VORTRÄGE

PLENARY SESSIONS

Plen1

Human Transgenerational Responses

Marcus Pembrey

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Transgenerational effects of maternal and even grand-maternal nutrition or other environmental 'exposures' are to be expected, although unpicking the influences is difficult. Effects down the male line were not expected because a novel inheritance mechanism would have to be postulated if genetic and social transmission can be excluded. Mammalian experiments now provide evidence of such male line transgenerational responses to diet or stress that implicate transmission of epigenetic marks or ncRNAs. The few human observational studies suggest (male line) transgenerational responses exist that are unlikely to be due to cultural and/or genetic inheritance. Historical studies of ancestral food supply in Sweden and ongoing UK cohort studies of parental early life smoke exposure reveal exposure sensitive periods during development and sex differences in transmission and offspring outcomes. Human observational data will be presented that suggest early-life parental or ancestral exposures do indeed contribute to developmental variation in the population; and so confound present studies of common complex diseases (Pembrey et al. J Med Genet. 2014 Sep;51(9):563-72).

Plen2

Transcriptome dysregulation and single cell analysis in Trisomy 21

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Trisomy 21 is the most frequent genetic cause of cognitive impairment. To assess the perturbations of gene expression in trisomy 21, and to eliminate the noise of genomic variability, we studied the transcriptome of fetal fibroblasts from a pair of monozygotic twins discordant for trisomy 21. We have shown that the differential expression between the twins is organized in domains along all chromosomes that are either upregulated or downregulated. These gene expression dysregulation domains (GEDDs) can be defined by the expression level of their gene content, and are well conserved in induced pluripotent stem cells derived from the twins' fibroblasts. Comparison of the transcriptome of the Ts65Dn mouse model of Down's syndrome and normal littermate mouse fibroblasts also showed GEDDs along the mouse chromosomes that were syntenic in human. The GEDDs correlate with the lamina-associated (LADs) and replication domains of mammalian cells. The overall position of LADs was not altered in trisomic cells; however, the H3K4me3 profile of the trisomic fibroblasts was modified and accurately followed the GEDD pattern. These results indicate that the nuclear compartments of trisomic cells undergo modifications of the chromatin environment influencing the overall transcriptome, and that GEDDs may therefore contribute to some trisomy 21 phenotypes.

The study of gene expression in mammalian single cells via genomic technologies now provides the possibility to investigate the patterns of allelic gene expression. We used single-cell RNA sequencing to detect the allele-specific mRNA level in 203 single human primary fibroblasts over 133,633 unique heterozygous single-nucleotide variants (hetSNVs). We observed that at the snapshot of analyses, each cell contained mostly transcripts from one allele from the majority of genes; indeed, 76.4% of the hetSNVs displayed stochastic monoallelic expression in single cells. Remarkably, adjacent hetSNVs exhibited a haplotype-consistent allelic ratio; in contrast, distant sites located in two different genes were independent of the haplotype structure. Moreover, the allele-specific expression in single cells correlated with the abundance of the cellular transcript. We observed that genes expressing both alleles in the majority of the single cells at a given time point were rare and enriched with highly expressed. Overall, these results have direct implications in cellular phenotypic variability. Single cell transcriptome analysis in trisomy 21 will be discussed.

SYMPOSIA

S1-01

Mechanisms of aging associated stem cell mutations and cancer formation

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Aging is characterized by an accumulation of DNA damage and the evolution of diseases and cancer. Increasing evidence indicates that stem cells are the cell type of origin of cancer formation. In the hematopoietic system it was shown that stem cell mutations and clonal hematopoiesis precede cancer formation. Molecular mechanisms that contribute to the induction of mutations and clonal selection of mutant stem cell and progenitor cells during aging remain yet to be delineated. In previous work we showed that DNA damage limits the self renewal of HSCs by inducing BATF-dependent stem cell differentiation. During my talk I will present novel mechanisms that contribute to the induction of mutations and transformation of HSCs in response to aging and DNA damage accumulation.

S1-02

Autophagy and longevity

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Spermidine is a ubiquitous polycation that is synthesized from putrescine and serves as a precursor of spermine. Putrescine, spermidine and spermine all are polyamines that participate in multiple known and unknown biological processes. Exogenous supply of spermidine supresses necorsis and prolongs the life span of several model organisms including yeast (Saccharomyces cerevisiae), nematodes (Caenorhabditis elegans) and flies (Drosophila melanogaster) and significantly reduces age-related oxidative protein damage in mice, indicating that this agent may act as a universal anti-aging drug. Spermidine induces autophagy in cultured yeast and mammalian cells, as well as in nematodes and flies. Genetic inactivation of genes essential for autophagy abolishes the life span-prolonging effect of spermidine in yeast, nematodes and flies. These findings complement expanding evidence that autophagy mediates cytoprotection against a variety of noxious agents and can confer longevity when induced at the whole-organism level. We hypothesize that increased autophagic turnover of cytoplasmic organelles or long-lived proteins is involved in many age associated diseases.

S2-01

Selfish mutations in the testis: implications for genetic disease

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The clonal expansion of pathogenic mutations in somatic tissues is associated with phenotypes such as tumour and tissue overgrowth; but such ‘acquired’ mutations are typically not inherited. However, the situation is different when a similar oncogenic process takes place in the special context of the testis. In this case, clonal expansion of pathogenic mutations may lead not only to the formation of testicular tumours but also to elevated levels of specific mutations in sperm, and consequently an increased risk of transmission of pathogenic alleles to the next generation. This process, which we termed selfish spermatogonial selection, has been described to explain the paternal age-effect and the high birth prevalence observed for some rare spontaneous disorders, such as Apert syndrome (FGFR2) or achondroplasia (FGFR3). We will review our current understanding of this selfish process and discuss its potential contribution to genome heterogeneity and the pathology of other disorders – including cancer predisposition and neurodevelopmental disorders (i.e. schizophrenia and autism). Given that this process, which affects all men as they age, is anticipated to be associated with an increased risk of transmission of functional (pathogenic) alleles, it is likely to be particularly relevant for ageing reproductive populations.

S2-02

Genetic mosaicism in neurological disease

Annapurna Poduri

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Genetic mutations that cause human disease are conventionally considered to be inherited from one's parents and present in all of the cells of the body. We know, however, that most mutations that cause cancer arise somatically, and we have become increasingly aware of mutations that cause other diseases and that arise de novo, meaning they are undetectable in the parents. Some such de novo mutations arise in the gamete of a parent, but others arise after fertilization during embryonic development, generating post-zygotic, or "somatic" mutations that are present in a "mosaic" state, with only some cells carrying the mutations and others not. Somatic mutations occur in several neurodevelopmental diseases associated with epilepsy and intellectual disability, although their broader relevance for neurological disease is unknown. We will discuss some key examples, including the developmental brain disorders hemimegalencephaly and focal cortical dysplasia, recently shown to arise from both germline and somatic mutations.

S2-03

Mosaicism in dermatological diseases

Rudolf Happle

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The concept that all nevi represent mosaics has now been corroborated at the molecular level. For example, the various types of epidermal nevi originate from at least 11 different postzygotic mutations, whereas the CHILD nevus reflects lyonization. – Epigenetic mosaicism explains the patterns of lyonization and some linear pigmentary disturbances reflecting autosomal monoallelic expression. – In the category of genomic mosaicism, the concept of lethal genes surviving by mosaicism was proven in 10 different phenotypes including Proteus syndrome and giant melanocytic nevus. – In autosomal dominant skin disorders, a type 2 segmental manifestation has today been confirmed by molecular analysis in six different traits including PTEN hamartoma syndrome and Gorlin syndrome. – In some types of epidermolysis bullosa, a postzygotic back mutation may result in patches of healthy skin. Such revertant mosaicism may help develop new therapeutic strategies. – An example of allelic didymosis (twin spotting) is cutis tricolor, representing a heterogeneous group of disorders. – Possible clinical examples of superimposed segmental manifestation of polygenic skin disorders include psoriasis, atopic dermatitis, and many other common cutaneous diseases. Molecular analysis comparing the tissue of pronounced segmental involvement with the remaining skin may yield new insight into the complex genetic predisposition to such disorders.

S3-02

The analysis of gene and chromosomal interactions by 3C/4C

Britta Bouwman, Carlo Vermeulen, Geert Geeven, Peter Krijger, Michael Eyres, Atze J. Bergsma, Marjon Verstegen, Yun Zhu, Yori Schell, Patrick J. Wijchers, Elzo de Wit and Wouter de Laat

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Chromosome conformation capture (3C-based) technologies enable uncovering the folding of chromosomes inside the cell nucleus at unprecedented resolution. They have shown that genomic functioning can follow form, with for example enhancers physically looping towards their target genes, in the mammalian genome over distances sometimes larger than a megabase. 3C-based methods have also demonstrated that chromosomes are structurally organized into topologically associated domains (TADs): chromosomal segments within which sequences preferentially contact each other. TADs are thought to serve as structural molds that direct physical contacts between enhancers and the target genes that are contained within the same TAD, while preventing regulatory contacts and gene activation across TAD borders. While these topological units impose relevant constraints on the wiring of the transcription regulatory network, there is also evidence for regulatory DNA interactions across TAD borders and even between TADs on different chromosomes. For example, we showed in the past that an ectopic super enhancer on one chromosome can activate a natural target gene on another chromosome. This however was apparent only in selected cells that happened to accommodate this inter-chromosomal contact, resulting in variegated gene expression or transcriptional noise. Beyond the level of TADs, chromosomes fold such that active and inactive TADs each

cluster among themselves and therefore physically separate in the nuclear space, with inactive TADs often occupying more peripheral nuclear positions and active TADs positioned more towards the interior. In this lecture we will present novel strategies that we are developing and new insight into the 3D genome of mammalian cells.

S4-01

The sperm epigenome (and its implications for the embryo)

Klaus Steger

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Epigenetic mechanisms regulate which genes will be expressed at a specific time point in a distinct cell-type. While it is well-known that mother´s lifestyle influences the distribution pattern of epigenetic marks which may in addition be transmitted to her offspings (1), it has recently been reported that also father´s lifestyle may have an effect on gene expression in the offsprings (reviewed in 2) suggesting that sperm must transmit epigenetic information to the oocyte at fertilization.

