3 months, a Wilms tumor of the left kidney was removed. In addition, a cyst of the right kidney was documented. At 5 months exomphalos-macroglossia-gigantism (EMG) syndrome was diagnosed clinically by Professor Wiedemann. However, at that age it was documented that some features were not typical for EMG syndrome like rather small hands with short fingers and notably a supernumerary nipple on the left side. At the age of 6 years a tongue reduction surgery was performed and a submucous cleft palate was corrected. Motor and speech development were delayed, and the boy received special education.
The patient's clinical history was reviewed again at the age of 21 years, after the clinical diagnosis of BWS could not be confirmed by molecular genetic testing for molecular mutations in 11p15 by MLPA. At this time, his height was 2,04 m and his frontal head circumference was 68 cm. He is of normal intelligence, after successfully passing his A-Levels he started with engineering studies. Physical examination showed pectus excavatum, 2 supernumerary nipples, several areolar skin tags and a large mandible, resulting in marked facial dysmorphism. Based on the clinical features the diagnosis of BWS was withdrawn and SGBS suspected. Sequencing of the GPC_3 gene subsequently revealed a hemizygosity for the nonsense mutation p.Tyr81X (c.243C>G) in exon 2. In comparison to the initial diagnosis of BWS, this finding has an important implication for the recurrence risks in the family.

We will demonstrate the evolution of the phenotype in SGBS from infancy to adulthood and compare the clinical features of SGBS and BWS. Despite the advances in molecular genetic testing, an accurate clinical diagnosis still has an important place in genetic syndromology.

P-ClinG-105

Blepharophimosis-ptosis-epicanthus inversus syndrome plus

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Blepharophimosis-ptosis-epicanthus inversus syndrome (BPES; OMIM #110100) is a rare autosomal dominant syndrome, which is characterized by the main and partly name-giving features: blepharophimosis, ptosis, epicanthus inversus and lateral displacement of the inner canthi with normal interpupillary distance (telecanthus). Two types of BPES can be recognized: type I BPES with female infertility due to premature ovarian failure, and Type II BPES, presenting with eyelid abnormalities only.
Our patient was born after an uneventful dicygotic twin pregnancy at 38 weeks of gestation. Birth weight was 1195 g(<10%), length 39 cm (<10%) and head circumference 26 cm (<10%). She had the typical facial features, and in addition abducted thumbs. Her development was delayed. She sat at 18 months, and walked with one hand held at 20 months with a broad-based gait. She spoke her first real words at 20 months and followed one-step commands. At 20 months her height was 78 cm (3-10%), weight 8,8 kg (<3%) and CFC 43 cm (<3%). Karyotype and sequence analysis of the FOXL2-gene were normal, but array-CGH revealed a 10 Mb microdeletion [arr 3q22.1q23(130.724.444-141.632.618) x1], spanning more than 70 genes, including FOXL2.
BPES is caused by FOXL2 mutations, subtle FOXL2 deletions or 3q23 microdeletions, or deletions of the FOXL2 regulatory region. Patients with larger deletions more often present with associated anomalies, like mental retardation, microcephaly or short stature. We discuss the molecular mechanisms underlying the features of our patient.

P-ClinG-106

Molecular investigations in a family with 3-methylglutaconic aciduria, optic atrophy and facioscapulohumeral muscular dystrophy (FSHD) phenotype

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Different syndromes have been described in which mutations affecting components of the mitochondria leading to increased urinary excretion of the metabolite 3-methylglutaconic acid. The affected gene products play important roles in mitochondrial structure, function, and dynamics; Mitochondrial dysfunction typically affects tissues with a high energy demand like muscle, brain, and nerves.
The aim of this project was to find the disease causing mutation in a consanguineous family with increased levels of 3-methylglutaconic-acid in urine and plasma. The four patients also show optic nerve atrophy and a neuromuscular phenotype resembling facioscapulohumeral muscular dystrophy (FSHD) with onset in early adulthood. Homozygosity mapping revealed a candidate interval on chr. 11, whole-exome sequencing failed to detect any disease-causing variant and no copy number variantion was found in Array-CGH.
Currently, our investigations focus on non-coding and potential regulatory regions close to described mitochondrial genes within the linkage interval.

P-ClinG-107

Identification of potential causative gene loci for an autosomal recessive novel syndrome with brain anomalies, vision impairment and distinct facial dysmorphism.

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In a large family of Turkish origin, two affected offspring's were born. Both children, a girl (10 years old) and a boy (6 years of age) show similar clinical anomalies, like a severe mental retardation with corpus callosum agenesis, ataxia, moderate microcephaly, square face, hypertelorism, bilateral ptosis, arched eyebrows, epicanthal folds, downslanting palpebral fissures, strabismus, amblyopia, broad nasal bridge, low set ears, a relatively short philtrum and downturned corners of the mouth. Pediatric and neurological examinations including brain MRI, myography and electroneurography of both patients did not reveal any additional disorder and a hereditary motor sensory neuropathy (HMSN) could be ruled out. Since the mothers of both patients are first degree cousins and both fathers are second degree cousins too, pedigree analysis allowed concluding that an autosomal recessive mode of inheritance of this particular syndromic disorder is very likely. Based on these findings we performed a 250 k Affymetrixs SNP array analysis using DNA of both patients for homozygosity mapping. By this approach it was possible to reduce the homozygous genomic regions that are shared by both patients to only 4 segments ranging from about 3.05 Mb to about 8.84 Mb in size. Because the number of potential candidate genes in these genomic regions (hg19) according to the obtained RS-loci on chromosome 1 (1p36.22), chromosome 2 (2q22.3), chromosome 3 (3p14.1) and chromosome 9 (9q21.13) is still considerable, we will as a next step apply a whole exome next generation sequencing approach to identify homozygous mutations in both patients and will then be able to focus just on the 4 genomic segments mentioned above. Since none of these segments to the current knowledge contains any known appropriate disease causing gene and although the phenotype of our patients shows some similarities with Toriello-Carey syndrome and Charlevoix disease or Andermann syndrome, it clearly does not resemble these syndromes, so we think that our patients exhibit a new syndromic disorder. As soon as we have a causative gene mutation identified, it will be of highest priority to find another non related family with this new syndrome and an independent mutation in this gene as well.

P-ClinG-108

Dominant mutations in beta-catenin 1 (CTNNB1) appear to be a frequent cause of intellectual disability

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Intellectual disability (ID, IQ<70) affects up to 3% of the general population. Until recently, the underlying reason of ID was unclear in about half of the affected individuals, also due to its high locus heterogeneity. The introduction of whole exome sequencing (WES) techniques enables elucidating the underlying genetic background of ID.
We performed WES in a cohort of 250 individuals with unexplained ID in a trio design with their unaffected parents. We identified and confirmed heterozygous de novo CTNNB1 (beta-catenin 1) mutations in four unrelated individuals (three of Caucasian and one of Asian

descent) – two frameshift mutations, one stop mutation, and one splice mutation.
All four patients have a severe motor delay and profound speech impairment. In addition they have a hypotonia of the trunk and hypertonia of the legs. The craniofacial phenotype comprises a microcephaly in three of four patients (in two of them already present at birth) and some consistent facial features - a broad nasal tip, small alae nasi, a long philtrum and a thin upper lip vermillion.
Beta-catenin is a key downstream component of the canonical Wnt signaling pathway, and acts as a negative regulator of centrosome cohesion. Whereas somatic gain-of-function mutations in CTNNB1 have already been found in various tumor types, germline loss-of-function mutations were suspected in animal models to influence neuronal development and maturation. This was supported by the finding of dominant inactivating CTNNB1 mutations as a cause of ID in three out of 865 patients (0.35 %) (de Ligt et al., NEJM 367:1921-1929, 2012). The clinical phenotype was characterized by absent or limited speech, microcephaly and spasticity with severely impaired ability to walk.
Our finding of four individuals in our cohort of 250 with ID (1.6 %) suggests that CTNNB1 loss-of-function mutations might be a more frequent cause of ID than estimated from the data of de Ligt and colleagues. Our data further emphasize the importance of Wnt signalling in human brain development and/or function.

P-ClinG-109

Targeted next-generation sequencing identifies two novel TRIM32 mutations in a patient with LGMD phenotype

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Limb-girdle muscular dystrophy (LGMD) is a genetically heterogeneous group of muscular disorders. Causative mutations in about 30 genes have been identified over the last 10 years. LGMD2H is a rare autosomal recessive disorder characterized by proximal weakness, atrophy and mildly to moderately raised levels of creatine kinase (CK) with an onset between the first and fourth decade. Besides a slowly progression, some of the symptoms include facial weakness and respiratory weakness. LGMD2H results from mutations in the gene encoding the tripartite motif-containing protein-32 (TRIM32) on chromosome 9q33.1.
We report on a 45-year-old male patient with a general weakness and a decreased load-carrying capacity for about 3 years. The fact that the CK level is between five and seven times the upper limit of normal and a conspicuous muscle biopsy leading to LGMD as a suspected diagnosis. We performed targeted next-generation sequencing (NGS) with a panel including 37 genes that are associated with limb-girdle muscular dystrophy. We identified two heterozygous novel mutations in the TRIM32 gene, namely c.626_627delCT and c.1901A>G (p.Asp634Gly). The deletion is leading to a frameshift and most probably to a truncated protein. The substitution changes the amino acid aspartic acid to glycine and effects the C-terminal NHL region, like every known mutation in the TRIM32 gene that causes LGMD2H. Further substitutions in TRIM32 have been reported as causative before. Both mutations were confirmed by Sanger sequencing.
Therefore gene-panel diagnostics with next-generation sequencing is an appropriate method to detect causative mutations in case of diseases with clinical and genetic heterogeneity.

P-ClinG-110

Partial MED13L deletion in a patient with developmental delay, muscular hypotonia, dysmorphic features, and minor heart anomalies: further evidence for a recognizable MED13L haploinsufficiency syndrome

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MED13L (mediator complex subunit13-like) is a component of the Mediator, a multiprotein complex that is involved in transcriptional regulation. A balanced translocation disrupting the gene was previously identified in a patient with intellectual disability and transposition of the great arteries (TGA). Subsequent mutation analysis of patients with isolated TGA revealed three heterozygous missense mutations and led to the assumption that MED13L is involved in early brain and heart development. Recently, haploinsufficiency of the MED13L gene in two patients has been associated with a phenotype including hypotonia, moderate intellectual disability, facial dysmorphism, and congenital heart defects (CHD).
Here we report on a boy who was referred to us for evaluation of his developmental delay. He was born after 29 weeks of gestation following a twin-pregnancy. His twin sister is healthy and shows normal psychomotor development. In the neonatal period he had respiratory distress and sucking weakness. Echocardiography revealed a persistent ductus arteriosus botalli and patent foramen ovale; later mild pulmonal and tricuspid valve insufficiency and supraventricular extrasystoles were detected. At the age of 2 years and 9 months, he showed significant motor and speech delay, strabism, severe muscular hypotonia and dysmorphic features including facial hypotonia, hypoplastic alae nasi, micrognathia as well as large ears. Oligo/SNP array analysis (Affymetrix CytoScan HD) revealed a 100 kb deletion in chromosomal region 12q24.21 encompassing exons 4 to 9 of the MED13L gene. MLPA (multiplex ligation dependent probe amplification) analysis of the patient and his parents confirmed the deletion and showed a de novo occurrence.
The striking similarities between our patient and the previously reported deletion patients support the hypothesis of a distinct syndrome with recognizable facial features caused by haploinsufficiency of the MED13L gene. However, the cardiac phenotype of MED13L haploinsufficiency appears to be variable since the boy presented here shows only minor cardiac anomalies compared to CHD reported in previously published cases.

P-ClinG-111

A patient with partial trisomy 16q: Case report and review of the literature

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Duplications of the long arm of chromosome 16, leading to a partial or complete trisomy 16q, are a rare group of disorders with a highly variable phenotype. Variable intellectual disability, behavioural abnormalities, dysmorphic features, malformations, and often limited survival have been reported. It has been suggested that the length of the 16q duplication correlates with clinical phenotype and survival - other studies suggest that the site of the duplication correlates with the clinical phenotype.
We present an 8 year old boy with facial dysmorphism consisting of downward slanting palpebral fissures, hypertelorism, thin everted upper lip, dysplastic helices, facial and scull asymmetry, as well as

moderate to severe intellectual disability, muscular hypotonia, structural heart defect, hydronephrosis and unilateral postaxial polydactyly. Karyotyping revealed a de novo duplication of part of the long arm of chromosome 16: 46,XY,dup(16)(q11.2q23). In addition we performed Array-CGH (180K, Bluegnome) to determine the exact size and localisation of the duplication.
We present the clinical and molecular-cytogenetic information of our patient and a review of the literature of 16q trisomy including a discussion of possible genotype-phenotype correlations.

P-ClinG-112

Novel calmodulin (CALM2) mutations associated with congenital arrhythmia susceptibility

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Background: Genetic predispositions to life-threatening cardiac arrhythmias such as in the congenital long-QT syndrome (LQTS) and (CPVT) represent treatable causes of sudden cardiac death in young adults and children. Recently, mutations in calmodulin (CALM1, CALM2) have been associated with severe forms of LQTS and CPVT, with life-threatening arrhythmias occurring very early in life. Defects in calmodulin function alter crucial calcium-dependent mechanisms involved in cardiac membrane ion channels activity, suggesting novel molecular basis for life-threating conditions occurring at an early stage of life.
Methods: We employed next-generation sequencing approaches (targeted exon capture, exome sequencing) along with conventional targeted mutation screening in three cohorts of genotype-negative LQTS probands.
Results: We identified three novel de novo missense mutations in CALM2, in two subjects with LQTS and one subject with clinical features of both LQTS and CPVT.
One mutation (p.D132E) was discovered by targeted screening of calmodulin genes. The proband was an adult German female with a history of perinatal bradycardia and neonatal LQTS as well as clinical features consistent with CPVT in later childhood.
A second CALM2 mutation (p.D134H) was detected by targeted candidate gene sequencing of 11 Japanese LQTS probands. This mutation was found in a girl who had suffered at age 19 months a syncopal event associated with long QTc (579 ms) followed by several episodes of cardiac arrest during exertion.
Another mutation (p.N98S) was discovered by analyzing exomes of 190 genotype-negative Japanese LQTS probands. This mutation was present in a 5 year-old boy with multiple episodes of exercise-induced syncope and dizziness along with a QTc of 478 ms. Interestingly, p.N98S mutation in a different calmodulin gene (CALM1) was previously associated with CPVT in an Iraqi child.
All mutations affect highly conserved residues located within the third (p.N98S) or forth (p.D132E, p.D134H) calcium binding loops in the carboxyl-terminal domain, and mutant calmodulin proteins exhibited reduced calcium binding affinity.
Conclusions: CALM2 mutations can be associated with less severe forms of LQTS and with overlapping features of LQTS and CPVT, suggesting the possibility of multiple ion channel targets of dysfunctional calmodulins.

P-ClinG-113

A family with microcephaly, visual problems, mild intellectual disability (ID) and a mutation in KIF11

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Mutations in KIF11 cause the autosomal dominant condition microcephaly with or without chorioretinopathy, lymphoedema, or mental retardation (MCLMR, MIM 152950). It is a rare condition and so far only a few families with mutations in KIF11 have been reported (Ostergaard et al., 2012, Jones et al., 2013, Hazan et al., 2012).
Here we present a mother and her daughter, presenting with congenital microcephaly, visual problems and mild intellectual disability (ID). The mother had chorioretinopathy and the daughter myopia. They did not show any signs of lymphoedema. They shared the same dysmorphic features with a prominent nose, thickened eyebrows, thick lower lip and a prominent chin. These dysmorphic features are consistent with those patients previously described (Vasudevan et al., 2006, Ostergaard et al., 2012, Jones et al., 2013). Molecular analysis of the KIF11 gene confirmed a nonsense mutation (c.1159C>T, p.Arg387*) in the mother and her daughter.
We present the clinical and molecular data from this family and give a short review on this rare condition characterized by microcephaly with or without chorioretinopathy, lymphoedema, or mental retardation (MCLMR) with a wide clinical spectrum.

P-ClinG-114

A novel hemizygous OFD1 mutation leads to aberrant splicing in a five year old boy with Joubert syndrome

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Joubert syndrome (JBTS) is characterized by a distinctive midhindbrain malformation. The corresponding hallmark of JBTS in axial MRI imaging is the 'molar tooth sign'. Postnatal breathing abnormalities, muscular hypotonia and developmental delay are common, additional symptoms such as retinal dystrophy, cystic kidney disease, hepatic fibrosis and polydactyly may be present. JBTS is genetically heterogeneous with mutations in more than 20 causative genes reported to date. Whereas inheritance is mostly autosomal recessive, mutations of OFD1 (OMIM 300170) cause the only known X-linked JBTS subtype (OMIM 300804). We present a 5 year old boy with severe developmental delay, muscular hypotonia, absent speech development, epilepsy, and bilateral polydactyly. In addition he has dysphagia with frequent aspiration. MRI scan showed cerebellar hypoplasia with molar-tooth-sign, arachnoid cyst, and right sided abnormal gyration with polymicrogyria and pachygyria. By targeted massively parallel sequencing of all known JBTS genes, we identified a novel OFD1 mutation: c.2488+5G>T, which affects the extended splice donor motif of intron 18. The mutation

was inherited from the unaffected mother. Sequencing of OFD1 RT-PCR products showed no wild-type transcript but revealed aberrant transcript variants with skipping of exon 18, exons 18/19, and exons 18/19/20. All aberrant transcript variants disrupt the reading frame and very likely lead to mRNA degradation due to nonsense-mediated decay (NMD) or a truncated non-functional protein. In any case, they were all present besides the wild-type transcript in the patient's mother. In conclusion, a constitutive c.2488+5G>T splice site mutation in OFD1 is pathogenic and causes X-chromosomal JBTS in the patient reported here.

P-ClinG-115

Case-control study of the MTHFR polymorphism C677T in case-mothers of children with neural tube defects and control-mothers of Pakistani origin.

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Introduction: Neural tube defects (NTDs) are congenital malformations accompanied with low birth weight, which result from a failure of the neural tube to close during the fourth week of embryogenesis. Genetic studies examining the gene coding for the folate metabolising methylenetetrahydrofolate reductase (MTHFR) enzyme suggest that the functional 677C/T thermolabile polymorphism contributes a genetic risk to NTDs. Previous studies suggest that not only the fetal genotype but also the maternal genotype might have an impact on the fetal development. Here we carried out a case-control study of case-mothers and control-mothers of Pakistani origin.
Patients and Methods: We examined 109 case-mothers of children born with NDTs and of 100 control-mothers without history of NTDs in their offspring. Both, case-mothers and control-mothers were of Pakistani origin. Genotype comparisons were carried out using Fisher's exact test.
Results: Genotype analysis of case-mothers and control-mothers revealed 11(10.09%) and 2(2%) respectively to be homozygous for the MTHFR 677T allele,furthermore 32(29.35%) case-mothers and 26(26%) control-mothers to be heterozygous (two-tailed p-value 0.0331).
Conclusion: We found the homozygous and heterozygous MTHFR 677TT/CT genotypes to be more frequent among case-mothers compared to control-mothers. Combined analysis of these results with the information of folate supplementation during the periconceptional period of case-mothers and control-mothers is warranted to further elucidate the role of the MTHFR genotype and folate supplementation per se in the risk of NTDs in pregnancies of women of Pakistani origin.

P-ClinG-116

Incidental detection of chromosomal aneuploidy by targeted NGS for retinitis pigmentosa

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With more than 80 causative genes identified to date, retinitis pigmentosa (RP) is a genetically extremely heterogeneous condition. We have established targeted next-generation sequencing (NGS) of more than 120 genes associated with non-syndromic retinal dystrophy. Besides mere sequence analysis, we routinely quantify NGS reads to detect structural rearrangements such as deletions comprising one to multiple exons. Through this approach, we are able to identify the causative mutations in the majority of patients with RP and related conditions. Here, we report a 4 bp deletion in an X-linked recessive RP gene, RP2, predicted to result in a loss-of-function allele, in a male simplex patient with early-onset and rapidly progressive RP. Surprisingly, the wildtype sequence was also present in about half of the reads, and the heterozygous state of the mutation was confirmed by Sanger sequencing. Quantitative readout for all X-chromosomal genes in our panel comparing the patient with male and female controls consistently indicated the presence of additional X-chromosomal material in the patient. Chromosome analysis revealed a 47,XXY karyotype in all cells, consistent with Klinefelter syndrome. Clinical manifestation of RP2 mutations in females (and very likely, in individuals with Klinefelter syndrome) is unusual. X chromosome inactivation assay revealed a skewed X inactivation pattern. Therefore, RP in our patient likely results from preferential expression of the mutant RP2 copy. Combined sequence analysis and quantification of NGS data was instrumental in identifying the complex cause of RP (sex chromosome aneuploidy, X-linked point mutation and skewed X-inactivation) in this patient. We suggest that patients should be informed that NGS aimed at the identification of mutations for Mendelian diseases may incidentally reveal sex chromosome aneuploidies that would have remained undiagnosed otherwise.

P-ClinG-117

The updated PKHD1 mutation database for autosomal recessive polycystic kidney disease (ARPKD) meets the ARegPKD multinational European ARPKD patient registry

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Mutations in the PKHD1 (Polycystic Kidney and Hepatic Disease 1) gene on 6p12 are causative for autosomal recessive polycystic kidney disease (ARPKD). The incidence of ARPKD is about 1 in 20,000 live births. It is characterized by massively enlarged bilateral polycystic kidneys and congenital hepatic fibrosis. The PKHD1 gene is complex due to its genomic size (longest ORF 66 exons) and its transcript structure. The detection rate of about 90% for at least one pathogenic variant in our patient cohort is in line with data from the literature. The mutations are distributed over the whole coding region and the adjacent intronic sequences. Common mutations account for only 10–20% of all PKHD1 mutations whereas the remaining mutations are largely unique to individual families (private mutations). Our PKHD1 mutation database (http://www.humgen.rwth-aachen.de) has been estab-

lished in 2003 to catalogue all changes detected in the PKHD1 gene in a disease specific database. Almost 750 PKHD1 pathogenic mutations, variants of unknown significance and polymorphisms are currently listed. We continuously update the database and include new data and information to further improve the data quality. In detail about 150 variants were added within the last two years. To strengthen the accuracy of predictions regarding a possible pathogenicity of variants the frequency in control chromosomes data were revised. Furthermore the minor allele frequency (MAF) was extracted from the dbSNP137 database and included if available. Additionally data regarding segregation analyses, the evolutionary conservation among six species and rs-numbers in case of dbSNP137 annotated variants have been included now. The bioinformatic validation with web-applications was reevaluated with PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/) and the MutationTaster tool (http://www.mutationtaster.org/).
Further plans encompass the inclusion of mutations documented in the international ARegPKD patient registry. ARegPKD was recently established with support of the German Pediatric Nephrology Association (GPN) in cooperation with the European Study Consortium for Chronic Kidney Disorders Affecting Pediatric Patients (ESCAPE Network) with the aim to collect data on the clinical courses of ARPKD patients. A multicenter multinational approach has been chosen to achieve deep phenotyping of a well-described cohort to increase the knowledge about this severe kidney disorder.
To sum up: details on the mutation database update are presented and the ARegPKD patient registry is introduced.

P-ClinG-118

Evaluation of Results of Patients Subjected to Cardiovascular Disease (CVD) Panel in South-East Region of Turkey

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Cardiovascular disease (CVD) risk factors, such as arterial hypertension, obesity, dyslipidemia or diabetes mellitus, as well as CVDs, including myocardial infarction, coronary artery disease or stroke, are the most prevalent diseases and account for the major causes of death worldwide. In the present study, 4,709 unrelated patients subjected to CVD panel in south-east part of Turkey between the years 2010–2013 were enrolled and DNA was isolated from the blood samples of these patients. Mutation analyses were conducted using the real-time polymerase chain reaction (RT-PCR) method to screen six common mutations (Factor V G1691A, PT G20210A, Factor XIII V34L, MTHFR A1298C and C677T and PAI-1 -675 4G/5G) found in CVD panel. The prevalence of these mutations were 0.57%, 0.25%, 2.61%, 13.78%, 9.34% and 24.27% in homozygous form, respectively. Similarly, the mutation percent of them in heterozygous form were 7.43%, 3.44%, 24.91%, 44.94%, 41.09% and 45.66%, respectively. No mutation was detected in 92 (1.95%) patients in total. Because of the fact that this is the first study to screen six common mutations in CVD panel in south-east region of Turkey, it has a considerable value on the diagnosis and treatment of these diseases. Upon the results of the present and previous studied, a careful examination for these genetic variants should be carried out in thrombophilia screening programs, particularly in Turkish population.

P-ClinG-119

Screening of Common and Novel Familial Mediterranean Fever Mutations in South-East Part of Turkey

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Familial Mediterranean Fever (FMF) is an autosomal recessive autoinflammatory disorder (MIM#249100), particularly common in populations of Mediterranean extraction. MEFV gene, responsible for FMF, encoding pyrin has recently been mapped to chromosome 16p13.3. In the present study, 3,341 unrelated patients with the suspicion of FMF in south-east part of Turkey between the years 2009–2013 were enrolled and genomic sequences of exon 2 and exon 10 of the MEFV gene were scanned for mutations by direct sequencing. We identified 43 different type of mutations and 9 of them were novel. DNA was amplified by PCR and subjected to direct sequencing for the detection of MEFV gene mutations. Among the 3,341 patients, 1,598 (47.8%) were males and 1,743 (52.1%) were females. The mutations were heterozygous in 804 (62.3%), compound heterozygous in 188 (14.5%), homozygous in 281 (21.8%) and mutations had complex genotype in 17 (1.32%) patients. No mutation was detected in 2,052 (61.3%) patients. The most frequent mutations were M694V, E148Q, M680I (G/C) and V726A. We could not find any significant differences between the two common mutations according to the gender. Molecular diagnosis of MEFV is a useful tool in clinical practice, thus a future study relating to genotype/phenotype correlation of FMF in more and larger group in Turkish population involving the whole MEFV gene mutations is necessary.

P-ClinG-120

Single Nucleotide Polymorphisms of the Glucocorticoid–Receptor Gene Influence the Outcome of Cardiac Surgery

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Cardiac surgery triggers a systemic inflammatory response which is associated with postoperative morbidity and mortality. Hydrocortisone is sometimes used to blunt this phenomenon and is known to influence the outcome of cardiac surgery. Glucocorticoid effects are mediated by glucocorticoid receptors (GRs) for which a number of common single nucleotide polymorphisms (SNPs) exist that influence GR sensitivity to cortisol. We selected three common SNPs of the GR gene that are known to affect GR sensitivity and analyzed these data in relation to early outcome variables in patients undergoing cardiac surgery. We tested the effects of the following GR-SNPs: rs4123247 (BcII, increased cortisol sensitivity), rs33388 (cortisol hypersensitivity) and rs10052957 (FPB5, cortisol resistance). The GR-SNP rs12054797 with no known effect on cortisol sensitivity was used as a control. Hydrocortisone was administered according to a previously validated algorithm. All study personal was blinded with regard to the patients` genotype. Study endpoints (primary outcome variables) were the required dosages of hydrocortisone during surgery and in the Intensive Care Unit. Secondary outcome variables were the maximal dosage of vasopressors (norepinephrinemax and epinephrinemax) required to achieve hemodynamic stability, postoperative plasma concentrations of cortisol and interleukin-6 and the duration of Intensive Care Unit therapy. The study was approved by the Ethical Committee of the University hospital of Munich and all patients gave informed consent. The study population consisted of 95 patients. There was no significant difference in demographic variables, ASA scores or the duration of cardiopulmonary bypass between genotypes. Homozygous carriers of alleles associated with increased GR sensitivity (BclI *G, n=10 and rs33388 *G, n=25) required significantly lower dosages of hydrocortisone in the Intensive Care Unit than non-carriers of the respective alleles. Homozygous individuals (n=6) for the TT–allele of the FPB5–SNP required significantly higher dosages of hydrocortisone during surgery and in the Intensive Care Unit than heterozygous carriers. They also needed significantly higher norepinephrinemax and epinephrinemax dosages in the Intensive Care Unit to achieve hemodynamic stability and showed a significantly longer duration of Intensive Care Unit therapy than het-

erozygous or non-carriers of the T-allele. GR–SNPs had no direct effect on p. op. plasma levels of cortisol or interleukin-6. The SNP rs12054797 with no known effect on GR sensitivity did not influence any of the outcome variables. In summary, hydrocortisone dosages administered according to evidence based algorithm to cardiac patients during and after cardiac surgery are influenced by SNPs in the GR.

P-ClinG-121

A Large Homozygous Microdeletion in the RAB3GAP1 Gene causes Warburg Micro Syndrome

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Warburg micro syndrome (WARBM) is characterized by microcephaly, severe intellectual disability, neocortical malformation, dysplasia of the corpus callosum, specific ocular manifestations, and hypothalamic hypogonadism. This rare and genetically heterogeneous, autosomal recessive syndrome is caused by biallelic mutations of the RAB3GAP1 (RAB3 GTPase-activating protein 1), RAB3GAP2, or RAB18 (RAS-associated protein RAB18) gene. Here, we delineate the so far largest intragenic homozygous RAB3GAP1 gene microdeletion of 50.8 kb encompassing exons 4-15 in two siblings of a consanguineous family of Kurdish-Armenian descent. The two affected children present with a severe phenotype of WARBM1 (MIM #600118). This mutation was identified by Sanger sequencing and confirmed by array CGH. The reported deletion of about 45% of the coding gene sequence does not provoke a specific phenotype. Rather, the severe phenotype is consistent with previous descriptions of the syndrome. This finding supports the hypothesis that RAB3GAP1 mutations mostly cause nonsense-mediated decay of the corresponding mRNA or a severe truncation of the RAB3GAP1 protein with loss of function.

P-ClinG-122

Exome Sequencing Identifies Compound Heterozygous Mutations in C12orf57 in Two Siblings With Severe Intellectual Disability, Hypoplasia of the Corpus Callosum, Chorioretinal Coloboma and Intractable Seizures

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In patients with genetically heterogeneous disorders such as intellectual disability (ID) or epilepsy, exome sequencing is a powerful tool to elucidate the underlying genetic cause. Homozygous and compound heterozygous mutations in C12orf57 have recently been described to cause an autosomal recessive syndromic form of intellectual disability, including hypoplasia of the corpus callosum, optic coloboma and intractable seizures. Here, we report on two siblings from non-consanguineous parents harboring the two compound heterozygous truncating mutations c.1A>G, p.Met1 and c.184C>T, p.Gln62* in C12orf57 detected by exome sequencing. Both mutations could be confirmed by subsequent Sanger sequencing. C12orf57 shows it's most abundant expression in fetal brain amongst others, but very little is known about its specific function.

Up to now, 21 patients in eight families have been identified with homozygous or compound heterozygous mutations in C12orf57. All patients showed developmental delay. In more than half of the cases the degree of intellectual impairment was further specified as severe ID. Almost all patients developed seizures. Cranial imaging showed either hypoplasia or agenesis of the corpus callosum in most of the patients. In addition, one third of the patients were found to have optic coloboma.

P-ClinG-123

Interstitial deletion affecting the region 7q31.2q32.3, but not the FOXP2 gene, in a boy with global developmental and speech delay

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Chromosomal microdeletions and microduplications play a significant role in the etiology of intellectual disability, developmental delay, congenital anomalies and autism spectrum disorders.
In 1998, Fisher et al. used molecular genetic studies in a large pedigree, the KE family, to assemble a critical interval for speech and language disorders (SPCH1, #602081) to a 5.6-cM region of 7q31 between D7S2459 and D7S643 encompassing the FOXP2 gene. On further studies by O`Brien et al. (2003), the FOXP2 gene was confirmed to be associated with speech and language impairment, including autism.
We report on a 2,5-year-old boy presented to us with growth and mental retardation, muscular hypotonia and facial dysmorphism. Further clinical examination revealed overlapping of the third and fourth toe, but no other skeletal anomalies. Examination at birth showed no malformations of inner organs, but some mild abnormalities on cerebral MRI. Additionally, in early childhood he suffered recurrent respiratory infections. The boy has a severe communication disorder with considerably delayed speech and language development. He is the second child of healthy, non-consanguineous parents and has a phenotypically normal sister.
G-banded chromosomal studies (resolution 550 bands) revealed an interstitial deletion within the long arm of the chromosome 7 confirmed by FISH analyses. The karyotype is: 46,XY,del(7)(q31).ish del(7)(q31q31)(D7S486-). Array-CGH analysis using a CytoChip ISCA 4x180K v1.0 (181.873 Oligonucleotide, BlueGnome, Cambridge, GB) disclosed an interstitial deletion within 7q of about 15,2 Mb (arr [hg19] 7q31.2q32.3(115,523,306-130,699,177)x1; ISCN 2013). The deleted region contains 66 OMIM genes (including CFTR, FLNC, WNT2), 24 of them listed in the OMIM database of genetic disorders. The FOXP2 gene, which is also located within 7q, was not deleted.
Parental karyotyping including FISH studies and molecular analysis for mutations on the remaining CFTR allele are in progress.
Finally, we review the small number of reported cases with interstitial deletion of 7q31.2q32.2 and present the clinical and molecular data of our patient in comparison to the literature.

P-ClinG-124

Phenotype associated methylation differences in monozygotic twins – a genome wide approach

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Monozygotic twins are genetically and epigenetically nearly identical. However differences in the epigenetic profile between the twins increase with age due to environmental influences. Nevertheless they provide an unequivocal source to study disease related changes, as they are perfect matched controls. Recent epigenome-wide association studies of disease-discordant twins revealed an association between the phenotypes and differentially methylated regions for several traits.

In the presenting study we investigated the methylome of 24 monozygotic twin pairs (n=48) using Illumina's Infinium HumanMethylation450 BeadChips. Illumina's Bead Chip interrogates 485577 single CpG's distributed over the whole genome and returns the methylation state of each individual investigated cytosine in terms of percentage. Statistical analysis was conducted to identify differentially methylated regions between the twin pairs with respect to different phenotypes like discordances in birth weight, birth height or blood pressure. The top loci showing differential methylation further underwent validation experiments using targeted deep amplicon sequencing on an Ion Torrent.

Statistical evaluation of both the BeadChip derived as well as the Ion Torrent derived data revealed the presence of unique methylation patterns and differentially methylated loci between the different investigated phenotypes. Although differential methylation between the twins was highly significant, it was also shown that the difference in methylation intensities was for many CpGs below 10%.

Consequently, the conducted epigenome-wide association study confirmed differential methylation in phenotype-discordant monozygotic twins. In the future longitudinal studies would be needed to identify the role of epigenetic characteristics in disease.

P-ClinG-125

Mutations in CERS3 cause autosomal recessive congenital ichthyosis in humans.

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Autosomal recessive congenital ichthyosis (ARCI) is a rare genetic disorder of the skin characterized by abnormal desquamation over the whole body. In this study we report four patients from three consanguineous Tunisian families with skin, eye, heart, and skeletal anomalies, who harbor a homozygous contiguous gene deletion syndrome on chromosome 15q26.3. Genome-wide SNP-genotyping revealed a homozygous region in all affected individuals, including the same microdeletion that partially affects two coding genes (ADAMTS17, CERS3) and abolishes a sequence for a long non-coding RNA (FLJ42289). Whereas mutations in ADAMTS17 have recently been identified in autosomal recessive Weill-Marchesani-like syndrome in humans and dogs presenting with ophthalmologic, cardiac, and skeletal abnormalities, no disease associations have been described for CERS3 (ceramide synthase 3) and FLJ42289 so far. However, analysis of additional patients with non-syndromic ARCI revealed a splice site mutation in CERS3 indicating that a defect in ceramide synthesis is causative for the present skin phenotype of our patients. Functional analysis of patient skin and in vitro differentiated keratinocytes demonstrated that mutations in CERS3 lead to a disturbed sphingolipid profile with reduced levels of epidermis-specific very long-chain ceramides that interferes with epidermal differentiation. Taken together, these data present a novel pathway involved in ARCI development and, moreover, provide the first evidence that CERS3 plays an essential role in human sphingolipid metabolism for the maintenance of epidermal lipid homeostasis.

P-ClinG-126

Non allelic homologous recombination during meiosis leading to a variable pattern of SHOX deletion or duplication in familial dyschondrosteosis

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Leri Weill dyschondrosteosis is caused by haploinsufficiency of the SHOX gene and its downstream transcriptional regulatory region. The clinical characteristics include disproportionate short stature, mesomelic limb shortening and Madelung deformity. The phenotype of disorders associated with SHOX anomalies is characterized by high inter- and intrafamilial heterogeneity and a lack of correlation with genotype. Thus it is possible that affected members of the same familiy develop variable clinical signs ranging from absent, mild to severe forms of dyschondrosteosis.

We report on an unusual case of familial dyschondrosteosis. MLPA analyses and FISH were performed to detect the deletions and duplications within the SHOX region.

The index patient was found to carry a classic SHOX deletion in combination with a partial deletion of the regulatory region proximal of the SHOX gene on his Y-chromosome. His phenotype was remarkable for short stature and mesomelic limb shortening. His daughter (5 years) and his son (3 years) were subsequently shown to carry an extended deletion including the entire SHOX gene and the complete proximal regulatory region. Both children presented with borderline short stature and mild to moderate mesomelic limb shortening. Interestingly, his third child, a newborn daughter, carried a duplication of a part of the regulatory region of the SHOX gene, which was not seen in the father. This child developed no symptoms yet. These findings suggest, that nonallelic homologous recombination during male meiosis leads to different patterns of SHOX deletions or duplications even within the same family.

P-ClinG-127

Fragile X- like phenotype in a boy with maternally inherited microduplication 7q11.23 syndrome

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Background. Language impairment is considered to be a complex disorder with several gene loci interacting with one another and influenced by environmental factors. Somerville et al described 2005 for the first time a syndrome associated with reciprocal duplication of the Williams syndrome microdeletion region. In affected patients expressive language presents the area of greatest weakness. Recent reports estimate this microduplication to be clinically recognizable. Here we report a case with high similarity to a fra X syndrome phenotype, tall stature and vertical transmission.

Clinical report T.Z. is the first child of unrelated parents.The patient's mother showed mild intellectual disability but normal speech, the father is healthy.He was born after full-term pregnancy without any neonatal problem. All parameters of the neonate were in the upper normal range. Motor development was slightly delayed, unaided walking became possible with 14 mo. At the age of 3a severe delay in expressive language was noted, as well as behavioural disturbance with attention deficit disorder, and a clinical-genetic investigation was initiated. At this time, the patient presented with tall stature (Pc 97), but normal weight and OFC, broad forehead, large protruding and simply formed ears, happy mood, joint laxity and hyperagility. Receptive language was adaequate, expressive language however completely lacking. The syndromatic aspect was highly suggestive for fragile X-syndrome.

Lab investigations.Lymphocyte culture (GTG banding) resulted in a normal male karyotype. PCR amplification of the CGG repeat of the FMR-1 gene revealed a product with normal allel size. MicroArray analysis (Affimetrix) showed an interstitial 1.5 Mb duplication in 7q11.23 including the whole region of the 7q11.23 duplication syndrome. Lastly, FISH and MLPA investigations revealed the identical duplication in the patient's mother.
Discussion The most common clinical feature of 7q11.23 microduplication is expressive speech delay. This hallmark is in contrast to the ability to speak in Williams syndrome-patients. In addition, some dysmorphic features and behavior characteristics show a contrary phenotype to Williams syndrome. In our patient the facial features, the relatively tall stature and somewhat autistic behaviour were suggestive for fragile X syndrome. The maternal transmission of the duplication was reported so far only in singular cases (Dixit, 2013). Given the still limited number of patients known to be affected with this particular microduplication and the variable phenotypic expression it seems reasonable to consider this probably underdiagnosed condition in all similar fraX-negative cases by array investigations.

P-ClinG-128

Clinically Significant Variant (CSV) Analysis

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With the ingression of next generation sequencing (NGS) into clinical laboratories, a new level of medical diagnoses has been reached. NGS is assumed to be a fast and easy way for in depth analyses at the molecular level. Clinicians require diagnoses of genetic diseases and send their requests to the laboratory with a delineation of the suspected illness. In the laboratory, the genes involved in the clinical presentation have to be depicted and an in silico synthesis of an appropriate primer panel for NGS is started. This process needs some iterations because the sequencing coverage of the genes sought must be optimized. Once successful sequence analysis is completed, a variant list of the deviations from the reference sequences can be compared with several databases for clinically significant variations. Consequences of variations without curated clinical significance can be predicted, but their meaning for the referring clinician is doubtful.
Thus, NGS still presents substantial efforts for laboratories not specialized in just particular genetic diseases. Therefore, for a more general approach, we have come up with CSV analysis (as proposed by A. R.). To this end, we have devised a primer panel covering all OMIM curated clinically significant variations (CSV) for NGS. By utilizing only this panel, we can optimize the performance of this panel, it can be adapted at will, and the interpretation of the results is streamlined. All variations found are curated and meaningful for the clinician. The search for genes involved in the clinic of a particular genetic disease is omitted, but mutations in exactly these respective genes will still be found. Of course, care must be taken to only report variations that are specific to the referral in order not to violate ethical considerations and judicial regulations concerning genetic analyses. With the upcoming ClinGen database, this primer panel can be further optimized to cover only variations that are explicitly causing genetic diseases.

P-ClinG-129

Clinical variability of MFN2 mutations, the major cause of axonal Charcot-Marie-Tooth neuropathy

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Charcot-Marie-Tooth (CMT) neuropathy is clinically and genetically heterogeneous. MFN2 gene mutations are responsible for autosomal dominant CMT2A and account for 10-30% of axonal CMT disease. Rarely, biallelic mutations are seen confirming autosomal recessive neuropathy. Several studies imply that CMT2A is characterized by an early onset in childhood and severe impairment with a large proportion of patients becoming nonambulatory before age 20. Additional features include pyramidal tract sings, optic atrophy, and brain MRI abnormalities. The estimated rate of de novo mutations is high (about 20-30%) and reflects the clinical severity. Few families have a late onset between 20 and 40 years and mild disability, suggesting a bimodal distribution of severity. Familial concordance indicates that severe and mild phenotypes are determined mainly by the position of mutations in the MFN2 gene.
Within a large series of unrelated axonal CMT patients who underwent genetic testing in our laboratory (n=289), we detected 20 patients out of 14 families with MFN2 gene mutations, representing 5% of all patients with axonal CMT or 8% of those with dominant inheritance (n=89). Age at onset and severity showed a broad spectrum with 7 patients starting with symptoms under age 10. Four patients had an age at onset of 10-20 years, 4 patients received the diagnosis at age 40-70 years being mildly affected relatives of index patients, and in 4 patients no clinical data were available. One patient was a marathon runner up to his late fifties and started with peroneal weakness at age 70-75. When examined at age 82, he had calf muscle atrophy and used a walking stick. Eight patients had a negative family history, 2 of whom had a de-novo dominant mutation confirmed. Patients with an early onset and negative family history had mutations upstream or in the GTPase domain of MFN2 (p.R94W, p.R94Q, p.T206I, p.Q235H), however, this also applied to the patient starting in his 70ies (p.R250E). Most patients had a clinical picture similar to other CMT types, apart from one patient with mild spasticity and increased tendon reflexes, none had optic atrophy or other features pointing towards CMT2A. One patient with early onset used a wheelchair at age 19 for longer distances, but all other patients, of whom clinical details were available, were still ambulatory at the time of examination (median age 13 years, range 4-82 years). Electroneurographic results were documented in 9 patients who generally showed normal motor nerve conduction velocities (median nerve 46-60 m/s), while no motor nerve potentials were recordable in the lower extremities in 3 patients.
To conclude, MFN2 gene mutations are rare causes of hereditary neuropathies but correspond clinically well to other more prevalent types. If reduced nerve conduction velocities are present, CMT2A is highly unlikely. NGS based gene panels will facilitate genetic diagnosis in the heterogeneous group of CMT disease.

P-ClinG-130

Dominant forms of Hereditary Motor and Sensory Neuropathy type I: spectrum of mutations and phenotype for Belarusian patients

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Hereditary motor and sensory neuropathy I type (HMSNI) is genetically heterogeneous group of peripheral nervous system disorders with distinct prevalence of dominant forms: HMSN subtype IA (MIM 118229) and IB (MIM 118200) showed autosomal dominant (AD), HMSNIX (MIM 302800) - X- linked dominant (X-D) inheritance.
We presented a whole spectrum of mutations identified in selective group of the patients with presumably HMSNI. Results. DNA investigations of 3 most frequently involved genes - PMP22 (location 17p11.2), MPZ (location 1q22.1-q23) and GJB1 (location Xq13.1) - made for patients whose disease manifested by symmetrical progressive weakness, atrophy of distal limb muscles, foot deformity, diminished or absent tendon reflexes, sensory disturbances, reduced nerve conduction velocities (NCV<38 m/s) revealed HMSNI in 101 cases in 51 families (46 males, 55 female). Ratio of subtypes is HMSNIA 82.4% (42/51), HMSNIX 13.7% (7/51), HMSNIB 3.9% (2/51). 23 families showed 2-4

affected persons. Prenatal DNA diagnostics performed in one family detected healthy fetus. HMSNIA (AD). DNA study of PMP22 gene using locus-specific markers D17S2218, D17S2220, D17S2223, D17S2226, D17S2229 identified dup17p11.2 in 76 patients from 42 families. HMSNIB (AD). DNA resequencing of 6 exons of MPZ gene detected 2 point mutations (IVS1-2A C*; Thr124Met) in 2 and 3 exons accordingly with following myelin protein Po defect in 5 patients from 2 families. HMSNIX (X-D). Mutational analyses of GJB1 gene identified 7 mutations (Ser50Cys*, Leu90Ile*, Trp133Stop*, Tyr135Cys, Val181Met, Glu208Lys, Stop284Leu*) in 20 patients from 7 families. Phenotypes were compared with purpose to delineate the clinical peculiarities of HMSNI subtypes for the DNA diagnostics selection. Patients with HMSNIA showed common course and clinical picture of peripheral neuropathy with demyelinating findings (median NCV <32 m/s). In adult HMSNIB patients the disease manifested by symmetrical distal motor and sensory disturbances, tremor, ataxia, demyelinating-axonal electrophysiological signs, visceral abnormalities. Additional features: myopia, hearing impairment, scoliosis, urethrohydronephrosis, enuresis, neurogenic uric bladder. Affected HMSNIB offspring (boys aged 3 and 5 years old) showed reduced nerve conduction velocities at earlier stage of disorder. Males suffering from HMSN1X showed more severe phenotype than affected female. Inter- and intra-familial variability was found in all HMSNI subtypes. Diagnostics. Genetic counseling of patients with suspected HMSNI for clinical, genealogical, NCV data → DNA studies: PMP22 gene (dup17p11.2) > GJB1 gene>MPZ gene> HMSNI confirmation → Genetic prognoses → Prenatal DNA diagnostics. Conclusion. Detection of the mutation makes it possible to calculate genetic risk for outcome and to perform prenatal DNA diagnostics of HMSNI in the affected families.
* - mutations described for the first time for Belarusian patients

P-ClinG-131

Autosomal recessive congenital ichthyosis: Mutation screening by Next Generation Sequencing

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Autosomal recessive congenital ichthyosis (ARCI) constitutes a heterogeneous group of keratinization disorders characterized mainly by abnormal skin scaling over the whole body. These disorders are mostly non-syndromic and limited to skin; a total of 60–70% of patients present with severe symptoms, including a collodion membrane at birth. The main skin phenotypes are lamellar ichthyosis and congenital ichthyosiform erythroderma, although phenotypic overlap in the same patient or in patients of the same family can occur. The different forms of ARCI can be caused by mutations in 8 genes (TGM1, ALOXE3, ALOX12B, NIPAL4 (ICHTHYIN), ABCA12, CYP4F22, PNPLA1 and CERS3). Consequently, the genetic diagnosis of this disease has so far been very complex. Stepwise analysis of various genes with traditional Sanger sequencing is elaborate, time-consuming and expensive. Therefore, next generation sequencing (NGS) technologies are the most promising approaches to identify mutations in ARCI.
We established amplicon-based high-throughput sequencing for ARCI. A sample of 17 patients with typical clinical symptoms of the disease was included to DNA mutational screening. Multiplex PCR were performed by the use of primers composed by a gene-specific part and a universal tail. Each primer was labeled by a molecular identifier (barcode), which serve to identify specific patient´s DNA sample.
Depending on the initial clinical diagnosis, we identified causative mutations in 71% of the ARCI patients by this comprehensive genetic testing. The genes ALOX12B and NIPAL4 were more frequently affected than others. In 29% of patients with autosomal recessive congenital ichthyosis no mutation could be identified. NGS-based mutation analyses are reliable and cost-efficient approaches in gene diagnostics of genetically heterogeneous diseases like ARCI.

P-ClinG-132

A NCAM2 deletion in a patient with autism

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An 8-year-old boy with autism spectrum disorder, speech delay, behavioural problems, disturbed sleep and macrocephaly presented in our genetics clinic. He is the first child of non-consanguineous parents. Chromosomal analysis had revealed a normal male karyotype 46,XY, and testing for fragile X syndrome and PTEN sequencing was inconspicuous.
Array-CGH analysis indicated a microdeletion of 1.6MB: arr 21q21.1-q21.2(22444986-24047363)x1. This region of chromosome 21 contains the entire NCAM2 gene and no other functional genes. His mother also carries this microdeletion and is macrocephalic. Results of psychological assessments of the mother revealed no behavioural features of autism. Three maternal blood relatives are reported to have speech problems. However, two of these do not carry the NCAM2- deletion. Instead, the deletion was present in at least two non-autistic family members, as shown by MLPA.
Autism spectrum disorder (ASD, OMIM 209850) encompasses different forms of autism with a broader phenotype. Two-thirds of all patients with ASD suffer from mental retardation. Among the genes involved, NCAM2 has been assumed to play a role in the development of ASD because of its function in neurites (outgrowth, bundling). In the literature, there is one report of an autistic boy with an 8.8 MB-microdeletion involving 19 genes including NCAM2 and another autism-related candidate gene, GRIK1. Our results indicate, that although the heterozygous deletion of NCAM2 might play a role in the development of ASD, there must be other genetic and/ or non-genetic variants leading to the presentation of clinical symptoms in our patient.

P-ClinG-133

Diagnosing hereditary ataxias in a cohort of consanguine patients using a Next-Generation-Sequencing panel

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Background: Hereditary ataxias impose a relevant challenge when molecular diagnosis is sought. While more than 100 genes are involved in Mendelian diseases with ataxia, only a small proportion of these genes have been systematically tested in cohorts of patients with a consanguine family history. With the advent of next-generation-sequencing (NGS) a massive sequencing approach can be implemented with relatively ease. We investigated the occurrence of disease causing variants sequencing a cohort of closely related patients recruited for the EUROSCA and NEUROMICS EU projects respectively. The families originated mainly from the Mediterranean area. Each patient was strictly selected to avoid sequencing of persons suffering non hereditary kinds of ataxia or ataxia due to triplet repeat enrichment.
Methods: We have established a selector-based enrichment method (HaloPlex, Agilent) specifically targeting 140 known ataxia genes as

well as genes causal for rare diseases possessing a phenotypic overlap with ataxia. The panel covers most known genes causal for pure ataxia, mitochondrial ataxia and metabolic ataxia as well. A total of 582kb genomic DNA is specifically enriched and sequenced by Illumina MiSeq (2x 150 bp paired-end). Data analysis is accomplished using an in house bioinformatics pipeline based on ANNOVAR.
Results: Although massive parallel sequencing usually brings up a couple of variants (Ø 384 ± SD 16), filtering for rare variants (in our own NGS database and in 1000g, ESP6500) and for functional relevance (ns,ss,indel) reduced this count to Ø 20 ± SD 4. A statistical evaluation of the panels performance shows superior coverage (Ø > 96 % cov 20X ± SD 1,8) and target enrichment values (Ø 178 ± SD 48 mapping depth on target) as well. Several disease causing mutations could be identified in genes like APTX, FGF14, NPC1, PLEKHG4, SACS, SETX, SIL1, SPTBN2, SYNE1 and many others.
Conclusion: A panel sequencing approach offers a cheap and fast possibility to screen large patient cohorts for rare disease causing variants. Focusing on patients with a consanguine family background allows the discovery of rare and new variants for ataxia in a relatively high frequency.

P-ClinG-134

CHARGE and Kabuki syndrome. Two related syndromes?

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CHARGE syndrome (coloboma, heart defects, atresia of the choanae, retarded growth and development, genital hypoplasia and ear anomalies) is an autosomal dominant malformation syndrome due to mutations in the chromodomain helicase DNA-binding member CHD7. Kabuki syndrome, a congenital malformation syndrome, as well, is characterized by developmental delay, typical facial features (long palpebral fissures and ectropion of the lateral third of the lower eyelids), prominent digit pads, and skeletal and visceral abnormalities. The underlying cause of Kabuki syndrome are mutations in the MLL2 gene, which encodes for a H_3K_4 histone methyl transferase.
Here, we describe a patient who was initially diagnosed as having CHARGE syndrome based on the spectrum of inner organ malformations like choanal hypoplasia, heart defect, anal atresia, vision problems and conductive hearing disorder. However, mutation screening of CHD7 revealed no mutation, while sequencing analysis of the MLL2 gene identified the heterozygous de novo nonsense mutation c.5263 C>T (p.Gln1755*). Because of the remarkably overlap between the symptoms seen in CHARGE and Kabuki syndrome patients we performed interaction studies.
Indeed, we could describe by Co-immunoprecipitation and Duolink Pla II method proteins which interact with both, MLL2 and CHD7. Therefore, we propose that CHD7 and MLL2 are working in the same chromatin remodelling and chromatin modification machinery, regulating a subset of same genes which might explain the overlapping phenotype of both syndromes.

P-ClinG-135

From entire short arm to submicroscopic duplications of chromosome 16 - is there a common phenotype?

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Patients with duplication of the whole short arm of chromosome 16 as well as those with pure interstitial trisomy 16p13.3 are both very rare with only a few cases reported in the literature. When the first patients with entire 16p duplications were reported in the 1970s only standard karyotyping could be used to detect the chromosomal aberration. Today, even submicroscopic 16p duplications can be analyzed by array techniques.
Here we report the case of a female infant with de novo whole arm duplication of chromosome 16p detected by chromosomal analysis. Fluorescence in situ hybridization and array CGH were performed to characterize the duplication in more detail. Our patient displayed the typical clinical findings of patients with 16p duplication including dysmorphic features with proximally placed hypoplastic thumbs, severe psychomotoric retardation and constitutional growth delay. In addition, she exhibited evidence of neonatal hemochromatosis. We compared the malformation spectrum in our patient with that of patients with much smaller 16p13.3 duplications described in the literature and report on the overlapping clinical features.

P-ClinG-136

AmplideX FMR1 Process Control facilitates broader access to fragile X testing by enabling calibration of repeat sizing across electrophoresis platforms.

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Introduction Fragile X syndrome is caused by the expansion of CGG repeats in the 5' untranslated region of the fragile X mental retardation (FMR1) gene. Clinical phenotypes are apparent in both premutation carriers (55-200 repeats) and full mutation individuals (>200 repeats) with links to autism, intellectual disability, anxiety, seizures, ADHD and adult onset disorders including infertility (FXPOI) and ataxia (FXTAS). For research and routine testing, laboratories rely on PCR and different electrophoresis platforms to determine the number of CGG repeats. Therefore, a method is needed to improve sizing accuracy that can expand access to fragile X testing and to standardize results between laboratories.
Methods PCR products produced using AmplideX® FMR1 PCR reagents were compared between different electrophoresis platforms: a 3500xl Genetic Analyzer and 310 Genetic Analyzer (Life Technologies), a 2100 Bioanalyzer (Agilent Technologies) and a FlashGel™ DNA System (Lonza). The repeat lengths were standardized between platforms using the AmplideX® FMR1 Process Control which was formulated as a mixture of cell line DNA comprised of alleles corresponding to 18, 30, 32, 56, 85, 116 and >200 CGG. CGG repeat lengths for samples were derived from a linear fit of the size of the process control amplicon peaks on each platform to their expected repeat length. A combination of 5 cell line DNA and 41 clinical samples were tested with the 310 Genetic Analyzer and compared to Southern blot analysis. Two samples in this set, a male CVS and normal female, failed PCR due to low quality DNA. A separate set of 97 clinical samples was compared between the 3500xL, Bioanalyzer and FlashGel systems.
Results. The concordance was 100% for samples tested on both the 310 and 3500xL and >98% between the Bioanalyzer and the 3500xL. Samples that differed by a single CGG repeat could be distinguished on the 310 and 3500xL, whereas lower resolution and sensitivity was observed on the Bioanalyzer and Lonza gel systems. Clinical samples tested on the 310 were 100% concordant to Southern blot results or results from previous testing. Triplet repeat primed PCR products could be analyzed on the 310 or 3500xL but only gene specific PCR products could be analyzed on the other systems.
Conclusions. We demonstrate that pooled cell line controls such as the AmplideX® FMR1 Process Control can serve as a calibration tool, allowing fragile X alleles to be accurately sized and compared across

multiple platforms. In this study, higher resolution, accuracy and sensitivity were observed using capillary electrophoresis. Overall, our findings expand FMR1-based screening and diagnostic testing options for researchers and laboratories.

P-ClinG-137

Identification of a Mutation causing Hereditary Tyrosinemia type 1 in an Infant with Parainfluenca and Pneumonia

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Hereditary Tyrosinemia type 1 (HT1; OMIM 276700) is an autosomal recessive disorder resulting in the accumulation of tyrosine in body fluids and tissues due to deficiency of fumarylacetoactase. It is caused by mutations in the fumarylacetoacetate hydrolase (FAH) gene (OMIM 613871). Clinical manifestations in untreated infants and young children are severe liver and renal tubular dysfunction, abdominal pain, neurological deficits and/or respiratory problems associated with growth failure. The most severe complications are hepatic cancer and acute neurological crises. Typical biochemical findings include increased succinylacetone concentration in blood and urine, elevated plasma concentration of tyrosine, methionine and phenylalanine. Without therapy (diet low in phenylalanine and tyrosine and NTBC for tyrosinemia) this disorder is fatal.

We report a six-month-old girl of non-consanguineous healthy parents, presenting with severe pneumonia associated with parainfluenca and influenca A infection (H1N1). The clinical findings were hepatomegaly, liver cirrhosis, renal tubular acidosis and impaired blood coagulation. Urine organic acid analysis showed elevated succinylacetone, consistent with the diagnosis of HT1. Analysis of the FAH gene identified a homozygous mutation c.1062+5G>A. This patient responded well to intensive respiratory and antiinfection therapy, tyrosine-free formula and oral treatment with NTBC [2-(2nitro-4-trifluoromethylbenzoyl)-1,3- ciclohexanedione. This resulted in correction of renal tubular acidosis and improved liver function.

HT1 usually presents in infancy with features suggestive of liver disease or sepsis like symptoms. All patients need for life long dietary and pharmalogical therapy.

Neonatal screening is essential for the early diagnosis and timely therapy of this treatable disease, that otherwise may be lethal.

P-ClinG-138

UBR1 Deletions in Johanson-Blizzard Syndrome

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Johanson-Blizzard syndrome (JBS; MIM #243800) is a rare disorder that is inherited in an autosomal recessive manner. The major clinical characteristics are exocrine pancreatic insufficiency, hypo-/aplasia of alae nasi and oligodontia of permanent teeth. Further anomalies include hearing loss, scalp defects, and cognitive impairment of variable degree. JBS is caused by homozygous or compound-heterozygous mutations of the UBR1 gene. UBR1 encodes for a ligase of the N-end rule pathway, called ubiquitin-protein ligase E3 component N-recognin 1. Up to now, all known mutations have been point mutations or small deletions detected by Sanger sequencing.

Our cohort consisted of 63 patients from 52 unrelated families with a clinically well defined JBS phenotype. We detected 98 mutations in 104 alleles by Sanger sequencing of the complete UBR1 gene, which equals a mutation detection rate of 94%. To discover larger deletions and duplications, we designed MLPA probes for each of the 47 exons. The MLPA analysis was performed using reagents and enzymes that were provided by MRC-Holland (Amsterdam, The Netherlands).

In two patients with an unambiguous JBS phenotype, Sanger sequencing revealed a mutation in only one allele; two further patients were not found to harbour any mutations by sequence analysis. Those four patients were additionally analysed by MLPA technology. In patient 1, a heterozygous nonsense mutation was detected when sequencing the UBR1 gene. MLPA revealed an additional heterozygous deletion of three exons; thus, we were able to detect two pathogenic mutations in the patent's two UBR1 alleles. Patient 2 presented with a heterozygous missense mutation in UBR1. We were not able to detect a second mutation by using MLPA analysis, so one out of two alleles of patient 2 remains unresolved. In patient 3, who was born to consanguineous parents, no pathogenic alteration of the UBR1 gene was detected by Sanger sequencing; MLPA revealed a homozygous deletion of one exon. Patient 4 also had no identifiable mutations using the sequencing approach. A heterozygous deletion of four exons could be demonstrated by applying MLPA technology. In the second allele, an alteration has not been detected so far.

We were able to identify four UBR1 deletions (including one homozygous deletion due to parental consanguinity) by MLPA analysis in the six alleles that did not show a pathogenic alteration by sequencing. This raises the mutation detection rate from 94% (Sanger sequencing) to 98% (additional MLPA analysis). Those results are in line with other findings in autosomal recessively inherited disorders. The two expected, but undetected, mutations are assumed to affect the promoter or to be inversions or insertions that cannot be detected by the employed methods. Applying both Sanger sequencing and MLPA analysis, we could detect at least one pathogenic mutation in each of the clinically well-defined JBS cases, giving further proof that JBS is a non-heterogeneous disorder.

P-ClinG-139

A new case of de novo 22q11.2 distal deletion syndrome in a dysmorphic girl without congenital heart defects

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The 22q11.2 distal deletion syndrome is characterized by a rare microdeletion localized adjacently distal to the common 22q11.2 deletion (DGS/VCFS). It´s occurrence derives from a high number of low copy repeats (LCRs) within this region. While the DGS deletion is caused by recombination between LCRs in the regions LCR22-2 to LCR22-4, the uncommon distal 22q11.2 deletion arises from recombination of two LCRs from LCR22-4 to LCR22-8, with LCR22-4 being the most proximal LCR in almost all cases. The phenotypic features reported for the 22q11.2 distal deletion syndrome are developmental delay, especially of expressive language, learning disability, microcephaly, prenatal and postnatal growth retardation, congenital heart defects, minor skeletal anomalies, prematurity, and relatively common facial dysmorphic features such as upslanting palpebral fissures, dysplastic ears, long smooth philtrum and thin upper lip.

We report on a 3-year-old girl, who is the second child of healthy, non-consanguineous parents. The pregnancy was uneventful. She was born in the 41st week of gestation. The birth length was 50 cm (25th centile), the birth weight was 2610 g (<3rd centile) and the head circumference was 34 cm (25th centile). The further course showed developmental delay, postnatal microcephaly and recurrent infections. Echocardiography did not show any heart defects. We saw the girl at the age of 3 years in the clinical genetics department. Clinical examination revealed

dysmorphic findings: slightly rotated ears with Darwinian tubercles, narrow upslanting palpebral fissures, long philtrum, thin upper lip, flat midface and flat forehead. Her body length was normal (50th centile), the head circumference was 47 cm (-2SD).
We performed chromosomal analysis, FISH analysis to exclude DGS and array CGH analysis (Agilent® Sure Print G3 Human CGH 180K Microarray).
Conventional chromosomal analysis was normal and FISH analysis for DGS did not yield pathological findings. Molecular karyotyping revealed a 1.16 Mb interstitial deletion of 22q11.21q11.22 (21.798.705-22.963.000) containing 19 genes including HIC2, MAPK1 and TOP3B. FISH analyses (using a deletion-specific probe for 22q11.21) of the parents were unremarkable which is suggestive for a de novo deletion in the affected girl.
MAPK1 is discussed as a potential candidate gene for congenital heart defects that are often observed in 22q11.2 distal deletion syndrome. Here we report on another rare case of 22q11.2 distal deletion including MAPK1 gene in a patient not affected by cardiac defects.

P-ClinG-140

Causative mutations in two Turkish families with Retinitis pigmentosa as identified by exome sequencing

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Abstract
Purpose: Retinitis pigmentosa (RP) comprises a group of inherited retinal diseases, which is genetically highly heterogeneous. Typical symptoms are night blindness and loss of peripheral and the central visual field, eventually leading to blindness. The study's purpose is to identify the causative mutations in two Turkish families with RP using whole exome sequencing.
Methods: The exomes of three female members of two Turkish families were sequenced using the Illumina platform. All candidate genes for retinal degeneration were screened, because the inheritance mode has been less than clear at the outset. PCR, Sanger sequencing, fragment length analysis and denaturing high performance liquid chromatography were used to verify the results.
Results: Mutation screening in pedigree 1 revealed a 4 bp deletion (p.Glu555Glyfs*14) in exon 14 of RP GTPase regulator (RPGR) gene on the X chromosome which leads to a frameshift and a stop codon. The screening of pedigree 2 showed a 1 bp deletion in the VCAN gene on chromosome 5 (p.Ser1707Valfs*44). The mutations were detected in all affected individuals and were excluded in all healthy family members. In addition, 160 German and 60 Turkish controls showed the wild type for both mutations. The RPGR deletion has already been described, the VCAN mutation has not been reported before.
Conclusions: The mutation in exon 14 of RPGR causes a severe form of RP in males and females. A female carrier did not exhibit RP symptoms which can be explained with non-random X-inactivation, variable expressivity of RPGR mutations and/or modifying genetic factors. All these phenomena have already been described in association with X-linked RP. VCAN has been described as a candidate gene for retinal dystrophies, the mutation identified here leads to a RP phenotype with an autosomal dominant inheritance mode.

P-ClinG-141

Reporting incidental findings of carrier status for recessive disorders, and the need for trio testing: Results of a study using a comprehensive NGS panel for intellectual disability

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Intellectual disability (ID) is characterized by extensive genetic heterogeneity. Apart from chromosome disorders, mutations in several hundred genes that follow autosomal dominant, autosomal recessive or X-linked modes of inheritance have been associated with ID to date. In patients with syndromic ID, additional clinical findings can be specific enough to suspect causative mutations in a small subset of genes. However, the majority of ID patients provide no clinical clues for targeted analyses, which renders parallel screening of all known ID genes the method of choice.
We have applied an NGS-based panel including 1222 genes (the so-called Kingsmore panel) to a cohort of 40 patients with mostly sporadic and non-syndromic ID. As a result, we detected unambiguously causative mutations in genes such as SYNGAP1, ASXL3 and CTSD, which proved the suitability of this panel. The interpretation of variants in dominant genes was, however, hampered by the fact that only the patients, but not their parents (i.e., no trios) were analyzed, which necessitated parental testing of potential de novo variants by conventional Sanger sequencing. In routine diagnostics, trio testing should therefore be taken into consideration in spite of the initially higher costs.
We also detected deleterious heterozygous mutations in recessive genes in a large proportion of patients. Those mutations were in most cases not related to the clinical problems of the respective patients, but they potentially posed risks for the offspring of family members. Strategies for dealing with such incidental findings that take pre-analytical counselling, severity and prevalence of the respective disorder and characteristics of the variant into account will be discussed and illustrated with selected examples.

P-ClinG-142

Clinical spectrum of females with HCCS mutation: cell selection mechanisms determine the phenotype of microphthalmia with linear skin defects (MLS) syndrome

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Background: Segmental Xp22.2 monosomy or an heterozygous HCCS mutation is associated with the microphthalmia with linear skin defects (MLS) or MIDAS (microphthalmia, dermal aplasia, and sclerocornea) syndrome, an X-linked disorder with male lethality. HCCS encodes the holocytochrome c-type synthase involved in mitochondrial oxidative phosphorylation (OXPHOS) and programmed cell death.
Methods: We characterized the X-chromosomal abnormality encompassing HCCS or an intragenic mutation in six new female patients with an MLS phenotype by cytogenetic analysis, fluorescence in situ

hybridization, sequencing, and quantitative real-time PCR. The X chromosome inactivation (XCI) pattern was determined and clinical data of the patients were reviewed.
Results: Two terminal Xp deletions of ≥11.2 Mb, two submicroscopic copy number losses, one of ~850 kb and one of ≥3 Mb, all covering HCCS, 1 nonsense, and one mosaic 2-bp deletion in HCCS are reported. All females had a completely (>98:2) or slightly skewed (82:18) XCI pattern. The most consistent clinical features were microphthalmia/anophthalmia and sclerocornea/corneal opacity in all patients and congenital linear skin defects in 4/6. Additional manifestations included various ocular anomalies, cardiac defects, brain imaging abnormalities, microcephaly, postnatal growth retardation, and facial dysmorphism. However, no obvious clinical sign was observed in three female carriers who were relatives of one patient.
Conclusion: Our findings demonstrate a wide phenotypic spectrum ranging from asymptomatic females with HCCS mutation to patients with a neonatal lethal MLS form. Somatic mosaicism and the different ability of embryonic cells to cope with an OXPHOS defect and/or enhanced cell death upon HCCS deficiency likely underlie the great variability in phenotypes.

P-ClinG-143

Panel Diagnostics for Deafness Disorders using Next-Generation Sequencing

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Hearing impairments can be classified in many different ways and have an incidence rate of approximately one in 1000 births and additionally affect 50% of the population at age 80 or higher. There are multiple genetic or non-genetic causes for hearing loss. The non-genetic factors include ototoxic drugs, perinatal infections or traumas. In most cases both exogenous factors and mutations in one or more genes contribute to the phenotype. 80% of all familial, non-syndromic deafness cases are inherited in an autosomal recessive manner. In half of them mutations in the GJB2 (gap junction protein connexin 26) are responsible for the phenotype. Sometimes heterozygous mutations in the GJB2 can occur in combination with a heterozygous deletion del(GJB6-D13S1830) in GJB6 (gap junction protein connexin 30). Furthermore there are over 70 genes known which can cause different types of deafness (autosomal dominant, autosomal recessive, X-linked recessive or mitochondrial).
In a pilot study we designed a deafness gene panel comprising 75 nuclear genes and 6 mitochondrial genes associated with hearing impairment. In addition to 3 positive and 7 negative controls we sequenced 2 related patients with non-syndromic deafness. Conventional diagnostics by GJB2 sequencing and GJB6 deletion analysis was exhausted and yielded no results. Sequencing was performed on the Illumina MiSeq Next-Generation Sequencing platform. Data analysis was performed using CLCbio workbench (v6.5) and custom developed Perl scripts. The target regions, in total encompassing 463,987bp, were enriched via in-solution oligonucleotide hybridization and capture (Illumina TSCE). On average, 95- 97% of the reads could be mapped to the human genome (build hg19), of which between 69- 71%, respectively were on target. On the basis of the above mentioned controls a variant calling pipeline was established and validated.
Using that pipeline the 2 patients were analyzed. Mother (age 47) and daughter (age 7) are both affected with hearing impairment showing variability in the individual clinical appearance. The mother's disease onset was at the age of 4. Her hearing loss is more severe than her daughter's. The daughter was diagnosed with 'minimal peripheral hearing loss': she presented with slight delay of speech and behavioral problems. At age 5 hearing threshold measurement was conspicuous, showing decreased bone conduction threshold. Her symptoms improved over the last years. Any intake of ototoxic drug was not investigated. After no causal mutation in the nuclear chromosomes was detected, the A7445G mutation of the mitochondrial genome was found. This mutation is known in the literature to be associated with deafness induced by ototoxic drugs or independent of them. Any intake of ototoxic drug was not investigated. The mutation was confirmed independently by Sanger sequencing.

P-ClinG-144

SCN9A related pain-disorders: expanding the mutation spectrum of this channelopathy

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Pain stimuli are detected by nociceptors - neurons that transmit sensory information via long axons from the body periphery to the spinal cord. Nociceptor function critically depends on voltage-gated sodium ion (NaV) channels that are essential for electrogenesis in excitable cells. Nine pore-forming α-subunits of such channels (NaV1.1–NaV1.9) have been identified in mammals and three of them (NaV1.7, NaV1.8 and NaV1.9) are preferentially expressed in peripheral neurons. NaV1.7, encoded by SCN9A, is a "chameleon" concerning the clinical phenotype associated with mutations in the gene. Mutations in the gene have been linked to human pain disorders ranging from a congenital insensitivity to pain (CIP) on the one hand to extreme pain disorders termed primary erythromelalgia (PE) and paroxysmal extreme pain disorder (PEPD) on the other hand. Recently, mutations in the channel have also been identified in Small Fiber Neuropathy, a neurodegenerative disorder of small sensory nerve terminals.
Using next-generation-sequencing in patients with different pain disorders we identified novel mutations in SCN9A. In a sporadic case of PE, characterized by burning pain of the lower extremities, we identified a heterozygous de novo missense-mutation (c.2623C>G, p.Q875E) in SCN9A. Heterologous expression of the mutant channels shifted the activation of NaV1.7 in a hyperpolarized direction, allowing the mutant channels to open in response to a weaker depolarization. This gain-of-function effect results in hyperexcitability of nerve cells. In a large family with severe episodic rectal pain, which is typical for autosomal dominant PEPD, we identified a novel causative gain-of-function missense-mutation (c.4880T>C, p.M1627T) that segregated with the disease phenotype in four generations. Finally, we identified novel biallelic loss-of-function mutations in SCN9A in a patient with congenital indifference of pain, who suffers from recurrent injuries, burns and fractures. He also shows anosmia, a feature that is part of the SCN9A-related CIP. Deletions in both copies of SCN9A in this patient (c.3309delC and c.5340delC) result in loss-of-function and prevent action potential generation of nociceptors. The findings illustrate the central role of the NaV1.7 molecule in a broad range of pain-spectrum disorders.

P-ClinG-145

A comprehensive approach for identifying mutations involved in non-syndromic hearing loss

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The compounded nature of non-syndromic hearing loss (NSHL) stems from both genetic and environmental origins. Genetic causes account for approximately half of NSHL, have an affection rate of approximately

1 in 1000 newborns, exhibit genetic heterogeneity, and typically follow standard Mendelian inheritance patterns, whereby monogenic mutations confer dominant or recessive phenotypes.
We engaged in a comprehensive study to evaluate genetic mutations involved in NSHL using a variety of techniques, each with their specified detection limits to interrogate copy number and nucleotide variation. We analyzed 93 NSHL patients using the Illumina Omni-1 Quad SNP array, whereby we could solve a number of clear cases, particularly, one X-linked POU3F4 (MIM: 300039) hemizygous deletion and one DFNA24 (MIM: 606282) heterozygous deletion case, as well as two homozygous STRC (Stereocilin, MIM: 603720; DFNB16) deletions, through informative copy number variation (CNV). Remarkably, the importance of CNVs in the context of disease becomes realized when array CGH/SNP arrays are combined with other methods. For instance, recurrent STRC heterozygous deletions fueled further investigation of the mutational fallout of this gene; however, orderly mutational analysis was hindered due to a neighbouring pseudogene with 98.9% genomic and 99.6% coding sequence identity, prompting the development of a carefully designed Sanger sequencing assay for clean pseudogene exclusion. This combined array and Sanger sequencing approach solved an additional three cases and highlights a limitation of next generation sequencing (NGS) investigation of disease-relevant genes that have pseudogene counterparts.
We continued with 32 NSHL individuals from the microarray cohort composing a total of 25 index cases for targeted gene panel sequencing. We were able to further solve or identify a probable mutation in 14 out of 25 cases and found NGS particularly helpful for quickly sequencing large genes that would otherwise be too time and resource intensive to resolve through classical Sanger sequencing approaches, including a microarray patient with both a heterozygous deletion and compound missense mutation in the 72 exon gene, USH2A (MIM: 608400). Targeted panel NGS also provides the advantage of correcting initial clinical misdiagnosis and is invaluable for the exclusion of additional mutations in cases with previously identifiable mutations.
Herein, we present a summary of our experience using a variety of approaches for NSHL diagnostics and describe how we circumvented pseudogene sequence in a gene in which many of our patients presented mutations and ultimately resulted in the development of a new sequencing method for routine diagnostics. We present and suggest an optimal diagnostic algorithm for comprehensive NSHL diagnostics that has contributed to our solve rate of approximately one in three patients already screened for GJB2 and GJB6 mutations.

P-ClinG-146

A pitfall of the molecular diagnostic of congenital adrenal hyperplasia

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We herein present a case of a 26 year old pregnant woman with hirsutism, hyperandrogenemia, and oligomenorrhea, who was presumed to be affected with congenital adrenal hyperplasia (CAH) by an external genetic analysis. CAH is an autosomal recessive disorder, which comprises several inherited metabolic diseases of the adrenal cortex. The major cause of CAH is a 21-hydroxylase deficiency (21OHD), due to inactivation of the highly polymorphic 21-hydroxlyase gene (CYP21A2). CAH is characterized by reduced glucocorticoid and mineralocorticoid, and increased androgen production. This can lead to an altered development of primary and secondary sex characteristics. Hence, the woman was already treated with dexamethasone to prevent virilization in case of a female affected fetus. Reanalysis of the CYP21A2 gene in our lab during the pregnancy indeed confirmed the heterozygous nonsense mutation (p.Q319*). However, MLPA (Multiplex ligation-dependent probe amplification) analysis additionally revealed three copies of the gene. Based on the molecular testing of her parents, a paternal inherited CYP21A2 gene duplication with the p.Q319* mutation on one gene copy was identified. Therefore, no pathogenic allele could be detected in the pregnant woman. These findings do not support the clinical diagnosis of CAH. Furthermore, the analysis of her partner displayed the presence of the p.Q319* mutation and a duplicated CYP21A2 gene on both alleles. Since both the pregnant woman and her husband do not carry a pathogenic allele, there is no increased risk for CAH for the fetus. Because of these findings, the prenatal treatment with dexamethasone was discontinued. Thus to exclude misdiagnosis, it is important to consider that even a pathogenic mutation can result in no phenotypic effects due to a duplication of the gene. In conclusion, these results highlight the importance of gene copy number analysis to complement the mutation screening in CAH-patients.

P-ClinG-147

Turcot- syndrome associated with glioblastoma and café-au-lait spots in a young girl

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Turcot-syndrome (TS, OMIM 276300) is a genetically ill defined rare genetic disorder of DNA mismatch repair. It is characterized by the occurrence of primary tumors of the central nervous system and of the colon during the first and second decades of life, associated with café-au-lait spots, axillary freckling and hypopigmented spots.TS is usually caused either by a mutation of the APC gene (OMIM 611731,Adenomatous polyposis coli)or of mismatch repair genes associated with the different genetic forms of Lynch syndrome (OMIM 120435). Whereas medulloblastoma usually is associated with an APC mutation, a glioblastoma usually occurs in one of the mismatch repair gene mutations. Molecular diagnosis may contribute to the appropriate diagnoses and appropriate care of affected patients. We present a 14-year-old girl with TS. She presented with signs of a tumor of the central nervous system (glioblastoma multiforme) and café-au-lait spots without adeno-carcinoma of the colon or numerous adenomatous colorectal polyposis. Other symptoms were headache and vomiting. She had no history of headache or of single tonic seizures, weakness, and no loss of consciousness or memory disturbance. On her skin there were six café-au-lait spots >2 cm in diameter and areas of hypopigmenation. Colon endoscopy revealed no colorectal polyposis or carcinoma, however multiple unclear hyperpigmentations. Brain CT showed intraparenchymal hemorrhage with an infratentoriell tumor and multiple bilateral thalamus lesions. Histopathological findigs confirmed the glial origin of the tumor and a high rate of cell proliferation. The tumor was diagnosed as glioblastoma with giant cell features WHO grade IV. Our patient underwent right parietotemporal craniotomy and gross total resection of the tumor. A molecular study of the tumor tissue showed micro-satellite instability of MSH 6, which is rarely present in Lynch syndrome. We found in the genetic analysis of lymphocyte DNA a compound heterozygous mutation in the MSH6 gene (OMIM 600678), c.1665A>G; and c.3226C>T.The family is negative for colonic cancer or colonic polyposis and café-au-lait spots.It is important to perform molecular testing and genetic counseling for early diagnosis of Lynch syndrome and preventive management in this family. This observation shows that glioblastoma, café-au-lait spots and a mutation in a mismatch repair gene (Lynch syndrome) may be associated with clinical manifestations of Turcot-syndrome.

P-ClinG-148

Differential DNA methylation of genes involved in neuronal gene regulation and function in the brain of patients with progressive supranuclear palsy (PSP)

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Progressive supranuclear palsy (PSP) is an atypical Parkinson syndrome characterized by abnormal eye movement, frequent falls, and memory loss in addition to parkinsonism. Histopathologically, neuronal and glial immunoreactive filamentous tau inclusions are found in the affected brain regions. In a previously published genome wide association study several genes were identified that contribute to PSP in combination with mostly unknown environmental factors.
In order to study potential environment-gene effects we analyzed the methylation pattern in forebrain DNA from PSP patients and controls. DNA was extracted from postmortem forebrains of n=94 PSP patients and of n=72 controls. After bisulfite conversion DNA was used to interrogate Illumina Infinium HD Methylation arrays (Illumina Human Methylation450_v1.1 Bead Chip). Bioinformatic analysis was done according to a previously published protocol that corrects for differences in dye saturation of the different Illumina bead versions and that provides corrected p-values for the methylation differences detected. Highly stringent criteria (>5% methylation difference at several sites at p<0.001) were applied.
Several genes met these criteria. Most significantly, hyper-methylation of genes involved in development and function of the forebrain such as DLX1 and SLIT1 were identified. Among the genes hypo-methylated in PSP, ZIC4 was the most significant. ZIC4 is a zinc family member gene. It encodes a transcription factor that is involved in the regulation of developmental processes. It appears to be mainly expressed in the cerebellum. Furthermore, two genes (HDAC4 and TRRAP) involved in epigenetic regulation at the histone level were also hypo-methylated. HDAC4 encodes a histone deacetylase and TRRAP codes for a component of a histone acetylase. Thus the potential upregulation of these two genes results in abnormal repression (HDAC4) or expression (TRRAP) of additional genes that may play a role in the pathogenesis of PSP.
Methylation differences found by analysis of Illumina methylation arrays were confirmed by pyrosequencing of relevant genes. Preliminary data demonstrate that expression of these genes is altered in PSP. In conclusion, the data reveal an essential role of epigenetically mediated dysregulation of specific genes in the molecular pathogenesis of PSP.

P-ClinG-149

Four novel mutations confirm the pathogeneity of POFUT1 in Dowling-Degos disease and illustrate a clinically variable phenotype

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Dowling-Degos disease (DDD) is an autosomal dominant form of a reticulate pigmentary disorder characterized by progressive and disfiguring hyperpigmentation at the flexures, large skin folds, trunk, face and extremities. We previously identified loss-of-function mutations in the keratin 5 (KRT5) gene and in POGLUT1, which encodes protein O-glucosyltransferase 1. A recent report of two Chinese families with DDD presented causative mutations in POFUT1, which encodes protein O-fucosyltransferase 1 from the Notch pathway.
In this study, we report four novel mutations in POFUT1 in simplex patients or families of different ethnical origins affected by DDD and describe the clinical manifestation of disease in our patients in relation to the patients reported in the Chinese study. Briefly, we identified two missense mutations and one nonsense mutation in three simplex cases from Germany, Denmark and India, respectively. All these mutations were identified by Sanger sequencing of POFUT1 in all DDD cases without mutations in KRT5 and POGLUT1. We also identified another novel nonsense mutation by exome sequencing of two affecteds in a Yemeni family comprising 7 individuals initially suspected to be affected by DDD which segregated in an autosomal dominant pattern. These novel mutations and the extensive clinical description confirm the pathogeneity of POFUT1 in DDD and illustrate a clinically variable phenotype.