Epigenetic processes influence gene expression via DNA-methylation, various histone modifications and short non-coding RNAs (reviewed in 3). DNA-methylation occurs at cytosine residues within CpG dinucleotides (CpG-islands). Apart from imprinted genes exhibiting a parent-of-origin-dependent gene expression, most gene promoters are hypomethylated resulting in transcriptional activation, while promoter hypermethylation is associated with gene silencing. There exists a variety of core histone modifications, however, the most extensively studied modifications are acetylation of lysines, mono-, di- or trimethylation of lysines and mono- or dimethylation of arginines. While histone acetylation is in general associated with chromatin expansion and gene transcription, the effect of histone methylation on gene expression can be either activating or repressing depending on the position of the amino acid and the number of methylated residues. In sperm, the replacement of histones by protamines represents an additional epigenetic mechanism, which results in chromatin condensation followed by gene silencing of the entire genome.

As histone-to-protamine exchange is incomplete, remaining histones carrying epigenetic marks have been hypothesized to be involved in the initiation of gene expression in the early embryo. Consequently, several studies performing deep-sequencing have been published within the last six years (reviewed in 4). Indeed, spermatozoal nucleosomes have been reported to be enriched at loci of developmental importance, such as imprinted gene clusters, microRNA clusters, HOX gene clusters and promoters of developmental transcription factors (5,6). Subsequently, retained nucleosomes have been attributed to GC-rich sequences lacking DNA-methylation (7,8). Recently, nucleosome retention in sperm was reported to be associated with distal intergenic regions and introns, centromere repeats and retrotransposons, whereas 5´ and 3´ untranslated regions as well as transcriptional start and termination sites have been demonstrated to reveal nucleosome depletion (9,10). Interestingly, the preparation method seems to be of pivotal importance for the outcome of deep sequencing studies (10).

References: (1) Wolff et al. 1998 FASEB J 12:949-957, (2) Hughes 2014 Nature 507:22-24, (3) Schagdarsurengin et al. 2013 Nature Reviews Urology 9:609-619, (4) Saitou & Kurimoto 2014 Dev Cell 30:6-8, (5) Hammoud et al. 2009 Nature 460:473-478, (6) Arpanahi et al. 2009 Genome Res 19:1338-1349, (7) Vavouri & Lehner 2011 PLoS Genetic 7e1002036, (8) Erkek et al. 2013 Nat Struct Mol Biol 20:868-875, (9) Samans et al. 2014 Dev Cell 30:23-35, (10) Carone et al. 2014 Dev Cell 30:11-22.

S5-02

Gene therapy in monogenic diseases

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The first gene therapy clinical trials were initiated more than two decades ago. In the early days, gene therapy shared the fate of many experimental medicine approaches and was hampered by the occurrence of severe side effects in a few treated patients. The understanding of the molecular and cellular mechanisms leading to treatment- and/or vector-associated setbacks has resulted in the development of highly sophisticated gene transfer tools with improved safety and therapeutic efficacy. Employing these advanced tools, a series of Phase I/II trials were started in the past few years with excellent clinical results and no side effects reported so far. Moreover, highly efficient gene targeting strategies and site-directed gene editing technologies has been developed and applied clinically. With more than 1900 clinical trials to date, gene therapy has moved from a vision to clinical reality. This presentation focuses on the application of gene therapy

for the correction of inherited diseases, the limitations and drawbacks encountered in some of the early clinical trials and the revival of gene therapy as a powerful treatment option for the correction of monogenic disorders.

S6-01

DNA polymerase proofreading mutations in cancer

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Three DNA polymerases - Pol α, Pol δ and Pol ε - are essential for DNA replication. After initiation of DNA synthesis by Pol α, Pol δ or Pol ε take over on the lagging and leading strand respectively. Pol δ and Pol ε perform the bulk of replication with very high fidelity, which is ensured by Watson-Crick base pairing and 3'exonuclease (proofreading) activity. Yeast models have shown that mutations in the exonuclease domain of Pol δ and Pol ε homologues can cause a mutator phenotype. Recently, we identified germline exonuclease domain mutations (EDMs) in human POLD1 and POLE that predispose to 'polymerase proofreading associated polyposis' (PPAP), a disease characterised by multiple colorectal adenomas and carcinoma, with high penetrance and dominant inheritance. Moreover, somatic EDMs in POLE have also been found in sporadic colorectal (3-5%) and endometrial (7-8%) cancers. Tumors with EDMs are microsatellite stable and show an 'ultramutator' phenotype, with a dramatic increase in base substitutions. Early evidence shows that these cancers have distinct somatic mutation profiles and clinical behaviours, such as a generally good prognosis. In the modern era, medical practice needs to identify small groups of patients such as the ultramutators and treat them appropriately.

S6-02

Familial Breast/Ovarian Cancer - more than BRCA1 and BRCA2

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It is now 20 years since the BRCA1 and BRCA2 genes were localized and identified. We know a tremendous amount about how these genes work, and great strides have been made in applying this knowledge clinically, most notably in the development of novel therapeutic approaches to cancers arising in mutation carriers. In addition, detailed, large-scale epidemiological studies have resulted in accurate estimations of cancer risks, and modifier genes are likely to be useful in the future to help stratify mutation carriers into clinically-useful risk categories. Despite these advances, 85% of the familial risk, and 60% of strongly heritable breast cancer remains unexplained, and similarly, a significant fraction of the genetic causes of ovarian cancer remains unexplained.

In this presentation, I will discuss newer breast and/or ovarian cancer susceptibility genes such as PALB2, ATM, CHEK2, RAD51C, RAD51D, BRIP1 and others. I will also weigh up the role of Lynch syndrome genes in breast and ovarian cancer risk. First, I will discuss the genes, their key functions and their contribution to breast/ovarian cancer risk. Then I will introduce gene panel testing for breast/ovarian cancer, and consider the candidacy of these genes in the setting of testing unaffected women in the medical genetics clinic. Following on from this, I will debate the pros and cons of population-based testing for BRCA1, BRCA2 and other related genes. Finally, I will review data that relates to the use of germ-line genetic information about breast/ovarian cancer genes in making therapeutic decisions.

Talk nach 12

Genom Austria: A project to explore the impact of genome sequencing on science and society (the talk and subsequent discussion will be in German)

Christoph Bock for Genom Austria

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‘Genom Austria’ explores the broader implications of personal genome sequencing. It is a joint project of the CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, the Medical

University of Vienna, and the PersonalGenomes.org foundation, and a member of the Global Network of Personal Genome Projects. Following the model of Harvard's Personal Genome Project, Genom Austria provides qualifying volunteers the opportunity to sequence their personal genomes and to share the data with the public. Participation is entirely voluntary and restricted to individuals who demonstrate adequate understanding of the implications of making their personal genomes publicly available. In its initial phase in 2015, Genom Austria will sequence the personal genomes of 20 selected volunteers who have consented to publish their genomes and related information openly and freely on the Internet. Genom Austria will also contribute to science education by organizing a school project and open science workshops. More generally, it creates a forum for interdisciplinary dialog among experts and the general public in areas such as biology, medicine, ethics, sociology, psychology, history, and the arts.

An introductory talk about personal genomes and Genom Austria will be followed by a plenum discussion on the genomic future of medicine and the shifting roles of genetics and genomics in broader medical and societal context, addressing for example some of the following questions:

- What will be the impact (and side effects) of widely available genome sequencing for society?
- Will genomes remain connoted mainly with diseases or will they become an acceptable topic for small talk?
- How is the role of the human geneticist going to change if genomes are becoming widely a discussed topic outside of the medical domain?
- Should we foster "genetic literacy" in the same way as schools teach "computer literacy"?
- How to protect the individual in the future when any shed hair could betray a person's DNA to pretty much any passerby?

Contact: cbock@genomaustria.at || http://personalgenomes.org/austria || http://genomaustria.at

SEL

sel-01

Disruption of Topological Chromatin Domains Causes Pathogenic Rewiring of Gene-Enhancer Interactions

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Mammalian genomes are organized into megabase-scale topologically associated domains (TADs) that have been proposed to partition the genome into large regulatory units. Here we demonstrate that the disruption of TAD structure can cause rewiring of functional interactions between genes and distant-acting enhancers, resulting in pathogenic phenotypes in humans and mice. We show that distinct limb malformations in human patients are caused by deletions, inversions or duplications altering the structure of the extended WNT6/IHH/EPHA4/PAX3 locus. To examine these variants in detail, we adapted CRISPR genome editing to generate mice with corresponding large rearrangements. 4C-seq in mouse limb tissue and patient-derived fibroblasts showed that the structural changes result in ectopic interactions between promoters and non-coding DNA across adjacent TADs. On the disease alleles, a cluster of limb enhancers normally associated with Epha4 is misplaced relative to TAD boundaries, causing it to interact with and drive ectopic limb expression of Wnt6, Ihh or Pax3, respectively. Taken together, our results demonstrate the critical importance of TADs for the functional orchestration of the genome in vivo and support their utility in predicting the pathogenicity of human structural variants.

sel-02

Mutations in ZAK cause autosomal recessive split foot malformation in humans and complex hindlimb defects in mice

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Split hand foot malformation (SHFM) is a clinically heterogeneous defect of the central rays of hands and feet that is characterised by locus heterogenity and different modes of inheritance with incomplete penetrance and variable expressivity in the predominant autosomal dominant forms. Genetic alterations affecting TP63, DLX5/6 and WNT10B are identified in a proportion of affected individuals and have provided insight into the role of these genes in limb development, but many patients remain without a molecular diagnosis. By using a combination of homozygosity mapping and exome sequencing in a consanguineous Pakistani family, we have identified a c.1103T>G / p.Phe368Cys mutation in ZAK (also known as MLTK) on chromosome 2q31.1 as the cause of an autosomal recessive split foot-hearing loss syndrome with highly variable expressivity. ZAK is a member of the MAPKKK family of signal transduction molecules with no known role in limb development. Screening of genetically unresolved SHFM cases identified an unrelated Tunisian individual with a homozygous intragenic ZAK deletion. Strikingly, the phenotype in both families consisted of split foot with normal hands, and both mutations affected the SAM domain of ZAK. SAM domains are protein-protein interaction domains that can dimerise or polymerise. Our biochemical studies using a super negative GFP gel assay show that the SAM domain of ZAK is monomeric. Notably, the p.Phe368Cys SAM domain missense alteration leads to unfolding of the protein causing aggregation due to loss of the alpha helical character. In situ hybridisation in mouse embryos revealed strong Zak expression in the heart as well as in the developing forelimb and hindlimb between embryonic days (E) 9.5 and 11.5. Consistent with a role for Zak/Mltk in cartilage and bone development morpholino oligonucleotide-mediated knockdown resulted in abnormal cartilage development in Xenopus laevis embryos. Finally we show that CRISPR-Cas mediated complete inactivation of Zak in mice causes lethality at E 9.5 due to severe cardiac malformation. The targeted deletion of the SAM domain in mice however was associated with a complex hindlimb malformation with some animals showing severe clefting of the whole hindlimb including the femur, tibia, fibula and the feet. Furthermore, expression analysis of mutant hindlimbs at E 10.5 and 11.5 showed a 60% decrease of Tp63 expression compared to wild type hindlimbs, suggesting a functional link between Zak and p63. Thus, through the identifiction of mutations affecting the SAM domain in autosomal recessive split foot we have identified an evolutionarily conserved role for ZAK in limb development.