P-ClinG-150

Compound heterozygous MPDZ mutations in a girl with congenital hydrocephalus and a complex cardiovascular defect

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We report on a 21-months-old German girl, the first child of her healthy and unrelated parents, with a combination of cerebral and cardiovascular malformations. Prenatal ultrasound at 30 weeks of gestation revealed macrocephaly due to severe congenital hydrocephalus internus as the result of aquaeductal stenosis. Amniocentesis with subsequent prenatal cytogenetic analysis revealed a normal female karyotype (46,XX). The girl was born by Caesarian section at 39th week of gestation. Birth weight was 3770 g (85th P.), length 52 cm (65th P.) and head circumference 45 cm (7.4 cm > 99th P.); APGAR score were 8-9-10. Beside macrocephaly the girl had facial dysmorphism with low set ears with pits, down slanting palpebral fissures and a deep nasal root. In the postnatal period a complex cardiovascular malformation was detected, consisting of juxta-ductal stenosis of the aortic isthmus, hypoplasia of the aortic arch, persistent superior vena cava, and atrial septum defect.
Hydrocephaly was treated post-partum by implantation of a ventriculo-peritoneal shunt. Within the first 6 months of life, the girl developed epilepsy, which was treated with levetiracetam. 180k microarray analysis detected a heterozygous 260-kb microdeletion at 9p23 affecting almost the entire MPDZ gene. The microdeletion was confirmed by quantitative RT-PCR.
Recently, a homozygous nonsense mutation in the MPDZ gene has been described to cause congenital hydrocephalus. MPDZ encodes the multiple PDZ domain protein that is primarily located at cellular tight junctions where it serves as a scaffold protein. As the microdeletion covering MPDZ was found in the heterozygous state in the patient, we sequenced the 46 coding exons of MPDZ and identified the hemizygous 4-bp deletion c.3853_3856delTCAG in exon 28. This mutation leads to a frameshift and introduction of a premature stop codon (p.(E1286Qfs*58)). Segregation analysis revealed the heterozygous 4-bp

deletion in the mother, while the father carried the 260-kb microdeletion in the heterozygous state strongly suggesting that the female patient is compound-heterozygous for the two MPDZ mutations.
To the best of our knowledge, this is the first report of compound-heterozygous mutations in the MPDZ gene causing congenital hydrocephalus. Our observation confirms an association of biallelic MPDZ mutations with congenital hydrocephalus. The role of MPDZ in proper formation of cellular tight junctions resembles the cell-cell adhesion function of the protein encoded by L1CAM, the gene which is associated with X-linked hydrocephalus and stenosis of the aqueduct of Sylvius. Thus, it is tempting to speculate that disturbance of cell-cell interaction is one pathophysiological mechanism underlying congenital hydrocephalus. Further investigations are necessary to determine the frequency of MPDZ mutations in patients with aqueduct stenosis and congenital hydrocephalus and its associated clinical features.

P-ClinG-151

The first gross genomic in-frame duplication of FBN1 in humans could define a new subclass of Marfan syndrome

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Marfan syndrome is an autosomal-dominant connective tissue disorder characterized by abnormalities primarily in the ocular, skeletal, and cardiovascular systems. Depending on the FBN1 gene mutations present, the phenotype may vary from minor isolated features to severe forms with neonatal onset.
We report on a 32-year-old woman suspected of having Marfan syndrome because of a spontaneous pneumothorax at the age of 18. The family history revealed no signs or symptoms indicative of the disease. The patient was of tall stature with a height of 187 cm, which is 9 cm above the 97th percentile. She had long slender limbs, a thin body habitus and weighed only 60 kg, corresponding to a BMI of 19. Her arm span was 185 cm and thus she had a normal armspan/height ratio of 0.99. The upper/lower body segment ratio of 1.03 was also normal. She exhibited arachnodactyly and slender feet, but had no other skeletal characteristics attributed to Marfan syndrome. The patient had a positive wrist sign and negative thumb sign. Her face did not exhibit marfanoid features. Two teeth had been extracted due to her narrow jaw. Cardiac evaluation, including echocardiography, was normal. Ophthalmological examination revealed myopia of -3 diopters as well as exophthalmos.
We performed DNA sequencing of the whole coding region of FBN1, as well as screening for gross genomic deletion or duplication by MLPA analysis. Two mutations, both have not yet been reported, were identified in a compound heterozygous state: The first mutation is a duplication of exons 10 to 24 (c.989_2854dup) resulting in an in-frame duplication of 622 amino acids. This mutation was inherited from the father. The second variant is the missense mutation c.3362C>G; p.P1121R in exon 28 of FBN1, which was inherited from the mother. The affected amino acid residue is highly conserved up to Tetraodon nigroviridis (considering 11 species). All four in silico prediction programs applied (SIFT, PolyPhen-2, AGVGD, MutationTaster) but AGVGD categorised the mutation as pathogenic. Both parents have a tall and thin habitus but no signs or symptoms of Marfan syndrome.
Whereas missense mutations are a frequent cause of Marfan syndrome, gross insertions or duplications in FBN1 have until now only been described in Tight skin mice. In these mice a heterozygous duplication of Fbn1 causes a larger than normal in-frame transcript, and fibroblasts secrete normal as well as mutant oversized Tight skin fibrillin-1 proteins that are stably incorporated into beaded microfibrilis with altered molecular organization. This alteration on the cellular level causes an accumulation of extracellular matrix. The skin thickens, adheres firmly to the hyperplastic subcutaneous tissue, lacks elasticity and has altered wound healing properties. Given the lack of Marfan syndrome symptoms in the parents, the missense mutation may act in a hypomorphic fashion and therefore result in phenotypic expression of the in-frame duplication.

P-ClinG-152

Overgrowth and developmental delay associated with a 200 kb deletion in 16p11.2 in two families

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Due to a high density of segmental duplications, the short arm of chromosome 16 is prone to a number of recurrent rearrangements. A common 600 kb microdeletion or –duplication in 16p11.2 has been associated with autism, intellectual disability, schizophrenia and mirrored weight and head circumference phenotypes. An adjacent, but separate distal 200 kb region in 16p11.2, which contains the SH2B1 gene, has been associated with isolated obesity as well as with developmental delay. Both aberrations go in hand with high variability and incomplete penetrance.
We now report on two families harboring the 200 kb deletion in 16p11.2. A 5 years 9 months old girl was referred to our clinics with suspected Sotos syndrome due to tall stature and developmental delay. Birth measurements were unsuspicious but at time of consultation her height and weight were above the 97th centile, and bone age was advanced by 18 months. She could walk with 18 months, started to speak with 20 months and attended special school due to learning disabilities at the age of 7 years. Whereas NSD1 testing revealed normal results, molecular karyotyping showed the 200 kb deletion in 16p11.2, which was inherited from the healthy mother.
The second family consisted of a 12 year old boy with unspecific mild to moderate intellectual disability and mild obesity and his 4 year old half-sister with severe obesity, tall stature, macrocephaly and mild motor delay. Due to suspected Sotos syndrome in the girl, NSD1 testing was performed and normal. Molecular karyotyping revealed the 200 kb deletion in 16p11.2 in both siblings. Interestingly, the girl, but not the boy, additionally harbored a microduplication 1q21.1, which has been recurrently associated with variable and incompletely penetrant developmental delay, ID, behavioural anomalies and large head circumference. Both aberrations were inherited from the mother, who was obese but otherwise healthy and without cognitive problems.
These two families further characterize the variable spectrum of phenotypes associated with the 200 kb microdeletion in 16p11.2. Our findings in family 2 also show that not even the co-occurrence of two ID-associated microaberrations necessarily leads to cognitive impairment.

P-Complex Genetics / Complex Diseases

P-ComplI-153

Large association study of exonic variants in idiopathic achalasia

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Idiopathic achalasia is a severe disorder of the lower esophageal sphincter (LES) with a lifetime prevalence of 1:10,000. The disease is characterized by the degeneration of neurons in the myenteric plexus leading to the development of a megaesophagus with irreversible loss of LES function. On the etiological level, achalasia is a multifactorial disorder with environmental and genetic factors being risk-associated. By testing markers in immune-relevant loci using the Illumina's Immunochip, we already identified strong association signals reaching genome-wide significance within the HLA-DQ complex indicating that autoimmune processes contribute to the pathophysiology of achalasia.
The aim of the present study was to determine the role of exonic variants in the development of achalasia. We performed an association study using Illumina's Exomechips which have been developed based on the data of 12,000 exomes. The chip contains more than 240,000 – mainly functional-relevant – markers. We genotyped 674 patients with idiopathic achalasia and 2,316 population-based controls from Central Europe and after quality control (QC) steps 106,417 markers remained for association testing.
The analysis yielded a strong association signal within the HLA region ($P < 5x10-08$). We carried out a conditional analysis adjusting for the variants within the HLA-DQ complex identified before within the Immunochip study. This analysis revealed that the HLA signal on the Exomechip is not independent of the already known achalasia risk variants within this region. Next, we focused on variants outside the HLA region and identified 139 markers reaching a $P < 10-03$. In total, 31 of the 139 markers are common variants (MAFcontrols > 5%, best hit $P = 1.55x10-05$). In contrast, 49 are low-frequent markers (MAFcontrols < 5%) and the minor allele is more frequent in patients compared to controls (best hit $P = 2.57x10-06$). Furthermore we used the INTERSNP-RARE software to test if there is an enrichment of rare, associated variants within specific genes. This analysis revealed an overload within the genes EDNRB and PLBD1 ($P < 8x10-5$).
The present study provides evidence that low-frequent and common exonic variants play a role in the pathophysiology of achalasia. Currently, we genotype a subset of the associated variants identified in this study in an independent sample of > 400 achalasia patients and > 1,000 controls in order to confirm the contribution of these variants to the development of achalasia.

P-Compl-154

Investigating the role of SHANK3 in Schizophrenia

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Recent studies have shown that mutations in the postsynaptic scaffolding proteins SHANK1, SHANK2 and SHANK3 are linked to a spectrum of neurodevelopmental disorders, including intellectual disability and autism spectrum disorders. To elucidate a putative contribution of genetic variants in SHANK3 to schizophrenia etiology, we sequenced the exons and flanking intronic regions of the gene in 500 affected individuals. Schizophrenia (SCZ) is a neuropsychiatric disorder with high variability in the clinical phenotype and is characterized by major impairments of perception of reality. Our sequencing results were compared to ancestrally matched controls from the EVS (exome variant server) and the 1000 genomes project. We identified a putatively deleterious missense variant in 40 SCZ individuals with a significantly higher frequency in cases compared to controls (P=0.00004, OR 1.96, CI 1.4 – 2.7). This variant is homozygous in two patients and heterozygous in 2 further patients where it was found in combination with a second rare deleterious variant. We identified deleterious SHANK3 missense variants in 0.9 % of the SCZ individuals that were not detected in controls. One deleterious missense variant, identified in our SCZ cohort, was previously found in a SCZ individual from a different study (combined data 2/685). The same variant was also detected in four individuals with autism spectrum disorder (4/1972) but has not been found in any of 6768 controls, proposing a genetic overlap for these two neuropsychiatric disorders. Our findings suggest that rare deleterious SHANK3 variants predispose to the development of schizophrenia.

P-Compl-155

Extending the population spectrum for nonsyndromic orofacial clefting: Recruitment and genetic analyses in an Arabian population from Yemen

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Nonsyndromic orofacial clefting (nsOFC) is among the most common of all congenital disorders and has a genetically complex etiology. Based on embryological and epidemiological data, the phenotype can be differentiated into nonsyndromic cleft lip with or without cleft palate (nsCL/P) and nonsyndromic cleft palate only (nsCPO), with nsCL/P being the most frequent form. Recent genetic research has identified numerous genetic susceptibility loci for nsCL/P. However, most studies have been performed in populations from Europe and Asia, and few data are available concerning genetic susceptibility to nsCL/P in Arabian populations. The present study investigated a large, newly recruited nsOFC sample from Yemen. Twenty-four single nucleotide polymorphisms (SNPs) representing all 15 currently known nsCL/P risk loci were genotyped in 242 nsCL/P cases and 420 healthy controls. Single marker association analysis revealed significant associations for four loci (8q24, 9q22, 10q25, 13q31). The strongest association was for the European high risk locus at 8q24 (Pcorrected = 5.09x10-4; heterozygous odds ratio (ORhet) = 1.74, 95% confidence interval (CI 95% CI) = 1.22-2.47, homozygous odds ratio (ORhom) = 2.47, CI 95% = 1.55 - 3.93). Five additional loci (1q32.2, 3q12, 8q21, 17q22, 20q12) showed nominal significance that did not withstand correction for multiple testing. Two loci (1p36, 2p21) failed to reach nominal significance but displayed a trend towards association with $P < 0.1$. Although the four remaining loci (1p22, 3p11, 15q22, 17p13) failed to reach nominal significance, the risk alleles were in the same direction as in the discovery studies. Due to insufficient sample size (n=49), we did not analyze the nsCPO sample.
This study represents the first investigation of the currently known nsCL/P genetic risk factors in a large Arabian case-control sample from Yemen. The results suggest that the majority of the 15 risk loci identified in European and Asian ethnicities also confer risk for nsCL/P in Arabian populations.

P-Compl-156

DDX3X 3´UTR splicing control in human male germ cells is impaired in men with AZFc deletion and severe hypospermatogenesis

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The human DDX3X DEAD-box RNA helicase is a master gene, functionally conserved from yeast to human, involved in nuclear cell cycle control and in the control of translation of a still unknown number of gene transcripts in the cytoplasm of soma cells. In mammals, its original autosomal chromosome location shifted to the sex chromosomes due to evolution of the X and Y chromosome structures. In human, this resulted in functionally restriction of its homologous Y gene copy, DDX3Y, to the testis, solely expressing its protein in premeiotic male germ cells. In contrast, the DDX3X protein is expressed in the testis solely in postmeiotic male germ cells. This suggests a specific translation control for DDX3X transcripts in the male germ line.

We found that DDX3X testis transcripts are mainly starting from a specific core promoter activated predominantly in germ cells after meiosis and processed for polyadenylation in the proximal 3´UTR. Interestingly, a minor fraction of these transcripts are processed in their 3´UTR, differently. Their primary 3´UTR first extends ~17kb, becomes then subsequently spliced at distinct sites to result in short 3´UTRs. We identified six different 3´UTR splicing variants by sequence analysis (I-VI; Rauschendorf et al. 2013, submitted).

We found that the expression pattern of these DDX3X 3´UTR splicing variants is severely impaired in infertile men with AZFc deletion. This microdeletion in the distal part of the Y long arm (Yq11.23) is known as the most frequent genetic lesion causing male infertility (Vogt et al. 2008). It usually cause hypospermatogenesis and a histological picture of mixed atrophy in their testis tubules; i.e., with no sperm in their ejaculate but still a low amount in some of their testis tubules isolated by testicular biopsy.

We, therefore wanted to analyse whether any AZFc gene, expressed after meiosis (i.e. CDY1; DAZ), and additional other genes also known to be involved in transcriptional and translational control mechanism(s) of germ line genes functional expressed first in spermatids (i.e. CREM), might be involved in the 3´UTR splicing control of the postmeiotic DDX3X transcripts.

We found an aberrant expression pattern of DAZ but not CDY1 transcripts, in all testicular tissue samples with severe hypospermatogenesis and a distorted DDX3X 3´UTR splicing process. It suggests that the functional expression of DAZ is required during the 3´UTR splicing process of the postmeiotic DDX3X germ cell transcripts with long 3´UTRs.

P-Compl-157

Nonsyndromic orofacial clefting and cancer – evaluating a possible common genetic background by analyzing GWAS data

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Nonsyndromic orofacial clefting is one of the most frequent congenital malformations. Based on epidemiological studies it has been suggested that nonsyndromic orofacial clefting and cancer may have a common etiology. Both nonsyndromic orofacial clefting and cancer have a genetically complex background. For nonsyndromic cleft lip with or without cleft palate (nsCL/P), the most common cleft subtype, and also for many cancer subtypes, molecular studies, mainly recent GWAS, have identified several susceptibility factors.

To test the hypothesis that frequent variants showing strong effects in one trait might confer a possibly less strong risk in the other trait we used unbiased genome-wide SNP data from large cohorts of patients with sporadic cancers and cohorts of individuals born with nsCL/P. We first searched for cancer entities that have been reported to be associated with nsCL/P based on literature data. This search revealed 10 studies, covering 11 different cancer entities (brain cancer, breast cancer, colon cancer, leukemia, liver cancer, lung cancer, lymphoma, neuroblastoma, prostate cancer, retinoblastoma, skin cancer). GWAS results were found for 9 of the 11 cancer entities.

We then performed two approaches: i) Data on genetic variants associated with nsCL/P were retrieved from our recently published meta-analyses of the two largest GWAS on nsCL/P (Ludwig et al., 2012). We analyzed 204 conclusively identified cancer susceptibility variants in this large genome-wide SNP dataset. One of the cancer-associated SNPs, rs6457327, on chr. 6p21.33, originally found to be significantly associated with follicular lymphoma, remained almost significant (P = 0.0528) after correction for multiple testing. ii) Corresponding authors of all cancer GWAS which we had used for SNP selection were contacted. For each of the 12 top SNPs from 12 nsCL/P risk loci, the authors were asked to retrieve association information in their cancer GWAS data sets. 29 different cancer sample sets that were available for the above named 11 cancer entities. We found an association for the nsCL/P risk locus at chr. 20q12 (rs13041247) with squamous cell cancer of the skin, and this finding remained significant after a conservative Bonferroni correction (P = 0.0016, data extracted from the Icelandic Cancer Registry). The risk allele for this SNP in squamous cell cancer of the skin, however, is not identical with the risk allele in the cleft patients.

Our study is the first to characterize possible pleiotropic risk loci for the two frequent disease traits nsCL/P and cancer, at an unbiased level, using large genome-wide datasets. Our results demonstrate a lack of a particular marker with very strong effects on both traits and rather suggest that variants strongly associated with one trait might act as modifier for the second one. Thus, although not entirely conclusive at the single-marker level, our study might serve as starting point for further projects.

P-Compl-158

Whole exome sequencing: Follow-up of a rare non-synonymous variant in grhl3 in a German family with nonsyndromic orofacial clefting

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Nonsyndromic cleft lip with or without cleft palate (nsCL/P) is one of the most common congenital malformations with a complex multifactorial etiology. Although many cases occur sporadically, families with multiple affected members also exist. In these pedigrees, dominantly inherited genetic alterations with high penetrance might be responsible for the presence of nsCL/P in affected individuals. In the context of a large collaborative study, whole exome sequencing (WES) was performed in two affected members of an extended nsCL/P family of

German origin. The entire pedigree comprised 11 affected individuals spread over three generations. The two index probands were distantly related cousins, and were expected to share 1/128 of their genetic information. After stringent filtering of variants shared between both individuals, one variant in the grainy-head like 3 (GRHL3) gene was identified as potentially causal. This variant, rs138381915, is located in exon 13 and the risk allele mediates an amino acid change from Arginine to Histidine at position 490 of the GRHL3 protein. Prediction programs (Polyphen, Mutation Taster) predict the alteration to be disease-causing. In sequencing datasets derived from the general population (ESP6500, dbSNP, 1000genomes), the frequency of the minor allele at rs138381915 is reported to be below 0.5 %. Literature research revealed GRHL3 as an interesting candidate gene for nsCL/P and has recently been shown to be activated by Interferon Regulatory factor 6 (IRF6) in the periderm and oral epithelium (de la Garza et al., 2013). Based on the hypothesis that rs138381915 might be the causal variant in the family studied, we first confirmed the presence of the variant in the two index probands by Sanger Sequencing. We then tested all family members for whom DNA was available for the presence of the putative risk allele. Of 29 members that were sequenced (11 affected, 18 unaffected), the risk allele was observed in a heterozygous state in seven individuals, only two of whom were affected. These two affected individuals' genotypes for this SNP were identical to the index probands analyzed in the WES study. Five individuals carried the putative risk allele but were not affected, while nine individuals did not carry the minor allele but were affected. These data suggest that rs138381915 is unlikely to be a causal variant regarding the nsCL/P phenotype in this particular family.

P-Compl-159

Next generation sequencing of a Parkinson specific gene panel as a powerful tool to identify rare variants in Parkinson patients.

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Multiple genetic and environmental factors are leading reasons in the etiology of Parkinson disease (PD) but only few patients (10%) have been identified with monogenic forms of PD. However, until now it is unclear whether there are simple genetic variations or polygenic cases which have an influence on disease risk, could explain cases of incomplete penetrance (LRRK2) or cases with only one heterozygous allele in autosomal recessive (AR) PD genes.

In order to identify additional genetic risk factors or digenic cases in patients with ambiguous genetic testing results we designed a PD specific HaloPlex gene panel including 13 monogenic PD genes, 17 susceptibility genes, 8 risk factors and 23 single nucleotide polymorphisms (SNPs). Therewith our study cohort of 207 MeFoPa samples from carriers with only one mutation in a recessive PD gene (PARK2, PINK1, DJ1,…) or with mutations and variants in LRRK2 and GBA were sequenced for the target region of 91.487 bp. This was done by use of an Illumina MiSeq next generation sequencing platform followed by the bioinformatics analyses using our in house pipeline.

By this approach, on average, >97% of the target region was covered by >20 reads with a mean coverage of 806 ± 211 reads. In 29 of 38 target genes the coding region was completely (100%) sequenced including almost every monogenic PD gene.

We confirmed 204 (91%) of all reported mutations with a sequence depth of ≥20 reads. The false-negatives are due to lack of coverage or the difficulties to identify heterozygous exon copy number variations (CNV) but they are just under investigation.

Within the subgroup of LRRK2 mutation carriers we additionally found 58 different rare variants in 25 different genes whereof three of them turned out as known disease causing alleles, two in GBA (p.E365K, p.N409S), one in LRRK2 (p.R1441G). We have also found the previously described risk factor p.M1646T (Lancet 2011) in 22 of 65 (33,8%) affected and in only 2 of 24 (8,3%) unaffected mutation carriers. Subsequent analyses identified a shared p.R1441G - p.M1646T haplotype of ~140kb ranging from Exon1 to Exon 49 in LRRK2.

This approach has shown to be robust for the efficient screen of large sample cohorts which should further help to decipher the complex genetic nature of PD.

P-Compl-160

Evidence for a polygenic contribution to androgenetic alopecia

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Male pattern baldness (androgenetic alopecia, AGA) is a highly heritable trait and the most common form of hair loss in humans. Genome-wide association studies (GWASs) including a meta-analysis and large-scale replication study have identified twelve genome-wide significant risk loci for AGA, which explain a major proportion of the genetic risk for AGA. However, the efficiency of contemporary GWASs for detecting disease associations is restricted by the burden of multiple testing due to the large numbers of single nucleotide polymorphisms (SNPs) considered in these analyses. Thus, only highly significant individual disease associations ($P<5\times10-8$) can be detected. It is therefore likely, that additional genetic risk factors with smaller individual effect sizes still await identification. Their sensitivity for detecting individual disease associations is limited but GWAS datasets can be used to analyze whether there is a collective contribution (polygenic contribution) of common SNPs that show disease association above the P-value threshold for genome-wide significance ($P>5\times10-8$). Irrespective of their small individual effect sizes, these loci might jointly explain a significant proportion of the overall genetic risk for AGA. Polygenic score analysis is a method for determining this collective contribution. This study used a German case-control sample for AGA, which comprised 581 severely affected patients and 617 controls, to determine the contribution of polygenic variance to genetic risk for AGA. The sample was divided evenly into discovery and test samples. An additive polygenic risk score was calculated from risk alleles with increasingly liberal P-values in the discovery dataset, which was then used to test for the enrichment of AGA risk score alleles in the independent test samples. The analysis provided significant evidence for the specific contribution of a polygenic component to AGA where the amount of variance explained was 1.4-4.5%. It is likely that the contribution of a polygenic

component and the large number of genes involved reflect the complexity of the AGA associated biological pathways. Further studies are required to progress from evidence for a polygenic contribution to understanding the specific genetic factors that comprise this polygenic component.

P-Compl-161

Identification of genetic variants in the schizophrenia candidate gene KCTD13 on chromosome 16p11.2

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Rare duplications in the chromosomal region 16p11.2 are an established risk factor for the development of schizophrenia. Deletions in the same region increase the susceptibility to autism and intellectual disability. The risk-bearing copy number variants (CNVs) have large effect sizes and span > 25 genes. Several of these genes are interesting candidate genes. However, the underlying risk gene/genes have not been identified, yet. Based on genetically modified zebrafish, Golzio et al. identified KCTD13 as the major driver of the neuroanatomical phenotype of CNVs in 16p11.2 (Golzio et al., 2012).

The aim of the study was to analyze, whether small changes in the DNA sequence contribute to the allelic spectrum of KCTD13. The identification of genetic variants in patients with schizophrenia would further support KCTD13 as a strong candidate gene for the disorder.

Targeted Sanger resequencing of all six exons was performed in 576 patients. All patients had a DSM-IV diagnosis of schizophrenia. Of these, 285 individuals had an early age-at-onset (< 21 years). Publicly available data from the 1000 Genomes Project and the Exome Variant Server were used to determine the frequency of the identified variants in population-based cohorts and individuals collected for studies focusing on lung and heart phenotypes.

We identified one point mutation in exon 1 and one point mutation in exon 5. In silico, both were predicted to be at least possibly damaging. Neither was listed in the 1000 Genomes Project or the Exome Variant Server. We were unable to check whether the mutation in exon 1 occured de novo as no DNA from the patient's parents was available. The identified mutation in exon 5 was probably inherited by the patient's father. No DNA of the father was available. However, the brother of the affected patient but not their mother carried the same mutation. The patient's brother did not fulfill the diagnostic criteria for schizophrenia but suffered from depression, agoraphobia and an eating disorder.

This is the first study that focused on the systematic detection of DNA sequence variants in the gene KCTD13. The identification of two novel point mutations provides additional support for it being a candidate gene for schizophrenia. Currently, we are analyzing whether small exon affecting deletions or duplications in KCTD13 are associated with schizophrenia.

P-Compl-162

Candidate gene sequencing of a 7-year old Fanconi anemia patient

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Known as a rare genetic disorder, Fanconi anemia (FA) is characterized by congenital malformations. Patients also often show progressive bone marrow failure and susceptibility to hematological and non-hematolocigal malignancies. Responsible for the disease are biallelic or hemizygous mutations in any one of at least 16 genes (FANC-A, -B, -C, -D1/BRCA2, -D2, -E, -F, -G, -I, -J, -L, -M, -N/PALB2, -O/RAD51C, -P/SLX4, Q/ERCC4) whose products interact with each other and with related proteins in the FA/BRCA DNA pathway for genomic maintenance. Few patients are left who cannot be assigned to any reported gene. One of these patients is a 7-year old German boy of non-consanguineous origin with a typical FA –phenotype. We performed haplotype reconstruction of the family and Whole Exome Sequencing (WES) and combined the resulting information. On the basis of segregation, function and suggested connection to the FA/BCA pathway we selected 26 genes apparently containing 73 compound heterozygous sequence variants, including RCC2, CDC14A, ZRNAB3, ALDH1L1, TOPBP1, ECT2, SMARCAD1, BAT3, AP5Z1, FBXO24, PRKCD, UBE2W, RAD54B, SMC5, APB1, MEN1, RECQL, UBE2N, PARPBP, PDS5B, RAD51B, HERC2, SLX4, SMARCA4, TOP3B and CDK5RAP3 for further analysis. All of the potential mutations were verified by Sanger sequencing but only the variants in SLX4, ALDH1L1, PRKDC, UBE2W and TOP3B were proved pathogenic. Unfortunately we could not detect any second pathogenic variant in these genes. Therefore further investigations will be necessary to resolve the gene defect in this patient. Nevertheless, our study nicely exemplifies ways of candidate gene exclusion by combinatorial genetic approaches.

P-Compl-163

Association studies at RUNX3 and ETS1 in psoriatic arthritis

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Psoriatic Arthritis (PsA) is a chronic inflammatory joint disease, mainly occurring in patients with the most common skin manifestation of psoriasis, psoriasis vulgaris (PsV). From the genetic point of view, both disease entities are complex diseases.

In a follow-up study of our genome wide association study, we had identified a new susceptibility factor for PsA at RUNX3 (Apel et al. Arthritis & Rheumatism 2013). The most significantly associated SNP (p=1.40x10-8) had been located in the first intron of the gene. RUNX3 codes for a transcription factor that is involved in CD8+-T lymphocyte differentiation, which is remarkable when considering PsA as a T-cell mediated disease.

Previously, variants in the 5' region of RUNX3 – located in the neighboring linkage disequilibrium (LD) block of our most significantly SNP in PsA - have been identified as susceptibility factors for ankylosing spondylitis (Evans et al., Nat Genet 2011), celiac disease (Dubois et al. Nat Genet 2010; Trynka et al. Nat Genet 2011) and PsV (Tsoi et al Nat Genet 2012). In order to test whether the susceptibility factor for PsA might be located within that LD block, we genotyped further three SNPs in our large European case-control-study. Interestingly, the ETS1 gene - coding for a transcription factor involved in regulation of RUNX3 - has been identified as a susceptibility factor for PsV (Tsoi et al. Nat Genet 2012). Therefore, we tested 5 SNPs at the ETS1 locus for association to PsA as well. The 5 SNPs were genotyped by using Taqman technology. In order to take into account the different origin of our German, Italian, Swedis, and British case-control-cohorts, a Cochran-Mantel-Haenszel test was performed.
The most significantly associated SNP at the ETS1 locus did not reach the level of genome-wide significance - though a notable p-value (4.27x10-5) -, while one of the SNPs at the 5' region of RUNX3 exceeded this threshold (p=2.68x10-8) and showed a similar odds ratio as our previously published SNP (1.26 [1.16-1.36]).
Using in silico analysis tools like Regulome DB (Boyle et al. Genome Research 2012), evidence for altered binding of transcription factors was minimal for the newly identified SNP at RUNX3. In contrast, for one of the SNPs being in strong LD, an effect on binding to transcription regulatory elements was indicated.
Further enlargement of our case-control-cohorts and analyzing them for the RUNX3-SNPs is ongoing to further fine-map the association signal. After the identification of the most promising SNP/ LD block at this locus, functional studies investigating e.g. effects on the transcription of RUNX3 will help to prove the pathogenicity of those variants in PsA.

P-Compl-164

Palmoplantar pustular psoriasis and its genetic background

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Palmoplantar pustular psoriasis (PPP) is a chronic inflammatory skin disease characterized by sterile pustules, erythema and hyperkeratosis on palms and soles. In at least 25% of PPP cases, psoriasis vulgaris (PsV) is also present and a significant proportion of patients suffers from psoriatic arthritis. Smoking and female sex are more frequent in PPP compared to PsV. So far, there are no confirmed genetic risk factors for PPP. Recently, in generalized pustular psoriasis, an extreme manifestation of pustular psoriatic disease, homozygous and compound-heterozygous mutations in the IL36RN gene have been identified to be causal. The same mutations have been described to be more frequent in a group of 139 PPP patients of European origin.
Here, we recruited a group of >140 PPP patients, most of them were female and smokers (>60%, respectively). About half of them had a manifestational age of ≤40 years.
We could confirm previous data showing that the frequency of the HLA-C risk allele, the major genetic risk factor for PsV, was comparable to the frequency of controls, indicating that PPP is genetically different from PsV.
We further analyzed IL36RN for mutations in coding exons as well as for intragenic deletions and duplications. We identified three heterozygous carriers of mutations and no carriers of copy number variants. Compared to a population-based control group of 4.300 European individuals, there was no significant difference in the frequency of mutations in IL36RN.
Our data indicate that PPP is genetically distinct both from PsV and generalized pustular psoriasis. Further effort is needed to identify genetic factors contributing to PPP.

P-Compl-165

Dissecting the Genetic Heterogeneity of Schizophrenia through Genome-Wide Association Analysis of Age at Onset in a German Sample

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Schizophrenia is a severe, disabling mental disorder affecting ~1 % of the population characterized by a heterogeneous phenotypic presentation- One major source of heterogeneity that may contribute substantially is age at onset (AAO). AAO of SCZ is highly variable and sex-specific, ranging from mid adolescence to late adult life. Previous studies suggest that AAO is under genetic control but few studies have systematically focused on AAO in SCZ in a genome-wide association study (GWAS).
In this study we performed a GWAS of AAO in SCZ using a German case-control sample. A total of 1187 cases and 1742 controls from the HNR, Kora, PopGen and MooDS Imaging studies were genotyped on Illumina BeadChips (Human 550K, 610K and 660K). AAO was defined as the age when the subject first suffered from a mental disease.
According to the statistical framework described by Power et al. in their recently published paper [Power et al. 2012] three distinct statistical methods were used to test for a genetic influence on AAO for Schizophrenia, each testing distinct hypotheses:
1) A time-to-event analysis with cases classified by their AAO and controls censored at their age of recruitment,
2) a case-control-analysis with subsets of cases compared to all controls using different AAO cutoffs, and
3) a quantitative trait analysis of AAO in cases only.
In addition, sex specific analyses were performed as a secondary analysis for the case-control analyses and the quantitative trait analysis. All tests were conducted with PLINK and R.
The analysis of AAO as a quantitative trait and using AAO specific cohorts provided novel association results, but no finding reached genome-wide significance in the primary analysis. The sex-specific analysis yielded slightly more robust findings for the quantitative trait

analysis in cases. However, larger sample sizes will be needed to investigate AAO in SCZ on a genome-wide level.

P-CompI-166

A family case of non-syndromic hearing loss with double-heterozygosity for mutations in the genes SLC26A4 and KCNJ10

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Hearing loss is the most common sensory disorder worldwide with a prevalence of 1 in 1,000 newborns. More than half of the congenital cases are due to genetic causes and of these 70% are non-syndromic and 30% are syndromic forms. Pendred syndrome (PS) as well as the non-syndromic enlarged vestibular aqueduct (EVA) are caused by homozygous or compound heterozygous mutations in the SLC26A4 gene. Additionally, two studies demonstrated digenic inheritance with double heterozygosity of mutations in the SLC26A4 gene together with mutations in the KCNJ10 in two patients with EVA and in the FOXI1 gene in one patient with EVA, respectively. However, this is regarded as a very rare condition since a digenic inheritance with pathologic mutations in both genes could not be supported in subsequent studies.

Here, we report a family case with three deaf patients. Both non-consanguineous parents are affected by hearing loss, the father bilateral since birth, the mother progressing since the age of three. The index patient, a 17-year-old daughter, shows a mixed hearing impairment with an inner ear component particularly on the left side.

Following a negative analysis of the connexin genes GJB2 and GJB6, we performed direct sequencing of the SLC26A4 gene in the three affected family members. The index patient carries the common mutation p.Leu236Pro (c.707T>C) in exon 6 which she has inherited from her father who is also heterozygous for the common mutation IVS8+1G>A (c.1001+1G>A) in intron 8. Hence, the father's hearing impairment can be explained by compound heterozygosity for these two SLC26A4 mutations. The mother was found to be heterozygous for the known SLC26A4 mutation c.1003T>C (p.Phe335Leu) in exon 9. To complete the diagnostic testing of the index patient and her mother we analyzed the genes KCNJ10 and FOXI1 and found the heterozygous missense variant p.Lys354Arg (c.1061A>G) in the coding exon of the KCNJ10 gene in both patients. This variant is listed in the NCBI dbSNP database (rs142596580), but its overall heterozygosity rate in the Exome Variant Server is only 0,1%. Likewise, a multiple sequence alignment revealed evolutionary conservation of the affected amino acid and the biometric tools "MutationTaster", "SIFT Sequence" and "PolyPhen-2" interpreted p.Lys354Arg as pathogenic. Only the tool "PMut" predicted it to be neutral but with a low reliability score of only 3. Accordingly, a pathogenic effect of the KCNJ10 variant p.Lys354Arg is very likely.

Our examined family presents two new cases of double heterozygosity for causative mutations in the genes SLC26A4 and KCNJ10 in patients with non-syndromic hearing loss. Both, the index patient and her mother, carry the probably pathogenic KCNJ10 mutation p.Lys354Arg (c.1061A>G) but different known pathologic SLC26A4 mutations. This is the second study supporting a digenic inheritance involving the SLC26A4 and KCJN10 genes in non-syndromic EVA (DFNB4).

P-CompI-167

Molecular haplotyping method of the ITPA and TPMT genes

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The thiopurine methyltransferase (TPMT) and inosine triphosphatase (ITPA) are crucial enzymes in metabolism of thiopurine drugs: azathioprine and 6-mercaptopurine which are used in the treatment of leukemia or inflammatory bowel diseases (IBD). Currently, the polymorphisms and changes frequency of the ITPA and TPMT genes in different populations are known. However, do not exist convenient methods for quick molecular haplotyping of most frequently detected changes: 460G>A and 719A>G in the TPMT gene and 94C>A and IVS2+21A>C in the ITPA gene.

The aim of this study was to establish a rapid and highly sensitive methodology for the easy determination of haplotype structure for the above-mentioned polymorphisms using Long ASO-PCR combined with RFLP for TPMT gene and ASO-PCR combined with sequencing for the ITPA gene. In the study we used DNA of 38 subjects with known genotype at position: 460G>A and 719A>G in the TPMT gene (8 samples) and in the ITPA gene at position 94C>A and IVS2+21A>C (30 samples). Among the tested samples, four were compound heterozygote for the both changes in the ITPA gene. Based on the analysis developed in this study, it was determined that they are located in trans position. Five of the TPMT samples have both changes in a heterozygous state. A result of Long ASO-PCR with sequencing showed that this changes are situated in a cis position (allele TPMT*3A).

After optimizing and initial validation we confirmed that the developed method can be successfully used as an efficient supplement for the TPMT and ITPA genes molecular analysis.

P-Cytogenetics / CNVs

P-CytoG-168

Clinically significant genomic alterations are enhanced in placentas from pregnancies with fetal growth restriction (FGR) - Preliminary results

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Inroduction: Advances in microarray technology allow high-resolution genome wide evaluation for DNA copy number variations: deletions or duplications. Fetal growth restriction (FGR) secondary to placental insufficiency is associated with substantially increased childhood and adulthood morbidity and mortality. The long term outcome is related to placental aberrations and intra-uterine programming.

Our aim was to demonstrate the usefulness of microarray testing in FGR placentas.

Methods: Using Affimetix (California, USA) genetic chip for chromosomal microarray (CMA), we performed an analysis of 10 placentas from pregnancies with FGR attributed to placental insufficiency. We analyzed 5 placentas from FGR below the 5th percentile and 5 placentas of FGR between 5 and <10th percentile. All the fetuses had a normal anomaly scan and normal karyotypes. The results were compared to 4 placentas from uncomplicated pregnancies with healthy neonates.

Results: Microarray analysis identified more clinically significant genomic alterations in FGR placentas compared to healthy controls. There was a correlation to the severity of the FGR (see the table). The genomic alterations were below the resolution of normal karyotyping. Genes that were altered in the severe FGR placentas are related to adult human height, stress reaction and also to cellular migration, differentiation and adhesion.

Conclusions: Though very preliminary, our data support placental evaluation using CMA for FGR placentas. Larger data sets are needed for further evaluation of our findings and its clinical implications.

FGR				
<5% FGR				
5% - <10% Control	P Value			
Normal CMA	20%	80%	100%	P<0.001
Gene gain 80%	0	0	P<0.001	
Gene loss 0	20%	0	P<0.001	
Average size	249±204.4	126.8	0	NS

P-CytoG-169

SPAG7 is a candidate gene for the periodic fever, aphthous stomatitis, pharyngitis, and adenopathy (PFAPA) syndrome

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Periodic fever, aphthous stomatitis, pharyngitis, and adenopathy (PFAPA) syndrome is an auto-inflammatory disease for which a genetic basis has been postulated. Nevertheless, in contrast to the other periodic fever syndromes no candidate genes have yet been identified. By cloning, following long insert size paired-end sequencing, of a de novo chromosomal translocation t(10;17)(q11.2;p13) in a patient with typical PFAPA syndrome lacking mutations in genes associated with other periodic fever syndromes we identified SPAG7 as a candidate gene for PFAPA. SPAG7 protein is expressed in tissues affected by PFAPA and has been functionally linked to antiviral and inflammatory responses. Haploinsufficiency of SPAG7 due to a microdeletion at the translocation breakpoint leading to loss of exons 2-7 from one allele was associated with PFAPA in the index. Sequence analyses of SPAG7 in additional patients with PFAPA point to genetic heterogeneity or alternative mechanisms of SPAG7 deregulation, such as somatic or epigenetic changes.

P-CytoG-170

Chromosomes in a genome-wise order change the landscape of Genetics in form and function

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Since our observations in the gsf, Munich, Abteilung Medizinphysik of PD Dr Georg Burger [†] in 1989 it was indicated in a series of publications that the two sets of human chromosomes are organized genome-wise. In our latest publication we demonstrated this order in nuclei in interphase and metaphase reflecting that this order is maintained during the cell cycle (Chaudhuri and Walther, Acta Haematologica 2013, 129:159-168). Application of the parental-origin-determination FISH (pod-FISH) supported the separation of the two parental genomes in a cell nucleus (Weise et al., Medizinische Genetik 2013, 25(1):155).

As a rule rather than an exception this genome-wise haploid order of chromosomes we find in a variety of samples from other species like different macaques or in mice and also in aberrant human karyotypes with triploidy, tetraploidy, uniparental disomy (UPD) and in samples with small supernumerary marker chromosomes (sSMC). The detailed analysis of 3 clinical cases, one with maternal heterodisomy 14 and an additional minute 14 chromosome in mosaic state, another case with maternal isodisomy 7 and an additional minute chromosome 7 in mosaic state, and a third case with an additional maternal inherited marker chromosome 22 shed light on the functional rule of this more general genome-wise order compared to a strict parental genome wise order that we introduced before (Medizinische Genetik 2013, 25(1):155). This genomic order may now substantially change the landscape of genetics, for example, by upgrading the tools of diagnosis (Chaudhuri et al., Anticancer Res 2008, 28:3573-78) and the understanding of genetic operations like epigenetics or codominance of the two alleles of a gene (Chaudhuri et al., Cellular Oncol 2005, 27:327-34). Similarly we may have to refine the terms Comparative Genomic Hybridization (CGH) or Loss of Heterozygosity (LOH) by specifying the involvement of the maternal and/or paternal genomes. Practical models may demonstrate the forthcoming advancements.

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P-CytoG-171

Variation of short arms of acrocentric chromosomes: benign polymorphism or relevant aberration?

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Short arms of acrocentric chromosomes are composed of heterochromatin, satellites, and satellite stalks containing multiple copies of the genes for ribosomal RNA (NORs - nucleolar organizing regions). Variations in size of the short arms are frequently observed in routine karyotyping. Staining techniques as C-banding, silver staining, or Fluorescence-in situ-hybridisation with appropriate probes may be performed to confirm the harmlessness of such variants.

Rarely, however, an enlarged p-arm of an acrocentric chromosome may involve euchromatic material resulting in a balanced or unbalanced karyotype, the latter resulting in a clinical phenotype.

Here we present six cases of an enlarged p-arm with suspicious appearance of chromosomes 13, 15 and 22. Partial trisomies were identified in four cases using molecular cytogenetic methods. 1) the unbalanced translocation der(13)t(10;13)(p12.3;p11.2) was found in the prenatal diagnosis because of a suspicious ultrasound scan; 2) chromosome analysis in a girl with developmental delay resulted in the unbalanced translocation der(15)t(9;15)(p13;p11.1.); 3) in a boy with microcephaly, failure to thrive, and developmental delay the unbalanced karyotype der(22)t(17;22)(p13.1;p12) was diagnosed; 4) the unbalanced karyotype der(22)t(18;22)(p11.1;p13) in a girl with mental retardation was identified.

Furthermore, prenatal diagnosis because of elevated maternal age revealed an enlarged short arm of chromosome 15. The karyotype der(15)t(Y,15)(q12;p11.2) confirmed the clinically irrelevant translocation of Yq-chromosomal heterochromatin onto the short arm of chromosome 15. Finally, in a man with azoospermia a balanced translocation t(13;21)(p13;q21.2) was identified. Obviously, spermatogenesis is severely impaired in this case.

In general, minute chromosomal aberrations may escape cytogenetic identification. This might be particularly true if chromosomal regions are involved which are known to be polymorphic like the short arms of the acrocentrics. Therefore, prominent or suspicious short arms of acrocentrics should be clarified using conventional and/or molecular cytogenetic methods.

P-CytoG-172

Molecular karyotyping significantly contributes to the molecular diagnosis in growth-retarded patients

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Recents studies provide evidence for a significant contribution of (sub) microscopic imbalances to the etiology of growth retardation syndromes without mental retardation, e.g. up to 20% of patients with features of Silver-Russell syndrome (SRS) carry pathogenic deletions or duplications. These aberrations have either been assigned to syndromes with a clinical overlap with SRS or they are unique for SRS. To further determine the spectrum of chromosomal imbalances in this group of patients, we performed molecular karyotyping with the Affymetrix SNP6.0 array in 15 patients ascertained as SRS but without defects on chromosomes 7 and 11 typical for SRS. In 4 patients, pathogenic copy number variations could be detected. They were all associated with known congenital disorders whose features overlap with SRS: one newborn carried a deletion of 4p16.3 (Wolf-Hirschhorn, WHS) and a duplication of 7q36, the other patients showed a deletion of 2q14.2 associated with holoprosencephaly (HPE), a deletion in 22q11.21 atypical for DiGeorge syndrome (DGS), and a 45,X karyotype (Ulrich-Turner, UTS). It is worthy to mention that these patients had initially not been clinically diagnosed as WHS, HPE, DGS or UTS, respectively, as they were either too young or represent ambiguous phenotypes. Summarising our data and those from precedent studies, a frequency of up to 20% for pathogenic deletions or duplications can be expected in growth-retarded patients with features reminiscent of SRS. As the different chromosomal imbalances in SRS cohorts as well as the clinical heterogeneity in these patients show, duplications and deletions have to be considered in patients with unspecific clinical findings consistent with SRS. In particular, the majority of the "SRS" cohort with pathogenic chromosomal imbalances illustrate that mental retardation is not an obligate attribute of microdeletion and –duplication carriers. In case of growth retardation, the identification of the basic genetic defect is needed for a personalized therapy, e.g. treatment with growth hormone. Therefore, molecular karyotyping should be implemented in the diagnostic algorithm in growth-retarded dysmorphic patients without mental retardation.

P-CytoG-173

Deletions of the cis-regulatory exonic enhancers 15 and 17 of the DYNC1I1 gene are associated with Split hand/foot malformation type I (SHFM1)

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The Split hand/foot malformation type I (SHFM1) disease locus maps to chromosome 7q21.3-q22, a region that includes the genes distaless homeobox 5 and 6 (DLX5/6). It has been shown that the double knock out of Dlx5 and Dlx6 in mice results in a split hand/foot phenotype. However except for one recessive DLX5 missense mutation no other coding mutations have been identified so far in individuals with SHFM1.

Recently several DLX5/6 associated cis-regulatory elements have been identified within the 1.2 Mb SHFM1 critical region on chromosome 7q21.3-q22. Interestingly, two limb specific enhancer elements are located within the coding exons 15 and 17 of the dynein cytoplasmic 1 intermediate chain 1 gene (DYNC1I1) termed "exonic enhancer" (eExons).

Here we analysed several individuals with SHFM by high resolution array-CGH. In two unrelated families presenting with an autosomal dominant SHFM phenotype we detected overlapping microdeletions of 167 kb and 202 kb respectively. The deletions encompass the two limb specific enhancer elements eExons 15 and 17 of DYNC1I1 as well as the last two exons of the SLC25A13 gene. Both genes have been shown not to be involved in limb development. Therefore we propose that deletions of the cis-regulatory eExons 15 and 17 of DYNC1I1 are the molecular cause of SHFM1 in the families investigated.

In this study we define a new minimal critical region for SHFM1 of 115 kb over 900 kb centromeric to DLX5/6 including the two limb specific cis-regulatory eExons 15 and 17 of DYNC1I1. Furthermore, we screened our cohort of SHFM individuals to determine the prevalence of copy number variations in this enhancer exons and their relevance for clinical diagnostics of SHFM.

P-CytoG-174

Interstitial 5.6 Mb microdeletion in the chromosome region 6q13q14.1 in a boy with intellectual disability and skeletal features

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Interstitial 6q deletions are associated with intellectual disability, hypotonia and mild facial anomalies, but the reported cases are rare and mostly represent large cytogenetically detectable deletions. We report on a male patient referred at the age of 18 months for evaluation due to developmental delay, hypotonia, mild facial dysmorphism and syndactyly II/III. Conventional karyotyping of lymphocyte- and fibroblast-cultures showed a normal male karyotype.

Subtelomere screening by fluorescence in situ hybridization as well as diagnostic analysis concerning Smith-Lemli-Opitz and Fragile X syndrome revealed also normal results. Upon reevaluation at the age of 13.5 years he showed intellectual disability and a distinctive pattern of skeletal anomalies (scoliosis, kyphosis, broad phalanges, foot anomaly). Molecular karyotyping using array CGH analysis showed an interstitial deletion in the chromosome region 6q13q14.1 with a deletion size of 5.6 Mb, encompassing amongst others the COL12A1 gene. We will present our data in comparison to the cases of the literature.

P-CytoG-175

Isodicentric Yq chromosome without AZF deletion in a patient with azoospermia and short stature

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The human Y chromosome contains large palindromes playing an important role for intrachromosomal recombination. Aberrant recombination events by homologous crossing over between opposing arms of these palindromes on sister chromatids can result in formation of isodicentric Y (idicY) chromosomes. Most recombination events occur within Yq resulting in idicYp chromosomes containing a doubled Yp and a breakpoint within Yq frequently resulting in deletions of the AZF regions of different extent. Rarely, the recombination event occurs within Yp resulting in idicYq chromosomes. The phenotype is depending on the presence of the SRY gene located immediately centromeric to the pseudoautosomal region 1. Deletions of SRY result in a female phenotype while deletions including the SHOX gene located in the

telomeric part of PAR1 may cause growth retardation. Moreover, idic Y chromosomes and particularly idic Yq chromosomes seem to cause meiotic and mitotic instability. A loss of substantial parts of PAR1 may cause a breakdown of meiosis during spermatogenesis resulting in azoospermia.
We report on a 37 year old male patient with azoospermia and short stature. A testicular biopsy displayed only few spermatids in few semniferous tubules. The patient displayed a body height of 150,5 cm differing substantially from his familial background but no further abnormalities. The microscopic chromosomal analysis showed an idic Yq chromosome in 81 %, a 45,X karyotype in 15 % and secondary aberrant Y chromosomes in 4 % of analyzed cells. To further characterize the observed idic Yq chromosome FISH analyses were performed using probes specific for the centromeric region, the SRY gene region and the SHOX gene region, respectively. The FISH of the centromeric region confirmed the presence of two centromeres on the aberrant Y chromosome but in approximately 80 % only one of the two centromeres was active. Furthermore, the FISH of the SRY gene region revealed two specific signals in the vast majority of the analyzed cells whereas the FISH of the SHOX gene region did not show a specific signal on the altered Y chromosome in any analyzed cell. An MLPA detected a deletion of approximately 700 Kb of the telomeric part of Yp while at least 75 % of PAR1 were preserved. In the molecular analysis of the AZFa, AZFb and AZFc regions, respectively, no deletion of any AZF gene was detected. We conclude that the aberrant Y chromosome is an isodicentric chromosome generated by a fusion of two Y chromosomal copies within the telomeric part of the pseudoautosomal region 1 resulting in a loss of the SHOX gene while the major part of PAR1 and the SRY gene are doubled. Thus, there could be some potential in the patient to generate few spermatozoa, although this question may depend on the stability of meiotic recombinations of the sex chromosomes. To answer this question further analyses of the spermatogenesis process from a new testicular biopsy to elucidate the meiotic potential may be helpful.

P-CytoG-176

Male patient with a non-mosaic idic Yp, short stature and infertility with azoospermia

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Isodicentric Y chromosome is the most common structural aberration of the Y chromosome. They are mitotically unstable and during cell division resulting in mosaicism with an additional cell line. Only 5% of the patients with dic(Y) were manifested in non-mosaic form. It is assumed that this event occurs probably during spermatogenesis.
Isodicentric Y chromosomes idic(Y) cause several sex-linked phenotypes. Patients carrying a dicentric Y chromosome have a wide range of somatic, genital, and gonadal phenotypic manifestations, depending on the structure of the dicentric Y chromosome, the breakpoints, the types of mosaicism and proportion of idic(Y)-containing cells in gonads and other tissues. As previously reviews have shown 41% of affected subjects were phenotypical females, 32% were phenotypical males, and 27% had different degrees of intersexuality.
We describe a 36-year-old male with a non-mosaic idic(Yp) chromosome, short stature and male infertility with azoospermia. Analysis, using G,Q,C banding, Fluorescence in situ hybridization (FISH) and Polymerase chain reaction (PCR) DNA techniques, revealed an abnormal non-mosaic karyotype of:
46,X,iso dic(Yp)(pter→q11.223::q11.223→pter)

P-CytoG-177

Human dicentric chromosomes and their centromeric activity

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Dicentric chromosomes are known in many species and are described in connection with mitotic instability. But since ~40 years stable dicentric chromosomes have been reported in human. A connection between their stability and the mitotic activity of both centromeres of the dicentric chromosome was assumed. This study presents the first large systematic study to characterize human dicentric chromosomes. Both large human dicentric chromosomes and dicentric small supernumerary marker chromosomes (sSMC) were investigated. Dicentric sSMC served as a model system for the analysis of centromere function, properties and centromere activity as they can be found more often than other human dicentrics.
After characterization on cytogenetic and molecular cytogenetic level, immunofluoresence experiments using antibodies against centromeric proteins were performed. 103 cases with dicentric sSMC and 41 large human dicentric chromosomes were investigated.
In dicentric sSMC, five different centromere activation patterns were detected and four different patterns in large dicentric chromosomes. The patterns varied in signal intensity and distribution as well as with respect to centromere structure. Two main parameters were found to influence the activity pattern: the chromosomal origin of the chromosome and the intercentromeric distance. This suggests a complex process of inactivation and stabilization at the centromeres influenced also by epigenetic parameters.
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P-CytoG-178

PhenogramViz: A tool for cross-species phenotype analysis and interpretation of copy-number variations

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Clinical geneticists are often confronted with lists of several copy number variants (CNVs) in a patient's genome containing hundreds of genes. Thus, at first, suspected pathogenic CNVs have to be separated from probably benign CNVs, e.g. by investigating the set of genes located in the affected regions. Often, further information on those genes is missing. Thus, model organism databases that record the phenotypic consequences of knocking out the homolog of a human gene are used. However, searching and aligning the model organism's phenotype to the patient's symptoms can be a laborious and time-consuming challenge, given the missing integration of phenotype information across species and the lack of dedicated software tools.
Cytoscape is a dedicated network visualisation software. We have implemented a Cytoscape app (plug-in) called PhenogramViz, specifically aimed at assisting the clinical geneticist in interpreting long lists of CNVs, given a set of phenotypes seen in a patient. The tool makes use of the integrated cross-species phenotype ontology ‚Uberpheno'.
PhenogramViz can easily be used to automatically visualise gene-to-phenotype relations by generating so-called phenograms. We show that a score based on these phenograms can be used to prioritize CNVs that are more likely to be responsible for the patient's phenotype. PhenogramViz is a user-friendly Cytoscape App that may help in routine diagnostics of CNVs by integrating and visualising phenotype information on the affected genes. PhenogramViz is freely available under http://compbio.charite.de/contao/index.php/phenoviz.html.

P-CytoG-179

46,XX,der(6)(pter-->q27::q27-->q24::q27-->qter) in a fetus with multiple malformations and complex mosaic in the placenta: unexpected cytogenetic findings, limitations in array-analysis and clinical findings.

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We present the findings of a malformed fetus of a 31 year old healthy female. It was her first pregnancy, conceived after ICSI. Short- and longterm culture after chorionic villus sampling (CVS) primarily showed a normal female karyotype. Because of multiple malformations the mother opted for termination of pregnancy. Chromosome analysis of fetal material revealed a morphologically abnormal chromosome 6, which was finally diagnosed as 46,XX,der(6)(pter-->q27::q27-->q24::q27-->qter). The fetal anomalies are concordant with formerly described cases in the literature. Interestingly, re-analysis of the former short- and longterm culture after CVS confirmed the results in the originally examined mitoses except for two. Additionally analysed cells of the CVS longterm culture showed a mosaic of normal female cells and at least 3 different cell lines with morphologically different abnormal chromosomes 6. Here we present the intriguing data from the microarray (duplication of a large part, quadruplication of a small part of 6q in the fetal material, possible mosaic state in the placenta) and FISH-banding analysis (multicolour banding - MCB) as well as a discussion of and model for the possible origin of this complex anomaly. This case strongly indicates, that only the use of these different technologies leads to clarification of such complex chromosomal alterations.

P-CytoG-180

Inverted segment size and the presence of recombination hot spot clusters matter in sperm segregation analysis

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As outlined by Anton et al. (2005), heterozygous carriers of chromosomal inversions have an enhanced risk of producing unbalanced gametes. This is due to the occurrence of recombination events within the inverted segment which leads to duplications/deficiencies. During meiosis pericentric inversions (PEI) can lead to at least four types of different meiotic configurations and for paracentric inversions (PAI) at least six different kinds of meiotic configurations can be hypothesized. Recombinant gametes can be generated if a single crossing over occurs within an inversion loop (Anton et al., 2005). Morel et al. (2007) suggested that in PEI, no recombinants are produced when the inverted segment size is <30%, a few recombinants are produced when the inverted segment size is within 30-50% and significant numbers of recombinants are produced when the inverted segment size is >50% of total length of the affected chromosome. However, for PAI, due to a limited number of reported cases with segregation analyses, a correlation between inverted segment size and the formation of recombinant gametes has not yet been done. We analyzed three own and 7 PAI cases from the literature and showed that the assumptions of Anton et al. (2005) and Morel et al. (2007) for PEI are not completely applicable to PAI. Our analysis reveals that the larger the inversion size in relation to the chromosome arm, the more likely recombination takes place. When the size of the PAI is smaller than 50% of the corresponding chromosome arm, the percentage of recombinant sperms is only between 0% and 3.72%. When the size is larger than ~50% then the percentage of recombinant sperms goes up to 10% and more (preliminary data). Such risk figures for the occurrence of recombination events within the segment involved in PAI carriers are urgently needed in genetic counseling. In summary, we hypothesize that the formation of recombinants in PAI can be correlated with at least three underlying principles: (i) GTG-band features (light or dark), (ii) the percentage of the chromosomal arm involved in the inverted segment, and (iii) to some extent the presence or absence of recombination hot spots around the breakpoint and/or the inverted chromosomal segment. Besides, the recently shown major influence of fragile sites on the breakpoint formation should also not been neglected (Liehr et al., 2011).

P-CytoG-181

Supernumerary ring chromosome 8 with complex rearrangement

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We report on a 26 year old female patient with mild mental retardation, illiteracy, behavioural problems and dysmorphic features. The patient is the second child of non-consanguineous parents. The parents and the sister are reported to be mentally retarded as well, but were not available for examination. Length, weight and head circumference were within a normal range. The patient presented with malar hypoplasia, retrognatism, high arched palate, asymmetric alae nasi, exophthalmos, kyphosis, pes valgus, hypoplastic toe nails and recurrent patellar luxation. During childhood, dislocation of the hip was described. Cytogenetic analysis revealed a female karyotype with a small supernumerary ring chromosome in approx. 71% of analysed lymphocytes. The ring chromosome was identified as a derivative of chromosome 8 by multi colour FISH analysis present in 55% of the analysed metaphases. Three signals were shown in approx. 50% of nuclei from buccal mucosa cells using a centromere 8 specific probe. Chromosome analysis of lymphocytes from the parents and the sister revealed normal karyotypes. To characterise the ring chromosome 8 in more detail, array-CGH analyses were performed on DNA from peripheral blood and buccal mucosa cells. In both tissues the amplified region was assigned to 8p11.22-q12.1. This region shows a complex rearrangement with altogether four triplications that alternate with four duplicated regions, with some of them may also be inverted. The complex aberration with frequent oscillation between the two copy number states might be due to chromosome shattering and rearrangement of part of the chromosomal segments resembling chromothripsis. Phenotypic manifestations of patients with supernumerary ring chromosome 8 depend mainly on the location of the duplicated chromosomal region and the degree of mosaicism. Several of the clinical features in our patient are also reported in cases of mosaic trisomy 8.

P-CytoG-182

CNV and Aneuploidy Detection by Ion Semiconductor Sequencing

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Ion Torrent™ semiconductor sequencing, combined with Ion AmpliSeq™ technology, provides simultaneous identification of copy number variants (CNVs), single nucleotide variants (SNVs), and small

insertions and deletions (indels) from a research sample by means of a single integrated workflow. 100% of assayed CNV regions (n=34) were detected using a reference set of 31 samples with known chromosomal aberrations. Low-pass whole-genome sequencing data, with approximately 0.01x read coverage, allowed the rapid ≤10 hour analysis of aneuploidies from research samples with extremely low initial input DNA amounts—even from a single cell. Using a control set of 10 samples with known chromosomal aberrations, 100% of the copy number changes were found, ranging from gains or losses of whole chromosomes to subchromosomal alterations tens of megabases (Mb) in size. The Ion PGM™ System minimizes the high cost and complexity of next-generation sequencing and, with Ion Reporter™ Software, facilitates user-defined CNV and aneuploidy detection, with three sensitivity options so that copy number analysis workflows can be tuned to achieve desired levels of sensitivity and specificity.

P-CytoG-183

Clinical variability in carriers of SHOX duplications

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Introduction: The SHOX gene is a member of the paired homeobox family and is located in the pseudoautosomal region 1 (PAR1) of the X and Y chromosomes. SHOX deletions are associated with idiopathic familiar short stature (MIM 300582) Leri-Weill Dyschondrosteosis (MIM 127300) and Langer mesomelic dysplasia (MIM 249700). In the literature, SHOX gene duplications are underrepresented compared to deletions and have been associated in rare cases with the Mayer-Rokitansky-Kuster-Hauser syndrome, which is characterised by malformations of the structures derived from the Müllerian ducts and associated with skeletal and urological abnormalities (1).
Clinical background: Here we report on 8 probands from four different families, including three unrelated boys (patients 1–3), two sisters (patients 4–5) and three clinically healthy relatives.
Patient 1, a 14 month old boy, presented with a diagnosis VACTERL association; his findings included Anal atresia, Cardiac defects (ASD, VSD, tetralogy of Fallot), Renal anomalies (multiple renal cysts), microcephaly and developmental delay.
Patient 2. a 5 year old boy, presented with a learning disability.
Patient 3, a boy aged 6 years, had bilateral hearing loss, speech and motor delay, and behavioural and concentration disorder.
Patients 4 (age 5 years) and 5 (age 3 years) were sisters, both showed an epicanthus. Both had pulmonal stenosis as newborns, and patient 5 also had an open ductus arteriosus. Some time ago cardiac controls showed rescue of all abnormalities for both girls. Furthermore, patient 4 had astigmatism, strabismus, and reduced social competence, and patient 5 had a bilateral double kidney anlage.
Genetic studies and findings: All patients showed normal karyotypes. All microduplications were identified using MLPA subtelomere screening (kits P036 and P070) and were confirmed and specified using microarray analysis (Affymetrix).
In patient 1, the duplication comprised substantial parts of the SHOX and CSF2RA genes; it had been inherited from the unaffected mother and was also present in the unaffected maternal aunt. In patient 2, the duplication included four genes (PLCXD1, GTPBp6, PPP2R3B and SHOX) and had been paternally inherited via the Y-chromosome.
In patient 3 the duplication comprised only the SHOX gene. The parents still have to be studied. Patients 4 and 5 showed identical duplications including only the SHOX gene. Results in the parents are pending.
Conclusion: There is an apparent heterogeneity in both the size of the duplicated area in the SHOX region and clinical phenotypes of carriers including not only the patients but also apparently healthy parents and other relatives. A disease causing relationship could not be established. However, the affected children should be closely surveyed for possible growth defects and their sexual and psychomotor development.
(1) Gervasini C. et al., Genet Med 2010:12(10):634-640

P-CytoG-184

Partial trisomy 5p associated with sagittal craniosynostosis

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Craniosynostosis is defined as the premature fusion of one or more cranial sutures resulting in characteristic skull deformities. An increased intracranial pressure may cause neurological impairments and midface hypoplasia leads to eye and respiratory problems. Craniosynostosis is a frequent craniofacial malformation and estimated to affect 1 in 2100-2500 newborns. Premature fusion of the cranial sutures can occur either as isolated malformation in non-syndromic craniosynostoses or as part of a syndrome. So far genetic causes have been identified mainly for syndromic craniosynostoses, i.e. mutations in FGFR2, FGFR3, TWIST1 and EFNB1. However, in more than 50% of cases the underlying genetic cause remains unknown.
Here we present a patient with premature closure of the sagittal suture. He underwent cranial surgery during the first year of life. Furthermore, the patient showed additional clinical features like posteriorly rotated ear, pectus carinatum, mild scoliosis, and mild intellectual disability. Initially, FGFR associated craniosynostosis was excluded by sequencing of FGFR1, FGFR2 and FGFR3. Because of the additional clinical features we performed an array CGH analysis using a 1M array (Agilent, Santa Clara, USA) to screen for submicroscopic copy number variations. Using standard analysis setting we detected a 13.1 Mb duplication on chromosome 5p [arr[hg19] 5p15.1p13.3(17,686,734-30,849,372) x3]. This region includes 6 OMIM genes. After visual inspection of the profile and changing of the analysis settings a considerably larger duplication extending further proximal on the short arm was detected [arr[hg19] 5p15.1p12(17,452,895-46,115,086)x3]. This 28.6 Mb duplication encompasses more than 40 OMIM genes. Investigation of the parents to determine the origin of the aberration is on-going.
We hypothesize that the partial trisomy 5p might be caused by a small supernumerary marker chromosome. To proof our hypothesis we intend to perform a conventional chromosome analysis in combination with FISH using probes located within the aberrant regions of chromosome 5p.
A review of published cases with partial trisomy 5p indicates that distal duplications (5p13.3-pter) are associated with a milder phenotype, whereas patients with proximal duplications (5p11-p13.2) show more severe phenotypes. Common clinical features are facial and limb malformations, cardiac defects, renal and intestinal malformations as well as mental retardation. In line with these data our patient with a proximal duplication presents with a relatively mild phenotype. A precise genotype-phenotype correlation remains difficult due to small sample size.
Interestingly, the duplication encompasses the gene encoding for FGF10, a ligand which has been shown to interact with FGFR2. Since FGFR2 mutations have been associated with craniosynostosis it is conceivable that an over/misexpression of FGF10 causes disturbed FGFR2 signaling resulting in premature fusion of the sagittal suture in our patient.

P-CytoG-185

CNV analysis in a cohort of 174 patients with bladder-exstrophy-epispadias complex

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The clinical presentation of the bladder-exstrophy-epispadias complex (BEEC) ranges from epispadias (E) and classical bladder exstrophy (CBE), to the most severe form, cloacal exstrophy (CE), often referred to as the OEIS complex. The birth prevalence for the complete spectrum has been reported to be 1 in 10,000 live births, with a male-to-female ratio of 2.4:1. Although the etiology for the majority of cases remains elusive, there are several lines of evidence, that de novo copy number variations (CNVs) represent a major genetic contributor.
Here we array-based molecular karyotyping in a large cohort of 174 BEEC patients, aiming to identify disease related de novo CNVs. For array-based molecular karyotyping we used the Illumina HumanOmniExpress-12v1.1 bead-chip, comprising a total number of 719,665 markers. All genotype data were analyzed by QuantiSNP using an Objective-Bayes Hidden-Markov model. To narrow down the computed number of 13,828 putative CNVs, we used different filter criteria and implemented various procedures for data analysis.
In total, 17 putative disease related autosomal CNVs ranging from 2,52 kb to 6,08 Mb in size, including one duplication in the Cat eye syndrome relevant region (22pter-22q11.21) remained. Validation of the CNVs and testing for their de novo occurrence with parallel investigation of the parents using quantitative PCR and MLPA is currently performed. Array-based molecular karyotyping furthermore identified triple X syndrome in an isolated CBE patient.

P-CytoG-186

Genetic stability within chondrocyte cultures using GTG, SKY, and locus-specific FISH

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The development of cell-based therapy raises the question whether the application of cell-based products to humans is safe. Therefore, it is important to determine whether the manufacturing process leads to chromosomal aberrations.
In a preclinical study, we analyzed 200 chondrocyte samples (40 adherent cultures and 160 spheroids) from three donors using Trypsin-Giemsa staining (GTG-banding), spectral karyotyping (SKY), and locus-specific fluorescence in situ hybridization (FISH).
Applying these techniques, the genetic analyses revealed no significant chromosomal instability for at least 3 passages. We detected clonal occurrence of polyploid metaphases and endomitoses with increasing cultivation time (passage 4-10). Y-chromosomal losses were identified in the two male donors with increasing frequency during the cultivation time. Interestingly, one donor showed trisomy of chromosomes 1,7,8,12, and translocation of chromosomes 7 and 9, which are also described for extraskeletal myxoid chondrosarcoma.
Our results attest to the necessity of (molecular) cytogenetic analyses at certain cultivation times in preclinical studies. More investigations are needed to evaluate the potential tumorigenic risk for osteoarthritic patients to an extension of articular chondrocyte implantation.

P-Genetic Counselling / Education / Genetic Services / Public Policy

P-Counse-187

Clinical utility of high-throughput sequencing in intellectual disability – the diagnostic implementation of an expanded „Kingsmore" gene panel with 1.222 disease-related genes

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Since de-novo mutations have been established as major contributor to intellectual disability in patients, sequencing has become a massive challenge for diagnostic laboratories (Vissers, 2009). Likewise, trio whole exome sequencing (WES) has become a standard detection tool with considerably high demands in sequencing capacities and bioinformatics resources. Here, we tested the diagnostic use of a targeted gene panel (Kingsmore gene panel, adapted by Ropers and colleagues) for the diagnostic implementation with special attention towards sensitivity, accuracy and efficiency.
Forty patients have been included and DNA has been enriched for exon-specific regions according to standard protocols (Illumina Custom TruSight Enrichment). Sequencing was done using Illumina SBS sequencing (Illumina, GA2x Sequencer) with an average of 41 million reads and a mean coverage of 160 reads per target base. Standard bioinformatics tools were used for mapping (stampy), annotation (annovar) and filtering to exclude frequent (non-pathogenic) variants.
For reporting, we evaluated 513 genes that have been published in more than one family with intellectual disability using OMIM datasets and the phenomizer database (http://compbio.charite.de/phenomizer/). Reducing target genes and increasing sequencing depth, as compared to standard exome sequencing metrics, reduced the fraction of low covered target bases with less than 20 reads to <5%. A total of 4.800 variants per patient decreased the potentially relevant variability to 12 novel, uncertain variants per patient on average. The latter includes potential de-novo mutations and other mutation types (recessive, X-linked). Several de-novo and inherited disease causing mutations have been identified among others in the SYNGAP1 and ASXL3 gene. As compared to WES, this approach offers a targeted diagnostic evaluation of published ID genes with high diagnostic sensitivity, a significantly reduced sequencing load, and a very limited risk for unsolicited findings.

P-Counse-188

Only consistent avoidance of acoustic triggers finally lead to absence of symptoms in a severely affected patient with long QT-syndrome type 2

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Introduction:
It is well established that in patients with long QT-syndrome (LQTS) type 2 due to a mutation in KCNH2 an acoustic startle response represents a specific trigger for the occurrence of ventricular cardiac arrhythmia.
We report of the vital importance of avoidance of the specific trigger "getting startled" for the course of the disease.
Case report:
A meanwhile 32year old female patient with a heterozygous mutation in KCNH2 (heterozygous 2bp-deletion, c.1275_1276delAC Thr425fsX517) suffered from countless syncopes due to cardiac arrhythmias since the age of 6 years. Despite optimal medical treatment (Metoprolol , resp. Propranolol 2-3 mg/kgKG and regular potassium and magnesium supplement) she experienced non-sustained and sustained ventricular arrhythmias leading to convulsive syncopes sometimes accompanied by considerable injuries caused by the falls (1) .
The patient was familiar with the known preventive measures for patients with LQTS (early antipyretic measures, avoidance of QT prolonging drugs, no loud alarm clock at the bedside, no competitive sports, no grapefruit juice) and followed them reliably, but nevertheless there was a recurrence of syncopes, resp. documented ventricular tachycardia.
For this reason, at the age of 28 an implantable cardiac defibrillator was implanted (11/2009). Her events were regularly triggered by startling from sudden loud noise (e.g. ringing of the telephone, alarm clock).
Since 02/2010 she paid attention to consequently avoid the known trigger, this means that telephones next to her were turned down, her alarm clock at the bedside was removed, and she knowingly does not relax completely in places where sudden loud noises can not be avoided.
Following those precautions she has now been asymptomatic for 3 years and 10 months.

Conclusion:
What initially appears to be a barely workable lifestyle modification can now be easily integrated into every day life by our patient. Since only getting startled by loud noises but not loud noises occurring while she was under "basic tension" (quote of the patient) led to syncope, she paid attention to stay awake and not doze off in public, e.g. on the subway. She´s working full-time as a graphic designer, does sports in a moderate intensity, feels hardly any restrictions in her leisure activities and wakes up without an alarm clock. Next to the medical treatment, which could not completely prevent syncopes, it was ultimately the avoidance of the trigger that led to a so far almost 4 year symptom-free interval in a severely affected patient.

(1) Feddersen et al., Lancet 2009

P-Counse-189

Adaptation of CUGC guidelines to next-generation sequencing (NGS) - a database for diagnostic NGS panels

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EuroGentest unit 2 „Genetic testing as part of health care" and the European Society of Human Genetics collaborate on the establishment and administration of Clinical Utility Gene Cards (CUGCs). These disease-specific guidelines evaluate the risks and benefits of the application of genetic tests in the clinical setting.
Each CUGC is authored by a multinational expert team; potential authors are identified based on e.g. their publication record and practical experience. Subsequent to the peer-review, the documents are published in the European Journal of Human Genetics (EJHG). EuroGentest commissions the establishment as well as the annual update of the guidelines. CUGCs mainly aim at clinicians, geneticists, referrers, service providers and payers in their decision to offer a genetic test to a person. Each CUGC is freely accessible.
With the long-term goal of adapting the CUGC format to next-generation sequencing (NGS) technologies, EuroGentest designed the NGS panel database. This data collection presents diagnostic NGS panels from different providers including panel name and tested genes. This information is further linked to the according disease(s) and genetic background, including OMIM genotype and Orpha number. On the one hand this database gives users the opportunity to quickly identify diagnostic options according to the different search terms: disease, gene, OMIM number, panel name, provider and Orpha number. On the other hand it determines any overlap or gap of tested genes between different panels. Based on these „core genes" the CUGC initiative can be expanded in order to cover NGS-based genetic test application in diagnostics. The according NGS guideline template is in progress.
NGS panel providers were identified through a web search using the web search engine Google. In order to specify the test information the respective laboratory websites were reviewed. We initially focused on European NGS panel providers. A prototype of our data collection is available at the EuroGentest website: https://eurogentest.eshg.org/index.php?id=668. As of November 26 2013, we identified 27 laboratories having launched a total of 853 clinical NGS tests covering 2254 genes.

P-Counse-190

De novo deletion at 16p11.2

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We report on a 12 year old boy with mental retardation, autism spectrum disorder (ASD), and growth parameters above 97th centile (length, weight, head circumference). Motor development milestones were significantly delayed and speech problems were observed.
Microarray-based comparative genomic hybridization analysis (CGH) was performed on this patient using the CGHTM HD v1.0 4-plex array which includes 180K oligonucleotide probes. qPCR detecting fragments in the chromosomal band 16p11.2 (ViiA 7 Real-Time PCR System) were used for investigation of healthy family members.
Fragile X syndrome was ruled out at the age of four years. Cytogenetic analysis revealed a normal male karyotype (46,XY). Array analysis showed a microdeletion of 75 oligonucleotide probes on chromosome 16p11.2 that spanned 535 kb, encompassing 17 genes.)
The microdeletion 16p11.2 is characterized by a spectrum of primarily neurocognitive phenotypes showing incomplete penetrance and variable expressivity. People with 16p11.2 deletion syndrome usually have developmental delay and intellectual disability. Most patients also have

at least some features of ASD being characterized by impaired communication and socialization skills, as well as delayed development of speech and language. Some affected individuals have minor physical abnormalities such as low-set ears or partially webbed toes (partial syndactyly). These features are not seen in our patient.
Signs and symptoms of the disorder vary even among affected members of the same family. Some people with the deletion have no identified physical, intellectual or behavioral abnormalities (reduced penetrance). The inheritance of 16p11.2 deletion syndrome is considered autosomal dominant. Most cases of 16p11.2 deletion syndrome are de novo. In our family only the mother´s carrier status was examined and revealed negative. The father was not available for testing. Both brothers of our index patient suffer from ASD. Therefore, they were tested and the microdeletion was ruled out.
In conclusion, patients with growth parameters above 97th centile and ASD should be investigated for 16p11.2 deletion syndrome.

P-Counse-191

First description of a patient with Vici Syndrome due to a mutation specifically affecting isoform 1 of EPG5

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Vici syndrome is a rare autosomal recessively inherited multisystem disorder characterized by agenesis of the corpus callosum, cataracts, cardiomyopathy, combined immunodeficiency and hypopigmentation [1, 2]. Cullup et al. recently showed mutations in the gene EPG5 to be causative for Vici syndrome [3]. EPG5 is involved in autophagy, an evolutionary conserved lysosomal degradation process, essential for cell homeostasis [4]. Following the first description in 1988 by Vici et al. [1], 24 other cases of Vici syndrome have been published with variable expression of the defining features [3, 5-10]. We report a further case of Vici syndrome with a homozygous truncating mutation of EPG5, identified by whole exome sequencing. The mutation in our patient is the first reported affecting only isoform 1 of EPG5 and presenting with typical clinical manifestations of Vici syndrome. Thus we assume that Vici syndrome is largely due to defects of isoform 1 of EPG5.
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P-Counse-192

De novo deletion of EXOC6B in a boy with intellectual disability and mild dysmorphism

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We describe a boy with intellectual disability (ID), language delay and minor dysmorphic features with a heterozygous de novo 459 kb deletion in chromosomal region 2p13.3 (ranging from 72,555,328-73,013,836 bp, hg 19), involving only parts of the gene EXOC6B. This gene is nearly ubiquitously expressed and encodes the exocyst complex component 6B which is part of a multiprotein complex required for targeted exocytosis. Little is known about the effect of EXOC6B haploinsufficiency. Recently, a heterozygous deletion of EXOC6B along with a deletion of the CYP26B1 gene has been reported in a boy with ID, language delay, hyperactivity, facial asymmetry, a dysplastic ear, brachycephaly, and mild joint contractures. In 2008, a patient with a complex syndromic phenotype, including left renal agenesis, neutropenia, recurrent pulmonary infections, long bone diaphysis broadening, growth, and developmental delay (DD) was found to carry a de novo translocation t(2;7) involving the genes TSN3 and EXOC6B. Further characterization of the translocation indicated that disruption of TSN3 may be responsible for the phenotype. Recently, disruption of EXOC6B by a de novo balanced translocation t(2;8) has been described in a patient with DD, epilepsy, autistic, and aggressive behavior. This is the first report of a de novo deletion affecting only EXOC6B in an individual with ID. In conclusion, based on our findings and recent data from literature, there is evidence that EXOC6B may play an important role in intellectual development.

P-Counse-193

Differentiation of stem cell derived cardiomyocytes of the pacemaker system

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The rhythmic beating of the heart is initiated by a population of pacemaking cells located in the sinoatrial node. The investigation of pathways controlling pacemaker function is of crucial relevance to our understanding of cardiac conduction disorders, e.g. arrhythmia.
Based on previous analysis using different animal models (Mouse, Zebrafish, Xenopus), we have established a functional link between the homeodomain transcription factor Shox2 and the development of the sinoatrial node as well as arrythmogenic phenotypes including bradycardia. Using Shox2 as a molecular tool, we are now investigating molecular pathways regulating early cardiac conduction differentiation using a refined embryonic stem (ES) cell based model system. We have isolated pluripotent Shox2+/+, Shox2+/- and Shox2-/- murine ES cells from blastocysts of Shox2 deficient mice to address the following questions: 1) Differentiation of mouse ES cells into ES cell derived cardiomyocytes followed by enrichment of sinus-node like cells, 2) Morphological and electrophysiological characterization of Shox2 dependent phenotypes during cardiomyocyte differentiation and 3) Identification of Shox2 dependent signaling pathways by comparing expression profiles of differentiated Shox2+/+ and Shox2-/- ES cells using RNA-Seq technology.
In summary, we have successfully established a cardiac differentiation model based on ES cells isolated from Shox2 deficient mice. This model will contribute fundamentally to the current knowledge of early pacemaker development and provides a basis for future biomedical applications.

P-Counse-194

Sequencing of the PTF1A gene and 1.5 kb of its promoter region in neural tube defects (NTDs).

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Objective: Recently a retrotransposon insertion in the 5‘ regulatory domain of Ptf1a was found to result in ectopic gene expression and multiple congenital defects in Danforth‘s short tail mouse (Sd) including neural tube defects (NTDs). We speculated that gain-of-function mutations in human PTF1A or its promoter would be involved in the formation of human NTDs.
Patients and methods: Molecular genetic analysis of the PTF1A gene and 1.5 kb of its 5’ flanking sequence was performed in blood samples of 72 aborted fetuses with severe NTDs.
Results: Examination of all PTF1A exons and their adjacent splice sites failed to reveal any likely causative sequence variant. A total of three variants (non-synonymous coding in exon 2: rs7918487; non-coding in the 3’UTR: rs10828415, rs149560393), deposited in dbSNP (Build 138) were detected. These SNPs showed similar frequencies as reported in the databases and are all unlikely to be causative for human NTDs.
Conclusion: Mutations in PTF1A or its promoter region are not a frequent cause for human NTDs. Due to its role in the Sd mouse we speculate that mutations in one of the genes targeted by this transcription factor might contribute to human NTDs. In mice, the complex tempospatial expression pattern of Ptf1a was reported to be controlled by at least three regions occupying around 30 kb in the 3’ and 5’ flanking regions of the gene. Hence, a more extended sequence analysis may also allow for detection of variants involved in the formation of human NTDs.

P-Counse-195

Mutational and phenotypic spectrum of MTFMT and lessons learned from a combined exome sequencing and candidate gene screening study

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Defects of mitochondrial oxidative phosphorylation (OXPHOS) are associated with a wide range of clinical phenotypes and time courses. Diagnostic strategies rely on the biochemical analysis of tissue samples and may reveal isolated or combined respiratory chain deficiencies. OXPHOS deficiencies can be caused by mutations of nuclear genes that are involved in mitochondrial protein translation. Due to their genetic heterogeneity such disorders cannot be diagnosed on clinical grounds alone. Hence next generation sequencing (NGS) provides a distinct advantage over candidate gene sequencing to discover the underlying genetic defect in a timely manner. One recent example is the identification of mutations in MTFMT that impair mitochondrial protein translation through decreased formylation of Met-tRNAMet.
Here we report the identification of eight additional patients from seven families who were affected with Leigh encephalopathy or white matter disease, microcephaly, mental retardation, ataxia, and muscular hypotonia. In three patients, the causal mutations were identified by exome sequencing followed by stringent bioinformatic filtering. In one index case, exome sequencing identified only a single heterozygous mutation, while the second one in the non-covered first exon was later detected by Sanger sequencing. High-resolution melting curve-based mutation screening of MTFMT identified pathogenic mutations in another three index cases, one of them previously unsuccessfully investigated by exome sequencing.
Patients’ fibroblast cell lines showed a severe decrease in MTFMT protein and reduced steady-state levels of complex I and IV subunits. We provide detailed clinical descriptions on ten MTFMT patients and review five previously reported cases. Together, this study expands the clinical phenotype and contributes to the analysis of genotype-phenotype relations.

P-Counse-196

Targeted high-throughput sequencing provides additional evidence for the causal relevance of new candidate genes in colorectal adenomatous polyposis identified by CNV analysis

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Background: Adenomatous polyposis syndromes are characterized by multiple colorectal adenomas and a high lifetime risk of colorectal cancer. Germline mutations of the APC and MUTYH genes cause the autosomal dominant Familial Adenomatous Polyposis (FAP) and autosomal recessive MUTYH-associated Polyposis (MAP. However, in up to 50% of families no germline mutation can be identified. To uncover new causative genes we performed a genome-wide copy number variation (CNV) analysis followed by high-throughput sequencing of candidate genes.
Methods: By CNV analysis we identified 97 candidate genes in 221 patients with unexplained colorectal adenomatous polyposis. To validate the functional relevance of the affected genes, we performed a systematic screening for point mutations in a validation cohort of 192 unrelated cases (145 mutation negative polyposis patients and 47 familial colorectal carcinoma cases with microsatellite stable tumors meeting the Amsterdam I or II criteria) using a targeted next generation sequencing approach (TruSeq enrichment protocol, Illumina). Data analysis was done by standard protocols using the VARBANK pipeline (CCG, Cologne, https://anubis.ccg.uni-koeln.de/varbank/).
Results: We verified 15 different truncating point mutations in 11 genes. In two genes, multiple mutations were found; CNTN6 shows a different truncating mutation in each of four patients and FOCAD shows a different mutation in two cases. All other truncating mutations were found only once. Although KIF26B shows just one frameshift mutation, it harbors 10 rare missense variants, five of which are predicted to

be deleterious by three in-silico programs. The genes have previously been reported to be involved in cell adhesion and Notch signaling or to be related to early-onset colorectal cancer.
Conclusions: We found truncating point mutations in 11 % of candidate genes identified by CNV analysis providing additional support for their functional relevance in unexplained adenomatous polyposis. A segregation analysis was not possible in any of the families. To confirm the causality and phenotype spectrum of these genes, larger sample sizes and functional studies are needed.