sel-03

Spotlight on the pathogenesis of Kabuki syndrome

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Kabuki syndrome (KS) is a genetic disorder characterized by developmental delay and congenital anomalies that include a characteristic facial gestalt. KS is caused primarily by dominant mutations in KMT2D (MLL2), a methyltransferase that promotes active transcription and, more rarely, in KDM6A, a lysine 27 demethylase of the KMT2D complex. Although the functions of many chromatin modifying proteins have been well studied, the physiological systems regulated by them are largely unknown. In This study we shed a light on the molecular pathogenesis of KS: Using whole exome sequencing we found a mutation converted to homozygosity by uniparental isodisomy (UPD) in RAP1A in a patient with a KS phenotype, and a de novo dominant mutation in the closely related gene RAP1B in a second patient. In vivo and in vitro studies showed that suppression of each of RAP1 (RAP1A and RAP1B), KMT2D, and KDM6A leads to defective convergent-extension (CE) movements and to context-dependent MAPK signaling dysfunction. We further show that RAP1 interacts genetically with KMT2D and that RAP1B expression is downregulated in both kmt2d morphant zebrafish (zf) embryos and KMT2D deficient patient cells. In view of the frequent skeletal abnormalities in KS patients, we also demonstrate that depletion of KS genes affects the layout of the pharyngeal skeleton in zf by disturbed F-actin polymerization and cell-cell intercalation. Interestingly, the CE and the skeletal defects could be rescued in vivo by a small molecule MAPK inhibitor, offering a potential treatment paradigm for some aspects of KS. Taken together, our genetic and functional data (i) reveal the first evidence that defective MEK-ERK signaling is a common molecular driver for KS, (ii) suggest that KS is a member of the RASopathy spectrum, and (iii) provide a potential direction for treatment design.

sel-04

Circulating tumor DNA sequencing reveals cancer driver genes and their plasticity in focal amplifications

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In cancer genetics focal amplifications, i.e. copy number increases of restricted regions, are of great interest because they may contain overexpressed dominantly acting cancer genes and may be amplified to very high copy-numbers. Moreover, the small size of these regions facilitates the identification of a specific aberrant cancer target gene. Here we analyzed circulating tumor DNA (ctDNA) of 125 plasma samples which were derived from 38 and 36 patients with metastasized breast and prostate carcinoma, respectively. We calculated copy-number aberrations directly from plasma by applying read-depth analyses to next-generation sequencing data. Subsequently, we identified focal amplification and deletion events from this dataset by applying stringent criteria and filtering steps to raw copy-number profiles. We were able to show that known overexpressed cancer genes frequently reside within focally amplified regions identified by our approach. These genes provide insights into altered pathways. For example, we demonstrate that focally amplified regions in breast cancer are related to members of the PI3K family whereas amplified genes in prostate cancer are related to the Src family. Moreover, we conducted serial analyses in 42 patients and observed the evolution of novel focal amplifications in 23.8% of these patients. Many of these focal amplifications contained established tumor genes and had highly relevant therapeutic relevance. In one prostate cancer patient we

even observed the transition from an adenocarcinoma to a neuroendocrine prostate cancer (NEPC), which was associated with gross changes of the tumor genome. Identification of focal amplifications in ctDNA provides novel insights into the evolution of metastasized cancer, altered pathways, the plasticity of tumor genomes, and has an impact on the clinical management of cancer patients. As our analyses can be completed within less than 48 hours, our study is an important step for bringing ctDNA genomics into the clinic.

W1 CLINICAL GENETICS I

W1-01

Bainbridge-Ropers syndrome caused by mutations in ASXL3 – a recognizable condition?!

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Intellectual disability (ID, IQ<70) affects up to 3 % of the general population. Until recently, the underlying cause was unclear in about half of the affected individuals. The introduction of whole exome sequencing (WES) techniques enables further elucidating the genetic background of ID. We performed WES in a cohort of 250 individuals with unexplained ID with and without additional features and identified heterozygous de novo ASXL3 mutations in three unrelated individuals. Three further affected individuals were obtained by clinical collaboration from different WES cohorts. All six patients have severe muscular hypotonia with feeding difficulties in infancy, significant motor delay and profound speech impairment. Their craniofacial phenotype is characterized by a long face, arched eyebrows with mild synophrys, downslanting palpebral fissures, a prominent columella, small alae nasi, a high, narrow palate and relatively little facial expression. Beside five patients with loss-of-function mutations (four stop mutations, one frameshift mutation), one patient carried a missense mutation [(c.5408C>T, p.(Pro1803Leu)] which was predicted by different in silico algorithms to be probably damaging/disease-causing (PolyPhen2, SIFT, MutationTaster).

Truncating ASXL3 mutations were first described in 2013 by Bainbridge et al. as cause of intellectual disability, identified by WES in four children with overlapping phenotypes, and included in OMIM as 'Bainbridge-Ropers syndrome' (#615485). A fifth child with a truncating ASXL3 mutation was published by Dinwiddie et al., 2013. With the knowledge of only five affected individuals, all of them carrying truncating ASXL3 mutations, this seemed to be a rather rare condition. Our finding of three individuals in our primary cohort of 250 (1.2 %) suggests that ASXL3 mutations might be a more frequent cause of ID. We broaden the mutational spectrum by adding a missense mutation in ASXL3 which we regard as causative for Bainbridge-Ropers syndrome in a girl with characteristic features.

In conclusion, we further delineate Bainbridge-Ropers syndrome and emphasize that ASXL3 mutations lead to a recognizable clinical phenotype.

W1-02

POLD1 germline mutations in patients with atypical Werner syndrome

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Segmental progeroid syndromes are rare, genetically heterogeneous disorders characterized by clinical signs of premature aging affecting several tissues or organs. The prototype example is the Werner Syndrome (WS), mostly caused by biallelic germline mutations in the Werner helicase gene (WRN). While LMNA mutations are also found in a few atypical cases of WS, we have previously shown that 10-15 percent of

patients with suspected WS do not harbor mutations in WRN or LMNA - individuals we operationally call atypical Werner syndrome patients. Heterozygous mutations in POLD1 have recently been reported as the cause of a mandibular hypoplasia, deafness, progeroid features (MDP) syndrome. These findings prompted us to analyse the entire POLD1 gene in our previously established cohort of fifty atypical WS patients. We identified six patients from five pedigrees bearing a recurrent POLD1 heterozygous in-frame deletion (c.1812_1814delCTC, p.Ser605del). In addition, we identified a rare heterozygous missense mutation (c.1519C>T, p.Arg507Cys) in one additional individual. The same alteration was only recently reported in another MDP patient. Clinical variability was observed among our patients, including premature hair greying and hair loss, early cardiac death, lack of joint contractures and lack of hearing deficit. Further, we report the first cases of vertical transmission of the mutation, suggesting that POLD1 mutations do not affect female fertility. Moreover, functional characterization of patient´s primary cell lines revealed prolonged recovery after replication stressors indicating a defect in DNA synthesis as putative pathomechanism. We observed no chromosomal instability nor hypersensitivity towards replication-related genotoxic agents. The latter, together with the clinical findings seems to indicate that these patients may not have a significantly increased cancer risk. Taken together, our findings provide further support for POLD1 mutations as the genetic cause of atypical progeroid syndromes and confirm the existence hotspot mutations. Moreover, we further expand the clinical spectrum suggesting that POLD1 testing should be extended to patients with unclassified segmental progeroid syndromes.

W1-03

Metabolic and genetic research into early onset epileptic encephalopathies

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Introduction

Early onset epileptic encephalopathies (EE) represent a heterogeneous group of rare disorders that constitute a major diagnostic and therapeutic challenge and the majority of patients still remain without a clear diagnosis. Recent research has unravelled a growing number of inherited inborn errors of metabolism but also sporadic de novo mutations in neuronal genes as the underlying causes of EE. Due to the rarity of the single disease entities knowledge on clinical phenotypes is very limited. We apply a combined "omics" approach to unravel the etiologic background of EE.

Methods

Study inclusion criteria were defined as following: i) onset of epilepsy before age 4 years, ii) difficult to treat seizures for more than 6 months iii) mental retardation iv) normal microarray and normal sequencing of the SCN1A gene. Exclusion criteria constitute morphological changes involving brain cortex. From April 2013 until December 2014, we included 63 patients with early onset EE of unclear etiology. The division of Child neurology at the Kinderspital Zurich cares for the majority of these patients and holds detailed long-term records of seizure semiology and ictal EEGs. Neurometabolic analysis included evaluation of biomarkers including aminoacids in plasma, alpha-aminoadipic semialdehyde, pipecolic acid, vitamine compounds, a culture of lymphoblast cell line for further studies and an untargeted metabolomics analysis. Genetic analysis included high-resolution chromosomal microarray testing and whole-exome sequencing in index patients and parents by means of next generation sequencing.

Results

Detailed clinical and pedigree analysis was collected from all patients and entered into the study database. Targeted biochemical analysis and newly established vitamin B6 profiling and AASA testing revealed normal results for the study cohort. Untargeted plasma metabolome analysis was so far performed in 36 patients and identified a novel plasma biomarker for spermine synthase deficiency.