P-Counse-197

PCR- and Southern blot-based analysis of the C9orf72 hexanucleotide repeat in different motor neuron diseases

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The GGGGCC-hexanucleotide repeat expansion in C9orf72 is the most common genetic cause of familial amyotrophic lateral sclerosis (fALS) and frontotemporal dementia (FTD). Here we report the frequency of C9orf72 repeat expansions in different motor neuron diseases (ALS, motor neuron diseases affecting primarily the first or the second motor neuron and hereditary spastic paraplegia (HSP)). Whereas most studies on C9orf72 repeat expansions published so far rely on a PCR-based screening we applied both PCR-based techniques and Southern blotting. Furthermore, we determined the sensitivity and specificity of Southern blotting of the C9orf72 hexanucleotide repeat in DNA derived from lymphoblastoid cell lines (LCLs). C9orf72 repeat expansions were found in 27.1 % of familial ALS patients, only once in 68 sporadic ALS patients, and not in 61 HSP patients or 62 patients with motor neuron diseases affecting clinically primarily either the first or the second motor neuron. We found hints for a correlation between C9orf72 repeat length and the age of onset. Somatic instability of the C9orf72 repeat was observed in LCLs compared to DNA derived from whole blood from the same patient and therefore caution is warranted for repeat length determination in immortalized cell lines.

P-Counse-198

Extended in vitro maturation of bovine oocytes affects methylation of the oocyte-specific isoform DNMT3Lo and embryonic development

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Correct DNA methylation patterns are essential for normal mammalian development. The DNA methyltransferase 3-like (DNMT3L) gene is expressed during germ-cell developmental stages where genomic imprints are established. Here we used the bovine model to study the epigenetic reprogramming during oocyte development, maturation and fertilization. We analyzed the methylation and mRNA expression of several developmentally important genes (H19, SNRPN, OCT4, DNMT3A and DNMT3Lo) in oocytes from three different follice size (<2 mm, 3-5 mm and >6 mm) and two different in vitro maturation groups (standard IVM for 24 h versus extended maturation for 48 h). The matured oocytes were then in vitro fertilized and in vitro cultured. In the three different size groups of premature oocytes we could not find any significant methylation differences but a reduced transcript abundance in oocytes from <2 mm and >6 mm follicles. When comparing oocytes matured for 24 h versus 48 h, we detected a significantly (p=0.03) increased methylation of the oocyte specific promotor of DNMT3L (DNMT3Lo) in the 48 h group. Although oocyte fertilization rates did not differ significantly, we observed a reduced cleavage rate (24 h: 51.9±3 vs. 48 h: 38±7%) and a reduced number of embryos developing to the blastocyst stage (24 h: 21.1±2.5% vs. 48 h: 0.4±0.35%) in the 48 h group. Our findings suggest that an extended maturation time reduces the developmental potential of the oocyte, which may directly or indirectly be related to abnormal DNMT3Lo methylation.

P-Counse-199

Ichthyosis prematurity syndrome in Germany: Results from ichthyosis registry NIRK

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Introduction: Autosomal recessive congenital ichthyosis (ARCI) refers to a heterogeneous group of keratinization disorders. One important differential diagnosis of this ichthyosis group is the Ichthyosis Prematrity Syndrome (IPS). Children are born prematurely and are at high risk for neonatal asphyxia due to occlusions in the bronchial tree from amniotic fluid debris. The autosomal recessive disease is caused by mutations of the FATP4 gene.
Objective: Pilot study of the disease frequency and clinical course of patients in Germany.
Method: Databank and clinical record analysis of the registry of the Network for Ichthyoses and related Keratinization Disorders (NIRK).
Results: Within one year >3 patients with the diseases have been registered in Münster. The disease was unequivocally diagnosed by the distinct ultrastructural phenotype (EM type 4) and confirmed by mutation analysis (if done so far). One of the patients was affected by moderate cerebral impairment due to birth complications/asphyxia. Others survived without any complications. All of them shortly after birth showed a rapid healing of the ichthyosis, but had a residual form of dry skin.
Conclusion: Considering the critical neonatal period in IPS, we would like to raise the awareness for the disease, e .g. the prenatal observation of dense amniotic fluid should be taken as an early sign for risk of prematurity with severe but transient complications at birth.

P-Counse-200

A Twenty-Year Follow-up of a Familial Chromosomal Translocation resulting in Trisomy 3q13.3-qter leading to Infantile Death

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In 1994 I was contacted by the DRK Kinderklinik Siegen for evaluation of a 4-week-old male infant with multiple congenital malformations and partial trisomy 3q13-qter und partial monosomy 4q35 (46,XY,t(3;4) dup(3)(3q13.3-qter),del4(q35-qter)). This imbalanced karyotype was due to a maternal balanced reciprocal translocation (46,XX,t(3;4)(q13.3;q35)). The infant (R.A.T. 120894/94E1266/6934) was born at 35 weeks gestation with a birth weight of 2090 g, length of 42 cm and OFC 31 cm, Apgar 7/8/10. Dysmorphic facial features included upward

slanting palpebral fissures, broad depressed nasal bridge, anteverted nostrils, micrognathia, low set ears, short neck, and short extremities. He had a high arched palate without clefting. A large ventricular septal defect and a type II atrial septal defect were diagnosed, but no signs of cardiac insufficiency. X-ray signs of a skeletal dysplasia were absent. The infant was discharged in satisfactory general condition at the age of two months with a weight of 2850 g, length 49 cm and OFC 34.8 cm. He had left facial paralysis and developed opisthotonus and respiratory stridor. At home he responded to external stimuli. Respiratory stridor and breathing difficulties increased. At the age of 5 months the infant died at home in his sleep. The parents did not consent to an autopsy. I presented a preliminary report at the 21st Arbeitstreffen Klinische Genetik Nordrhein on October 10, 1995 in Düsseldorf.
Prior to the birth of this infant the mother had had three spontaneous abortions at 7, 11, and 28 weeks gestation and had given birth to a normal girl, at that time five years old. Chromosomal analysis in Essen confirmed the imbalanced karyotype in the infant and revealed the presence of the balanced reciprocal translocation in the mother and the daughter. The maternal grandmother had a normal karyotype, the paternal grandfather was not available for study. The parents of the patient were informed of the situation by genetic counseling and my written reports. These emphasized that their 5-year-old daughter with the balanced translocation should be reexamined and receive genetic counseling when she reached adulthood. I marked the record of this family not to be discarded after ten years. In addition, I sent an informatory letter to the family in 2001, six years after the patient had died.
In November 2013 the patient's sister, now age 25 years, contacted me in Leipzig for genetic counseling to aid in her family planning. Chromosomal analyses of her and her partner are being done, and various options for prenatal diagnosis will be discussed with the couple on December 4, 2013. The size of the patient's duplication 3q appears to be the largest documented in a live-born infant, albeit without a distinctive phenotype. This observation emphasizes the importance of providing long-term follow-up in disorders that can re-occur in consecutive generations in one family.

P-Counse-201

Integrated care for high-risk patients with breast and ovarian cancer in the Regensburg Centre for Hereditary Breast and Ovarian Cancer

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In 1996, the Deutsche Krebshilfe (Dr. Mildred Scheel Foundation) established 12 Centres for Hereditary Breast and Ovarian Cancer in Germany. The major goal was to develop standards for interdisciplinary counselling, genetic testing, patient care and therapy in families with hereditary breast and ovarian cancer. In 2011, three additional centres were affiliated with the Consortium, including the Centre for Hereditary Breast and Ovarian Cancer Regensburg.
The Regensburg Centre brings together genetic counsellors, molecular geneticists, gynaecologists, onco-psychologists and radiologist and offers an integrated care for patients with increased risk for breast and ovarian cancer. Catchment area is a population of approximately 2 Mio. from the Upper Palatinate and Lower Bavaria. For two years now our Center is firmly established in the structure of the German Consortium for hereditary breast and ovarian cancer and provides the genetic and gynecological counseling, molecular diagnostics, early detection, preventive measures, treatment and care in high-risk patients based on state-of-the-art knowledge in breast and ovarian cancer. Rising demand in counselling was met with the effect that waiting times for counselling sessions generally do not exceed 6 weeks. For molecular diagnostics BRCA1, BRCA2, and RAD51C gene analysis is offered by applying Next Generation Sequencing technology. This approach has significantly reduced the turn-around time for DNA testing to less than 5 weeks. Together, our Centre has been well received in the area and demonstrates the urgent need of integrated care in cancer predisposition syndromes.

P-Counse-202

Is Prenatal Diagnosis an Eugenic Question to Society? Growth of Genome Testing Needs Debate

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The number of tests for prenatal diagnostics is steadily increasing, more and more.
This raises the question, in which way this will influence the future of mankind and society.
1) Since 1958, when I. Douglas made for the first time an ultrasonography of an unborn foetus, there is an increasing number of non-invasive and invasive (amniocentesis 1966) methods for prenatal diagnosis. The aim is till today to detect hereditary diseases, mutations or abnormalities. In the first years prenatal diagnosis was only performed, when there was a tentative diagnosis.
Eugenic ideas rose at the beginning of the 20th century and the aim was the improvement of mankind (Galton). Prevention of hereditary diseases is one way of improving mankind.
2) The availability of many different methods offers new possibilities for prospective parents to get a lot of information of their unborn child. This depends not on the question, whether this facts are important for therapy. Nowadays a foetus's entire DNA sequence can be determined by sequencing its fragments of the mother's blood. This is not needed to avoid diseases that are genetic determined. Nowadays prenatal diagnosis is an individual decision and not forced by any law like before 1945 in several states of the USA and in Germany by NS-government: the significant difference to eugenics before 1945.
3) Another aspect are offered by Next Generation Sequencing (NGS) that allow to develop new and simple tests like the so called Praena-Test for testing on trisomy 13, 18 and 21. The result is not necessary for therapy, only to decide, whether abortion should be performed. This is continuity to abortion because of eugenic reasons before 1945. At this time eugenic ideas focussed on the societal value, after Second World War this changed to individual ones.
4) Neither societies nor governments are prepared for the availability of an increasing number of genetic information. Today the genome of an unborn child can be analysed; this creates new individual and societal questions: Prospective parents can influence the genome of their child according to their own beliefs. Today we have no idea, what risks this will create in the future. Also, ff parents get an unfit child, their relatives may ask the question, whether this had been necessary.
New opportunities are offered by prenatal diagnostic, but also create new ethical questions and need to be discussed of as many people as possible.
• Prenatal diagnostic will improve mankind in the future and therefore it is eugenic.
• The consequences of prenatal diagnostic must be discussed within the scientific community but also with the lay public, because those will influence society.
• Legal regulations must base on consent in society and are not only a legislative question.
Therefore, the possibilities of prenatal diagnosis ask emphatic for a debate of a great amount of people.
This is task of public policy.

P-Counse-203

Beyond BRCA1 and BRCA2: results from screening 94 genes in a large cohort of patients with familial breast and ovarian cancer.

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Background: Breast and ovarian cancer (BC/OC) predisposition, often seen in families with a high incidence of BC or young patients, has been associated with a number of high-, moderate-, and low-penetrance susceptibility genes. Prior to the introduction of next generation sequencing (NGS), only a small subset of these susceptibility genes (mostly BRCA1, BRCA2 and RAD51C) have been sequenced on a routine basis. With the advent of NGS-based panel sequencing, a routine screening of all genes currently associated with familial breast cancer became feasible. Here we report the results of panel-based screening of 94 genes that have been associated with hereditary cancer predisposition.
Method: Selection criteria for patients to be included in this study were defined by the German Consortium for Breast and Ovarian Cancer. They include, for instance, the number of cases within the family, the age of onset and the occurrence of ovarian cancer. High risk patients with previously excluded mutations in BRCA1 and BRCA2 were also included in the study. NGS was performed on an Illumina MiSeq sequencer, with 150 bp paired end sequencing chemistry. Target enrichment was performed with the Illumina TruSight cancer panel, which includes 94 genes associated with both common (e.g., breast, colorectal) and rare cancers (http://www.illumina.com/products/trusight_cancer.ilmn).
Results: In 28 % of the patients, BRCA1 or BRCA2 variations have been found. These were either clearly pathogenic protein truncating mutations (12 %) or very rare, unclassified missense variations with high probability of effect (16 %). In 39 % of the patients we found rare, unclassified missense variants in low penetrance susceptibility genes, especially NBN (nibrin) and ATM. In one case with early onset of breast cancer and no familial history, a putative splice relevant mutation in TP53 could be identified, which is currently being investigated on cDNA level. Despite the large set of 94 genes, 33 % of the patients did not reveal any convincing sequence variation. In order to complement the sequence variant detection by a comprehensive copy number analysis, a custom array has been designed that covers the same 94 target genes that are represented on the sequencing panel. Since many of the susceptibility genes are tumor suppressors, it is likely that exon losses or amplifications will also contribute to the mutation spectrum of these genes.
Conclusion: The extension of mutation screening beyond BRCA1 and BRCA2 reveals disease-causing mutations in high-penetrance genes, like TP53, as well as mutations in low-penetrance susceptibility genes. However, the enormous number of unclassified sequence variants and the detection of mutations and of carriers for hereditary diseases other than breast cancer predisposition poses a huge challenge for genetic counselling.

P-Counse-204

Epigenetic characteristics in inflammatory candidate genes in aggressive periodontitis: The role of interleukin 17C

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Background: Periodontitis has been described as a chronic inflammatory disease which is triggered by specific host dependent immune response. It is established that the immune response is influenced among others by a genetic predisposition. In recent times it was shown, that the gene expression is affected also by epigenetic modifications. Therefore, we investigated the CpG methylation pattern of 22 inflammatory candidate genes (ATF2, CCL25, CXCL14, CXCL3, CXCL5, CXCL6, FADD, GATA3, IL10RA, IL12A, IL12B, IL13, IL13RA1, IL15, IL17C, IL17RA, IL4R, IL6R, IL6ST, IL7, INHA, TYK2) in dependence of the periodontal status. Patients and methods: In this preliminary study 11 patients with aggressive periodontitis (54.5% males, 40.6+11.5years) and 10 periodontal healthy persons (40% males, 37.7+17.1years) were included. Gingival biopsies were obtained and immediately frozen in liquid nitrogen. After DNA isolation (QIAamp® DNA Micro Kit) the methylation pattern was quantified using EpiTect® Methyl II Signature PCR Array Human Inflammatory Response (Qiagen).
Results: In gingival inflamed tissues of patients with aggressive periodontitis there was a significant reduction in CpG methylation pattern of interleukin 17C compared with tissues of periodontal healthy persons (6.1% vs. 26.4%, p=0.007). The methylation pattern of all other genes investigated was not significantly modified regarding periodontal inflammation. Discussion: In our study we show for the first time a differential methylation pattern for IL17C in periodontitis. Interleukin 17C is an essential autocrine cytokine that regulates innate epithelial immune responses induced by bacterial challenge and inflammatory stimuli. The decrease in CpG methylation is presumably accompanied by an increase in gene expression. This could lead to a greater availability of interleukin 17C and the induction of epithelial immune response in inflamed oral tissue.

P-Counse-205

Targeted Resequencing of the schizophrenia candidate gene RB1CC1

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Schizophrenia is a severe neuropsychiatric disorder with heritability estimates of ~80%. In 2011, Xu et al. (2011) published the first exome-sequencing study focusing on de novo mutations in patients with schizophrenia. In 53 patients with schizophrenia, 22 unaffected controls and their parents a total of 40 de novo mutations were detected. The large number of genes reported to carry de novo mutations, together with the very low frequency of mutations among the patients, makes it difficult to pinpoint the genes that are relevant for disease pathogenesis. In order to provide additional genetic evidence for any of the genes suggested by the exome-sequencing study, we performed a follow-up study focusing on copy number variants.
We screened the genome-wide SNP array data from 1,637 patients with schizophrenia and 1,627 controls for the presence of copy number variants in any of the genes suggested by the exome-sequencing study. Duplications in RB1CC1 on chromosome 8 were overrepresented in patients. The duplications were followed-up in independent European samples. In the combined analysis, comprising of 8,461 patients and 112,871 controls, duplications in RB1CC1 were found to be associated with schizophrenia (P = 1.29 x 10-5; odds ratio = 8.58).
The aim of the present study was to further explore RB1CC1 as a candidate gene for schizophrenia.

The gene RB1CC1 consists of 24 exons. For two main reasons we focused our targeted Sanger resequencing on exon 15: (i) this exon contains > 30% of the gene's total protein-coding sequence, and (ii) Xu et al. (2011) identified a de novo frameshift deletion in this exon. A total of 1900 patients with the DSM-IV diagnosis of schizophrenia were included in our study. Publicly available data from the 1000 Genomes Project and the Exome Variant Server were used to determine the frequency of the identified variants in population-based cohorts and individuals collected for studies focusing on lung and heart phenotypes.
After quality control, the data from 1740 patients were available. Among 22 patients, a total of 17 different variants were identified and verified by sequencing the complementary strand. Of these, 10 were neither detected in the 1000 Genomes Project nor the Exome Variant Server. Currently, we are analyzing whether these variants co-segregate with a psychiatric diagnosis within the families of the affected probands. Furthermore, detailed phenotypic descriptions of the mutation carriers are being assembled.
RB1CC1 is a brain expressed gene and has been implicated in cell cycle progression and neurodegeneration. Our study is the first to systematically screen exon 15 in a large number of patients with schizophrenia for mutations that might be associated with the disorder. Currently, detailed statistical analyses (focusing on rare variants) are being performed. The results will be presented at the meeting.

P-Counse-206

Validation of a commercial 46 gene next generation sequencing assay for mutations causing cardiomyopathies

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Introduction: Genetic testing for mutations causing cardiomyopathy has recently entered international guidelines and is now widely accepted as a mean to nail down a suspected diagnosis and to timely identify pre-symptomatic carriers at risk. Due to the high degree of genetic heterogeneity, reliable and highly parallel sequencing techniques are required to allow cost-effective, high-yield mutation detection.
Objective: To determine the performance characteristics of next generation sequencing (NGS) using the Illumina TruSight Cardiomyopathy panel.
Subjects Materials and Methods: In experiment 1, DNA was extracted from peripheral blood of twelve samples previously shown to carry ten pathogenic mutations as well as 63 single nucleotide variants (SNVs) in the genes MYBPC3, MYH7, TNNI3, TNNT2 and TPM1. Exon sequences and adjacent splice sites of 46 genes previously linked to cardiomyopathy were enriched using the Illumina TruSight Cardiomyopathy panel. In experiment 2, two of the mutation-positive reference samples were enriched (same DNA sample) and analyzed again, together with six consecutive clinical samples (thus the run comprised eight samples instead of twelve in exp. 1). A proprietary NGS data processing pipeline was used.
Results: The average read depth was 210 (exp. 1) and 407 (exp. 2). A mean of 0.36 % (0.13 % in exp. 2) of the target region showed low coverage (<20X), corresponding to an average of 893 base-pairs (332) in 13 (5) genes. Of the 73 reference mutations/SNVs tested in Exp. 1, all were recovered, corresponding to an analytical sensitivity of 100% (95% CI: ≥95%). The analytical specificity was estimated by the evaluation of 73 exons known to be homozygous wild-type and was found to be 100% (95% CI: ≥95%). Of the 96 mutation/SNVs detected by NGS in two samples of exp. 1, all were recovered in exp. 2 and no additional mutation/SNV was found, indicating a high degree of intermediate precision. Across the six novel samples, an average number of 1.7 putative mutations were detected which required follow-up.
Conclusions: The determined performance characteristics indicate that NGS using the Illumina Cardiomyopathy panel is sufficiently accurate to be used as a clinical genetic test. More reference mutation/SNVs may be tested to increase the statistical power of the validation assay.

P-Counse-207

Interrogating the Clinically Relevant Genome with Targeted Exome Sequencing and Computational Phenotype Analysis

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Traditional methods of diagnosis for individuals affected by rare genetic disease fail to identify a definitive molecular cause for many individuals, often resulting in a so called diagnostic odyssey with extensive and often expensive and invasive clinical investigations. The advent of next-generation sequencing approaches and especially whole-genome and whole-exome sequencing (WGS/WES) have led to an unprecedented acceleration in the pace of disease gene discovery in research settings, and targeted NGS gene-panel investigations are now becoming routine in many countries. Nevertheless, the clinical utility of WGS/WES in routine diagnostics remains to be proven. A major difficulty involves the fact that WGS/WES identifies tens of thousands (WES) or millions (WGS) of variants, and bioinformatic filtering on variant rarity and predicted pathogenicity alone does not perform well. Furthermore, it may not be useful to identify a convincing variant in a candidate gene with no validated significance for human disease, meaning that WGS/WES interrogates more genes and other sequences than can currently be interpreted in a clinical setting. What's more, at typical coverage levels, WES typically does not provide enough read data for up to 20% of the enriched exons. For these reasons, we have developed a combined genomic and bioinformatic approach towards enriching those parts of the genome that can be clinically interpreted today at high levels, and prioritizing the identified variants using semantic similarity analysis based on data from the Human Phenotype Ontology project. We have performed a pilot project with 96 samples from individuals with known and unknown genetic diagnoses, using a custom SureSelect enrichment panel comprising 2875 genes (7.1 Mbp) currently known to have an associated Mendelian disease. Since about 98% of the targeted bases had a coverage of 20-fold or better, this resulted in sufficient coverage to identify all of the known disease mutations amongst the patients with known diagnoses. We will present our bioinformatic strategy of first filtering all identified variants for rarity and likely pathogenicity and then prioritizing the remaining genes based on similarity of the associated diseases to the phenotypic features observed in the patient being examined. Using this approach, approx. 15% of hard-to-diagnose cases could be solved. We will present the final study results as well as an online server that can be used to perform the bioinformatic analysis.

P-Monogenic Disease - From Gene Identification to Pathomechanism

P-MonoG-208

TALEN-mediated generation of a cell culture model for ichthyosis with confetti

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Ichthyosis with confetti (IWC) is a very rare genodermatosis. Patients affected by IWC are born with a generalized scaly erythroderma. During childhood they develop patches of normal skin in a confetti-like pattern. This type of ichthyosis is caused by heterozygous frameshift mutations in the keratin 10 (KRT10) gene resulting in an arginine-rich C-terminus of the aberrant K10 protein. The normal areas develop as a consequence of a gene conversion or mitotic recombination, which leads to an LOH at chromosome 17q where the mutated KRT10 allele is replaced by a copy of the wildtype allele. The exact mechanism underlying this progressive revertant mosaicism is currently unknown. Due to the rareness of IWC it is difficult to receive enough skin biopsies for studying the mechanism of this "natural gene therapy".
Transcription activator-like effectors (TALEs) are bacterial transcription factors which contain specific DNA-binding domains. These domains are composed of modules, each of which binds to one specific nucleotide. Recently, it was shown that TALE proteins also function when fused to a FokI nuclease domain, making these TALE nucleases (TALENs) a perfect tool for genome editing.
We engineered KRT10-specific TALENs and targeted the K10 locus in an immortalised keratinocyte line and introduced a known IWC-causing frameshift mutation - thus creating a model system which can be used to investigate the mechanism of LOH in IWC.

P-MonoG-209

Multi-gene-panel diagnostics detects MKS1- gene mutations in a boy with Joubert-Syndrome

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Background: Mutations in the MKS1 gene are known to be a major cause for Meckel-Gruber-Syndrome, a genetically heterogeneous condition and the most common form of syndromic neural tube defect characterized by occipital encephalocele, liver ductal plate malformation, polydactyly, and kidney cysts.
The MKS1 gene also accounts for a minor fraction of the total mutational load in Bardet-Biedl-Syndrome, a genetically heterogeneous syndrome characterized by rod-cone dystrophy, truncal obesity, postaxial polydactyly, cognitive impairment, male hypogonadotrophic hypogonadism, complex female genitourinary malformations, and renal abnormalities. To our knowledge MKS1-mutations have not yet been reported to cause classical Joubert-Syndrome phenotypes.
Clinical report: We report the phenotype of a five year old boy from Austria with episodes of apnoe during the first 7 months of life, severe hypotonia, psychomotor retardation, congenital nystagmus and a molar tooth sign detected in the MRI of the brain, which suggested the diagnosis of Joubert syndrome. The boy has additional symptoms that previously were described in children with Joubert syndrome, like facial dysmorphic features, agenesis of corpus callosum and slight changes in the retinal epithelium of the left eye. Renal abnormalities were not present and measurements of the head circumferences were in the normal range, as were length and weight. Ankyloglossia and symmetric camptodactyly of digits III + V was also detected.
Lab investigations: Lymphocyte culture (GTG banding) resulted in a normal male karyotype. Next-Generation sequencing based multi-gene-panel diagnostics from a blood sample of the boy revealed three mutations. Two mutations were located in the MKS1 gene on 17q23.2: The first mutation, c.1407-7_1408_35del29, has already been reported and is known to be a major cause for Meckel-Gruber-Syndrome in homozygous state. The second mutation in the MKS1 gene, a missense-mutation has not yet been reported in the medical literature and is not present in public databases. Five bioinformatic tools predict an alteration of protein function. We therefore assume that these two mutations in compound heterozygous state are causative for the phenotype in the boy. Multi-gene-panel testing additionally revealed a heterozygous mutation in exon 2 of the TCTN3 gene on 10q24.1, which is not reported in the literature yet and which is bioinformatically predicted to likely alter the protein function.
Discussion: Mutations in the MKS1 gene are primarily reported in patients with Meckel-Gruber-Syndrome, a phenotype which denotes the most severe (usually lethal) end of the spectrum of ciliopathies with occipital encephalocele and other early embryonic malformations. Here we describe a patient with classical Joubert syndrome which underlines the genetic heterogeneity in Joubert syndrome and illustrates the pleiotropy of MKS1 mutations.

P-MonoG-210

Deep next- generation sequencing as a tool in the diagnostics of McCune-Albright Syndrome

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McCune-Albright Syndrome is a sporadic disorder characterized by the classic triad of polyostotic fibrous dysplasia, café-au-lait skin pigmentation, and peripheral precocious puberty. Usually it is caused by postzygotic activating mutations in the guanine-nucleotide-binding protein alpha-subunit (Gsalpha), leading to a mosaic distribution of cells with constitutively active adenylate cyclase. We report of a 6 year old boy with monoostic fibrous dysplasia (FD), but only minor clinical signs of McCune-Albright syndrome (MAS). No mutation could be identified by Sanger sequencing of GNAS Exon 8 on DNA extracted from either whole blood or bone tissue. We subsequently reanalyzed this patient with deep next-generation sequencing (NGS). With a coverage of >100 000 we identified the causative missense mutation GNAS c.2531G>A (p.Arg201His) in 0.85% of sequencing reads (1536/180745 reads) in DNA isolated from peripheral blood. This frequency is far below the detection limit of most standard sequencing methods like e.g. Sanger sequencing. With NGS the mutation was detected in 20% of the reads (24953/123199 reads in DNA extracted from bone tissue) after whole genome amplification. Analysis of DNA isolated from sorted T- and B-lymphocytes, monocytes and granulocytes revealed variable degrees of mosaicism. Our report highlights the power of deep next-generation sequencing to identify low-frequency mutations in mixed cell populations that are missed by less sensitive approaches. In this case deep sequencing allowed the detection of the disease causing mutation in a primarily not affected tissue (blood and saliva). Thus deep sequencing might reduce the need of invasive tissue biopsies for molecular testing in selected disorders.

P-MonoG-211

Clinical and molecular characterisation of PYCR1-related Cutis laxa

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Autosomal recessive cutis laxa type 2B (OMIM #612940) is a segmental progeroid disorder characterized by intrauterine growth retardation, lax and wrinkled skin, a typical triangular face, and intellectual disability. This variable phenotype is often diagnosed as gerodermia osteodysplastica wrinkly skin, or De Barsy syndrome. Mutations in the PYCR1 gene encoding pyrroline-5-carboxylate reductase 1 were identified to be causative. This protein is part of a conserved metabolic pathway (proline-cycle) described to generate cytoplasmic NAD(P)+ via synthesis of proline.
In this study, we analysed 33 patients from 27 families. In comparison with all patients identified so far, we could further delinate the genetic and clinical spectrum and found PYCR1 to be the second most frequent disease-causing gene in individuals with autosomal recessive cutis laxa. A detailed analysis of the subcellular distribution revealed an exclusive mitochondrial localization of PYCR1. After in vitro RNAi-induced depletion of PYCR1 we found a severe fragmentation of the mitochondrial network, a decreased membrane potential and an increased apoptosis rate.
Thus, we conclude a role of PYCR1 in the regulation of the mitochondrial redox state, which influences mitochondrial dynamics and possibly metabolic activity. This combination of defects is likely to be a key event in the pathogenesis of ARCL2B.

P-MonoG-212

Screening for CDKN1C point mutations in growth retarded patients

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The significance of loss-of-function mutations in the imprinted CDKN1C gene (11p15) for the aetiology of Beckwith-Wiedemann syndrome (BWS; OMIM # 130650) is widely accepted. With the recent identification of gain-of-function mutations in growth retarded patients with IMAGe syndrome (OMIM # 614732) the central role of this factor in human growth could be impressively confirmed. Furthermore, a broad spectrum of opposite 11p15 mutations and epimutations has been identified both in BWS and growth retarded patients with Silver-Russell syndrome (SRS; OMIM # 180860) features. In SRS, single carriers of duplications affecting the maternal CDKN1C copy have been reported, and in these patients an increased expression of the maternally expressed growth-inhibiting factor CDKN1C has been detected. Considering the functional similarity of the CDKN1C alterations and the clinical overlaps between IMAGe syndrome and SRS (e.g. intrauterine growth retardation, prominent forehead, ear anomalies), we screened a cohort of SRS patients without one of the known molecular SRS defects (n=20) for point mutations in CDKN1C. As we did not detect any pathogenic mutation we conclude that mutations in CDKN1C are associated with the IMAGe syndrome features but that they can be neglected in SRS. We therefore suggest that testing for CDKN1C point mutations should be included in routine diagnostics of IMAGe syndrome and BWS but not of SRS. However, we cannot exclude that CDKN1C mutations account for single patients with SRS features.

P-MonoG-213

Classification of Fanconi-anemia cells using FANCD2 immunoblots

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Fanconi anemia (FA) is a rare autosomal or X-chromosomal recessive disease. It is characterized by symptoms including endocrine pathologies, hematological disorders, congenital malformations and tumors, with varying manifestation and severity. The cause of FA involves a DNA repair defect leading to genomic instability. The disease can be diagnosed by the hypersensitivity of FA cells against DNA-crosslinking agents. To date there are 16 FA genes have been reported: FANCA, -B, -C, -D1, -D2, -E, -F, -G, -I, -J, -L, -M, -N, -O, -P and -Q. The protein products of these genes are involved in the FA-BRCA pathway, which is regarded as exceedingly important for the repair of DNA interstrand lesions through Homologous Recombination. Monoubiquitination of FANCD2 and FANCI by the FA core complex plays a key role for FA-BRCA pathway activation in S phase. Together with its partner protein, FANCI, monoubiquitinated FANCD2 is then targeted to chromatin and facilitates DNA crosslink repair. Because of the critical monoubiquitination step we can classify cells into "upstream", "D2"or "downstream" defects, via D2 immunoblotting. On these blots the monoubiquitinated FANCD2 form is visible at 162 kDa (D2-L) in addition to the non-monoubiquitinated form at 155 kDa (D2-S). If there is almost no D2 protein detectable the cells can be assigned to the subtypes FA-D2. Is the monoubiquitinated FANCD2 protein present the disease causing mutation is located in one of the downstream FA genes. If only D2-S band is present on the immunoblot the patient can be categorized as "upstream" or FA-I. With this technique we have classified several patients.
For a more specific assignment further investigations are necessary, like MLPA, Whole Exome sequencing or target enrichment.

P-MonoG-214

Genetic studies on congenital forms of cataract in consanguineous families from Pakistan

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Cataract is defined as an opacity of the lens that impairs vision and congenital cataracts are a leading cause of childhood blindness worldwide. Hereditary cataracts show considerable heterogeneity, both clinically and genetically, and non-syndromic forms can be inherited as autosomal-recessive (AR), autosomal-dominant, or X-linked traits. To date, more than 40 genetic loci have been identified in humans, including 20 loci for autosomal-recessive non-syndromic cataracts, with the underlying gene identified at 14 of these loci. The purpose of this study was to investigate the genetic basis of congenital cataracts in consanguineous Pakistani families. We enrolled 23 consanguineous families with non-syndromic AR cataract with three or more affected individuals per family. Linkage analysis using flanking or intragenic microsatellite markers at known AR cataract loci was performed in all families. This resulted in linkage to a locus containing HSF4 in one family and the identification of a novel HSF4 nonsense mutation (c.1213C>T / p.R405*). In addition, we identified the same homozygous 93 kb deletion at the GCNT2 locus segregating with cataract in 6 independent families by combinations of targeted linkage analysis, long-range PCR, and breakpoint sequencing. The deletion encompasses exons 1B, 1C, 2 and 3 of GCNT2. We are currently investigating whether the deletion is

the product of a repeated Alu–Alu repeat-mediated non homologous recombination or a founder effect. In a family excluded for all known loci of AR cataracts, we performed genome-wide SNP-based genotyping and linkage analysis and identified a 22 Mb region on chromosome 22 with a maximum LOD score of 2.6. Exome sequencing in two affected individuals revealed 9 rare homozygous variants in the linkage region. Sanger sequencing of all variants in the family identified candidate mutations in three genes that co-segregate with cataracts in this family. In conclusion, a causative mutation was identified in 7/23 Pakistani families (30%), with a high prevalence of an intragenic GCNT2 deletion in this population. Absence of linkage to known AR cataract loci and the identification of a new chromosome 22 locus suggest even more extended genetic heterogeneity in AR cataract in Pakistan.

P-MonoG-215

Adrenal hyperplasia associated with biallelic Nf1 inactivation in adrenal cortex of Nf1Prx1 mice and in the NF1 patient.

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Background NF1 (MIM#162200) is a relatively frequent genetic condition, which predisposes to tumor formation. Main types of tumors occurring in NF1 patients are the benign cutanous neurofibromas, plexiform neurofibromas, optic pathway tumors, and aggressive malignant peripheral nerve sheath tumors. Less frequent are hematopoetic malignancies and the tumors of adrenal medulla, the pheochromocytomas. Apart of tumors individuals with NF1 often exhibit endocrine abnormalities, which are thought to underline such complications as precocious puberty (6% of NF1 patients), short stature (25% of NF1 patients) and possibly hypertension (4% of NF1 patients). Cases of precocious puberty in NF1 are frequently linked to optic gliomas and primary or secondary hypothalamic involvement. However multiple cases of precocious puberty in NF1 were described which occurred without hypothalamic involvement. Several cases of adrenal cortex adenomas have been described in neurofibromatosis type I patients suggesting NF1 might play a role in adrenal cortex development and homeostasis.
Methods We observed increased size of adrenal glands in the Nf1Prx1 mice, in which Nf1 is inactivated in the developing limbs and head mesenchyme as well as in adrenal gland cortex, but not adrenal medulla. We conducted histological and molecular analysis to determine if adrenal hyperplasia correlates with the changes in adrenal histology and gene expression as well as MAPK and cAMP signaling status. We also characterized genetically a single case of macronodular adrenal hyperplasia in neurofibromatosis type I patient who showed cortisol overproduction without indication of adrenal malignancy.
Results Mouse model data suggest that Nf1 is involved in the development and homeostasis of adrenal cortex. We also present a single NF1 patient with macronodular adrenal hyperplasia of the right adrenal gland and cortisol overproduction. The patient showed NF1 germ line mutation NM_000267:c.405 delG and the sequencing of the DNA isolated from the adrenal hyperplasia tissue revealed loss of heterozygosity (LOH) in NF1 locus, indicating somatic mutation occurred in the hyperplastic adrenal gland ablating second copy of NF1.
Conclusions Our study indicates that biallelic inactivation of NF1 in adrenal cortex in mouse model is associated with adrenal hyperplasia and ACTH independent, female specific corticosterone / aldosterone overproduction. The finding of biallelic Nf1 inactivation in adrenal macronodular hyperplasia of NF1 patient suggests that somatic inactivation of NF1 in adrenal cortex underlines the adrenal adenoma with overproduction of cortisol.

P-MonoG-216

Finding the genetic cause of rare Mendelian disorders by whole exome sequencing: first experiences from the Institute of Human Genetics in Hamburg

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Whole exome sequencing (WES) has been successfully applied to the discovery of mutations in rare Mendelian disorders. We established WES on the Illumina HiSeq2500 sequencing platform. During the last two years, we sequenced a total of 130 exomes; here we report data on three cases.
We enrolled a three-generation family with one affected individual in each generation. The index patient showed short stature, bilateral cataracts, sensorineural deafness, hypotrichosis and multiple freckles. He had significantly delayed ossification of carpal and tarsal bones. His mother and maternal grandfather were also affected. We did WES in the index patient, his affected grandfather and healthy brother and filtered for heterozygous variants in both patients that were absent in the unaffected child. Variants from dbSNP, 1000Genomes, and Exome Sequencing Project were filtered out. Sanger sequencing of eleven variants in the family ended up with one variant, c.44A>G (p.Q15R) in STX10 co-segregating with the disease. Another missense variant, c.125G>T (p.S42I) in STX18 was found in one healthy and three affected persons in the family. Both STX10 and STX18 encode syntaxins involved in vesicular trafficking. We conclude that either autosomal dominant or digenic inheritance may underlie the phenotype in the family.
The second individual had coarse face, gingival fibromatosis, intellectual disability, microcephaly, short stature, hypertrichosis, and brachydactyly. We performed WES in the patient, filtered for heterozygous variants absent in the databases and initially focused on variants in disease genes. We found the heterozygous missense mutation c.3493C>A (p.Q1165K) in SMARCA2 (SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2) which occurred de novo. Dominant SMARCA2 mutations cause the Nicolaides-Baraitser syndrome (NBS). Our patient has originally been diagnosed with Zimmermann-Laband syndrome (ZLS). Phenotypic overlap between NBS and ZLS suggest that ZLS may belong to the disease group converging on altered SWI/SNF chromatin remodeling.
The third patient had IUGR, microcephaly, progeroid facial appearance, cataracts, microphthalmia, hypotrichosis, and paucity of subcutaneous fat suggestive of the Hallermann-Streiff syndrome. He showed heart and brain malformations and lax skin on his neck and extremities. After parent-child sequencing we filtered for compound heterozygous mutations and detected the variants c.1910T>A (p.L637Q) and c.89-2A>G in ALDH18A1 in the patient. Biallelic ALDH18A1 mutations result in cutis laxa type IIIa suggesting that our patient had a severe form of this rare neurocutaneous disorder. Our data further underscore that WES allows efficient identification of coding mutations in

rare Mendelian disorders and should become clinically relevant in genetic diagnosis.

P-MonoG-217

MORF4L1: A member of the homologous recombination DNA repair pathway and a candidate gene for Fanconi anemia

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To date, 16 genes are known to cause Fanconi anemia (FA) if mutated. Yet there remain FA patients who do not reveal mutations in the reported FANC genes. This leads to the conclusion that there are still unknown members of the FA/BRCA DNA damage response network. Certain proteins represent FA candidates because they interact with authentic FA proteins. Therefore, we are screening unassigned FA cell lines for defects of those proteins. MORF4L1/MRG15 was characterized by Sy et al. (2009) as a novel PALB2/FANCN-interacting factor involved in homologous recombination. MORF4L1 also interacts with BRCA2, RAD51 and RPA1 suggesting a participation of that protein in the repair of DNA double-strand lesions (Martrat et al. 2011). Hayakawa et al. (2010) demonstrated that MORF4L1-deficient cells show reduced efficacy of homology-directed DNA repair and hypersensitivity to DNA interstrand cross-linking agents similar to PALB2- or BRCA2-deficient cells. They also found that MORF4L1 knockdown diminished the recruitment of PALB2, BRCA2 and RAD51 to DNA damage sites.
Based on the capability of FANCD2 monoubiqutination, cell lines derived from FA patients without mutations in the reported FA genes were classified as upstream or downstream. We examined 13 downstream FA lines unassigned to any complementation group by immunoblotting for MORF4L1 expression. Since PALB2, its interactor, is required for RAD51 foci formation, we included six RAD51 foci-negative FA lines. The levels of MORF4L1 protein in the FA lines were compared with normal controls. We did so by forming ratios of pixel counts, on the same blots, between MORF4L1 bands and those of the nuclear antigen P84 used as loading control. In the six FA lines deficient in RAD51 foci formation, we additionally screened MORF4L1 by Sanger sequencing of all exons and adjacent intron portions.
Protein extracts from 13 unclassified, FANCD2 monoubiquitination-proficient FA cell lines showed MORF4L1 expression levels similar to controls. This held in particular true for six FA lines deficient of RAD51 foci formation (FA ratio range, 0.9 – 1.6; control ratios, 0.4 – 2.0). Sequencing of gDNA from those six lines was performed to exclude any missense mutations that would not decrease protein expression levels. It revealed common SNPs, registered in the dbSNP database, and a few single-base substitutions and deletions deeper in introns in four of the six lines.
Our data suggest that MORF4L1 can be excluded as a candidate FA gene, at least in the studied group of patients.

P-MonoG-218

Characterization of large deletions in the DHCR7 gene

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Mutations in the DHCR7 gene cause the Smith-Lemli-Opitz Syndrome (SLOS, MIM 270400) which is an autosomal recessive congenital metabolic malformation disorder. Up to date more than 130 mutations in the DHCR7 gene have been described. All patients described in the literature have been submitted to the DHCR7 database (http://databases.lovd.nl/shared/genes/DHCR7). In approximately 4% of the patients the second mutations remains unidentified, in some cases due to regional deletions not detectable by DNA sequencing. Small deletions in the DHCR7 gene associated with a mild phenotype have been described earlier. Weaver et al. (2010) described the deletion of the 3rd and 4th exon in a fetus with a severe form of HPE (holoprosencephaly).
Here we present two unrelated fetuses with large deletions in the DHCR7 gene.
Case 1
At birth the girl was small for gestational age, had syndactyly of toes 2 and 3, an atrial septal defect, horseshoe kidney, and typical facial features. During the first year psychomotor retardation, muscular hypotonia, and feeding difficulties evolved. Plasma sterol analysis showed typical results for SLOS. Molecular analysis of the DHCR7 gene showed a heterozygous deletion of exon 3 to 6 inherited from her father. This large deletion is equal to a null mutation. The translation start is in exon 3, a second ATG in exon 4 cannot be used. On the second allele a c.1054C>T (p.Arg352Trp) substitution was detected. This common mutation was also detected in the patient's mother.
Case 2
The patient presented with high 7DHC levels and typical features of SLOS: cleft palate, secundum atrial septal defect, short phallus with hypospadias and undescended testes, bilateral 2/3 toe syndactyly, FTT, and typical facial features. Molecular analysis of genomic DNA showed a deletion of 1784 bp (c.-1065_-7+171del) encompassing the transcription start in exon 1 and exon 2. The DHCR7 promotor sequence lacks 788 bp upstream of exon 1. Therefore this allele will not be transcribed. This mutation has also been detected in the patient's mother. The paternal mutation is the common mutation c.278C>T (p.Thr93Met).
Diagnosis of SLOS was confirmed by molecular analysis. We were able to characterize the breakpoints on molecular level in both cases. Such large deletions in the DHCR7 gene have not been described before.

P-MonoG-219

SMCHD1 mutations cause FSHD type 2 and act as modifiers of disease severity

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Facioscapulohumeral muscular dystrophy (FSHD) is considered the most frequent autosomal dominant muscle disorder. Symptoms start in the second decade of life with muscular weakness in the face, shoulder girdle and upper arm muscles. Progression is mostly slow but disease severity varies widely ranging from asymptomatic carriers to wheelchair dependency.
The more common form FSHD1 is associated with a contraction of the D4Z4 repeat array on a FSHD-permissive chromosome 4 genetic background. The copy number variation ranges between 11-150 copies in the normal population and 1-10 in FSHD patients. Recently, a digenic inheritance has been reported to cause the rarer form FSHD2. These patients do not show the D4Z4 repeat contraction but also carry the FSHD-permissive chromosome 4 allele and a heterozygous loss-of-function mutation in the SMCHD1 gene on chromosome 18. As a common pathogenic mechanism, both FSHD types share a state of hypomethylation of the D4Z4 repeat array. In FSHD1, chromatin decondensation and hypomethylation are effects of the repeat contraction. In FSHD2, haploinsufficiency of the SMCHD1 protein results in hypomethylation of D4Z4. An open chromatin structure of the D4Z4 locus results in activation of the DUX4 gene whose expression is highly cytotoxic and causes skeletal muscle cell death.
In this study, we screened patients from 85 unrelated families (41 with a contraction in D4Z4, 44 without contraction, all showing the typical FSHD phenotype and the FSHD permissive haplotype 4qA161) for mutations in SMCHD1 by next generation sequencing (NGS). Further-

more, we developed a pyro-sequencing assay to determine the methylation status of the D4Z4 repeat.
We identified seven novel and one reported mutation in SMCHD1, ranging from missense mutations (p.Gly478Glu, p.Arg1449Lys, p.Val615Asp, p.Gln1463Pro, p.Pro1485Leu) and deletions of the 3' splice site (p.Val1093Leufs*26, p.Lys1092del) to the first nonsense mutation (p.Leu1663*). All mutations were confirmed by Sanger sequencing and predicted as pathogenic by the bioinformatics tools of Alamut (interactive biosoftware). The methylation status of the D4Z4 repeat array for FSHD2 patients (5-19 %) was found significantly lower than for healthy controls (30-65 %) and also lower than for FSHD1 patients (14-55 %). In addition to the mutation in SMCHD1, two of the patients showed a contracted D4Z4 allele and therefore have a double trouble situation for FSHD1 and 2.
Comparing the phenotype of the patients, all FSHD2 patients were mildly affected while patients with FSHD1 plus 2 were much more severely affected than expected from the number of repeat units (10 units) on the contracted allele.
Our findings confirm mutations in SMCHD1 as being causative of FSHD2 and a modifier of disease severity in FSHD1. With SMCHD1 mutations found in 9,4 % of the patients analyzed, FSHD2 cannot be considered a very rare disease. We therefore suggest including sequencing of SMCHD1 and methylation analysis in the diagnostic workup of FSHD.

P-MonoG-220

The role of the NR2A and NR2B subunits of the NMDA receptor in epileptogenesis

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NMDA receptors are tetrameric ligand-gated ion channels permeable to Na+, K+ and Ca2+ composed of two glycine binding NR1 subunits and two glutamate binding NR2 subunits (NR2A, NR2B, NR2C, NR2D) regulating synaptic plasticity.
Mutations in the NR2A and NR2B subunits encoded by the genes GRIN2A and GRIN2B have been associated with different phenotypes of intellectual disability. Mutations in GRIN2A were known to cause unspecific intellectual disability and epilepsy as well as other neurodevelopmental disorders, whereas mutations in GRIN2B have so far mainly been associated with autism spectrum disorders (ASD) but never with seizures.
We show for the first time that mutations of NR2 subunits of the NMDA receptor cause different and specific epilepsy phenotypes. NR2 mutations are involved in benign Rolandic epilepsy, the most frequent childhood epilepsy as well as in a variety of rare infantile epileptic encephalopathies, such as Landau-Kleffner syndrome and West syndrome.
Furthermore, we demonstrate distinct genotype-phenotype correlations. Severe encephalopathic phenotypes are significantly more often caused by truncating mutations in GRIN2A whereas missense mutations are by far more common in benign Rolandic epilepsy patients. For GRIN2B, the majority of ASD individuals presents with truncating mutations, whereas all epilepsy cases appear to have gain-of-function mutations. The severity of phenotypes correlates with the affected domain and the extent of activation of the receptor.
Our observations underline the so far underestimated role of dysregulated NMDA signalling in both frequent and rare epilepsy disorders and reveal promising pharmacologic targets for future therapeutic approaches.

P-MonoG-221

Genetic heterogeneity of congenital ichthyosis and the use of iPS cells to study rare genetic skin diseases

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Autosomal recessive congenital ichthyosis (ARCI) is a rare mendelian disorder of cornification and is physiologically characterised by skin barrier dysfunction, the main clinical symptoms being generalized scaling of the skin and variable erythema. Because it includes extensive clinical and genetic heterogeneity, a clear genotype/phenotype correlation has not yet been found in ARCI and 20% of patients have no mutations in any of the known genes associated with the disease.
Human keratinocytes have a short life-span when cultured, as they either terminally differentiate or enter senescence. Induced pluripotent stem cells (iPSC) technology is a promising way to overcome this problem, as keratinocytes can be differentiated in vitro from reprogrammed human fibroblasts, which are more often available and better suitable for expansion, producing an endless source for keratinocytes.
Hence, the aim of this project is to create an iPSC-based in vitro skin model for characterisation of the molecular mechanisms of ARCI and elucidate pathways for therapies. In parallel, genetic mapping including exome sequencing is being conducted in order to broaden the mutation spectrum of the disease as well as to identify unknown genes involved in ARCI.
We have generated an iPSC line from ARCI patient dermal fibroblasts, which is currently under characterisation. This cell line presents typical embryonic stem cell-like morphology and colonies were positive for Alkaline Phosphatase (AP) activity, which confirms their undifferentiated state. Immunocytochemistry was performed and positive signals were found for the pluripotency markers SSEA-4, TRA-1-60, TRA-1-81, OCT4, and NANOG. A normal karyotype was revealed after G-banding karyotype analysis. We are currently assessing mRNA levels of pluripotency marker genes by qRT-PCR as well as STR genotypes to test genomic stability.
The generation of further iPSC lines derived from ARCI patient fibroblasts is now in progress, using a non-integrating/excisable system consisting of the polycystronic "stem cell cassette" encoding the stemness factors Oct4, Klf4, Sox2, and cMyc (STEMCCA).This system provides a consistent reprogramming strategy that also addresses the safety concerns of transgene integration in iPS cells. Our approach promises straight forward cell models for the functional study of genetic variants

identified in a very heterogeneous group of diseases and of pharmacological interventions possibly leading to patient-specific therapies.

P-MonoG-222

Functional characterization of NAA10 gene missense mutations in N-terminal acetyltransferase deficiency with severe global developmental delay

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Recently we reported the identification of 2 de novo missense variants in the NAA10 gene by trio exome sequencing in 2 unrelated patients, a girl and a boy, with unexplained severe global developmental delay and without any major dysmorphisms. The girl is heterozygous for p.Val107Phe in exon 5 and the boy hemizygous for p.Arg116Trp in exon 6. A single hypomorphic missense mutation p.Ser37Pro has been previously associated with Ogden syndrome where it was reported in eight affected males from two different families. The phenotype in these boys was characterized by an aged appearance, craniofacial anomalies, hypotonia, global developmental delay, cryptorchidism, cardiac arrhythmias and death in infancy.

In an attempt to explain the discrepant phenotype we used in vitro N-terminal acetylation assays to show that the severity of the phenotype correlates with the remaining catalytic activity. Our functional analyses provide evidence of the severe heterozygous variant in the girl and the milder hemizygous variant in the boy being causative for their phenotypes. The mutation in the Ogden syndrome patients exhibited a lower activity than the one seen in the boy with intellectual disability, while the mutation in the girl was the most severe exhibiting only residual activity in the acetylation assays used. We propose that N-terminal acetyltransferase deficiency is clinically heterogeneous with the overall catalytic activity determining the phenotypic severity.

Our study adds to the growing evidence that candidate genes identified through next generation sequencing technologies require further replication studies and functional analysis to establish pathogenicity.

P-MonoG-223

Zinc- from a natural Cofactor to a Pharmacoperone in Doss Porphyria

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The vast majority of acute porphyria attacks are due to acute intermittent porphyria (AIP) which is inherited in an autosomal dominant manner with incomplete penetrance. In contrast ALAD/Doss porphyria constitutes a rare, autosomal recessive, highly penetrant type of acute porphyria with only 12 cases reported in the literature. Mutations in the ALAD gene cause profound deficiency of aminolevulinic dehydratase responsible for the second step in heme biosynthesis. If left untreated or in therapy refractory cases acute porphyria may become a life-threatening disorder resulting in tetraplegy and ventilator dependence.

WT-ALAD forms predominantly a high-activity homo-octameric structure, while human ALAD mutations commonly shift the equilibrium towards low-activity hexameric or dimeric quarternary structures.

At our hospital we follow a patient suffering from Doss porphyria with an unusual congenital onset of disease initially misdiagnosed as spinal muscular atrophy (SMA). His further medical course was complicated by endstage renal disease in adolescence which resulted in severe polyneuropathy due to the inability to excrete neurotoxic aminolaevulinic acid (ALA). Subsequent sequence analysis identified two missense ALAD variants (c.724G>A (p.V242I), c.838G>A (p.G280R)) in compound heterozygous state in our patient. ALAD activity and stability assays performed in RBCs of the family revealed a drastic reduction in the patient and surprisingly low values in the mother (G280R carrier) while the father (V242I carrier) exhibited values close to the normal range. Assessment of the paternal V242I variant by prediction programs was benign and the variant could be found at the EVS at a frequency of 2/6503.

Under the conception that Doss porphyria is mainly a conformational disease and against the background of his deleterious medical condition the patient received zinc substitution, the natural cofactor of ALAD, prior to living related kidney transplantation. 4 years after initiation of zinc treatment and 3.5 years after kidney transplantation the patient's general condition has improved dramatically.

Further analysis showed that zinc significantly increased ALAD activity in RBCs of the patient. As the main load of porphyrine synthesis occurs in the liver we expressed both variants in mouse liver. Low hepatic G280R-ALAD activity confirmed the pathogenic nature of this mutation. V242I-ALAD showed activity near wildtype, but decreased stability at incubation with 48°C (± ALA substrate). Interestingly native WB analysis of G280R- and V242I-ALAD in HEK293 cells could not detect the expected shift of morpheein equilibrium from high-activity octameric towards low-activity hexameric/dimeric ALAD as seen with most reported causative mutations. Apart from demonstrating the chaperone potential of zinc we propose a new pathomechanism and are currently investigating the effects of G280R/V242I oligomerization when coexpressed in mouse liver.

P-MonoG-224

HIBCH deficiency in a patient with phenotypic characteristics of mitochondrial disorders

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The catabolic pathway of the amino acid valine is dependent on a specific 3-hydroxyisobutyryl-CoA hydrolase (HIBCH), a nuclear-encoded mitochondrial protein. Only two patients with HIBCH deficiency and biallelic mutations in the HIBCH gene have been reported in the literature. Both of them demonstrated delayed motor development, muscular hypotonia, and early-onset deterioration of neurological functions. We report on a third patient, first child of healthy consanguineous parents, with a homozygous one-base pair insertion resulting in a premature stop codon (c.1129_1130insT, p.K377_S378delinsX) in the HIBCH gene (NM_014362). The mutation was detected by homozygosity mapping and whole-exome sequencing on a SOLID 5500xl platform. HIBCH activity in fibroblasts was below the limit of detection. The patient displays a variety of symptoms indicative of mitochondrial dysfunction; like severe muscular hypotonia, intellectual disability, Leigh-like hyperintensities of the basal ganglia in MRI, progressive brain atrophy, repeatedly elevated blood lactate, optic nerve atrophy,

seizures, and respiratory chain complex 1 deficiency with borderline depletion of mitochondrial DNA in non-frozen muscle tissue. Metabolic screening for aminoacidopathies and organic acidurias yielded normal results.
The phenotype of the patient further elucidates the clinical spectrum of HIBCH deficiency. Physical malformations do not appear to be a consistent feature of HIBCH deficiency. Instead, a mitochondrial dysfunction, presumably caused by an accumulation of toxic valine metabolites, seems to contribute to the phenotype. We suggest that HIBCH deficiency should be considered as a differential diagnosis in patients with suspected mitochondrial disorders, particularly with regard to the potential treatability of amino acid metabolism disturbances.

P-MonoG-225

Experiences in using target enrichment and next-generation sequencing in Fanconi anemia diagnostics

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Biallelic or hemizygous mutations in any of so far 16 identified FA genes (FANCA, -B, -C, -D1, -D2, -E, -F, -G, -I, -J, -L, -M, -N, -O, -P, -Q) which are components of the FA/BRCA DNA damage response pathway, are known to be causative for FA. Given the clinical variability among FA subgroups, early diagnosis and identification of FANC mutations is essential. While FA-A patients often have a relatively mild phenotype with later onset of bone marrow failure, patients with mutations in FANCD1 tend to have an earlier onset and show increased incidence of leukemia and solid tumors.
The diagnosis of FA is based on the defect in DNA repair. FA cells show increased chromosomal instability, reduced survival rates and accumulation in the G2 phase of the cell cycle, in particular in response to DNA interstrand crosslinking agents (ICLs), such as diepoxbutane (DEB) or mitomycin C (MMC). On the molecular level, diagnosis of FA is more challenging as it is time-consuming, labor-intensive and expensive to sequence all reported FA genes and it becomes even more demanding the more FA genes are identified.
The high demand for low-cost sequencing has driven the development of massively parallel sequencing technologies. Hence, we used target enrichment and next-generation sequencing to analyze all known FA genes as well as several promising candidate genes in parallel.
In a first approach we used a solution-based NimbleGen SeqCap EZ Choice library. During hybridization with specific probes patient DNAs were multiplexed and sequenced on a Roche GS Junior. Data analysis was performed using the NextGENe software. We were able to detect and confirm pathogenic mutations in the majority of our patients. Among the mutations in FANCA, -C, -D2, -L, -P and –Q, we found homo- and heterozygous single base pair substitutions, splice site mutations, an 18 bp duplication as well as several deletions up to 138 bp. We present our method and recent data.
Our results suggest that target enrichment combined with next-generation sequencing is a valuable tool for complementation group assignment and mutation analysis in FA diagnostics.

P-MonoG-226

Genetic heterogeneity of severe forms of congenital muscular dystrophy due to defective O-glycosylation of alpha-dystroglycan

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Dystroglycan (DG), encoded by the DAG1 gene, plays a central role as anchor for diverse extracellular proteins to the cytoskeleton. Posttranslationally it is cleaved into a transmembrane beta-subunit (beta-DG) and a highly glycosylated extracellular alpha-subunit (alpha-DG). Hypoglycosylation of alpha-DG has been shown to result in a wide spectrum of distinct congenital or limb girdle muscular dystrophies including the most severe clinical manifestations as Walker-Warburg syndrome (WWS) and Muscle-Eye-Brain disease (MEB). In addition to congenital muscular dystrophy with highly elevated CK values, patients with WWS or MEB present with a complex brain malformation including cobblestone lissencephaly, hypoplastic or absent corpus callosum and hypoplasia of pons, brain stem and cerebellum as well as various eye malformations. In WWS families, severe hydrocephalus today is commonly recognized prenatally as the first clinical manifestation during the second trimester. About 50-60% of those most severe clinical manifestations are currently explained by homozygous or compound heterozygous mutations in any of 12 WWS genes known to date. Only one primary dystroglycanopathy, resulting from a homozygous DAG1 missense variation p.Thr192Met within the extracellular alpha-subunit, has been described so far in a patient with a milder form of limb-girdle muscular dystrophy and hypoglycosylation of alpha-DG (Hara et al., 2011).
Over the last 10 years we have analyzed 39 WWS/MEB families with at least 2 affected siblings and/or consanguineous parents by linkage analysis and subsequent sequence analysis of candidate regions. In only 13 families obtained haplotypes were compatible with linkage to any of the tested loci (on average 5,8 loci/family), resulting in identification of the underlying mutation(s) in 6 families. Our linkage data support extensive genetic heterogeneity, more recently confirmed by the description of further genes associated with WWS/MEB.
Here we describe first results, obtained by a wider genomic approach including targeted NGS or exome sequencing for selected WWS/MEB families without linkage or mutations in any of the currently known genes including identification of the first mutation within beta-DG. The observed homozygous DAG1 missense mutation p.Cys669Phe affects a highly conserved cysteine residue, postulated to form an essential intrachain covalent disulfide bound within beta-DG. The two affected Libyan girls of non-consanguineous parents presented with a severe Muscle-Eye-Brain disease-like phenotype with the exceptional additional findings of macrocephaly and extended bilateral multicystic white matter disease, overlapping with the cerebral findings in megalencephalic leukencephalopathy with subcortical cysts (MLC). This novel clinical phenotype further expands the clinical spectrum of dystroglycanopathies and suggests a role of DAG1 not only for dystroglycanopathies but also for some forms of multicystic leukodystrophy.

P-MonoG-227

Novel mutations in the ENPP1 gene associated with Cole-disease

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Cole-disease is a rare autosomal dominant genodermatosis defined clinically by congenital or early-onset punctate keratoderma associ-

ated with irregularly shaped hypopigmented macules, which are typically found on the arms and legs, but neither on the trunk nor on acral regions. Histopathological examinations of hypopigmented macules reveal a reduction in melanin content in keratinocytes and a normal number of melanocytes whereas hyperkeratosis was present in the palmoplantar lesions. Ultrastructural studies support these findings showing a remarkable discrepancy between the amount of melanosomes in keratinocytes and melanocytes suggestive of a derogated melanosome transfer. Mutations in the gene for ectonucleotide pyrophoshatase/phosphodiesterase 1 (ENPP1), which is responsible for the generation of inorganic pyrophosphate, a natural inhibitor of mineralization, has recently been reported to cause Cole-disease (Eytan et al., 2013). Although an extracutaneous involvement for Cole-disease has not been described, mutations of the causative ENPP1 gene are associated with ectopic calcification in muscle and soft tissue as well as in case of an impaired somatomedin-B (SMB) domain of ENPP1 with an altered insulin-receptor interaction. Interestingly, insulin signaling plays a critical role in epidermal homeostasis and keratinocyte differentiation. To date, three different mutations in two unrelated French families and one Family from the United States have been published in the autosomal dominant ENPP1 gene.

Here, we identified two novel heterozygous ENPP1 mutations in two families with typical features of Cole disease. Family A, a large, three-generation family is of Spanish origin. Family B, which included one affected child, originates from Germany. All affected individuals displayed hypopigmented macules located over the extremities and hyperkeratotic papules over the palms and soles. In agreement with the previously published study, the two mutations identified affect highly conserved cysteine residues (p.Cys133Arg and p.Cys177Ser) within the somatomedin-B (SMB) domain. We confirmed co-segregation of the mutations with the disease phenotype in each of the two families.

Our study represents the second mutation report to date on patients with Cole-disease and supports that alterations in ENPP1 are causative for Cole-disease. The finding supports the role for the SMB domain in the pathogenesis and the genetic evidence of ENPP1 in the regulation of epidermal differentiation and pigmentation.

P-MonoG-228

Clinical study: Search for early neurobiological and neurobehavioral markers in Huntington´s disease

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Chorea Huntington is an autosomal-dominant inherited neurodegenerative disease leading to choreatic impaired movement, dystonia, loss of coordination, cognitive deterioration and disturbed behaviour. Age of onset (approx. 35-50 years) is inversely correlated to the length of the CAG-extension in the Huntingtin-gene. The timespan between diagnosis and death is 20 years on average. Clinical diagnosis is typically made at a stage where remarkable motoric problems (hyperkinetic movements, changes in muscular tone, bradykinesia, motoric tics, speech impediments and dysphagia) can be observed. However, the degeneration of neurons as well as substantial changes in different cerebral regions occur in a much earlier stage when no or only subtle symptoms can be noted. The putamen and the nucleus caudatus seem to be afflicted with atrophic changes first – up to 12 years before any apparent motoric symptoms. Additionally, neurons show a reduced detectability of proteins related to synaptic function and axonal transport in these early stages.

Clinical studies (Predict-HD, Track-HD, Enroll-HD) already have demonstrated that mutation carriers show subtle symptoms several years before clinical diagnosis. Features of this "pre-manifest phase" can include increased irritability and impulsiveness, difficulties in concentrating, forgetfulness as well as motoric unrest. Changes in personality, manic or psychotic episodes, depression and suicidal impulses are also often observed.

The precise pathophysiologic mechanisms leading to neurologic symptomatic in HD patients and the identification of therapeutic targets are subject of intensive research in our laboratory. To make a clinical control of therapeutic effects of potential medications possible, we are currently characterizing the pre-manifest phase more precisely: Firstly, we have developed a motion-sensor to carry on the wrist measuring involuntary/ spontaneous motoric activity. Probands are asked to wear it for 48 hours in their home environment to document subtle changes of motor activity in situations of daily life. Secondly, according to other diseases with autoimmune components, we are expecting specific antibody/antigene profiles in the early stage of the disease. These will be analysed using protein microarray techniques. Lastly, we have established an extensive battery of neuropsychological testings, including parts of the Unified Huntington´s disease rating scale (UHDRS), tests of the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) and memory tests of the Cambridge Cognition Board (CanTab®). To validate our system we will start with patients already severely affected and continue with pre-manifest probands. This study will contribute to the elucidation of early disease progression and pathogenesis in HD.

P-MonoG-229

Genotype and phenotype in six patients affected by ichthyosis with confetti and novel sequence variants at the C-terminus of KRT10

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Ichthyosis with confetti (IWC; also referred to as congenital reticular ichthyosiform erythroderma (CRIE)) was first described as ichthyosis en confetti in 1984. ICW is a very rare genodermatosis with only 16 patients genetically described to date, which includes the six patients described here. Patients are often noticed as newborns, with exfoliation of the skin and a generalized extensive erythema. Histological findings in the ichthyotic skin are an epidermal thickening and a disordered differentiation of the keratinocytes. During childhood numerous confetti-like patches of pale healthy-appearing skin begin to form, which enlarge very slowly over the years. Histology of the patches confirms, that the skin is indeed normal. The adult manifestation of IWC is a generalized scaly erythroderma interspersed with hundreds to thousands of confetti-like patches of healthy skin.

As shown by Choate et al. heterozygous defects in keratin 10 (KRT10) are responsible for the ichthyotic phenotype. Individuals affected by ichthyosis with confetti (IWC) carry small heterozygous deletions, insertions, or duplications in the C-terminus of the gene encoding KRT10 leading to a frame shift. As a consequence the altered proteins are predicted to be slightly shortened and to feature an arginine-rich C-terminus. It is assumed that this altered protein tail is responsible for the relocalisation of the protein from the cytoplasm into the nucleus and the nucleolus. In the healthy-appearing spots the pathologic mutation is found to be reverted to the wild type sequence by copy neutral LOH. By analyzing several confetti-like spots in the same individual it was shown that the expanse of the LOH differs in each spot: The LOH extends from different sites proximal to the centromere of chromosome 17q to the end of the chromosome. This might indicates separate gene conversion or recombination events during mitosis. The exact mechanism and point in time of this somatic revertant mosaicism is currently unknown.

Here we describe six patients with IWC in clinical detail along with their pathologic genetic mutations. We are hence able for the first time to make a correlation between genotype and phenotype of this rare disease and to describe minor as well as major disease criteria in a substantial number of patients. Furthermore we describe minor as well as major disease criteria for IWC. By analyzing the disease locus in fifteen control individuals without dermatologic manifestations we furthermore found an unexpected and presently undescribed genetic variance at the C-terminus of KRT10.

P-MonoG-230

Identification of two Bloom syndrome patients, novel BLM mutation and somatic reversion of them

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Bloom syndrome (BS, OMIM #210900) is an autosomal recessive disorder that is marked by pre- and postnatal growth retardation, inflammatory skin changes due to hypersensivity to UV light, telangiectatic, hypo- and hyper-pigmented skin flecks, and predisposition to malignancy. On the cellular level the syndrome can be characterized by a tremendously higher rate of sister chromatid exchanges (SCEs) compared to normal control cells. BS is caused by mutations in the BLM gene. Located on chromosome 15q26.1, which encodes a RecQ helicase, BS belongs to the group of genomic instability disorders with DNA repair defects. The mutational spectrum of BS includes many founder mutations in distinct ethnic groups.

We report on two BS patients, offspring of white British couples, with truncating BLM mutations. The first patient was diagnosed at age 10y. He had a histiocytic fibrosarcoma removed at 7 months and was since monitored for growth retardation. He revealed two previously reported compound heterozygous mutations that were also both listed as a SNP. One nonsense mutation in exon 7 (c.1642C>T, p.Gln548*, rs200389141) led to an immediate STOP and one delins mutation in exon 10 (c.2207_2212delinsTAGATTC, p.Tyr736Leufs*5, rs113993962) led to a STOP after 5 amino acids. These mutations were present in peripheral blood and a lymphoblast cell line and consist with an elevated SCE rate of 29,1 ± 10,4 in that line compared to normal lymphoblast lines (2,2 ± 1,5) (mean ± SD).

The second patient presented with ALL and treatment-related AML was diagnosed with BS at age 9y. He revealed a novel deletion in exon 7 (c.1624delG), which results in a shift of the reading frame and a premature STOP (p.Asp542Thrfs*2), and the previously described base exchange c.3415C>T in exon 18, which results in an immediate STOP (p.Arg1139*). Both mutations were present in genomic DNA and inconsistent with normal BLM function. A lymphoblastoid cell line showed the c.1624delG mutation but the c.3415C>T mutation was no longer detectable, implying total reversion in that cell type, consistent with a normal SCE rate of 4.1 ± 2.6 SCEs per metaphase similar to those in normal control lymphoblast lines. We also sequenced sequential samples obtained from bone marrow at diagnosis of the ALL after induction chemotherapy, and at diagnosis of the AML. At all stages of disease progression both mutations were detected, suggesting that the leukemia developed from cells with the BS phenotype.

P-MonoG-231

Developmental defects and premature ageing in lamin B receptor deficient mice

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The lamin B receptor (LBR) is a multifunctional inner nuclear membrane protein with structural impact on nuclear shape and chromatin organisation. Further, LBR belongs to the C14 sterol reductase family and has enzymatic activity in sterol metabolism. LBR mutations have been shown previously to cause dose-dependent hyposegmentation of granulocyte nuclei in heterozygous or homozygous Pelger-Huët-Anomaly. Heterozygous LBR mutations change blood morphology without causing any associated pathology. In contrast, homozygous mutations in LBR cause a spectrum of systemic malformations ranging from heart defects, brachydactyly and mental retardation, as occurs in Pelger-Anomaly, to severe cutaneous derangements, as seen in the recessive ichthyotic mice (ic), and finally to prenatal lethality, which is found in Greenberg dysplasia.

To elucidate the mechanisms responsible for the variety of disease manifestations in individuals with lamin B receptor deficiency, we studied prenatal and postnatal development in ic/ic mice (Spontaneous mutation Lbr icJ 1088insCC, which is a functional null mutation). We found first but modest differences in some of the homozygous embryos around mouse embryonic day E10. However, we noticed the highest mortality perinatally and around weaning. The survival varied between a few hours and several weeks or months. Generally, all homozygous mice display growth retardation and severe ic/ic skin defects. In addition to the previously described phenotypes ichthyosis, alopecia, nuclear hyposegmentation and occasional soft tissue syndactyly, we observed other manifestations as increased frequency of hydrocephalus, abnormal histology of heart and muscle cells and an abnormal fat distribution. The latter manifestations of Lbr deficiency overlap with those of Lamin A diseases, especially with Progeria, and with processes in physiological ageing. We therefore studied protein glycation as a biomarker of ageing. Compared to liver and heart of controls, ic/ic mice showed a significantly increased accumulation of intracellular glycated proteins Arg-pyrimidine, carboxyethyllysine and pentosidine. In contrast, we did not see changes in the accumulation of carboxymethyllysine as well as in advanced glycosylated end product (AGE) modification of the extracellular matrix.

Summarizing, manifestation in ic/ic mice start prenatally and are life threatening perinatally and around weaning. If homozygous mice survive these critical intervals, symptoms overlap with premature ageing. We conclude that the lamin B receptor is essential both for development and healthy ageing.

P-MonoG-232

Inheritance patterns and mutational mechanisms of the KATP-channel genes ABCC8 and KCNJ11 involved in congenital hyperinsulinism (CHI)

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Congenital hyperinsulinism (CHI) is a disorder of persistent hypoglycemia due to irregular insulin secretion in newborns and infants. Mutational analysis of 136 unrelated patients mainly from Central Europe was performed in the genes ABCC8 and KCNJ11 encoding the SUR1 and Kir6.2 subunits of the KATP-channel expressed in pancreatic ß-cells. Mutations in either ABCC8 or KCNJ11 were detected in 61 (45%) patients by conventional Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA) of ABCC8, which was applied when sequencing revealed a heterozygous or no causative mutation in either gene. Biparental recessive inheritance was observed in 34% and dominant inheritance in 11% of mutation-positive patients. One patient was compound-heterozygous for two mutations known for recessive and dominant inheritance modes, respectively. Paternal transmission of a mutation associated with a focal form of CHI was observed in 38% of mutation-positive patients. Pancreatic tissue was also analyzed by RT-PCR and sequencing and MLPA. Both, ABCC8 and KCNJ11, are located in proximity to the BWS imprinted critical region of chromosome 11p15. We demonstrate monoallelic expression due to somatic mosaicism for paternal uniparental isodisomy (UPD) for 11p15 associated with BWS specifically in focal pancreatic lesions but not in surrounding pancreatic tissue or blood cells. In conclusion, CHI is caused by recessive inheritance of either biparental or heterozygous paternal mutations in ABCC8 and KCNJ11 in the majority of patients of this cohort. A major second genetic event in focal form of CHI appears to be paternal UPD for 11p15.

P-MonoG-233

Implementation of an IT-platform for the multicenter analysis and clinical annotation of exomes of 250 children with intellectual disability

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The introduction of exome and whole genome sequencing into clinical diagnostics requires new structures for sequencing and data analysis to leverage the potential of automation inherent to these techniques. In principle, data analysis in this context is as simple as comparing a comprehensive list of variants identified in a patient with a comprehensive list of disease causing variants. In practice, this is currently limited by the substantial number of DNA variants with false annotation or uncertain significance.

As a pilot project, three cooperating diagnostic teams are investigating 250 trios consisting of patients with severe to mild ID as the only inclusion criterion together with their healthy parents for which high coverage (mean 110-times) exome sequences have been generated. In most individuals, causative copy number variations, FMR1 repeat expansions, and mutations of clinically plausible candidate genes had previously been excluded.

We set up an IT environment that supports central sequencing and automated primary data analysis and subsequently provides the results via a web interface to researchers and geneticists for manual curation, annotation and experimental validation.

Variant calling was performed with SAMtools (v0.18) for SNVs and short indels, with Pindel for larger indels and with ExomeDepth for CNVs. We further implemented algorithms for the detection of uniparental disomies and regions of homozygosity. Preliminary analysis of de novo variants revealed 1.7 de novo non-synonymous coding and canonical splice site mutations per patient which is above of the estimated average of de novo mutations per individual and generation. Of the de novo mutations, 24% were classified as gene-disrupting (nonsense, frameshift, splice site), 72% were missense mutations, and 4% were in-frame indels. 20% of the investigated cases carried a mutation in genes already known to cause ID. Interestingly, approximately half of the mutations in known ID genes are gene-disrupting mutations, indicating that loss-of-function is a common disease causing mechanism for ID. In addition, evidence for novel ID candidate genes is being generated from the detection of SNVs and indels in specific genes from known microdeletion regions.

In summary, we show that exome sequencing in a multicenter setting with an appropriate IT environment can efficiently be used to generate clinical diagnoses by integrating the advantage of standardized central sequencing and distributed evaluation and annotation of the resulting data in the clinical context.

P-Normal Variation / Population Genetics / Genetic Epidemiology / Evolutionary Genetics

P-NormV-234

Lactose intolerance is associated with colorectal cancer incidence in the Polish population

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During the last several years, a lot of efforts have been devoted to determine potential risk factors of colorectal cancer. One of such agents which might increase susceptibility to sporadic CRC is an ailment of digestive system called lactose intolerance, since it negatively affects functioning of intestines (after lactose consumption). It is caused by acidification of the lumen, osmotic balance disturbance, and alteration in intestinal bacteria composition. Primary lactose intolerance is a genetic disorder caused by several loci, from which, the most important for Caucasian population is LCT-13910T>C (C/C - lactose intolerant phenotype, C/T and T/T - lactose persistent). It is located in the 13 intron of MCM6 gene and operates as an enhancer of LCT gene. The major aim of the following studies was to check a correlation between incidence of lactose intolerance and increased risk of sporadic CRC development. The studies rest on genotyping of LCT-13910 loci in a group of control and 279 cases of sporadic CRC and comparison of frequencies of particular genotypes between those groups. Genotyping was performed by means of high resolution melting (HRM) analysis as a credible and fast genotyping method. Next, the results were subjected to statistical analysis by $\chi 2$ test of independence. The test, concerning association between lactose intolerance and sporadic CRC, achieved statistical significance. This observation may indicate the role of lactose intolerance as a risk factor for CRC (about 8% higher frequency of LCT-13910C/C genotype among CRC patients).

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P-NormV-235

Mutations in FKBP10 are a major cause of autosomal recessively inherited osteogenesis imperfecta in Iranian families

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Osteogenesis imperfecta (OI) is a rare genetic disorder of the skeletal system. The main clinical characteristic of OI is an increased bone instability causing high susceptibility to fractures. Depending on the severity of the disease, patients can present with a range of skeletal features such as reduced bone mineralization density, abnormal bone bending, short stature, scoliosis and hypermobility of the joints. The vast majority of OI cases carry autosomal dominant mutations in COL1A1 or COL1A2 coding for collagen I. Approximately 10% of OI cases are autosomal recessively inherited and a total of 15 genes have been yet identified, many of them encoding proteins involved in collagen I biosynthesis.

So far, little is known about specific distributions and gene frequencies of OI-associated genes in different populations. In our study, we investigated an Iranian cohort of 17 affected individuals of 15 consanguineous families diagnosed with a likely autosomal recessively inherited form of OI. We used a mixture of different mutation screening strategies combining multiplex microsatellite marker analysis, single gene Sanger-sequencing, and whole-exome sequencing.

We identified a total of 11 different mutations in five known OI genes in 14 of 15 OI families. In six families we found homozygous mutations in FKBP10 (p.Gly278Argfs*95, p.Met326Trpfs*39, p.Glu113Lys, c.392-3C>G) representing over 40% (6/14) of all detected mutations. The p.Gly278Argfs*95 (c.831dupC) mutation in exon 5 of FKBP10 was homozygously present in affected individuals from three families indicating that this mutation represents a hot spot mutation in the Iranian population. Furthermore, we identified one homozygous mutation in PLOD2 (p.Glu625Ala) in two unrelated families as well as one homozygous mutation in CRTAP (p.Leu67Pro). Remarkably, we identified five families with dominant mutations in COL1A1 (p.Gly248Arg, p.Pro417Ala, c.696+2T>G, c.2236-1G>A) and COL1A2 (p.Gly1012Ser) although we only tested consanguineous families in our study. Here, the majority of mutations arose de novo and determined the molecular cause in 35% of all investigated Iranian consanguineous OI families. Only in one family we did not observe any mutation in known OI genes analysed by whole-exome sequencing and studies are ongoing to identify a novel OI-associated gene in this family.

In conclusion, our study provides the first results on mutation frequencies in Iranian OI families. The detection of a major contribution of FKBP10 mutations is highly relevant for molecular diagnostic strategies in Iranian OI patients from consanguineous families. Moreover, the presence of high percentage of dominant COL1A1 and COL1A2 mutations in consanguineous families is critical and should be considered in further diagnostic testings.

P-NormV-236

Highly variable DNA methylation of ALU and LINE1 repeats in single sperm and their role in fertilization

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Epigenetic modifications, in particular DNA methylation plays an important role in gene regulation. We propose that sperm DNA methylation can influence expression of the paternal genome and developmental potential of the early embryo. We have used bisulfite pyrosequencing to determine the average methylation level of ALU and LINE1 repeats, representing 10% and 17% of the genome, respectively, in approximately 100 single sperms each of one fertile and one infertile male. Among individual sperms of the same donor, ALU methylation varied from 18% to 46% and LINE1 methylation from 57% to 94%, however there was no significant difference in the methylation distribution between the fertile (mean ALU methylation 26.0%, LINE1 80.9%) and the infertile donor (ALU 29.0%; LINE1 78.7%) or between sperms with normal (ALU 25.4%; LINE1 80.7%) and abnormal morphology (ALU 25.1%; LINE1 78.9%). In addition, plasmid bisulfite sequencing of individual sperms was performed for single molecule methylation analysis. Fully unmethylated, partially methylated, and completely methylated ALU repeats coexisted within the same sperm cell. On average, 15.5% of ALUs were completely unmethylated in sperm of the infertile and 13.9% in the fertile donor. Collectively, our results suggest a high variation in the methylation of individuals repeats within the same sperm cell as well as between different sperms of the same donor. The biological consequences of this varying repeat methylation for sperm function and embryo development remain to be elucidated.

P-NormV-237

Evolutionary origin and methylation status of human intronic CGIs not present in mouse

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CpG islands (CGIs) are clusters of CpG dinucleotides which meet specific sequence criteria. Most CGIs are located in the promoter region or in the transcription start site (TSS) of a gene and play a role in gene regulation. Former studies have shown that imprinting of the human RB1 gene is due to the presence of a differentially methylated CGI (CpG85) in intron 2, which is part of a retrotransposon derived from the PPP1R26 gene on chromosome 9. The murine Rb1 gene does not have this retrotransposon and is not imprinted. We have investigated whether the RB1/Rb1 locus is unique with respect to these differences.

For this we have compared the CGIs from human (n=27,718) and mouse (n=15,997) by in silico analyses. We serially numbered all CGIs from one organism with a unique ID and classified the location of each CGI with regard to protein coding regions. The following five classes to characterise the location were defined: TSS, 5'UTR, CDS(Exon), CDS(Intron) and 3'UTR, where a CGI can belong to one or more classes. We compared the sequences of 2,174 human intronic CGIs with the sequences of 579 murine intronic CGIs performing pairwise alignments (blast2seq) and found that there are 2033 human intronic CGIs that are not present in the mouse. A MEGA Blast search with human sequences has resulted in sequence similarities of 135 CGIs to sequences elsewhere in the human genome, which are suggestive of retrotransposition. By BLAT searches in seven primate genomes (chimpanzee, gorilla, orang-utan, gibbon, rhesus, marmoset and bushbaby), using the sequence of the additional human intronic CGI and flanking exons, we have determined the time points when these CGIs appeared during evolution. Most of the CGIs (72%) are present in all analysed members of the superfamily Hominoidea (human, chimpanzee, gorilla, orang-utan and gibbon). The methylation status of these CGIs was analysed in a monocyte methylome dataset from whole genome bisulfite sequencing. While most of the 135 CGIs appear to be fully methylated, five CGIs (including CpG85 of RB1) show differential methylation, which is suggestive of imprinting. The differential methylation was determined by methylation status analysis of each single read and single CpG dinucleotides. The five differentially methylated CGIs are located in introns of the following genes: ASRGL1, PARP11, RB1, PDXDC1 and MYO1D. Of these, RB1 is the only gene known to be imprinted.

Our study supports the notion that imprinting builds on host defence mechanisms by which the genome protects itself against retrotransposons and foreign DNA elements and that the epigenetic fate of a ret-

rotransposon depends on the DNA sequence and selective forces at the integration site.

P-Prenatal Diagnosis / Reproductive Medicine

P-Prenat-238

Further insights into the role of the M2/ANXA5 haplotype as recurrent micarriage factor

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M2 is a haplotype in the proximal core promoter region of the annexin A5 (ANXA5) gene and it is a confirmed risk factor for recurrent miscarriage (RM). Despite uncertainties concerning the exact pathology triggered by M2 carriage, the most favored explanation of its effects on early pregnancy losses points to a decreased expression of ANXA5 in placenta. ANXA5 is most abundant at the apical surface of placental villi, well in line with its extensively studied anticoagulant properties, important for the maintenance of hemodynamic balance during pregnancy. Since reduced ANXA5 expression should be largely tissue and cell specific, the development timing of the relevant pathology in the course of pregnancy is an important question. With the current study we sought to determine when in the course of gestation M2/ANXA5 is mostly associated with RPL and to confirm the role of paternal carriage.