Exome analysis was performed in 63 patients and identified relevant mutations in known disease genes in 58%, and in novel candidate genes in 25% of patients.

Conclusion

Identification of patients with known genetic defects will contribute to delineate the phenotypic spectrum of these rare entities. Targeted and untargeted biochemical analysis will identify treatable causes of metabolic encephalopathies and reveal novel diagnostic biomarkers.

Whole exome sequencing has the potential to identify novel genes involved in early onset epileptic encephalopathies, that can be further studied on a functional level in cell cultures. Ultimately, this study will translate into a better patient care due to improvements in disease recognition and management.

W1-04

Systematic evaluation of patients with idiopathic short stature using whole exome sequencing

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Shortness of stature is a common medical concern in childhood and has an incidence of 3% in the general population. Although body height is inherited as a highly polygenic trait, severe forms of shortness of stature are often monogenic in nature. After excluding defects of the growth hormone pathway and clinically recognizable syndromes the underlying cause remains unknown in approximately 70-80% of patients. This prevents potential treatment, failure to recognize a risk of recurrence and provide anticipatory prognosis in the majority of patients. With the advent of whole exome sequencing the identification of underlying genetic causes through unbiased genome-wide mutation screening without prior clinical clue pinpointing to candidate genes has become possible.

To address the hypothesis that the clinical spectrum of these syndromic forms might also include patients with idiopathic short stature we recruited a carefully characterized study group of more than 500 families with idiopathic short stature. We now selected 64 individuals where growth hormone defects, common genetic causes of short stature or copy number variants were excluded and performed whole exome sequencing. Variants were selected unbiased based on all modes of inheritance in agreement with the segregation in the families, population frequency below 0.1% and their potential effect on protein function (CADD score > 15) without prior selection of variants in known genes. We confirmed mutations in known short stature genes in 6 patients: One homozygous splice site mutation in CUL7 (3-M syndrome) in a consanguineous family with two affected children; a missense mutation in FGD1 (Aarskog syndrome) in a male patient with only mild learning disability; a missense mutation in COL2A1 in a patient with severe scoliosis; a known missense mutation in PTPN11 (Noonan syndrome) in a 1 year 6 months old child with normal development; compound heterozygous frameshift deletions in TRIM37 (Mulibrey nanism) in two affected children of non-consanguineous parents and a missense mutation in CUL4B in one patient without the reported mental retardation. All these syndromes have been reported to be associated with further clinical issues. In particular, directed prevention of the associated increased risk of nephroblastoma in the patients with Mulibrey nanism, screening for unrecognized congenital cardiac defects and specific developmental support in the other entities provides mandatory medical guidance for these patients.

In conclusion, whole exome sequencing identified the underlying genetic defect in genes already associated with shortness of stature in 10% of the patients with unsuspicious presentation investigated. This creates the prospect that other genes yet to be identified are underlying the shortness of stature in many of the remaining patients. Our data also highlight that the full clinical spectrum of most monogenic genetic disorders is yet to be explored calling for unbiased genetic analyses also in patients with idiopathic short stature.

W1-05

Molecular autopsies: should cardiovascular NGS-Panel testing be implemented in the workup of sudden unexplained death?

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Sudden cardiac death often has a strong genetic component or cause. A number of cardiomyopathies and channelopathies have a monogenic inheritance with high penetrance. The high degree of locus and allelic heterogeneity characteristic of both cardiomyopathies and channelopathies necessitates sequence analysis of the entire coding region of multiple genes, which has been a costly and lengthy process using traditional Sanger sequencing. In contrast, next generation sequencing (NGS) technologies allow now the simultaneous

testing of a large number of relevant genes and as a consequence NGS-based test panels are increasingly used in molecular genetics diagnostics. More than a year ago, we started NGS-based analyses of cardiologic patients and so far, more than 60 patients (not counting relatives tested for familial variants) were tested using our custom cardiologic NGS-panels covering 97 genes in the beginning and 117 genes in its current version. Due to overlapping clinical phenotypes we included genes for both structural as well as rhythmologic disorders. Although the tested collective was diverse in terms of clinical symptoms, suspected disease and family history, we could diagnose the cause with high confidence (at least one pathogenic variant) in about 60% of cases, in 30% we detected variants of unknown significance, and in the remaining 10% no genetic variants with a possible impact on the disease was identified.

However, an unsettling observation was that a relatively high proportion of positively tested patients had sudden unexplained deaths in their family history. In one index family, in which we identified a sequence alteration in the RyR2 gene in a 13 year old boy with nonspecific arrhythmias, we noted that his sister and an uncle both had died because of sudden cardiac death. Using autopsy material, we identified the same RyR2 mutation in the late sister. This case illustrates that the diagnosis and initiation of life saving means, such as implementation of an ICD, could have done years earlier in this family, if diagnostic work-up of the causes of sudden death had included appropriate molecular genetics testing. We envision that future autopsies will – depending on the cause of death- be extended by molecular genetics if potential results have a clear predictive value for the relatives. We will discuss the medical, legal, and ethical challenges in the context of such molecular autopsies.

W1-06

Dubious genetic testing – How responsible are we? - A case study.

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A Belgian patient of Armenian origin contacted our institute with the question of a genetic test. He presented with diffuse symptoms including abdominal pain, diarrhea, chest pain events, weight loss, intermittent “electric shock sensations” starting in the fingers and intermittent loss of muscle tone in the legs. Extensive investigations in several disciplines remained without any pathological findings.

We told the patient that we cannot see any promising basis for a genetic test in his situation. Two months later the patient informed us (6-page e-mail) that his symptoms had worsened. After three further months the patient called to make an urgent appointment. A foreign company “Gentle” had informed the patient that they had found a "life threatening" mutation in a genetic test which had been performed on request of the patient with a saliva test tube system without pretest counselling. Details would only be transmitted to a human geneticist after providing evidence of a consultation appointment.

On condition to know the exact findings in advance we made an appointment for the next day in order to avoid a possible treatable risk. According to an e-mail we received by the company a mutation in the SOD1 gene previously reported as pathogenic was found which does explain the patient’s clinical picture. With knowledge of this finding we canceled the appointment and informed the patient that the findings can hardly explain any of his symptoms nor can an acute threat to life be derived from them. He may turn to the company to get informed about the findings. A telephone conversation with the non-medical director of the company was unpleasant. In his view the described mutation does explain the symptoms and we as the geneticists named by "our" patient would have to inform the patient.

The patient, however, appeared next day without appointment. We arranged a meeting together with the local neurologist to inform him about the test results and offered the possibility to perform an electrophysiological examination. We did not disclose the results before the appointment but the patient already knew the findings at that time.

Meanwhile, an extensive literature search had shown that the pathogenicity of the putative pathogenic variant p.Asp90Ala of the SOD1 gene is unclear with reduced penetrance in gene carrier. The variant had been found in rare cases with classic ALS especially in homozygous carriers or in combination with additional changes in other genes, for example a clear pathogenic hexanucleotide repeat expansion in C9orf72. As expected the neurophysiological examination gave normal results. It was agreed not to pursue this finding.

A diagnosis of a severe somatoform disorder was suggested with the recommendation to contact a specialist.

Conclusion: This case illustrates a disastrous, reckless, dangerous and senseless genetic investigation and procedure.

Clear guidelines for management of similar requests and situations are needed.

W2 GENETIC VARIATION AND GENE EXPRESSION

W2-01

Deletion in the cis-regulatory landscape of FGF8 causes split hand/split foot malformation

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Fibroblast growth factor 8 (FGF8) located on human chromosome 10q24 encodes a key signaling factor, and its precise regulation is essential for normal limb and digit patterning. A complex cis-regulatory module has been identified that controls FGF8 expression during embryogenesis. In humans, duplications of this regulatory region on chromosome 10q24 lead to split hand/split foot malformation type 3 (SHFM3, also called ectrodactyly), a condition characterized by clinically heterogeneous defects of the central rays of hands and feet. The pathomechanism of the 10q24 duplications associated with SHFM3 is still unknown.

Here we report on a family with three affected individuals presenting with a split hand/split foot phenotype. Within this family the expressivity of the condition ranges from the classical ectrodactyly deformity to hypoplastic toes and mild syndactyly indicating the presence of a minor ectrodactyly component. We performed high-resolution array CGH and identified a novel 12 kb deletion located over 100 kb 3´ to FGF8 within the FBXW4 gene. The deletion removes three coding exons of FBXW4 and one known heart enhancer element. The F-BOX and WD40 domain protein 4 (FBXW4) also known as Dactylin, has been shown to play an important role in the Dactylaplasia mouse, a mouse model of SHFM3. However recent studies suggest that the lack of Dactylin expression does not seem to cause the ectrodactyly phenotype. Therefore we hypothesized that the deletion might disturb the cis-regulatory landscape of FGF8 resulting in misexpression of FGF8.

To investigate the cis-regulatory architecture of the FGF8 locus we performed chromosome conformation capture followed by high-throughput sequencing (4c-seq) in human skin fibroblasts. We could show that in fibroblasts the deleted fragment demonstrates a high interaction frequency with the promoter of FGF8. This could indicate the presences of important cis-regulators of FGF8 in the deleted region. To further examine the deletion in detail, we used CRISPR genome editing to generate transgenic mice with the corresponding deletion. The mice homozygous for the deletion showed a complex lower limb phenotype including clefting of the feet and polydactyly. Similar phenotypes have previously been observed in FGF8 misexpression studies in the chick. Thus, it is likely that the 12kb deletion associated with SHFM removes important cis-regulatory elements of FGF8.

Our data further highlight the role of cis- regulatory mutations in congenital disease and give new insights into the pathomechanism of the common 10q24 duplications associate with ectrodactyly.

W2-02

Mapping genetic and epigenetic factors influencing human hippocampal gene expression

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Genome-wide association studies (GWASs) have recently detected multiple loci associated with psychiatric disorders. The majority of these disease-associated variants are observed in noncoding regions and their functional effects are usually unclear. It is often suspected that at least part of these variants influence the expression of neighboring (cis) or distant (trans) genes. Novel methods allow to systematically investigate the regulatory effects of genetic variants by screening the genome for correlations between allelic variants and gene expression (expression Quantitative Trait Loci / eQTLs) or DNA methylation (meQTLs) in a tissue of

interest. Several studies have investigated the occurrence of eQTLs and meQTLs in human brain tissue. A major problem is the quality of the available eQTL and meQTL data sets from brain tissue as they are usually derived from post-mortem brain tissue and relatively small numbers of samples. As a consequence, the overlap of significant eQTLs and meQTLs between these studies is relatively low [McKenzie et al. 2014].

We employed 150 fresh frozen hippocampal biopsy samples derived from surgery of patients with chronic pharmaco-resistant temporal lobe epilepsy and performed genome-wide SNP genotyping, expression and methylation profiling. After stringent quality control, 4,250,386 imputed SNPs, 12,987 transcripts and 362,722 CpG islands were correlated in 115 brain samples using a linear regression model implemented in Bioconductors 'matrixQTL' package. We included surrogate variables consisting of age, sex, major principal components and neuronal proportion as covariates to limit the effect of confounding factors.

At a false discovery rate (FDR) threshold of 5%, 641 RNA transcripts and 19,953 CpGs showed a genetic regulation by common variants in cis (± 1 MB). The most significant eQTLs were observed at KCTD10, RPS26, IPO8, CRYBB2, HPR (eQTLs) and meQTLs at ADARB2, MYADML, ATPIF1, PMM2, KRTAP5-4 (meQTLs). In total, eQTLs of 272 RNA transcripts had not been reported by the largest brain or blood eQTL meta-analyses published to date [Kim et al. 2014, Westra et al. 2013].

In a next step, we tested for enrichment of our QTL data among the top hits from the public NHGRI GWAS catalogue. Preliminary results show enrichment for brain disorders including autism spectrum disorder, attention deficit-hyperactivity disorder, bipolar disorder, major depressive disorder, and schizophrenia.

In this study, we present an integrative large-scale functional genomic analysis to explore the effects of common DNA sequence variants on DNA methylation and mRNA expression. In contrast to all published studies, our samples were collected from fresh frozen and not post-mortem brain tissue. Therefore, the identified eQTLs and meQTLs provide an extremely valuable resource for functional annotation of SNPs and will help guiding the interpretation of GWAS hits in genetically complex brain disorders.

W2-03

De novo mutation suggests SEC31A as a new candidate gene for schizophrenia

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Schizophrenia (SCZ) is a common and severe brain disorder. It is puzzling the incidence rate of SCZ remains fairly stable over time while – at the same time – patients with SCZ have a reduced fecundity. One hypothesis is that the mutations that are selected out of the gene pool due to strong negative selection are replaced by de novo mutations. Genes hit by these de novo mutations are putative candidate genes for SCZ. So far, only a few exome sequencing studies focusing on de novo mutations in patients with SCZ were published. Interestingly, the de novo mutations identified affect different genes in each sample analyzed. So far only ~ 10 genes are reported to carry a pathogenic mutation in > two patients with SCZ. In order to unravel the complex genetic architecture of SCZ, more exome sequencing data are necessary.

40 clinically well characterized SCZ trios enriched for increased paternal age and an early age at onset were included in the study. Neither of the selected patients had a positive family history for SCZ or any other psychiatric disorder. All probands were of German origin according to self-reported ancestry. Exome DNA was captured from genomic DNA using NimbleGen in solution based capture. It was sequenced on an Illumina HiSeq 2000 sequencing instrument. The Varbank pipeline v.2.1 and interface were used for data analysis and filtering.

Only de novo mutations were analyzed. The allele frequency of the identified variants was checked in publicly available datasets from the 1000 Genomes Project, the Exome Variant Server, and the Exome Aggregation Consortium (ExAC) Data Set (> 65 000 individuals). Additionally, exome sequencing data from ~ 3 000 SCZ patients were checked for the presence of mutations in the genes identified in our study.

In total, we identified de novo mutations in 30 different genes. Six of these genes were reported in previous exome sequencing studies in patients with SCZ or autism. Particularly interesting is a de novo mutation in the gene SEC31A on chromosome 4. The mutation was predicted to be damaging by three in silico prediction programs. Additionally, our patient is the third patient with SCZ reported to carry a mutation in this gene.

Not each of the 30 genes identified plays a role in the development of SCZ. In order to prioritize the implicated genes for functional follow-up analyses, we gathered additional genetic evidence (both on common and rare variants) for an involvement of these genes in SCZ. The genes identified to carry a de novo mutation were (i) screened for copy number variants using our genome-wide SNP data set (1 637 patients with SCZ and 1 627 controls), and (ii) subject to set-based tests. With the latter an association between common variants

and SCZ in the largest published SCZ dataset (~ 150 000 individuals) was analyzed. The combination of our extensive genetic analyses allowed us to prioritize genes for functional follow-up studies. The full data will be presented at the conference.

W2-04

SHANK variants confer risk for schizophrenia

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The SHANKs are postsynaptic scaffolding proteins at glutamatergic synapses in the brain that are essential for proper synapse formation and function. The SHANK gene family (comprising SHANK1, SHANK2 and SHANK3) is linked to a spectrum of neurodevelopmental disorders, including intellectual disability and autism spectrum disorders (ASD). Schizophrenia (SCZ) is a neuropsychiatric disease with high variability in the clinical phenotype, characterized by major impairments in perception of reality and disorganized thought or behavior. Different studies have already pointed to an impairment of glutamatergic synaptic plasticity as an underlying cause of SCZ pathology.

To elucidate a putative contribution of genetic SHANK variants to the etiology of SCZ, we sequenced the genes SHANK2 and SHANK3 in 500 SCZ individuals and compared the sequencing results to ancestrally matched controls. In SHANK3, we found an association of 6 genetic variants, with study-wide significance ($P<0.001$). In addition, one rare amino acid exchange G>V, was found in 4/1543 SCZ patients and in 4/2147 individuals with autism spectrum disorders (ASD), but not in 9315 controls. In SHANK2, we found a significant increase in the total number of rare missense variants in SCZ individuals (6.9%) compared with controls (3.9%; $P=0.039$). Functional analysis of four SHANK2 missense variants (S610Y, R958S, P1119T, and A1731S) in primary hippocampal neurons revealed an impairment to various degrees. Overexpression of the A1731S variant in hippocampal neurons reduces the synaptic contacts and diminishes the actin polymerization rate. It was identified in four unrelated SCZ patients (0.83%) but not in any of the sequenced controls or public databases ($P=4.6x10-5$).

We conclude that the SHANK3 gene harbors several genetic variations predisposing to SCZ, ranging from common and uncommon variants to rare deleterious missense mutations. The SHANK3-G>V variant was found in both ASD and SCZ patients, pointing to an overlapping genetic contribution of SHANK3 to both neuropsychiatric disorders. Our results also provide first evidence of a causative role of rare SHANK2 variants in SCZ and underline the contribution of SHANK gene mutations to a variety of neuropsychiatric disorders.

W2-05

eQTL study of the 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 numerous genes that are regulated by expression quantitative trait loci (eQTLs) during immune response. 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. This study is one of the first to map eQTLs under immune stimulation and significantly enhance our knowledge on the innate immune system and its genetic determinants.

W2-06

Hair eQTLs - delineating the genetic basis of gene-expression in human hair follicle

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The human scalp hair plays an important role in our social and cultural life and its undesirable loss is often perceived as psychologically stressful in affected individuals resulting in a reduced quality of life. This has provoked many studies on the genetic and biological causes of hair loss. Candidate gene and genome-wide association studies have been successful in the identification of genetic risk factors that predispose to hair loss disorders such as alopecia areata and male-pattern baldness. However, the biological context in which these genetic risk factors exert their biological function is often unknown. Although the functional annotation of the human genome is becoming increasingly detailed, further experiments are warranted to elucidate the often tissue- and cell-specific biological effects. Here, the analysis of genetic variants that influence gene expression (eQTLs) has gained major importance. The aim of our present was to systematically map eQTLs in human hair follicle to enable functional annotation of genetic variants that influence hair-related traits and predispose to hair loss disorders. For this purpose we performed genome-wide genotyping of blood-DNA samples and transcriptome profiling of hair follicle RNA samples from 100 healthy male donors using the Illumina OmniExpress v1.1 and HT12v4 array. After imputation of genotypes (1000Genomes, Phase I, June 2014) and quality control, a total of 6,593,881 SNPs (impute proper info statistics>0.4; MAF>5%) and 11,407 expression probes (quantile normalized, detection P-value<0.05 in 5% of samples) of 97 individuals remained for eQTL analysis. The genome-wide analysis identified 2,684 independent cis-eQTLs. This corresponds to a genetic regulation of expression levels for ~25% of all genes analyzed. A comparison with published eQTL data sets from peripheral blood and brain revealed hair-follicle specificity for about ~40% of the cis-eQTLs (N=1,006). The strongest hair-follicle specific effects were observed for CNTN1 (rs10879019, P=2.5E-41), RPS26 (rs1131017, P=7.1E-39) and SEMA4G (rs55950013, P=1.4xE-37). Elaborate data analyses are currently underway to investigate the informativeness of this hair-eQTL data set for the interpretation of genetic findings for human diseases and hair-related phenotypes. Promising strategies include the interpretation of GWAS findings using the hair-eQTL data, tests for association of the limited set of functionally relevant hair-eQTLs in case-control samples for hair-related traits and disorders as well as pathway- and score-based approaches. It is hoped, that the integration of hair eQTL data with genetic association findings will help to pinpoint novel candidate genes and pathways and to further elucidate the biological mechanism that contribute to healthy hair growth and the development of hair loss disorders.

W3 MONOGENIC DISEASE I

W3-01

COQ4 mutations cause a broad spectrum of mitochondrial disorders associated with CoQ10 deficiency

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Primary Coenzyme Q10 (CoQ10) deficiencies are rare, clinically heterogeneous disorders caused by recessive mutations in several genes encoding proteins involved in CoQ10 biosynthesis. CoQ10, a lipoidal quinone, is an essential component of the electron transport chain (ETC), shuttling electrons from complex I/II to complex III. By whole exome sequencing we identified five individuals carrying biallelic mutations in COQ4. The precise function of human COQ4 is not known, but it seems to play a structural role in stabilizing a multiheteromeric complex, which contains most of CoQ10 biosynthetic enzymes. The clinical phenotypes of the five subjects varied widely, but four had a prenatal or perinatal onset with early fatal outcome. Two unrelated individuals presented with severe hypotonia, bradycardia, respiratory insufficiency and heart failure; two sisters showed antenatal cerebellar hypoplasia, neonatal respiratory distress syndrome, and epileptic encephalopathy. The fifth subject had early-onset but slowly progressive clinical course, dominated by neurological deterioration with hardly any involvement of other organs. CoQ10 amount was reduced in all available specimens from mutant subjects, often associated with decrease of CoQ10-dependent ETC complex activities and reduced oxygen consumption rate in cultured cells. The pathogenic role of all identified mutations was experimentally validated in a recombinant yeast model: oxidative growth, strongly impaired in strains lacking COQ4, was corrected by expressing a human wild-type COQ4 cDNA but failed to be corrected by expressing COQ4 cDNAs with any of the nucleotide variants identified in affected subjects. COQ4 mutations are responsible for early-onset mitochondrial diseases with heterogeneous clinical presentations associated with CoQ10 deficiency.