The prevalence of M2 was estimated in groups of German and Bulgarian RM patients, presenting at respective centers and compared to appropriate independent population control groups. Women were prescreened negative for carriage of the Factor V Leiden (FVL) and prothrombin (PTm) mutations and RM was defined as 2 or more losses. 236 RM Bulgarian women, 243 RM German women and 109 male partners thereof were included in this study. German population controls were from the PopGen biobank, UKSH Kiel (n = 533) and Bulgarian population controls were from the resource of the Naional Genetics Laboratory (n= 200), Sofia. According to fetal development at the time of miscarriage, patients were stratified into three subgroups: subgroup 1, embryonal losses, 5–10 gestational weeks (GW); subgroup 2, early fetal losses, 10–15 GW; subgroup 3, late fetal losses, >15 GW. Incidence of M2 carriage was estimated in the patients and control groups/subgroups, odds ratios were calculated and RPL risk was evaluated.

The meta-analysis of both populations confirmed a highly significant association of M2/ANXA5 with RM (combined OR, 1.6; 95% CI, 1.2–2.1; p=.003), Similarly, when compared with population controls, German male partners carrying M2 exhibited a trend towards a comparable RPL risk (OR, 1.5; 95% CI, 0.8–2.5). After meta-analysis of the German and Bulgarian samples, the ORs for subgroup 1 and subgroup 3 were 1.4 (95% CI, 0.9–2.0; p=.093) and 1.8 (95% CI,1.0–3.1; p=.049). However, the highest and most significant OR was found for subgroup 2 at 1.9 (95% CI, 1.3–3.0; p=0.003), with miscarriages in GWs 10–15.

M2/ANXA5 appears as an RPL risk factor in male and female carriers with most remarkable effects between the 10th and 15th week of gestation, which relates to a time in the course of pregnancy when vascular remodeling is most active to accomplish the transition from high- to low-resistance blood vessels.

This study provides relevant pointers to be considered for future potential therapies in large patient screens.

P-Prenat-239

The necessity to discuss combined PGD/PGS and the demonstration of its feasibility with currently available methods

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While Germany still awaits commencement of valid legal restrictions on preimplantation genetic analyses, the awareness of this reproductive option increases among ART-professionals and patients. Referring gynaecologists and couples ask for detailed description of associated clinical, technical and ethical issues. Genetic literacy develops at high speed and an increasing number of requests are made for combined testing for a single gene defect (PGD) and aneuploidy (PGS). Of note, with this our genetic counselling interview routine raises a topic that is yet rarely debated among experts.

The literature merely provides single case studies and thus mirrors our personal impression from exchange with other European centers: The clinical indication and motivation for a couple to ask for PGD/PGS are unique and are to be met on individual basis.

The procedure itself constitutes a diagnostic challenge. Blastocyst morphology does not allow every trophectoderm to be biopsied twice. Therefore it is not always possible to generate separate specimen to be assessed with each method specifically. For unification of protocols, we established a combined amplification of DNA from single cells with multiple displacement (MDA) and random priming (REPLI-g Single Cell Kit, Qiagen, Hilden, Germany and SurePlex Amplification System, BlueGnome, Cambridge, UK). Optimization was done utilizing cells isolated from buccal swabs of reference individuals. For validation, PCR products were generated from 15 pg genomic DNA each of 20 products of conception harboring various trisomies as previously determined by cytogenetics. Hybridization onto 24sure V3 BAC arrays resulted in profiles that were well within quality parameters provided by the manufacturer (BlueGnome). For each sample, there was sufficient MDA-product of optimal molecular weight to perform PGD as well. Thus, we demonstrated that PGD/PGS can be conducted with already well implemented means.

The advent of new methods utilizing high resolution SNP haplotyping or next generation sequencing promises to revolutionize simultaneous testing of mutations of interest and aneusomy on single cell level. Initial reports suggest, that the methodological fusion of PGD and PGS will become technical standard very soon. Novel genome-wide PGD approaches provide higher quality than locus- and family-specific assays. At the same time they deliver a PGS-result.

There is a serious need for professional discussion on how to deal with the outcomes of such analyses. Since technical progress has already outrun the legal regulation for Germany before it came into effect, clinicians and geneticists should debate on indications for PGD/PGS, communication of the physical and psychological demands a couple has to face, as well as consideration of the ethical and financial burden that comes with it. We contribute to this with the presentation of our experience from appeals to our center and the technical implementation of PGD/PGS in our genetic lab.

P-Prenat-240

Poly(A) tail length of maternal-effect gene mRNAs in murine oocytes is influenced by postovulatory aging

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The maternal effect (ME) is the influence of the mother's genotype on the phenotype of her offspring. In the zygote and during the first cell divisions after fertilization the embryonic genome is not active and therefore early embryonic development depends on the ME gene mRNAs and proteins of the oocyte. It has been shown before that postovulatory aging of oocytes from Xenopus tropicalis led to developmental defects which appear mainly due to age-dependent deadenylation of maternal transcripts. To investigate if such processes are also relevant for the reduced developmental potential of aged oocytes in mammals, poly(A) tail dynamics of selected ME genes were analyzed in postovulatory aged mouse oocytes.

For isolation of in vivo matured oocytes, 5 to 8 weeks old C57/Bl6J mice were superovulated and oocytes were either directly frozen after isolation from the ampullae or aged in vitro for 12 or 24 hours. For in vitro maturation of oocytes, preantral follicles were isolated from the ovaries of 12-day old prepubertal C57Bl/6J x CBA/Ca F1 hybrid mice, and cultured for 12 days in presence of rFSH to the antral stage before being stimulated by rEGF/rhCG for in vitro ovulation. Postovulatory aging of in vitro matured oocytes was for 12 hours in culture. qRT-PCR-analysis was performed with random-hexamer primed cDNA to quantify total amount of mRNA of specific ME genes or with oligo(dT)16 primed cDNA to indicate poly(A) tail length. To specify changes in poly(A) tail length, qPCR results were confirmed by ePAT (extension Poly(A) test) for two representative genes (Zar1 and Dnmt1).

Postovulatory aging of in vivo matured oocytes led to a decrease in total mRNA amount of the selected ME genes as well as in poly(A) tail length. After 12 hours of aging only Nlrp5 of the 10 genes investigated showed a trend in poly(A) tail reduction. Additional aging for a total of 24 hours resulted in a stronger decrease of total mRNA amount and poly(A) tail length affecting 6 genes (Tet3, Trim28, Dnmt1, Nlrp5, Nlrp14 and Oct4). In vitro matured oocytes appeared more susceptible to postovulatory age-related decrease of mRNA amount and poly(A) tail length of most of the ME genes investigated (Tet3, Trim28, Zfp57, Dnmt1, Nlrp5, Zar1) already after 12 hours of aging.

In conclusion, postovulatory aging of oocytes may not only lead to a decrease in total mRNA amount but also to a poly(A) tail reduction of specific ME gene transcripts that are developmentally relevant. The age-dependent deadenylation of the poly(A) tail appears progressive and independent on egg activation, and seems to be more pronounced in postovulatory aged in vitro matured mouse oocytes compared to in vivo matured oocytes. This may contribute and predispose to developmental failures after fertilization in both systems.

P-Prenat-241

Preovulatory aging of murine oocytes affects transcript levels and poly(A) tail length of maternal effect genes

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Embryonic development between fertilization and zygotic genome activation (ZGA) is regulated by maternal effect (ME) genes of the maternal genome, which are expressed in the oocyte. Transcription in oocytes ceases at the onset of maturation and is resumed only after ZGA. The synthesis of new proteins during this period is regulated at the posttranscriptional level, for example by modification of poly(A) tail length of mRNA. We could show in a previous study that preovulatory aging, caused by delayed ovulation, is associated with developmental defects in mice. The molecular and genetic mechanisms underlying these defects are unknown. Here, we investigated the transcript levels and poly(A) content of mRNA from 10 ME genes in preovulatory aged murine metaphase II oocytes. Oocytes were obtained from C57Bl/6J mice after superovulation (in vivo maturation). Preovulatory aged oocytes were generated by delaying ovulation for 3 days using the GnRH antagonist cetrorelix. Transcript levels were determined by qRT-PCR using random hexamer-primed cDNA. qRT-PCR of oligo(dT)-primed cDNA was taken as an indicator of poly(A) tail length. Random hexamer priming showed significantly decreased transcript levels of in vivo aged oocytes compared to controls for Brg1 and Tet3. Small or no effects were found for Trim28 (Kap1/Tif1β), Zfp57, Dnmt1, Nlrp2, Nlrp5 (Mater), Nlrp14, Oct4 (Pou5f1), and Zar1. Using oligo(dT) priming, we did not observe any significant effects of aging, although there was a trend towards increased poly(A) mRNAs indicated by increased cDNA levels for Brg1, Tet3, Trim28, Zfp57, Dnmt1, Nlrp2 and Nlrp5. This could be a sign of continuing polyadenylation and precocious recruitment of maternal mRNAs during preovulatory in vivo aging, while overall transcript levels decline. In a second set of experiments, oocyte overmaturity was studied after in vitro growth and maturation of follicles in a preantral follicle culture system. For the control group, preantral follicles were isolated from prepubertal mice and cultured to the large antral stage for 12 days in vitro. For the aged group, follicles were cultured for 14 instead of 12 days before initiation of ovulation. During in vitro preovulatory aging of oocytes, transcript levels did not change significantly, whereas poly(A) content decreased for most of the transcripts. Taken together, these data suggest that preovulatory aging affects transcript levels and poly(A) tail length of selected ME genes. Also, aging of oocytes in vitro shows adenylation changes opposite to those observed after preovulatory aging in vivo. Since in vivo preovulatory aging can occur during prolonged hormonal treatment in the course of assisted reproduction, and cryopreservation of follicles followed by in vitro culture is handled as an option for fertility preservation in women diagnosed with cancer, our results may be of clinical relevance.

P-Prenat-242

Studying Paternal Age Effects on the Sperm Epigenome via Deep Bisulfite Sequencing

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Epidemiological studies provide evidence for an influence of paternal age on the prevalence of certain diseases in the offspring, including autism and cancer. Genome-wide sequencing techniques revealed that the offspring of older men carry more genetic mutations. It is well known that the number of germ cell divisions in males increase with age; therefore, the higher de novo mutation rate in the offspring of older fathers may have originated in sperm. We speculated that in addition to an increased number of genetic mutations, the sperm of older males contain more epigenetic perturbations when compared to their younger counterparts. The inheritance of sperm-specific epimutations may contribute to the etiology of disorders showing a "paternal age effect". To explore this possibility, we performed deep bisulfite sequencing analysis of well-selected candidate genes to detect rare epimutations in young and old males with normal sperm parameters. Deep bisulfite sequencing using the GS Junior permits methylation analysis of several amplicons at single molecule level (representing one sperm) with a high coverage depth. Single molecules are clonally amplified and sequenced at single base resolution. This permits the identification of very rare methylation aberrations affecting a few cells (needle-in-a-haystack). Using deep bisulfite sequencing, we analyzed the promoter methylation of 2 imprinted genes (MEST and KCNQ1OT1), 4 tumour suppressor genes (BRCA1, PTEN, RAD51C, and NF1), an achondroplasia associated gene (FGFR3), one alzheimer's related gene (PSEN1), and one autism linked gene (SHANK3). On average; we had 1462 reads for

BRCA1, 1290 reads for PTEN, 780 reads for RAD51C, 406 for MEST, 249 for PSEN1, 322 for LIT1, 262 for SHANK3, 703 for FGFR3, and 496 reads for NF1 per sperm sample. We detected several fully methylated alleles in MEST, KCNQ1OT1, and FGFR3; nevertheless no association with paternal age was evident. NF1 displayed a significant increase in CpG errors ($p<0.0001$) when comparing the sperm of young versus old males. NF1 is a negative regulator of the RAS pathway which is implicated in selfish spermatogonial stem cell propagation. These results provide insight on the epigenetic effect of paternal aging on sperm that might have consequences on the offspring of older fathers.

P-Prenat-243

Identifying genes in early development: cross species phenotyping confirms mutations in KIF14 to cause a fetal lethal ciliary phenotype

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Gene discovery using whole exome sequencing (WES) has focused on postnatal phenotypes such as well-characterized syndromes or intellectual disability. Little attention has been paid to using WES in fetal disorders that are lethal in utero, because they often appear to be sporadic and have not been recognized to be Mendelian. The prenatal clinical data are frequently imprecise. Even if autopsy data are available, associating a fetal multiple congenital anomaly (MCA) phenotype to a specific clinical diagnosis is often difficult. These issues considerably limit WES approaches.

We used WES in a family with a recurrent pattern of an undescribed fetal MCA syndrome, diagnosed in the 2nd trimester by ultrasound, which we expected to be lethal because of distinct brain and kidney malformations. The clinical signs confirmed by autopsy include severe flexion arthrogrposis, IUGR, severe microcephaly, complex brain malformations and renal cystic dysplasia/ agenesis and overlap with the characteristic of ciliopathies, but were not diagnostic of a known condition. We hypothesized biallelic disruption of a gene leading to a defect related to primary cilia.

Family-based WES identified novel truncating autosomal recessive mutations in KIF14. Kinesins are microtubule-dependent molecular motors, and KIF14 depletion was shown to induce cytokinesis failure. Recent studies in mice demonstrate that autosomal recessive mutations in the same gene recapitulate the human phenotype. In our kif14 zebrafish morpholino knockdown we investigated assays for ciliary defects and confirmed the role of cilia in the disease mechanism, corresponding to our prior phenotypic prediction. We provide a further example of the emerging relation between cytokinesis and the primary cilium.

WES can be used in individual families with undiagnosed lethal MCA syndromes to discover responsible autosomal recessive mutations, provided that prior to data analysis the fetal phenotype can be correlated to a particular developmental pathway in embryogenesis. Cross species phenotyping allows to validate genes involved in those extremely rare phenotypes and increase our knowledge about normal and abnormal human developmental processes. Ultimately, families will benefit from the option of early prenatal diagnosis.

P-Prenat-244

Partial rescue of the KIT deficient embryonic phenotype in TSPY transgenic B6;NMRI-KitWv/KitWv mice

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The TSPY gene is conserved in placental mammals and encodes the testis-specific protein, Y-linked. It is assumed that TSPY plays a role in proliferation and/or meiotic differentiation of testicular germ cells. Recently, TSPY copy number variations and mutations within the first exon have been identified as modifiers of spermatogenesis and risk factors for male infertility. Because the laboratory mouse is carrying only a single-copy Y-chromosomal Tspy pseudogene (Tspy-ps), we generated a TSPY transgenic mouse line on a NMRI-outbred strain genetic background (NMRI-Tg(TSPY)9Jshm) that carries a human TSPY gene in approximately 50 copies on the mouse Y chromosome. TSPY transgenic B6;NMRI-KitWv/KitWv mice on a mixed B6;NMRI genetic background are able to partially rescue spermatogenesis and fertility of homozygous KitWv-mutant males. Whether this effect is mediated by a function of TSPY on the survival and/or proliferation of primordial germ cells and gonocytes during embryonic development or caused by an effect of TSPY in proliferation and differentiation of differentiated type A spermatogonia in the postnatal testis, is currently unknown.

To analyze whether the human transgene exercises a proliferative and/or apoptosis protective effect on embryonic or early postnatal male germ cells, we analyzed the testes of 15,5 dpc and 4,5 dpp old TSPY transgenic B6;NMRI-KitWv/KitWv mice and non transgenic B6;NMRI-KitWv/KitWv controls immunhistochemically by means of Ki-67 or activated caspase-3 antibodies and TUNEL-assay. We observed neither for 15,5 dpc nor for 4,5 dpp old TSPY transgenic KIT deficient testes significant differences in Ki-67, caspase 3 and TUNEL labelled tubular cells in comparison to controls. However, quantification of gonocytes in TSPY-transgenic and non transgenic embryos revealed a notably increased number of gonocytes and testis cords with gonocytes in TSPY transgenic B6;NMRI-KitWv/KitWv testes in comparison to B6;NMRI-KitWv/KitWv testes. These findings point to a promoting effect of TSPY on early foetal development of male germ cells.

P-Prenat-245

Parallel prenatal testing for trisomies (-13, -18 and -21), turner syndrome, 22q11.2 del/dup-syndromes (e.g. DiGeorge), Smith-Lemli-Opitz syndrome and Noonan syndrome in fetuses with increased nuchal translucency using the AmpliSeq™ technology (Life

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Increased nuchal translucency (NT) is a common ultrasound finding in fetuses with an abnormal karyotype (mainly trisomies 13, -18, -21 and turner syndrome).

Beside of gross chromosomal defects increased NT is also a feature seen in a wide range of rare genetic syndromes. In most cases prenatal testing strategies in fetuses with increased NT has so far been restricted to karyotyping and/or microarray analysis.

Recently, it has been suggested to perform diagnostic prenatal testing for Noonan syndrome in cases with increased NT and a normal karyotype. Noonan syndrome is an autosomal dominant condition characterized by short stature, congenital heart defects, skeletal abnormalities and developmental delay. The incidence is 1:1500 live births. Noonan syndrome can be the result of mutations in several genes of the RAS-MAPK pathway, therefore genetic testing for all Noonan syndrome genes is time and money consuming.

During the last years high throughput next generation sequencing (NGS) has been implemented into genetic diagnostics allowing paral-

lel testing of many genes in a short time and for lower costs. Moreover, NGS data can be also used for the detection of gain and/or loss of genetic material by counting the relative number of reads mapped to the targeted region.
Consequently, NGS is a perfect tool for prenatal testing in fetuses with increased nuchal translucency covering much more relevant genetic aberrations than karyotyping.
We decided to use the AmpliSeq™ technology (Lifetechnologies) on the Ion PGM platform to test for mutations in genes of the RAS-MAPK pathway (PTPN11, BRAF, SHOC2, MAP2K1, MAP2K2, HRAS, RAF1, NRAS, KRAS, SOS1), for the multiple pterygium syndrome (CHRND, CHRNA1, CHRNG) and for the Smith-Lemli-Opitz syndrome (DHCR7). Additionally, our design included genes on the chromosomes -13, -18, -21, X and Y. Another gene was located within the critical region of the DiGeorge syndrome on 22q11.2. Only the number of reads mapped to these genes, were used to detect gains or losses of these regions.
Our first validation experiments on these disorders showed that our approach is able to detect PTPN11 point mutations, 22q11.2 deletions, Turner syndrome and trisomies 13, -18 and -21 with high sensitivity.
In conclusion, our results demonstrate that our customer-designed AmpliSeq™ systeme is able to detect point mutations (e.g. in the PTPN11 gene) as well as deletions (DiGeorge syndrome) or trisomies in a diagnostic setting. Our approach can extend the scope of prenatal testing in fetuses with increased nuchal translucency.

P-Prenat-246

Prenatal manifestation of HNF1B (TCF2) mutations – four cases

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Background:
HNF1B (TCF2, OMIM 189907), the gene encoding for hepatocyte nuclear factor 1beta, is involved in early renal development. HNF1B mutations can cause the ‚renal cysts and diabetes syndrome' (RCAD), an autosomal dominant disorder comprising renal disease and maturity-onset diabetes of the young (MODY). Additional findings, derived from HNF1B mutations, are anomalies of the genital tract, pancreatic anomalies, liver function abnormalities and hyperuricemia. Patients with a microdeletion or microduplication of the chromosomal region 17q12, encompassing the HNF1B gene, may also show dysmorphism, developmental delay, learning difficulties, autism spectrum disorders, psychiatric disorders or other malformations.
The HNF1B associated nephropathy is highly variable and includes mainly cystic renal dysplasia, renal agenesis, horseshoe kidney, tubular anomalies and hydronephrosis. Prenatal ultrasound may show hyperechogenic and cystic kidneys. In most cases, the diagnosis of HNF1B associated nephropathy is made postnatally.
Objective:
We report four cases of HNF1B derived renal disease, three of them diagnosed prenatally because of fetal renal abnormalities and one diagnosed in the neonatal period. Prenatal phenotypes and outcomes are presented. Similarities and differences between cases are discussed and compared to current literature data.
Methods and Results:
Fetal renal disease was detected by ultrasound in the second/third trimester of pregnancy. All four cases showed cystic renal dysplasia with hyperechogenic parenchyma and slightly enlarged kidneys, not typical for autosomal dominant or recessive polycystic kidney disease. One case presented in the first trimester with fetal hydrops.
In three cases microdeletion 17q12, encompassing the HNF1B gene, was identified by aCGH, in one case a point mutation in the HNF1B gene was detected by Sanger sequencing.
Conclusion:
Fetal HNF1B associated nephropathy should be suspected in cases with cystic renal dysplasia not typical for ADPKD and ARPKD. Prenatal aCGH is of crucial importance for the genetic diagnostics.

P-Prenat-247

Non-invasive prenatal testing (NIPT) for common fetal trisomies in multiple gestations and a case report of a discordant result due to a vanishing twin

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Objective
PrenaTest, a non-invasive molecular genetic prenatal test (NIPT) for the most common fetal autosomal trisomies based on massively parallel sequencing of cell-free fetal (cff) DNA in maternal plasma, has been widely used in singleton pregnancies since its commercial launch in August 2012. Multiple pregnancies as well as vanishing twins had to be excluded from the clinical routine due to the lack of validation data for such cases. Thus, the purpose of this study was to validate the PrenaTest for multiple pregnancies.
Methods
40 blood samples from women pregnant with multiple gestations (mono-, di- and trichorionic) have been consecutively collected for research & development (R&D) purposes between November 6th 2012 and November 16th 2013. Two blood samples came from women pregnant with triplets, the remaining 38 samples came from twin pregnancies. In 15 cases the pregnancy was caused by assisted reproduction, thereof one case using an egg donation. The cffDNA fraction has been determinded by a quantitative PCR assay (QuantYfeX). The samples have been analysed within about two weeks after receipt of the blood samples and the results have been communicated to the pregnant women by their responsible doctor as non-validated R&D result. Only in positive cases the test results have been confirmed by invasive diagnostic methods.
Results
The blood samples have been taken in pregnancy week 9+3 to 23+0, with the median at 14+2. The required minimal cffDNA fraction of 4% in monochorionic and 8% in dichorionic twin pregnancies has been failed in four cases. In another four cases the cffDNA fraction has not been determined. There were two positive results for trisomy 21 in twin pregnancies, one monochorionic and one dichorionic. Both have been confirmed by invasive diagnosis. All other results were negative.
Case report
For a PrenaTest in a pregnancy designated as singleton a reputed false-positive result for a trisomy 21 has been reported back by the responsible doctor. Methodical failure of the PrenaTest has been ruled out by analysis of a second and third blood sample, taken at later stages in the pregnancy, all with the same, positive result for trisomy 21. Investigation of the placenta after birth revealed a fetus papyraceus, proven to be positive for trisomy 21 by karyotyping.
Conclusions
The PrenaTest NIPT method is regarded as reliable also for twin pregnancies and probably as well for multiple pregnancies if cffDNA fractions are about 4% per fetus. Undisclosed vanished twins can contribute a sufficient proportion to the total cffDNA fraction to cause discordant NIPT results, thus being a limitation to the NIPT method.

P-Prenat-248

Preimplantation genetic diagnosis for monogenic disorders – impact of different biopsy forms and transfer time points on the rate of transfer cycles and pregnancies

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In Germany, preimplantation genetic diagnosis (PID) will legally be restricted to the analysis of pluripotent cells obtained by trophectoderm biopsy (TEB) on day 5 and/or 6. TEB more recently has been established as a relatively new, but promising biopsy technique, providing higher sample sizes with more than one embryonic cell, lower costs of genetic testing due to the limited number of available blastocysts per cycle and presumably lower rates of mosaicism.

At our center in Regensburg so far polar body diagnosis (PBD) has been applied for monogenic disorders for more than 12 years. In addition, during a legal time window between 2010 and 2011 we have established and performed add-on TEB in 9 PGD cycles for monogenic disorders.

We here compare our PBD results in Regensburg to the current PGD results for monogenic disorders, reported so far after blastomere biopsy and TEB with a special focus on the main outcome parameters: percentage of transfer cycles and obtained clinical pregnancy rates.

So far, 147 diagnostic PBD cycles have been performed at our center for a total of 71 independent families with autosomal recessive (n=27 families), X-linked (n=23) or rare autosomal dominant (n=21) conditions. At our center in Regensburg a clinical pregnancy could be obtained for 37 of the 71 families (52,1%) with 2,07 oocyte retrieval cycles per family. A genetic diagnosis could be established for 56% of mature oocytes, 245 embryos have been transferred in 122 diagnostic cycles.

Within the wide time window of embryo transfer (ET) after PBD, clinical pregnancy rates per ET at our center were: 31,65% (ET at day 2/3), 34,38% (ET at day 5) and 13,64% (cryo with ET in subsequent cycle), respectively. However, the implantation rate per embryo was higher after ET on day 2/3 (19,9%) when compared to day 5 (15,5%).

Our overall clinical pregnancy rate per PBD cycle to ET (31,97%) is comparable to the pregnancy rate after PGD for monogenic disorders published by the ESHRE PGD consortium mainly performed by day 3 biopsy + day 5 transfer (29,17% - data collection I-XI), with similar proportions of PGD cycles to ET in 81,5% (ESHRE cohort) and 83,0% in our overall PBD cohort.

Only very limited data are currently available on TEB, indicating some benefits for cycles with higher numbers of available blastocysts as well as drawbacks of the long tissue culture, late biopsy and the frequently required cryo conservation with transfer in a subsequent cycle.

Preliminary TEB data on PGD for monogenic disorders, published by McArthur et al. 2008 (177 OR cycles), suggest an expected substantially lower ET rate after TEB (63,84%) with higher implantation and clinical pregnancy rates per ET, resulting in an overall clinical pregnancy rate per OR cycle of 32,7%. Additional prospective studies will be necessary to determine, which couples might ultimately benefit from blastocyst culture und TEB to perform PGD for monogenic disorders.

P-Prenat-249

Non-invasive prenatal testing (NIPT): Laboratory experiences of PrenaTest®

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Non-invasive prenatal testing (NIPT) is an emerging new option in prenatal care. According to the International Society for Prenatal Diagnosis (ISPD) NIPT is an advanced screening method for women at increased risk of common fetal aneuploidies. The commercially available PrenaTest® has been introduced in Europe in August 2012 with focus on Germany, Switzerland and Austria and exhibits sensitivities and specificities around 99%. The laboratory experiences from routine application of NIPT service are reported, and test accuracy, quality parameters as well as patient profiles are discussed. PrenaTest® for common fetal trisomies is done by massively parallel sequencing (MPS) of cell-free DNA from maternal plasma. Presented data were collected during laboratory routine from August 2012 to January 2013 for the detection of trisomy 21 only, and from February 2013 onwards for the detection of trisomies 13, 18 and 21.Within one year about 5,600 successful PrenaTest® analyses have been reported, with 98.0% negative results, 1.5% positive for trisomy 21, 0.4% positive for trisomy 18 and 0.1% positive for trisomy 13. In about 0.2% of cases, no result could be reported even after repeat of the analyses, if requested. According to ad hoc feedback from the ordering specialists there were no false-negative results up to now. In eight false-positive results reported hitherto (one trisomy 13, five trisomy 18 and two trisomy 21) the result from NIPT is suspected to be discordant from the result of amniocentesis because of confined placental mosaicism or an undiscovered vanished twin. Currently, the main indications for the application of the PrenaTest® are an increased maternal age and an increased risk for aneuploidy based on primarily first trimester screening test. Particularly women in week 12+0 through 13+6 - the period in which the nuchal translucency measurement is done - opt for the PrenaTest®. Interestingly, a second peak at week 17+0 to 17+6 is seen, which coincides with the period when amniocenteses are usually carried out. This might be an indication that women who would otherwise have chosen an amniocentesis to obtain information about the health of their child now select the PrenaTest®, instead. In the near future, to characterize the limitations of NIPT more precisely, it is important to analyse thoroughly the discrepancies between NIPT results and the results of conventional karyotyping. It is intriguing that the majority of recently reported "false" positive NIPT results seem in fact to be the consequence of fetal vs. extra fetal cytogenetic discrepancies, thus of biological origin as expected from our knowledge collected from chorionic villi analyses. These findings suggest that biological reasons rather than methodical failures play the major role, emphasising the highly important collaboration between geneticists and gynecologists specialized in ultrasonography.

P-Prenat-250

Meckel Gruber syndrome – Mutational spectrum and double encephalocele as an underdiagnosed characteristic feature

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Meckel Gruber syndrome (MKS) is a rare autosomal recessive disorder characterized by multicystic kidney dysplasia, central nervous system malformations (usually occipital encephalocele), ductal plate malformation of the liver and postaxial polydactyly. Death occurs often before or shortly after birth. MKS is genetically heterogeneous with so far of nearly 12 known disease causing genes. Identifying the underlying genetic defect is therefore often challenging. However, there is a considerable clinical and genetic overlap between the ciliopathies MKS, Joubert syndrome and related disorders and severe manifestations of Bardet-Biedl syndrome.

Among 43 cases with Meckel syndrome we found mutations in 33 cases (77%) in the following genes: MKS1 (33%), MKS3 (39%), MKS4 (18%) and MKS6 (9%), which is in accordance with frequencies reported in literature. Exact data, however, are highly variable and are difficult to assess because of heterogeneous study groups.

On the background of a broad overlap of features of other syndromes a thorough evaluation of cases is important. On this background we

will present a case with a double encephalocele, a typical finding in MKS with two truncating mutations in the MKS3 gene. The fetus additionally showed microcephaly with a hemorrhagic necrotic occipital meningoencephalocele and a second smaller skin-covered meningoencephalocele of cerebellar tissue protruding through a defect of the lower occipital defect with inclusion of the foramen magnum. Furthermore autopsy revealed large polycystic dysplastic kidneys and hypoplastic lungs. No polydactyly was present. Histological examination of the kidney displayed cysts in medullary and cortical region and fibrosis of interstitial connective tissue. The liver showed increased bile duct formation in portal tracts and mild duct proliferation was also found in pancreas and epididymis.
It is well known, that a double encephalocele is a common and typical finding in MKS which, however, is often not diagnosed. The demonstration of a double encephalocele makes the diagnosis of MKS in addition to other findings likely (Rehder and Labbé, Prenatal Diagnosis, 1981).
Because a detailed phenotypic description of affected cases with MKS is often not available in a routine clinical setting we want to emphasize a NGS-based diagnostic algorithm including the four mentioned genes to achieve a complete coverage of these "core" genes followed by diagnostic exome sequencing.

P-Prenat-251

DNA methylation analysis of in vitro matured human oocytes retrieved from small antral follicles

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Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age. When undergoing standard assisted reproductive technologies (ART) for infertility treatment, PCOS patients have a high risk of developing ovarian hyperstimulation syndrome (OHSS), which is due to an increased response to ovarian stimulation. To avoid hormonal stimulation and OHSS, immature oocyte-cumulus complexes can be retrieved from 2-10 mm follicles without an ovulatory dose of hCG and matured in vitro.
ART coincides with genome-wide epigenetic reprogramming during late oocyte and early embryo development. Due to the increased vulnerability of the oocyte to adverse environmental factors during in vitro culture and maturation, it is important to evaluate whether in vitro maturation (IVM) interferes with epigenetic reprogramming of maternal methylation patterns.
To investigate the influence of IVM on the epigenetic status of human oocytes, we examined 61 in vitro and 40 in vivo matured oocytes from PCOS patients. We analysed four imprinted genes (maternally methylated LIT1, PEG3, SNRPN and paternally methylated GTL2) as well as three developmentally important non-imprinted genes (DNMT3Lo, OCT4 and NANOG), using limiting dilution bisulfite pyrosequencing. This technique allows one to determine the methylation patterns of individual alleles of several genes in single cells. Both in vitro and in vivo matured oocytes showed only few abnormal alleles, consistent with epimutations. Although the number of abnormally (de)methylated imprinted alleles was slightly higher in the in vitro group, this difference was not statistically significant. The observed abnormalities may be due to the fact that the in vitro matured oocytes were not yet completely matured. We also found slightly increased numbers of abnormal alleles in immature in vivo matured oocytes, compared to mature in vivo oocytes. We conclude that the human IVM technique by itself does not significantly alter the epigenetic status of oocytes.

P-Prenat-252

Prenatal molecular genetic diagnosis of skeletal dysplasias. A single center experience

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Skeletal dysplasias are a large, heterogeneous group of conditions involving the formation and growth of bone. Some skeletal dysplasias are associated with additional abnormalities in other organ systems.
Prenatal diagnosis relies primarily on fetal ultrasound findings, but molecular analysis is used to confirm the presumptive diagnosis and to determine the recurrence risk.
The diagnosis of a substantial number of the most frequent skeletal dysplasias can be confirmed in a short period of time by molecular genetic analysis of the involved genes (e.g. thanatophoric dysplasia, diastrophic dysplasia, campomelic dysplasia, Ellis-van Creveld syndrome or hypophosphatasia).
We present a retrospective analysis of 300 prenatally diagnosed cases seen in a single tertiary center between 1985 and 2013 by an expert team of experienced sonographers and human geneticists.
Thus, we demonstrate clinical findings and molecular genetic data and construct work-ups for the diagnosis of „difficult" cases (e.g. short rib-polydactyly syndromes, Filamin B associated skeletal dysplasias) including suggestions for gene panel or whole exome investigations.
We were able to establish a final diagnosis in 50% of all cases by molecular genetic testing. Some cases are exampflified with clinical, radiological, pathological and molecular genetic data.
We want to point out the importance of molecular genetic diagnosis for confirming the clinical diagnosis of skeletal dysplasias and providing exact information for genetic counselling.

P-Prenat-253

The proximal promoter of the pluripotency gene Oct4 displays strain-specific DNA methylation differences in mouse spermatocytes

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Global analysis of the mouse methylome revealed striking similarities in DNA methylation patterns of promoter regions between the highly specialised sperm cell and different pluripotent cell types. Thus, the promoter epigenome of spermatocytes is already largely reprogrammed towards a pluripotent state. It is also well known that different mouse strains respond significantly different to standardized in vitro-fertilization (IVF) protocols. The question arises whether intraspecific differences in epigenetic modifications of mouse gametes contribute to this phenomenon.
We used bisulfite pyrosequencing to quantify the DNA methylation levels of several imprinted genes (H19, Igf2r, Plagl1 and Snrpn) and the pluripotency marker gene Oct4 in spermatocytes of Mus musculus domesticus (C57B6/J) and Mus musculus castaneus (CAST/Ei) male mice. DNA methylation of the repetitive short interspersed element (SINE) B1 served as an indicator of global methylation levels. We found no significant differences in the methylation levels of the above mentioned imprinted genes and the B1 elements between the two strains. Surprisingly, however, we could detect an almost two-fold higher methylation level of the proximal Oct4 promoter in CAST/Ei (~40%) compared to C57B6/J (~20%) males. To verify if our finding is a general phenomenon applying to regulatory regions of pluripotency genes, we will extend our methylation analysis to other pluripotency genes and

other regulatory elements at the Oct4 gene locus, like the distal and proximal enhancer. In addition, we will include spermatocytes of further mouse inbred strains in our studies.
Our data support a model, in which the regulation of pluripotency factors in spermatocytes has a major impact on the outcome of assisted reproduction techniques.

P-Prenat-254

„Meckel-like" prenatal presentation of Bardet-Biedl syndrome

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Bardet-Biedl syndrome (BBS) is a heterogeneous autosomal recessive ciliopathy mainly associated with obesity, postaxial polydactyly, retinal dystrophy, learning disability and male hypogenitalism. According to the literature an impaired kidney function can be diagnosed in about 80 % of patients over the age of 16 either as a result of tubulointerstitial lesions or also secondary to persisting hypertension and/or diabetes. Sever cystic kidney dysplasia (CKD) has also been described but is not a very frequent finding in BBS. On average the patients are diagnosed as BBS at the age of about 10 years. However the prenatal ultrasonographic detection of a postaxial polydactyly often in combination with kidney pathology has lead to prenatal clinical confirmation of the syndrome in some cases often with a known family history.
So far, at least 15 different causative BBS genes have been identified. Genetic and clinical overlap with other primary ciliopathies exists, e.g. to Joubert syndrome (JS) or - in case of a prenatal CKD (with or without enlarged kidneys and oligohydramnios) - to Meckel syndrome (MKS).
The latter severe clinical presentation is rare and has been termed as "Meckel-like" phenotype. In affected fetuses in addition to polydactyly, CKD and hepatic fibrosis neural tube/brain abnormalities can be present e.g. as a Dandy Walker malformation. On molecular level, mutations in the genes BBS2 (n=3/13), BBS4 (n=2/13), BBS6 (n=1/13), BBS10 (n=4/20) and recently in BBS1 (n=1/1) have been detected in fetuses with "Meckel-like" features.
We report on 7 "Meckel-like" BBS patients. We could identify mutations in all cases. Whereas 4 cases carried mutations in the already known "Meckel-like" genes BBS1 (n=1/7), BBS4 (1/7) and BBS10 (n=2/7), 3 patients carried mutations in the BBS12 gene, which has not yet been known to cause this phenotype. In addition to these molecular data we will present clinical features of our patients to illustrate the phenotype. Furthermore we will present our diagnostic algorithm in this phenotype on the basis of two affected sibs. In that family the diagnostic workup included SNP array based homozygosity testing followed by direct sequencing of the BBS4 gene leading to the identification of a previously not described splice site mutation.
Clinical and genetic data of severe manifestations of fetuses with the features of the Meckel/Joubert/BBS spectrum give strong evidence that a delineation of a clinical basis alone is not useful.

P-Prenat-255

Mutational analysis of the NR5A1 (SF1) gene in infertile males

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The steroidogenic factor 1 (SF1) protein, encoded by the NR5A1 gene (OMIM 184757), plays a central role in gonadal development and steroidogenesis. Mutations in NR5A1 were first described in patients with primary adrenal insufficiency. Subsequently, NR5A1 mutations were also identified in patients with 46,XY disorders of sexual development as well as in men with hypospadias, bilateral anorchia and micropenis and women with primary ovarian insufficiency. Recently, heterozygous missense mutations were found in 4% of infertile men with unexplained reduced sperm counts living in France, but all mutation carriers were of non-Caucasian ancestry. Therefore, we sequenced the NR5A1 gene in 488 predominantly Caucasian patients with azoo- or severe oligozoospermia. 237 men with normal semen parameters were sequenced as controls. In addition to several synonymous variants of unclear pathogenicity, three heterozygous missense mutations predicted to be damaging to SF1 protein function were identified. Functional studies of these three missense mutations indicated only minor consequences on transactivational activity using a luciferase assay. The andrological phenotype in infertile but otherwise healthy mutation carriers seems variable. In conclusion, mutations altering SF1 protein function and causing spermatogenic failure are also found in men of German origin, but the prevalence seems markedly lower than in other populations.

P-Prenat-256

DMRT1 mutations are rarely associated with male infertility

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Male infertility is a heterogeneous disorder including genetic causes such as chromosome aberrations or AZF deletions of the Y chromosome. So far only few monogenetic causes for male infertility could be identified. In vivo experiments with knockout mice demonstrated that the transcription factor DMRT1 plays a pivotal role in testis development and spermatogenesis by influencing the differentiation of Sertoli cells and cell cycle control of germ cells. Analyses of male Dmrt1-/- mice showed severe testis hypoplasia, disorganized seminiferous tubules and non-differentiated Sertoli cells. In addition, germ cells were missing. In humans, deletions of DMRT1 are associated with XY gonadal dysgenesis, XY ovotesticular disorder of sexual development (DSD) but also with impaired spermatogenesis. Obviously, deletions of DMRT1 can cause a wide spectrum of disorders of gonadal development. We assumed that missense mutations in DMRT1 in males may rather cause infertility than gonadal dysgenesis.
Therefore, we performed sequence analysis of DMRT1 in a total of 163 patients with severe oligozoospermia (sperm concentration < 5 Mill. / ml, N = 86) or non-obstructive azoospermia (N = 77). The latter group encompasses 63 patients with SCO (Sertoli-cell-only)-syndrome and 14 patients with meiotic arrest, while 86 of the oligozoospermic patients had a mixed atrophy testicular phenotype. The control group contained 215 fertile men.
In total, we identified 4 putative pathogenic mutations in the DMRT1 gene in 6 patients. One of the variants, c.132C>T, found in a patient with spermatogenesis arrest, causes no amino acid exchange and is not listed in dbSNP. However, the Alamut Splicing prediction algorithms showed in 2 of the 5 cases that this variant possibly generates an alternative donor splice site. A second variant, c.671A>G (p.Asn224Ser, rs140506267) was found in two patients with SCO syndrome and

is listed in dbSNP with a very low MAF (0.0023). The third variant c.991G>C (p.Asp331His, rs139434590) was found in another patient with SCO syndrome. Both variants are predicted to be damaging for DMRT1 protein function. The putative mutation c.991G>C was also detected in heterozygous state in one of the control samples. The variant c.783C>T (rs34946058) was detected in two patients with mixed atrophy, but also in one of the fertile control samples. This variation causes no amino acid exchange (p.Pro261Pro). While in silico analysis showed no indication for the creation of alternative donor or acceptor splice sites, this variant entirely diminishes the exonic splicing enhancer (ESE) motif for SF2/ASF.
In summary, we detected two variants which are predicted to be damaging for DMRT1 protein function and also two variants which possibly generate an alternative donor or acceptor splice site. Because of these findings we suggest that DMRT1 mutations are rarely associated with male infertility.