W3-02

Mutations in GTPBP3 cause a mitochondrial translation defect associated with hypertrophic cardiomyopathy, lactic acidosis and encephalopathy

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Respiratory chain deficiencies exhibit a wide variety of clinical phenotypes resulting from defective mitochondrial energy production through oxidative phosphorylation. These defects can be caused by either mutations in the mtDNA or mutations in nuclear genes coding for mitochondrial proteins. The underlying pathomechanisms can affect numerous pathways involved in mitochondrial physiology. By whole exome and candidate gene sequencing we identified eleven individuals from nine families carrying compound heterozygous or homozygous mutations in GTPBP3, encoding the mitochondrial GTP-binding protein 3. Affected individuals from eight out of nine families presented with combined respiratory chain complex deficiencies in skeletal muscle. Mutations in GTPBP3 are associated with a severe mitochondrial translation defect, consistent with the predicted function of the protein in catalyzing the formation of 5-taurinomethyluridine (τm5U) in the anticodon wobble position of five mitochondrial tRNAs. All cases presented with lactic acidosis and nine developed hypertrophic cardiomyopathy. In contrast to individuals with mutations in MTO1, the protein product of which is predicted to participate in the generation of the same modification, most cases with GTPBP3 mutations developed neurological symptoms and MRI involvement of thalamus, putamen and brainstem resembling Leigh syndrome. Our study of a mitochondrial translation disorder, points towards the importance of post-transcriptional modification of mitochondrial tRNAs for proper mitochondrial function.

W3-03

Mutations in NDUFB11, encoding a complex I component of the mitochondrial respiratory chain, cause the microphthalmia with linear skin defects (MLS) syndrome

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The microphthalmia with linear skin defects (MLS) syndrome is an X-linked male-lethal disorder also known as MIDAS (microphthalmia, dermal aplasia, and sclerocornea). Additional clinical features may include neurological and cardiac abnormalities. MLS syndrome is genetically heterogeneous as heterozygous mutations in HCCS or COX7B have been identified in MLS-affected females. Both genes encode proteins involved in the structure and function of complexes III and IV, which form the terminal segment of the mitochondrial respiratory chain (MRC). However, not all cases with typical features of MLS syndrome carry a mutation in either HCCS or COX7B gene. The majority of MLS-affected females has severe skewing of X chromosome inactivation suggesting that mutations in HCCS, COX7B and other, as yet unidentified X-linked gene(s), cause selective loss of cells in which the mutated X chromosome is active. By applying whole exome sequencing and filtering for X-chromosomal variants we identified a de novo nonsense mutation in the NDUFB11 gene (Xp11.23) in one female individual and a heterozygous 1-bp deletion in a second individual, her asymptomatic mother and an affected aborted foetus of the patient's mother. NDUFB11 encodes one of 30 poorly characterized supernumerary subunits of the NADH:ubiquinone oxidoreductase, the first and largest enzyme of the MRC. By shRNA-mediated NDUFB11 knockdown in HeLa cells, we demonstrate that NDUFB11 is essential for complex I assembly and activity. These results demonstrate that X-linked genetic defects leading to the complete inactivation of complex I, III or IV underlie MLS syndrome. Our data reveal an unexpected role of complex I dysfunction in a developmental phenotype, further underscoring the existence of a group of mitochondrial diseases associated with neurocutaneous manifestations.

W3-04

Neu-Laxova Syndrome Is a Heterogeneous Metabolic Disorder Caused by Defects in Enzymes of the L-Serine Biosynthesis Pathway

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Neu-Laxova syndrome (NLS) is a rare autosomal recessive disorder, which presents with a recognizable pattern of severe malformations leading to prenatal or early postnatal lethality. Homozygous mutations in PHGDH, a gene involved in the first and limiting step in L-serine biosynthesis, were recently identified as the cause of the disease in three families (Shaheen et al. 2014). By studying a cohort of 12 unrelated families affected by NLS, we provide evidence that NLS is genetically heterogeneous and can be caused by mutations

in all three genes encoding enzymes of the L-serine biosynthesis pathway. Consistent with recently reported findings, we could identify PHGDH missense mutations in three unrelated families of our cohort. Furthermore, we mapped an overlapping homozygous region on chromosome 9 containing PSAT1 in four consanguineous families. This gene encodes phosphoserine aminotransferase, the enzyme for the second step in L-serine biosynthesis. We identified six families with three different missense and frameshift PSAT1 mutations fully segregating with the disease. In another family, we discovered a homozygous frameshift mutation in PSPH, the gene encoding phosphoserine phosphatase which catalyzes the last step of L-serine biosynthesis. Interestingly, all three identified genes had been previously implicated in serine deficiency disorders, characterized by variable neurological manifestations. Our findings expand our understanding of NLS as a disorder of the L-serine biosynthesis pathway and suggest that NLS represents the severe end of serine deficiency disorders, demonstrating that certain complex syndromes characterized by early lethality may indeed be the extreme end of the phenotypic spectrum of already known disorders.

W3-05

Novel autosomal recessive intellectual disability syndrome with manganese deficiency, muscular hypotonia, and cerebellar atrophy caused by mutation of the SLC39A8 transporter

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We ascertained two Egyptian siblings of consanguineous parents both presenting with severe intellectual disability, hypotonia and hyporeflexia, strabismus, short stature, early-onset epilepsy or absences, and normal head circumference with brain atrophy in one child. Further examinations were unremarkable. Autozygosity mapping and exome sequencing revealed a candidate mutation in SLC39A8 (encoding the protein ZIP8) in a homozygous status that leads to an alteration on protein level; p.Gly38Arg. In silico simulations and molecular modelling showed that this is a highly conserved residue and that the alteration is probably pathogenic. Independently, a further family was identified within the FORGE (Finding of Rare Disease Genes) Canada project, a national initiative to identify genes for rare childhood diseases, carrying the exact same variant. Five children of 5 families of Hutterite descent, an Anabaptist group living in North America with a small founder population and continued genetic isolation, were also found homozygous for the p.Gly38Arg alteration. The patients presented severe developmental delay, severe hypotonia, short stature, cerebellar atrophy with normal head circumference, mild skeletal dysplasia, and connective tissue abnormalities.

SLC39A8 is a member of the solute carrier gene (SLC) superfamily. The encoded protein ZIP8 is a Zn/HCO symporter, and also transports Mn and Cd across the plasma membrane. Evaluation of trace element levels in affected patients revealed low blood Mn levels in all the Egyptian and the Canadian patients, and low Zn levels in the Canadian patients, further supporting the pathogenicity of the alteration.

Cell cultures of several tissues taken from hypomorphic Slc39a8(neo/neo) mice exhibit diminished zinc and iron levels. Consequently, Slc39a8(neo/neo) homozygotes from gestational day(GD) 11.5 onward are pale, growth-stunted, and die between GD18.5 and 48 h postnatally. Defects include severely hypoplastic spleen, hypoplasia of liver, kidney, lung, and lower limbs.

We conclude that the variant we identified in SLC39A8 is pathogenic, and that it probably leads to a distinguishable autosomal recessive, severe intellectual disability syndrome with Mn deficiency, short stature, and muscular hypotonia. Further symptoms are brain atrophy with normal head circumference, seizures, strabismus, and osteopenia. Based on the severity of the symptoms of our patients and on the results of the hypomorphic mouse model, we think - at this stage - that the variant we identified in SLC39A8 influences the functions of the protein, but is not a loss of function. This is the first gene associated with a human Mn deficiency syndrome and this finding also provides insight into the role of Mn homeostasis in development and health.

W3-06

Haploinsufficiency of the NFIB gene in patients with mild intellectual disability

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Recent reports have highlighted significant roles for members of the nuclear factor one (NFI) family of transcription factors in development of a number of organ systems particularly in neuronal development. It was recently shown that deletions or mutations in the family members NFIA and NFIX lead to human phenotypes including malformations and intellectual disability (ID). There is evidence to suggest also a role in numerous embryonic developmental processes including regulation of cortical development and callosal formation for NFIB. However in the current literature there are no reports of a human phenotype caused by anomalies of the NFIB gene.

Here we present six unrelated patients with overlapping microdeletions in the chromosomal region 9p23p22.2. De novo occurrence of the deletion could be proven in the 5 cases from which parental samples were available. The identified deletions have different breakpoints and range in size from 225 kb to 4.3 Mb. The smallest region of overlap of the six deletions narrows down the critical region to a genomic segment containing only the NFIB gene. Furthermore we present one patient with a de novo missense mutation within NFIB, predicted to be pathogenic by in silico prediction programs (SIFT/ Mutation Taster).

All patients presented with a similar phenotype of mild ID, muscular hypotonia, speech delay, attention deficit, and variable behavioral anomalies. For all patients growth parameters (body height, head circumference) were above the mean; one individual had absolute macrocephaly. The patients showed mild facial anomalies. Variable mild structural brain anomalies were observed including corpus callosum agenesis.

Based on these findings and the previously published functional data obtained from animal model we propose NFIB as a novel causative ID gene. We assume haploinsufficiency as the common disease-causing mechanism of the presented NFIB variations.

W4 CLINICAL GENETICS II

W4-01

Cohesinopathies are branching out: Mutations in chromatin-associated factors as genetic cause of CdLS-overlapping phenotypes

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Cornelia de Lange syndrome (CdLS) is a genetically heterogeneous disorder manifesting extensive phenotypic variability. To date, mutations in *NIPBL, SMC1A, SMC3, RAD21* and *HDAC8* , which encode subunits or regulators of the cohesin complex, are found in about 70% of patients. While mutations in the *NIPBL* gene can be identified in the vast majority of patients with typical CdLS phenotypes, mutations in the other four genes result in more CdLS-overlapping phenotypes but which can be highly atypical.

We utilized gene-panel as well as trio-based exome sequencing for genetic analyses of five unrelated individuals with the diagnosis of CdLS, who had previously been screened for intragenic mutations in the known CdLS genes and for genomic deletions or duplications.

Thus we could identify a heterozygous de-novo mutation in each of the patients. Interestingly, all five mutations affect different chromatin-associated factors and were predicted to result in a loss of protein function. Two of the patients carry a nonsense mutation or a 4-bp frame-shift deletion in the *ANKRD11* gene which have been linked with KBG syndrome. The other three mutations affect components of the SWI/SNF chromatin-remodeling complex which is associated with Coffin-Siris and Nicolaides-Baraitser syndrome. These mutations

include a missense exchange in *SMARCB1*, a frame-shift deletion in ARID1A and a larger deletion including the entire ARID1B gene.

Our results nicely support very recent molecular findings that could show an intimate link between the cohesin and the SWI/SNF chromatin-remolding complex. Besides, the *ANKRD11*-encoded as ankyrin repeat-containing cofactor 1 (ANCO-1) is known to associate with different histone deacetylases to fulfill its function as transcriptional regulator. Hence our findings further contribute to the hypothesis that CdLS and overlapping "cohesinopathy phenotypes" result from alterations in the developmental and tissue-specific gene expression profiles.

In summary, mutations in *ANKRD11* and components of the SWI-SNF complex can result in a CdLS-overlapping phenotype. Sequencing analysis of the respective genes has to be considered for those patients with the suspected clinical diagnoses of CdLS who were tested as mutation negative in the five known CdLS genes.

W4-02

Exome sequencing unravels unexpected differential diagnoses in individuals with the initial diagnosis of Coffin-Siris and Nicolaides-Baraitser syndromes

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Coffin-Siris syndrome (CSS) and Nicolaides-Baraitser syndrome (NCBRS) are rare intellectual disability (ID)/congenital malformation syndromes that represent distinct entities but show considerable clinical overlap. They are caused by mutations in genes encoding members of the SWItch/Sucrose NonFermentable (SWI/SNF) complex. However, there are a number of patients with the clinical diagnosis of CSS or NCBRS in whom the causative mutation has not been identified. In this study, we performed trio-based whole exome sequencing (WES) in ten previously described but unsolved individuals with the tentative diagnosis of CSS or NCBRS and found causative mutations in nine out of ten individuals. Interestingly, our WES analysis disclosed overlapping differential diagnoses including Wiedemann-Steiner syndrome, Kabuki syndrome, and Adams-Oliver syndrome. In addition, causative de novo mutations were identified in GRIN2A and SHANK3. Moreover, trio-based WES detected SMARCA2 and SMARCA4 deletions, which had not been annotated in a previous Haloplex target enrichment and next generation sequencing of known CSS/NCBRS genes emphasizing the advantages of WES as a diagnostic tool. In summary, we discuss the phenotypic and diagnostic challenges in clinical genetics, establish important differential diagnoses, and emphasize the cardinal features and the broad clinical spectrum of SWI/SNF complex disorders and other disorders caused by mutations in epigenetic landscapers.

W4-03

Filippi syndrome is a heterogenic disorder with a high proportion of CKAP2L mutations

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Filippi syndrome is a rare, presumed autosomal recessive, disorder characterized by microcephaly, pre- and post-natal growth failure, syndactyly, and distinctive facial features, including a broad nasal bridge and underdeveloped alae nasi. Some affected individuals have intellectual disability, seizures, undescended testicles in males, as well as teeth and hair abnormalities. We performed homozygosity mapping and whole-exome sequencing in a family from Sardinia with two affected children and identified a homozygous frameshift mutation c.571dupA (p.Ile191Asnfs*6) in CKAP2L encoding the protein cytoskeleton-associated protein 2-like. The function of this protein was unknown until it has been re-discovered in the mouse as Radmis (radial fiber and mitotic spindle) and shown to play a pivotal role in cell division of neural progenitors. Sanger sequencing of CKAP2L in a further eight unrelated individuals with clinical features consistent with Fillippi syndrome revealed biallelic mutations in four cases. In contrast to wild-type lymphoblastoid cell lines (LCLs), no CKAP2L was detectable at the spindle poles of dividing cells in LCLs established from the individuals homozygous for the c.571dupA mutation. Furthermore, in cells from the affected individuals, we observed an increase of the number of disorganized spindle microtubules owing to multipolar configurations and defects in chromosome segregation. The observed cellular phenotypes are in keeping with data from in vitro and in vivo knockdown studies performed in human cells and mice, respectively. Our findings show that loss-of-function mutations of CKAP2L are a major cause of Filippi syndrome.

We are exploring the functional domains of CKAP2L and investigate interacting binding partners during embryonic development to explore the pathways involved in the etiology of the Filippi syndrome. Whole-exome sequencing of additional Filippi patients, including non-consanguineous families from Europe are in progress to identify further genes associated with this heterogenic rare disorder.

W4-04

Homozygous and Compound-Heterozygous Mutations in TGDS Cause Catel-Manzke Syndrome

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Catel-Manzke syndrome is characterized by Pierre Robin sequence and a unique form of bilateral hyperphalangy causing a clinodactyly of the index finger. We describe the identification of homozygous and compound heterozygous mutations in TGDS in seven unrelated individuals with typical Catel-Manzke syndrome by exome sequencing. Six different TGDS mutations were detected: c.892A>G (p.Asn298Asp), c.270_271del (p.Lys91Asnfs*22), c.298G>T (p.Ala100Ser), c.294T>G (p.Phe98Leu), c.269A>G (p.Glu90Gly), and c.700T>C (p.Tyr234His), all predicted to be disease causing. By using haplotype reconstruction we showed that the mutation c.298G>T is probably a founder mutation. Due to the spectrum of the amino acid changes, we suggest that loss of function in TGDS is the underlying mechanism of Catel-Manzke syndrome. TGDS (dTDP-D-glucose 4,6-dehydrogenase) is a conserved protein belonging to the SDR family and probably plays a role in nucleotide sugar metabolism.

W4-05

Phenotypic and molecular insights into CASK-related disorders in males

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Background: Heterozygous loss-of-function mutations in the X-linked CASK gene have been described to cause progressive microcephaly with pontine and cerebellar hypoplasia (MICPCH) and severe intellectual disability (ID) in females. Facultative features include hypo-/hypertonia, movement and behavioral disorders, and seizures in about 40% of cases. CASK encodes the calcium/calmodulin-dependent serine protein kinase which belongs to the membrane-associated guanylate kinase protein family. CASK is involved in organizing synapses, trafficking ion channels to the synapse and regulating transcription by interacting with transcription factors in the nucleus. Different CASK mutations have also been reported in males. The described phenotypes are diverse and range from ID with or without nystagmus, FG syndrome, MICPCH, to Ohtahara syndrome and early myoclonic epilepsy. However, a systematic evaluation of the phenotypic spectrum in males in relation to their genotype has not been performed to date.

Methods: We identified a CASK alteration in 8 novel unrelated male patients by targeted Sanger sequencing, copy number analysis (MLPA and/or FISH), genome-wide array CGH analysis and whole exome sequencing, and performed transcript (RT-PCR followed by sequencing) and protein analysis

(immunoblotting). The clinical phenotypes and natural history of the 8 patients and all CASK-mutation positive males reported previously were reviewed and correlated with available molecular data.

Results: CASK alterations in the 8 patients include one nonsense mutation, one 5-bp deletion, one mutation of the start codon, and 5 partial gene deletions or duplications; 7 were de novo, including 3 somatic mosaicisms, and one was familial. In 3 subjects, transcript analysis revealed the presence of specific mRNA junction fragments indicating an in tandem duplication that disrupted the integrity of CASK. The 5-bp deletion at the 3' end of exon 7 resulted in multiple aberrant CASK mRNAs. In fibroblast cells derived from patients with a CASK loss-of-function germline mutation, no CASK protein could be detected, while individuals who are mosaic for a severe CASK mutation or carry a hypomorphic mutation still had detectable amount of wild-type protein.

Conclusions: CASK alterations in males are associated with 3 distinguishable although overlapping phenotypic groups: (i) MICPCH with severe epileptic encephalopathy caused by hemizygous loss-of-function mutations, (ii) MICPCH associated with inactivating alterations in the mosaic state or a partly penetrant mutation, and (iii) syndromic/nonsyndromic mild to severe ID with or without nystagmus caused by CASK missense and splice mutations that leave the CASK protein intact but likely alter its function or reduce the amount of normal protein. Our findings facilitate focused testing of the CASK gene and interpreting sequence variants identified by next-generation sequencing in cases with a phenotype resembling either of the three groups.

W4-06

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

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We have mapped the strongest human QTL modifying cardiac repolarization (QT interval) to OLFML2B and NOS1AP in 1q23.3 using a GWAS approach. 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 24 variants 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 24 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 -10% to -50% 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).

W5 STRATEGIES AND TECHNOLOGIES

W5-01

The Human Phenotype Ontology: Semantic unification of common and rare disease

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Deep phenotyping, the precise and comprehensive analysis of individual phenotypic abnormalities for the purpose of translational research, diagnostics, or personalized care, depends on computational resources to capture the phenotype of patients or diseases and integrate it with other relevant information such as genomic variation. The Human Phenotype Ontology (HPO) is widely used in the rare disease community for differential diagnostics, phenotype-driven analysis of next-generation sequence variation data, and translational research, but a comparable resource has not been available for common disease. Here we present disease models for 3,145 common human diseases comprising a total of 132,006 annotations to terms of the HPO. Together with previously existing resources for rare disease, the HPO now comprises over 250,000 phenotypic annotations for over 10,000 rare and common diseases and can be used to examine the phenotypic overlap amongst common diseases that share risk alleles, as well as between Mendelian disease and common disease linked by genomic location.