P-Prenat-257

Loss of Msy2-mRNA interaction might affect transcript level and poly(A) tail length of maternal effect genes after postovulatory aging in mouse metaphase II oocytes

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Introduction: Mammalian oocytes are structured with functional domains like the subcortical RNP domain, subcortical maternal complex, or spindle-chromosome-complex (SCC), discussed in control of gene expression (1). mRNA stability and storage is regulated by the germ cell-specific RNA binding protein Msy2 (2). Knock-down of Msy2 in oocytes decreased mRNA and caused sub-fertility(2). Postovulatory ageing (PostOA) affects the transcript level and poly(A) tail length of select maternal effect genes (MEGs)(3).
Study question: Does PostOA affect the subcellular localization and abundance of Msy2 in in vivo and in vitro grown and matured oocytes exposed to PostOA, or trimethylation of histone H3K9?
Methods: Metaphase II oocytes were isolated 18h post the ovulatory stimulus after 13 days of preantral follicle culture4, or 12 hours later (PostOA, in vitro group), or after ovulation in vivo, or PostOA for 24h in M2 medium (PostOA, in vivo group).
Spindle, chromosomes, pattern of H3K9 trimethylation and distribution and abundance of Msy2 was analyzed by confocal microscopy(4,5) and Western Blot.
Results: PostOA in vitro caused an increase in spindle aberrations and unaligned chromosomes. Trimethylation of H3K9 was significantly decreased. Msy2 was in ooplasm, and enriched in the subcortical RNP domain and in the SCC in controls. Msy2 becomes redistributed to the central ooplasm upon PostOA in vivo. There is a shift in abundance and molecular weight of Msy2 protein upon maturation in Western Blot, and a dramatic decrease in Msy2 level during PostOA.
Conclusions: Epigenetic alterations in H3K9me3 were implicated in susceptibility to meiotic errors and epimutations (5), and in chronological aging of GV oocytes6. In accordance, H3K9me3 is also decreased in PostOA. Phosphorylation of Msy2 triggers maternal mRNA degradation during mouse oocyte maturation (2). PostOA in vivo for 24h and in vitro for 12h reduced the total mRNA content and poly(A) tail length of several MEGs essential for developmental competence (3). It is shown for the first time that Msy2 is enriched in the spindle at metaphase II. Similarly, Dnmt1 message is enriched at the spindle1 and reduced poly(A) mRNA was present after PostOA. Msy2 localization at SCC and in the subcortical RNP domain possibly provides for protecting and regulating MEG mRNAs and translation. Further studies have to show whether the mRNA deadenylation and degradation of MEGs is causally related to spindle alterations and phosphorylation, and redistribution and decrease in Msy2 protein during PostOA, thus contributing together with alterations in chromatin to changes in gene expression during development, increased susceptibility to meiotic errors, and reduced developmental potential.
1Romasko et al 2013; Genetics
2Medvedev et al 2008; DevBiol
3Dankert et al 2013; Abst. 7th IntConf FemRepTract
4Trapphoff et al 2010; HumRep
5Trapphoff et al 2013; FertilSteril
6Manosalva et al 2008; Theriogenology
(Supported by DFG)

P-Prenat-258

Gene Expression Patterns in a disturbed Karyotype: Keys to the Clinical Conundrum of Klinefelter Patients

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Background: Klinefelter Syndrome (KS) is the most common chromosome disorder in men (47,XXY), exhibiting a phenotype with marked variation, frequent hypogonadism and increased mortality. It is unclear whether and to what extent the genetic impact of the supernumerary X-chromosome contributes to the pathology.
Methods: EXAKT (Epigenetics, X-chromosomal features and Clinical Applications in Klinefelter syndrome Trial) is a Münster-based as yet largest prospective non-interventional project involving 132 Klinefelter men and their parents assessing a wide range of cardiovascular, inflammatory and metabolic factors in comparison to age-matched male (n=50) and female controls (n=50) in relation to genetic investigations. The main objective was to elucidate whether differential gene expression patterns could be detected in KS patients and whether these patterns would be related to inherent pathologies.
Results: Gene-expression was substantially disturbed in patients with KS vs. male and female controls, respectively. This dysregulation with differential expression of 36 not only X-chromosomal but also autosomal genes puts these phenotypical males into a genetic framework located between men and women with normal karyotypes. A range of these genes has previously been attributed to gender-specific modulations of immune responses. Simultaneously, the EXAKT KS cohort exhibited increased insulin resistance/inflammatory status, a procoagulatory state, higher waist circumference, dyslipidemia and an altered cardiac rhythmogenic setting (shorter QT-interval being partly located within the pathological range) vs. controls. Affiliatingly, the extent of clinical dyshomeostasis was associated with the degree of expression of dysregulated genes in KS. Paternal origin of the supernumerary X-chromosome was an additional enforcing confounder regarding insulin resistance and cardiac phenotype. In testosterone-treated KS patients, the pathophysiological pattern persisted in general, albeit depending on inflammatory-regulating gene expression.
Conclusions: In KS patients, the supernumerary X-chromosome contributes to a number of pathologies by altering the pattern of gene expression: insulin resistance, dyslipidemia, enhanced inflammation markers as well as altered cardiac rhythmogenic setting are involved; this was observable independently from testosterone substitution treatment which may have attenuated responses in KS.
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P-Technology and Bioinformatics

P-Techno-259

Ex-Exome - Sequencing in Human Genetics.

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The selection of genes based on the principle of cause and effect in hereditary diseases was and is still a reasonable paradigm in human genetic diagnostics. On this basement, the advent of Next Generation Sequencing (NGS) was consequently first evolved to simultaneously target and analyze comprehensive lists of known genes that have already been described as the cause of a specific genetic disease. The main limitation of these by definition termed "diagnostic panels" is, that design, improvement and maintenance of a diagnostic panel for a specific disease was and is hard to realize and can be obtained by Research and Development (R&D) teams only. Technically, the uniformity and completeness of coverage is hard to achieve, and logistically, new genes need to be included as soon as they are discovered. The initial genome- or exome- partitioning technologies for targeting these genes and their related costs are mostly stable nowadays, in principle still consume the same cost for targeting 1 Mb, 7 Mb, or even 30 Mb of the genome. Instead the sequencing cost dropped dramatically and will further decrease in the near future.
We utilized a highly flexible diagnostic Ex-Exome approach by using commercially available Exome contents paired-end sequenced on an Illumina machine, and build up a diagnostic pipeline focused on the selection, reliability and completeness of the analysis of those genes, that are in the range of a specific disease. National law for the diagnostic setup and analysis pipeline were addressed, as well as promises, limitations and validity of the technology regarding mutation types, error rate, sensitivity and specificity.

P-Techno-260

Computational prioritization of disease genes by network analysis

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In recent years, the knowledge about genetic variation in humans has increased substantially with the advent of next generation sequencing (NGS) methods. For instance, whole exome sequencing (WES) has been successfully established as a tool for discovery of new disease genes and diagnosis of patients with unknown syndromes. When the exome of a single human individual is sequenced and compared to a reference genome, 20.000 to 50.000 variants are usually identified. After removing synonymous and non-coding variants and presumably non-pathogenic variants present in public databases, several hundreds still remain. Because further manual filtering of all variants in question is very time-consuming, a variety of computational methods to predict disease-causing genes have been suggested, so-called gene prioritization methods. Among these, network analysis of protein-protein interaction networks has previously been successful in the prediction of disease genes. Here, we propose a new method for the prediction of causative genes based on 1.) the STRING database's protein-protein interaction (PPI) network and 2.) a set of seed genes derived from the individual patient's phenotypes, using associations between genes and phenotypes available as part of the Human Phenotype Ontology (HPO). After construction of the PPI network, the distribution of the ratio of the number of seed genes to the total number of genes in the vicinity of a candidate gene (i.e. at different distances from the corresponding network node) is analyzed. Distribution shapes are compared for verified disease genes (obtained from the OMIM database) and random genes. To predict the causative role of a candidate gene in an individual patient, the distribution of seed genes specific for the patient's phenotype is then compared to previously obtained distributions of verified disease genes. The method is analyzed for accuracy and applied to real patient data.

P-Techno-261

High throughput sequencing in a diagnostic laboratory: Pros and cons of enrichment technologies

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Molecular diagnosis of complex human genetic diseases is still challenging because in most cases multiple genes harboring putative deleterious mutations have to be analyzed. So far in most diagnostic laboratories Sanger sequencing is still used as the golden standard but capillary sequencing is excessive time-consuming and expensive at least for the screening of multiple genes. However, recently there was a shift away from Sanger sequencing after introduction of high-throughput sequencing methods, which are often collectively referred to as "next-generation" sequencing (NGS) which have facilitated substantial increases in sequencing content while dramatically decreasing the cost per base.
But because these technologies are originally introduced especially for large sequencing projects it is difficult to scale down this technology for screening disease causing genes in a diagnostic laboratory with its specific needs and requirements. To fill this gap table top NGS Systems have been introduced by Life Sciences (Ion Torrent PGM and Proton) by Illumine (MiSeq) and by Roche (GS Junior). While lllumina and Roche launched sequencer adapted to established technology Ion Torrent introduced a sequencing device using a sequencing technology based on the detection of hydrogen ions that are released during the replication of DNA. To validate this technology in respect to usability, software requirements and accuracy we tested several gene panels comprising between 3 and 420 genes covering between 100 and 16000 exons. Regions of interest were enriched in different ways: single PCR; multiplex-PCR (AmpliSeq: Ion Torrent; GeneRead NGS System: Qiagen) or HaloPlex custom designed kits (Agilent).
Our validation showed that technically multiplex PCR seems to be the superior technology for target enrichment because of its easy workflow, but that severe problems can emerge for proper detection of sequence variants if the user is not aware of its limitations. Especially the formation of sequencing blocks of the target region prevents the detection of complex variants at the ends of these blocks facilitating false negative claiming. This serious problem might impair the overall use of PCR amplification for target enrichment at least as long as software solutions cannot handle the problem.

P-Techno-262

Next-Generation Sequencing in the Molecular Diagnostics of Rare Diseases using a Gene Panel Approach

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The implementation of Next-Generation Sequencing in a clinical diagnostic setting opens vast opportunities through the ability to simultaneously sequence all genes contributing to a certain indication at a cost and speed that is superior to traditional sequencing approaches. Especially in the case of rare, heterogeneous disorders this may lead to a significant improvement in diagnostic yield. On the other hand, the practical implementation of NGS in a clinical diagnostic setting involves a

variety of new challenges which need to be overcome. Among these are the generation, analysis and storage of unprecedented amounts of data, strict control of sequencing performance, validation of results, interpretation of detected variants and reporting.
We present a panel approach for the molecular diagnostics of rare disorders. Exonic regions of more than 250 custom selected genes are enriched in parallel by oligonucleotide hybridization and capture (Illumina TSCE), followed by massively-parallel sequencing on the Illumina MiSeq instrument. During analysis, only genes from the requested indication (grouped in subpanels) are selected to limit analysis to relevant genes, while simultaneously minimizing the possibility of unsolicited findings. Data analysis is performed using the CLC Genomics Workbench (v.6.5.1, CLCbio) and custom developed Perl scripts. Target regions which fail to reach the designated coverage threshold of 20X are re-analyzed by Sanger sequencing. Additionally, identified candidate mutations are independently confirmed. All detected variants are imported into an in-house relational database scheme which may be queried via a web interface for dynamic data analysis and filtering. Information from all 250 genes is used in an anonymized way for internal variant frequency calculation, quality control and the detection of potential sequencing artifacts.
We have applied this approach to more than 200 samples from a variety of different disorders. In particular we use the outlined approach for the diagnostics of arrythmogenic cardiac disorders (LQTS, HCM, DCM), connective tissue disorders (EDS, TAAD), rare kidney disorders (Nephrotic Syndrome, CAKUT), neurological disorders (Noonan syndrome, Microcephalies), metabolic disorders (MODY diabetes) and coagulopathies.

P-Techno-263

Approaches to increase diagnostic yield for clinical genomic sequencing

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Exome and genome sequencing is increasingly utilized to diagnose individuals where other genetic testing has been unsuccessful or would be cost-inefficient. However, diagnostic yield estimates from clinical exome testing remain low (~25%). We have developed a comprehensive approach aimed at improving accuracy and completeness in genomic sequencing with specific improvements made in sequencing, variant calling, and interpretation to increase diagnostic yield.
Our approach has three parts:
1. Increased sequence coverage over regions of biomedical importance with the aim of creating a finished medical exome. On many samples, we quantified average coverage over all exons and developed specialized sequencing libraries to improve performance over regions with low (<20x) or absent coverage. We also targeted regulatory regions and other non-exonic regions known to contain disease and pharmacogenetic-associated variation.
2. Development of an approach integrating four orthogonal methods to identify and rank SVs based on their predicted likelihood of causing disease.
3. Implementation of a novel, knowledge-based ranking system to rank detected variation by likelihood of being causally related to the observed phenotype. Samples with described clinical features and known causative variants representing a broad range of conditions and variant types (including SVs) were obtained, sequenced, and our proprietary pipeline was used to align reads and call variants. Our internal database linking clinical features to genes was used to identify and rank candidate genes for each sample. We identified all possible inheritance patterns, and allowed for de novo events and non-penetrance, ranking genotype expectations by likelihood.
Our approach results in greater sensitivity to detect causal variation:
1. We increase the number of biomedically-relevant genes covered at >99% sensitivity for variant detection from 2000 to >3000.
2. We have substantially greater sensitivity (96.27% compared to 55.6% average), and a lower false discovery rate (1.37% compared to 27.55% average) on SV detection in genome sequence than any of the four methods used independently.
3. We reduce the number of candidate variants requiring manual review and in all cases tested the known causative variant was ranked first by our approach.
By increasing sequencing coverage over genes and variants of biomedical importance, improving detection of structural variation, and developing ranking approaches to highlight those variants most likely to be causally related to phenotype, we increase the likelihood that the underlying genetic etiology of cases submitted for exome or genome sequencing will be determined.

P-Techno-264

Similarity metrics for filtering duplicate entries in databases of genomic sequence variants

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Removing frequently detected variants is one of the most effective approaches to reduce the number of candidate mutations in the data analysis of next-generation sequencing studies. The incidence of a rare disorder in a population serves as an upper bound for the allele frequency or genotype frequency that can be used as a filter for dominant or recessive disorders. However, the frequentist inference requires that genotypes of a single individual are represented in the database only once. With many and decentralized data submitters the risks increases that samples of the same individual are sequenced multiple times and are contributed independently under different pseudonyms. We developed a metric that computes the distances to reference samples of the 1000 genomes project. The distance profile of a sample is a unique signature that may be used to assess whether a list of sequence variants has already been submitted. We show that this distance signature is highly specific for a sample but still error tolerant. This allows the identification of replicates from different enrichment procedures, sequencing platforms and bioinformatics pipelines. Furthermore the distance signature of a sample provides also the possibility to identify a pseudonymized sample
without using the sequencing variants itself and might help to protect medically sensitive patient information.

P-Techno-265

Framework based on Shannon-entropy of SNP-marker combinations for sample tagging in re-sequencing projects

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Sample tagging is designed for identification of accidental sample swap, which is among the major problems in re-sequencing studies. In this work, combinations of SNP-markers are evaluated in terms of Shannon-entropy, so that the fixed-length combinations approach the maximal entropy. The test results show that the optimized combinations of 23 SNP-markers can differentiate the individuals in the simulated samples with the comparable size to the present world population. The average Hamming distances among random individuals by the optimized 23-SNP-marker and 48-SNP-marker combinations, are 17 and 41, respectively. This scheme of samples re-identification and discrimi-

nation is proved robust with large sample size, including different ethnic groups. The optimized sets of SNP-markers are designed for Whole Exome Sequencing (WES), the burgeoning branch of re-sequencing projects, and a software is provided for customer-design. The sample tagging plan based on this framework will improve re-sequencing projects in a reliable and cost-effective way.

P-Techno-266

Integrated sequence analysis pipeline provides one-stop solution for identifying disease-causing mutations

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Defects of single genes play an important, previously underestimated role in the etiology of rare and common disorders, but the vast majority of such disease-causing changes are still unknown. Next generation sequencing (NGS) has greatly accelerated the search for these defects, and various bioinformatic tools have been developed to facilitate NGS-based variant detection, filtering, annotation and prioritization. Even for experts, however, the implementation of these tools can be a major challenge, and there is recent evidence that their performance is poorer than widely assumed.

To facilitate the processing and interpretation of NGS data in a clinical setting, we have developed a novel Medical Re-sequencing Analysis Pipeline (MERAP). MERAP assesses the yield and quality of sequencing results, and it has optimized modules for calling a broad spectrum of variants, including Single Nucleotide Variant (SNV), insertion / deletion (indel), Copy Number Variation (CNV), and other structural variants such as tandem duplications. MERAP identifies polymorphic variants as well as known disease-causing mutations by filtering against relevant public-domain databases, and it flags non-synonymous and splice site changes. MERAP uses its Logit score to estimate the likelihood that a given missense variant is disease-causing, by integrating various different pathogenicity scores. MERAP also considers other relevant information such as phenotype as well as interaction with known disease-causing genes and proteins. In various respects, MERAP compared favorably with GATK, one of the most widely used sequence analysis tools, e.g. because of its significantly higher sensitivity for detecting medium-sized indels, its easy and fast installation, minimal need for training and manual intervention and its economical use of computational resources. Upon testing more than 1200 individuals with mutations in known and novel disease genes, MERAP proved highly sensitive and specific, as illustrated here for 5 families with plausible, apparently disease-causing variants, including a novel ANKS1A mutation identified in a patient with autosomal recessive non-syndromic intellectual disability. We believe that the clinical implementation of MERAP will expedite the diagnostic process as well as the identification of many disease-causing gene defects that are hitherto still unknown.

P-Techno-267

Combined High Throughput Assay Design and Analysis Pipelines for DNA methylation analyses

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Epigenetics refers to the study of heritable changes in gene expression that are flexible enough to respond to environmental influences without changes in the underlying DNA sequence. Occurring epigenetic abnormalities are playing an important role in e.g. cancer, genetic disorders, autoimmune diseases, pediatric syndromes and aging. Therefore, epigenome-wide, mainly DNA methylation studies are increasing to complement genome-wide association studies and to search for novel disease genes, as well as clinically relevant biomarkers. However, feature selection in highly multivariate data (e.g. from genome-wide screening methods) often leads to a large proportion on false positive results. Thus, it is critical to put forward a large number of identified features for independent validation in larger sample cohorts. As a result we have developed several validated assay design pipelines for 100-1000 targets from high-throughput experiments. The pipelines provided can be used for primer design of methylation-sensitive restriction enzyme-based qPCR (MSRE), methylation-specific PCR (MSP), targeted deep bisulfite sequencing (TDBS), SNP-testing (SNP qPCR) and targeted resequencing. Furthermore, we have developed assay analysis pipelines for visual (methylation level, assay quality and database links) and statistical evaluation of qPCR results from MSRE, MSP, TDBS, SNP and targeted resequencing. All pipelines are integrated in the XworX platform, a user-friendly workflow-based software which can be downloaded from the XworX website (http://www.xworx.org). This systematic approach to epigenomic-wide screening by using bioinformatics pipelines can significantly increase the speed and success rate of DNA methylation studies, and significantly improve the ability to evaluate and compare laboratory results.

P-Techno-268

Infinium Methylation Assay Analyses Pipeline – A Key for genome-wide DNA methylation analyses

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Epigenome-wide association studies and technologies hold promise for the detection of new regulatory mechanisms that may be susceptible to modification by environmental and lifestyle factors affecting susceptibility to disease. DNA methylation as one of the epigenetics mechanisms plays a critical role in the regulation of gene expression and is known to be an essential factor in e.g. age-related diseases, cancer and some chronic diseases. The "Infinium HumanMethylation450 BeadChip" is a whole genome approach to interrogate methylation sites per sample at single-nucleotide resolution and several bioinformatic approaches for analyses are made. Nonetheless, one of today's challenges is the analysis of big data. Therefore, this pipeline aims on combining several approaches and introducing a graphical user interface, as well as adding additional statistical methods and annotation, for personalized, easy and fast data processing. The pipeline includes analyzing and visualizing of Illumina's 450k array data, normalization steps, peak correction, batch removal, as well as analysis of methylation patterns and gene expression leading to significant probe lists depending on user's choices. Thereby, we focus on transparency of data and methods by including quality reports of each analysis step and warnings if a statistical analysis step may not be appropriate. The pipeline is integrated in the XworX platform, a user-friendly workflow-based software which can be downloaded from the XworX website (http://www.xworx.org). This systematic approach to epigenomic-wide screening by using bioinformatics pipelines can significantly increase the speed and success rate of biomarker development.

P-Techno-269

Mutational Screening of BRCA1 and BRCA2 using the Ion AmpliSeq technology

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The introduction of benchtop Next generation sequencing (NGS) technologies in diagnostic laboratories offers a faster, more comprehensive

and cost-effective methodology for mutational screening than Sanger sequencing.
In this study, we present a strategy for the diagnosis of hereditary breast and ovarian cancer using Ion AmpliSeq multiplex PCR technology on the Ion Torrent PGM system (Life Technologies). A global consortium consisting of seven laboratories was created. The technical protocol was developed and piloted by centers in the Netherlands and Portugal, as well as tested and validated in five additional centers including our own laboratory. Non-overlapping primers were designed to cover all coding regions and exon-intron boundaries of the two tumor suppressor genes BRCA1 and BRCA2 resulting in a multiplex PCR approach of 167 amplicons using 30 ng of genomic DNA. 165 patients (~30 patients per center) with germline BRCA mutations previously detected by Sanger sequencing were selected, with the aim to assess the ability to detect and call the full spectrum of mutation types. Both BRCA1 and BRCA2 genes were amplified using the designed Ion AmpliSeq primer panels and sequenced on the Ion Torrent PGM system by loading eight barcoded samples onto an Ion 316 chip. Data from all PGM runs of each study center were processed using Ion Torrent Suite 3.1. Ion Reporter Software was used for mapping and variant calling. The target regions were covered 100% with a minimum coverage of 100x. Only one amplicon in exon 23 of BRCA2 gene was covered less (>60x) in some runs. All selected mutations including point mutations and deletions/insertions as well as mutations in homopolymer stretches were reproducibly detected, and no true variant was missed.
These BRCA gene analyses using Ion AmpliSeq multiplex PCR technology on the Ion Torrent PGM system are successfully performed in our routine molecular diagnostics. Barcoding enables the simultaneous analysis of ten patients on an Ion 316 chip. The SeqNext module of the Sequence Pilot software (JSI medical systems) is used for variant calling. Amplicons with insufficient coverage (<50x) are resequenced by the Sanger method. All identified disease-causing variants are validated using Sanger sequencing as well. Additionally, MLPA analyses are performed to identify exon deletions/insertions in the BRCA genes. To date, we have screened about 100 breast and/or ovarian cancer patients using this methodology. A disease-causing mutation has been identified in approximately 25% of the patients indicating a high sensitive strategy.
Furthermore, Ion AmpliSeq custom panel sequencing on Ion Torrent platforms provides a time- and cost-effective strategy for the identification of mutations and can be applied to other breast cancer genes and adapted to several disease groups. Additionally, the integration of copy number variation detection into this methodology will enhance the efficiency in the future.

P-Techno-270

High Resolution Melting (HRM) Analysis in Noonan Syndrome

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Noonan syndrome is an autosomal dominant disorder characterized by short stature, heart defects, developmental delay and typical facial appearance. Mutations causative for this syndrome have been found in genes encoding protein components of the RAS/MAPK pathway. Currently 8 genes are known to cause mainly Noonan syndrome, but 4 more genes are known that may cause one of the other RASopathies. It may be difficult to clinically distinguish Noonan syndrome from other RASopathies, especially in young children. Thus, up to 12 genes, comprising about 140 coding exons, may need to be analyzed when comprehensive genetic testing is considered. Sanger sequencing for such a number of amplicons is laborious and relatively expensive. Therefore we explored an alternative possibility of using High Resolution Melting (HRM) analysis to screen for mutations in patients with Noonan syndrome.
24 samples of patients with known mutations in different exons of the genes PTPN11, SOS1, RAF1, SHOC2 and KRAS as well as 267 patients with a phenotype suggestive of Noonan syndrome were included in this study. Genomic DNA was extracted from blood samples or saliva using different methods. Some samples required whole genome amplification (GenomiPhi V2) before usage, because very little DNA could be obtained. Mutation hot spot amplicons were selected from the 5 genes to include 32 HRM amplicons covering all known mutations in the respective genes, except for exons 1 and 4 in PTPN11 and KRAS respectively. High Resolution Melting analysis was performed on a Roche Light Cycler 480 instrument. In all of the 24 DNA samples with a known mutation the amplicon carrying the mutation was correctly identified by HRM. In the study cohort, only amplicons that produced curves deviating from normal were further analysed by Sanger sequencing, which is necessary to determine the specific mutation. Following this strategy, only 962 of the 8544 amplicons (11,3%) had to be sequenced. 126 patients (47,2%) were found to have a mutation in one of the amplicons and of those 88 (69,8%) had a mutation in the PTPN11 gene, 21 (16,7%) in SOS1, 11 (8,7%) in RAF1, 2 (1,6%) in KRAS and 4 (3,2%) in SHOC2.
High Resolution Melting was successfully used to pre-screen patients for the hot spot regions of some of the known RASopathy genes. HRM had been used for PTPN11 mutation screening in patients with Noonan syndrome before (Lo et al., Clinica Chimica Acta 2009; 409:75–77), but this is the first large scale project to have screened so many patients. Using HRM reduced the costs by 60% in our laboratory compared to standard bidirectional sequencing. HRM analysis is a robust method that works well with differently extracted DNAs and also whole genome amplified samples and saliva DNA. The method had 100% sensitivity in the cases with previously known mutations. We can conclude that HRM analysis is an alternative to screen for mutations in Noonan syndrome and the other RASopathies.

P-Techno-271

Development of a generic fully automated sequencing workflow using the Ion Torrent PGM as a conceivable replacement of Sanger sequencing in routine diagnostics

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Sanger sequencing has been the workhorse in molecularly working laboratories for more than three decades. With the implementation of NGS technologies, completely new possibilities are emerging, enabling to sequence several genes, and even complete exomes and genomes simultaneously. Most DNA laboratories are using these new technologies besides conventional Sanger sequencing, and choose between the different technologies dependent on their needs. However, it is also possible to essentially substitute the old Sanger sequencing technology, without any need for a major re-design of the sequencing approach. In our department, we offer, next to diagnostic exome sequencing, around 800 different genes for routine DNA diagnostics using a fully automated Sanger sequencing workflow. In addition, we are currently offering DNA diagnostics using the Ion Torrent PGM for the breast cancer genes BRCA1 and BRCA2, for the calcium-release channel gene RYR1, and for mitochondrial DNA. For each of these approaches, a specific design was developed in order to obtain most optimal results: the breast cancer genes are enriched using an AmpliSeq design, the RYR1 gene using an amplicon design optimized for PGM, and the mitochondrial DNA using a long-range PCR. Although these approaches are working well, we find that establishing and optimizing a new design for each gene or panel to be studied by NGS is costly and inefficient. We therefore investigated the possibility to transfer the complete automated Sanger sequencing workflow, which processes ~600.000 amplicons a year, into a completely automated NGS workflow using the Ion Torrent PGM. We here describe that it is possible to use the original Sanger-optimized amplicons, without any need for a re-design, and

sequence them on the PGM instead of performing Sanger sequencing. A special pooling strategy, pooling unique amplicons in common pools and recurrent amplicons in different pools, allows to perform a limited number of library preparations, and thereby reduces sequencing costs dramatically when compared to Sanger sequencing. Data analysis of the such generated sequencing data using the commercially available software SeqNext resulted in a sensitivity of 99.59% and specificity of 99.99%, claiming that the PGM is as good as the gold standard Sanger sequencing. Given these data, we are confident to replace our current fully automated Sanger sequencing workflow by a new, fully automated semiconductor sequencing workflow, and we believe that this will be initiating the end of the Sanger sequencing era.

P-Techno-272

MutAid - A NGS based integrated and user-friendly mutation screening and decision support system for human molecular diagnostics

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Recent development of high throughput, next generation sequencing (NGS) technology has revolutionized the molecular diagnosis of human genetic disease. The ability to generate enormous amount of sequence data in a short time at an affordable cost makes this approach ideal for a wide range of applications from sequencing a group of candidate genes, all coding regions (exome sequencing) to the entire human genome. The technology brings about an unprecedented application to the identification of the molecular basis of genetic disorders caused by rare and common genetic variants. However, the benefits offered by NGS technologies come with a number of challenges such as NGS data management, quality control, mapping, variant calling and their annotation via a robust and user-friendly pipeline that must be adequately addressed before they can be transformed from research tools to routine clinical practices.

We have developed a mutation screening and decision support pipeline for analysis of data generated by the most common sequencing platforms: Illumina, 454, Ion Torrent, and traditional Sanger Sequencing. The system furthermore allows the clinician to identify causal mutations through NGS data analysis and suggests which regions need to be validated with ABI Sanger sequencing. The analysis detects SNVs, insertions, deletions, copy number variations and translocations. Multiple patients can be analyzed in parallel and data is managed efficiently which allows easy and fast inter-patient multi-platform analyses and comparisons. One example is its use in breast cancer risk assessment by analyzing sequencing data (patients and family background) produced by a commercial BRCA1/2-genes test kit. Another is the targeted amplicon re-sequencing data analysis. Support is offered in decision-making as mutations are visualized using the UCSC genome. Corresponding information of common SNP-databases is linked. By the integrated report-generation module our system facilitates and improves the whole process of decision-making and the elaboration of diagnostic findings. This helps improving diagnosis quality and saves valuable time of the clinician. All sequence variants are automatically identified in a robust pipeline developed for specific sequencing platform and annotated with human reference genomes. In order to keep the records all operations and manipulations on the imported data is tracked transparently. MutAid therefore enables efficient and well documented analyses of complex sequencing data, paving the way towards efficient whole genome sequence analysis.

P-Techno-273

DMD deletions comprising partial exonic sequences not detected by MLPA analyses

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Duchenne muscular dystrophy (DMD) is caused by mutations in the X-linked DMD gene encoding dystrophin, a large rod-shaped protein interacting with integral membrane glycoproteins of the sarcolemma in skeletal muscle fibers. The most common causes are exon-spanning deletions or duplications typically leading to frame-shifts and premature stop codons in the dystrophin transcript. These copy number variations (CNVs) are usually detected in patients by multiplex ligation-dependent probe amplification (MLPA).

Here, we report on two DMD patients with highly elevated creatine kinase (CK) levels, in whom no CNVs in the DMD gene could be detected by MLPA analyses. Amplification of all 79 exons of the DMD gene for direct sequencing revealed no PCR products for exon 12 in patient 1 and for exon 46 in patient 2. Multiplex PCR analyses in these patients confirmed the loss of exon 12 and exon 46, respectively, which disagrees with the results of the MLPA analyses. As the adjacent introns of the respective exons are very large, a primer walking strategy followed by Sanger sequencing was performed in order to detect the breakpoints of the deletions in the DMD gene of both patients. In patient 1, a deletion of 258 bp was detected comprising 188 bp of intron 11 and 70 bp of exon 12. According to Alamut (Interactive Biosoftware), this deletion alters the acceptor splice site of exon 12 and skipping of this exon is very likely. In patient 2, extensive primer walking revealed a fragment consisting of a sequence segment located deeply in intron 45 and a part of exon 46. Sanger sequencing of this fragment showed a large deletion involving about 13,000 bp of intron 45 and 17 bp of exon 46 combined with an insertion of 50 bp. This indel also leads to an alteration of the acceptor splice site, presumably resulting in skipping of exon 46. Most likely, both deletions detected are causative for the muscular dystrophies in patients 1 and 2.

Although only the 5'-parts of exons 12 and 46 are deleted, amplifications of these exons completely failed because the forward primers for sequencing and multiplex PCRs are located within the deleted intronic regions in both cases. MLPA analyses in contrast didn't detect these deletions because the respective probes are located in the non-deleted downstream regions of exons 12 and 46, respectively. A recently performed next generation sequencing (NGS) approach on an Illumina MiSeq after enrichment of 37 muscle disease genes by Nextera Rapid Capture (Illumina) confirmed the breakpoints of both CNVs. In patient 1, the deletion and its breakpoints could directly be identified using the software GensearchNGS (PhenoSystems), while in patient 2 the more complex CNV required some additional bioinformatic analyses, especially BLAST.

Generally, MLPA analyses and time-consuming primer walking strategies for detailed characterization of CNVs could now be replaced by more convenient enrichment methods based on hybridization combined with NGS technology.

P-Techno-274

Rapid exome sequencing using the Ion Proton™ System and Ion AmpliSeq™ Technology

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Exome sequencing reveals important genetic variation through interrogation of the interpretable part of a genome via enrichment strategies that target all the coding regions of a genome. Ion AmpliSeq™ technology has been expanded to target the human exome to create an easy-to-

implement, cost-effective, flexible exome sequencing workflow using the Ion Proton™ System with the Ion PI™ Chip, and data analysis using Ion Reporter™ Software. The simplicity and speed of PCR allows the most rapid method of exome enrichment with a total library construction time of <6 hours, requiring the least amount of hands-on time and the fewest pipetting steps compared to alternative capture methods.
The technology enables the enrichment and sequencing of over 19,000 genes in 294,000 amplicons in a two day workflow. Results show high uniformity with >92% of targeted bases covered at 20x, with two exomes per Ion PI ™ v2 chip; 88% coverage at 20x for 3 exomes samples per chip. Exome-specific workflows in Ion Reporter™ Software enabled the identification of high confidence loss-of function variants through trio analysis.

P-Techno-275

Micro-RNA depletion protocol for next generation sequencing technology in blood samples

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Micro-RNAs (miRNAs) are a class of small non-protein coding RNAs playing a role in post-transcriptional gene regulation. Deregulation of miRNA expression has been observed in numerous human pathologies as cancer, inflammation, intellectual disability, neurodegeneration and metabolic or organic diseases. Within the last decade miRNA expression analyses via microarrays as well as next generation sequencing techniques have become an important tool in clinical research where blood represents a widely used and easily obtained sample type. However, expression profiling of blood samples implicate many challenges since it is composed of heterogeneous cell types, and the different methods for handling and processing blood may cause changes in expression profiles ex vivo.
Another challenge that comes along with mRNA expression profiling in whole blood samples is the strong expression of globin that results in decreased Percent Present calls, decreased call concordance, and increased Signal variation in microarray analysis and a saturation of the amount of sequenced reads in next generation sequencing techniques. Globin reduction protocols have been established to overcome this issue.
We have discovered that a similar problem arises with miRNA-486-5p, applying next generation sequencing technology on miRNA expression profiling in blood samples. miRNA-486-5p appears to be a vast abundant miRNA in RNA samples deriving from blood and has been considered to be a plasma miRNA biomarker expressed by blood cells. miRNA-486-3p is actually involved in the regulation of γ–Globin expression in human.
According to globin reduction protocols we have developed a new protocol for miRNA-486-5p depletion based on bead technology. Using a biotin labelled antisense oligonucleotide and streptavidin beads we could reduce miR-486-5p expression up to 300 fold. This depletion was confirmed via qRT-PCR. Possible off-target effects were analyzed by microarray and next generation sequencing experiments. The miRNA-486-5p reduction achieved in this protocol is sufficient to perform next generation sequencing technology with blood samples successfully.

P-Techno-276

Exomate: an easy to use exome sequencing analysis pipeline

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High throughput exome sequencing is a widely used technology for deciphering mutations in the coding regions of a genome at relatively low cost. While bioinformatics analyses of exome sequencing data mostly agree on best practices regarding the analysis steps (e.g. using the GATK guidelines), called genomic variants rely on the set of parameters and applied filtering.
We present Exomate, a software that combines a best practices variant calling workflow with a web frontend. By storing the called variants in a PostgreSQL database, Exomate allows filtering and parameter tuning in almost real time. This enables iterative tuning of thresholds, or the selection of different samples for filtering by non computer scientists via the web interface. The web interface presents metadata, annotations, quality control data and statistics to facilitate a comprehensive data analysis on different levels. So far, the software has been used in several successful studies, e.g. [1,2,3].
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[2] Czeschik et al. „Clinical and mutation data in 12 patients with the clinical diagnosis of Nager syndrome". Human Genetics 2013
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P-Therapy for Genetic Disease

P-Therap-277

Development of a new therapeutic strategy for glutaric aciduria type 1

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Glutaric aciduria type 1 is an autosomal recessive metabolic disorder caused by mutations in GCDH. It is characterized by gliosis and neuronal loss in the basal ganglia and a progressive movement disorder that usually begins during the first year of life. The only available treatment is a protein reduced diet, which reduces the accumulation of the neurotoxic metabolites glutaric acid, 3-hydroxyglutaric acid, and glutaryl-CoA. The analysis of glutaric aciduria type I is part of the newborn screening in Germany and revealed 52 patients between 1999 and 2009. The outcome of these patients was evaluated in relationship to therapy. The treatment lowers the risk of movement disorder, but less than half of patients follow the strict recommendation. Recently, by exome sequencing of individuals with 2-aminoadipic and 2-oxoadipic aciduria we identified mutations in DHTKD1, coding for dehydrogenase E1 and transketolase domain-containing protein1. We could show that this enzyme acts directly upstream of GCDH in the L-lysine-degradation pathway, mediating the last unresolved step in the L-lysine-degradation pathway.
Since patients with DHTKD1 mutations showed only a mild phenotype with developmental delay, this opened the possibility to treat glutaric aciduria type1 patients with DHTKD1 inhibitors. In order to verify this treatment option, we created a Dhtkd1 knock-out mouse using a TALENs (transcription activator-like effector nucleases) approach. These nucleases bind to the desired DNA sequence and disrupt gene function by small insertions or deletions with high specificity. Our initial characterization of the Dhtkd1 mouse model revealed no obvious neurological phenotype.

An existing Gcdh mouse is known to develop a severe phenotype upon lysine-rich diet. To clarify whether inhibition of DHTKD1 activity can rescue the severe phenotype of GCDH deficiency we are currently generating the Gcdh/Dhtkd1 double knock-out mouse.
This approach mimics the condition of a glutaric aciduria type 1 patient, who receives an inhibitor for DHTKD1 activity. In parallel, we are working on a DHTKD1 enzyme activity assay to investigate the effect of potential DHTKD1 inhibitors.
This study aims to develop a drug balancing the metabolic flow in the lysine degradation pathway, which should result in an improved clinical outcome of patients with glutaric aciduria type 1.

P-Therap-278

Molecular pathology of X-linked megalocornea and translational concept for regenerative medical therapies of corneal injuries

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Corneal lesions are a frequent cause for blindness worldwide. Advances in cellular regeneration concepts using autologous limbal stem cells or ex vivo cornea tissue engineering are becoming reasonable therapeutic options for allogenic transplantation. Thus, more knowledge about the regulatory gene network underlying corneal organogenesis is needed to target specifically cellular differentiation mechanisms. This prompted us to search for stem cell niche and differentiation factors in the cornea by unravelling the molecular pathology of monogenic cornea disorders with presumably disturbed differentiation processes.
By biometric corneal analysis of affected male members of a family with X-chromosomal recessive megalocornea we assumed that typical features of the disorder like the non pressure-associated cornea enlargement as well as the deep anterior eye chamber might be caused by accelerated cornea maturation during embryonic development. The identification of a disease causing frameshift mutation in the Chordin-like 1 (CHRDL1) gene which encodes for the bone morphogenetic protein (BMP) 4/7 antagonist Ventroptin supported this assumption as the affected TGFβ-signalling is essential for cellular differentiation. By expression analysis of Ventroptin and BMP4 in normal cornea samples from anatomical sections we found a protein distribution pattern consistent with the assumed Ventroptin-BMP4 antagonism especially in the corneal epithelium. While BMP4 was more or less uniformly distributed over the whole corneal epithelium Ventroptin was strongly expressed in the basal epithelium with a high-low expression gradient from the limbal stem cell niche to the mid corneal region.
By RT-PCR analysis using lymphocytes and granulocytes of the patients and controls we could demonstrate that two mutational effects have to be considered depending on the cellular context. While the frameshift mutation of CHRDL1 caused nonsense-mediated RNA decay in granulocytes, the mutated CHRDL1 mRNA was not completely abolished in lymphocytes most probably resulting in a truncated Ventroptin. In this case the remaining shortened protein is presumed to contain two of the three Cysteine-rich domains which are essential for the BMP4 binding affinity. At least this should lead to a partial reduction of protein function if there is not a higher impairment by protein misfolding. These findings could be a starting basis for understanding the phenotypic variability of X-linked megalocornea, but also contribute to insights into protein structure-function relations with regard to therapeutic approaches using recombinant Ventroptin to manipulate cellular differentiation processes.
In conclusion, our study shows that the elucidation of the molecular pathology of monogenic cornea disorders like X-linked megalocornea could contribute to the identification of stem cell niche factors of the corneal limbus which might be used as targets in regenerative medical therapies of corneal injuries.

P-Therap-279

Protein substitution in keratinocytes for enzyme replacement therapy in autosomal recessive congenital ichthyosis

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12R-LOX and eLOX-3 belong to the epidermis-type lipoxygenases (LOX) and are crucial enzymes acting in the same pathway involved in building up the skin barrier. Mutations in ALOX12B and ALOXE3 lead to autosomal recessive congenital ichthyosis (ARCI), a cornification disorder which is characterized by impaired skin barrier function, scaling and erythema. About 15% of ichthyosis-patients have mutations in one of these two LOX genes. So far only symptomatic treatment like moisturizing creams, bathing and mechanical removing of the skin scales are available to ameliorate the phenotype. Hence, there is a clear demand for new, causative treatment options. Substitution of the protein either via a cream formulation or using newly developed, advanced topical drug delivery systems will be an enormous benefit for the patients.
Therefore we have cloned the genes ALOX12B and ALOXE3 from differentiated keratinocytes and transferred into GFP- and His-tagged plasmid vectors, respectively. Using the GFP-tagged vectors two mammalian expression systems (HEK 293F-cells, CHO-cells) have been tested under different conditions in order to produce the active enzymes 12R-LOX and eLOX-3. Transfection efficiency was analysed with fluorescence microscopy and quantified with FACS analysis. ALOX12B showed the highest expression after 48-72 hours and cell lysates were collected at these time points. Protein expression was then determined by Western blot analysis. In a next step HEK cells are transfected with His-tagged 12R-LOX and eLOX-3 in a larger-scale setup in order to purify the enzymes from the whole cell lysates and to test them for their specific activity. Since the active enzymes 12R-LOX and eLOX-3 are needed for restoration of the LOX pathway in mutant keratinocytes and the products of these enzymes are unstable, activity assays are challenging. In addition to RP-HPLC analysis of LOX reaction products, we will thus analyse critical lipid components involved in keratinocyte differentiation and the formation of the epidermal barrier, and contributing to the pathophysiology of ARCI. Purified enzymes are packaged for drug delivery and applied to our in vitro patient skin models.
This approach of therapy is flexible and personalized and in comparison to the currently available symptomatic treatment it aims to substitute the missing protein. Once the principle is established we will adapt this type of therapy also to other known genes involved in the development of congenital ichthyosis.

Autorenregister

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