There are many SNPs that have been linked to more than one common disease by genome-wide association studies (GWAS hits). For instance, multiple ulcerative colitis and Crohn's disease susceptibility loci have been identified by GWAS that are common to both diseases, some of which are even shared with several other autoimmune disorders. We speculated that diseases that share phenotypic features might in general be more likely to also share GWAS hits. We showed using the new common disease phenotypic annotations that this is the case, with there being a statistically significantly greater degree of overlap than expected by chance ($p=2.29e-57$).

Similarly, we showed that the phenotypic overlap between rare and common disease linked to the same locus is higher than expected. As per the method described above, we examined 485 genes mapped between the complex (GWAS) and rare disease datasets. GWAS SNPs have been previously mapped to genes using SCAN DB. Similar to the common disease phenotype experiment, we then measured the phenotypic overlap between the complex diseases from GWAS Central and rare, Mendelian diseases associated with the genes in question. There was significantly higher overlap than expected by chance ($p=1.6e-7$).

Finally, we investigated the use of the new common disease annotations for computational differential diagnosis using the Phenomizer framework, and showed that the annotations presented in this work possess sufficient differential diagnostic specificity to be used in applications such as the Phenomizer

The annotations, as well as the HPO itself, are freely available.

W5-02

A significant proportion of de novo point mutations arise post-zygotically

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De novo mutations are recognized both as an important source of human genetic variation and as a prominent cause of sporadic disease. Mutations identified as de novo are generally assumed to have occurred during gametogenesis and, consequently, be present as germline events in an individual. However, Sanger sequencing does not provide the sensitivity to reliably distinguish somatic from germline mutations. Therefore,

the proportion of de novo mutations occurring somatically rather than in the germline remains unknown. To determine the contribution of post-zygotic events to de novo mutations, we analysed a set of de novo mutations in 50 parent-offspring trios using three sequencing techniques. We found that 8 out of 107 presumed germline de novo mutations (7.5%) were in fact present as mosaic mutations in the blood of the offspring and were therefore likely to have occurred post-zygotically. Furthermore, genome-wide analysis of de novo variants led to the identification of 5 de novo mutations in the offspring which were also detectable in the blood of one of the parents, implying parental mosaicism as the origin of the de novo mutation in 5 out of 50 trios. Remarkably, parental mosaicism detected by Whole Genome Sequencing was not validated by Sanger sequencing but was finally confirmed by deep sequencing. Our results show that a significant proportion of de novo mutations presumed to be germline in fact occurred either post-zygotically in the offspring or were inherited from low level mosaicism in one of the parents.

W5-03

Deletions, Inversions, Duplications: Engineering of Structural Variants Using CRISPR/Cas in Mice

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Structural variations (SVs) contribute to the variability of our genome and are often associated with disease. Their study in model systems was hampered until now by labor-intensive genetic targeting procedures and multiple mouse crossing steps. Here we present the use of CRISPR/Cas for the fast (10 weeks) and efficient generation of SVs in mice. We specifically produced deletions, inversions and also duplications at six different genomic loci ranging from 1.1 kb to 1.6 Mb with efficiencies up to 42%. After PCR-based selection, clones were successfully used to create mice via aggregation. To test the practicability of the method, we reproduced a human 500 kb disease associated deletion and were able to recapitulate the human phenotype in mice. Further, we evaluated the regulatory potential of a large genomic interval by deleting a 1.5 Mb fragment. The method presented permits rapid in vivo modeling of genomic rearrangements.

W5-04

strategies to improve the performance of rare variant rare disease association studies by optimizing the selection of controls

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When analyzing a case groups of patients with ultra-rare disorders the ethnicities are often diverse and the data quality might vary. The population substructure in the case group as well as the heterogeneous data quality can cause substantial inflation of test statistics and result in spurious associations in case-control studies if not properly adjusted for. Existing techniques to correct for confounding effects were especially developed for common variants and are not applicable to rare variants. We therefore analyzed different strategies to select suitable controls for cases that originate from different populations and differ in data quality. We developed an approach to build up a control group that is most similar to the individuals in the case group with respect to ethnicity and data quality by means of a metric that puts more weight on rare variants. We simulated different disease entities on real exome data and show that a similarity-based selection schemes can help to reduce false-positive associations and to optimize the performance of the statistical tests. We reanalyzed collections of unrelated patients with Kabuki make-up syndrome, Hyperphosphatasia with Mental Retardation syndrome and Catel-Manzke syndrome for which the disease genes were recently described. We show that rare variant association studies are more sensitive and specific in identifying the disease gene than intersection filters and should thus be considered as an favorable approach in analyzing even small patient cohorts.

W5-05

DNA-methylation- and autoantibody- biomarker development strategies for minimal invasive diagnostics

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An estimated 2.7 million new cancer cases and 7.6 million cancer related deaths were reported worldwide in 2008. It is well accepted that early cancer diagnosis can improve survival, thus there is a great need and anticipation to identify novel biomarkers for cancer diagnosis at the earliest possible stage, which can ideally be integrated in minimal-invasive diagnostic assays.

DNA methylation changes are a hallmark of cancer and these epigenetic changes in tumors can be used as markers for detection of circulating tumor DNA in serum/plasma as well in saliva samples. In addition cancer onset and progression produces mutated or aberrantly expressed proteins generally also termed as tumor associated antigens (TAAs) which are able to act as antigens and evoke an immune response which results in the production of autoantibodies. These autoantibodies and the early DNA methylation changes during neoplastic transformation are able to be detected months or years before the clinical diagnosis of cancer and can therefore be used as biomarkers for the early diagnosis of cancer. We have setup genome- and immunome-wide discovery technologies for elucidation of novel biomarkers. From microarray based discovery-studies we have defined cancer-specific multivariate classifiers with very good diagnostic performance, obtaining AUC-values of 0.9- 1 for the big 4 cancer entities. Efficient targeted multiplexed technologies were established for validation of findings. Using methylation sensitive restriction digestion based high throughput qPCR we can detect 0,1-1% of tumor-derived methylated DNA in the very limited amounts (10ng) of cell free DNA in plasma. As an example data we have tested 680 plasma samples, and could obtain AUC values of 0,84-0,91 for the 4 different lung cancer subtypes using a multiplexed methylation test. In addition we have tested these patients using autoantibody-profiles. Current developments of 100-plex Luminex-assays for testing candidate antigenic markers and multimodal analyses combining both autoantibody and DNA-methylation data for improving cancer diagnostics will be presented. In addition recent developments from protein-based to peptide based tools for autoantibody and immunome-analyses will be presented. Based on findings form different cancer-studies, both DNA methylation and autoantibody based strategies outperform the current clinical diagnostic methods and would be of high value for improving cancer diagnostics and patient management.

W5-06

A novel adenoviral system allows highly specific tumor cell detection in blood

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In recent years circulating tumor cells (CTC) have become an important prognostic biomarker in the field of cancer diagnostics. In addition the analysis of CTCs genomes does not only allow more personalized treatment approaches for the patients but also provides more detailed information on tumor evolution and heterogeneity.

Although there are currently a number of exciting methodologies for the identification and isolation of CTCs, there is no consensus on the best method. In order to establish a method for the identification of CTCs that allows subsequent molecular analyses on CTCs, we constructed a replication-deficient adenovirus that expresses the green fluorescence protein (GFP) in the E1 region of the adenoviral genome under the control of the hTERT promoter based on the existing replication-active TelomeScan adenoviral system from Oncolys BioPharma. Various amounts of cells from colorectal cancer cell lines were spiked into heparinised blood samples of healthy donors and enriched with the RosetteSep CTC Enrichment Cocktail Containing Anti-CD56 (StemCell Technologies). Viral transduction of the enriched fraction revealed GFP expressing cells which were isolated and analyzed by Next Generation Sequencing (NGS). Isolated cells revealed specific copy number changes of the parental cell line. In negative control experiments no GFP positive cells were detected indicating that we developed a highly specific CTC detection system.

Compared to the already commercially available TelomeScan, we produced a safer, replication-deficient adenoviral vector system for the detection and isolation of CTCs which allows a comprehensive genomewide analysis of single cells by NGS.

W6 MOLECULAR MECHANISMS AND THERAPY

W6-01

Methylation of microRNAs

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Methylation of N6-adenosine (m6A) has been observed in rRNA, tRNA, snoRNA and mRNA. In order to find out whether microRNAs (miRNAs) also are modified, we have analysed the small RNA fraction from HEK293 cells by RNA immunoprecipitation with an antibody against 6mA, followed by high-throughput RNA sequencing (RIP-seq). In a control experiment we used unspecific IgG. We found that 239 miRNAs were enriched more than 2-fold in the anti-m6A sample compared to the control sample. Many miRNAs (n=124) were enriched more than 10-fold and 17 miRNAs were enriched more than 100-fold. Of the 239 miRNAs, 119 (49.7%) contained the RNA methyltransferase target sequence RAC, whereas among all miRNAs the numbers were 922/2555 (36.1%). Fisher's exact test showed that the motif is very significantly enriched in the immunoprecipitated miRNAs (p-value: 5*10E-6). For an unbiased search for the best discriminating IUPAC consensus motifs between immunoprecipitated miRNAs and the complementary set, we used two complementary methods: the MoSDi (Motif Search and Discovery) suite and Fisher's exact test on all IUPAC motifs of reasonable size. Both methods agreed on the results: For length 3, RAH (202/239 = 84.5% vs. 66.7%, Fisher p-value: 10E-10) and YGA (125/239 = 52.3% vs. 36.2%, p-value: 10E-7) together discriminated the immunoprecipitated miRNAs from the others (RAH contains RAC). All Fisher p-values remain highly significant even after conservative Bonferroni multiple testing correction. The finding of the methyltransferase target sequence in the precipitated miRNAs supports the notion that certain miRNAs are methylated.

For finding out whether there is a link between m6A and miRNA levels, we knocked down the ubiquitously expressed m6A demethylase FTO in HEK239 cells. After the knock-down, 222 /239 methylated miRNAs had reduced steady state levels, suggesting that methylation affects the biogenesis and/or stability of miRNAs. We tested three miRNAs (let-7e, miR-7-5p and miR-22-3p) by qRT-PCR and confirmed decreased levels of the mature miRNAs, but not of the primary transcripts. On the other hand, 42 miRNAs had increased steady state levels with a fold-change >2. Almost all of these were unmethylated. Possibly, certain miRNAs live longer as a consequence of attenuated gene silencing, caused by methylation of the target mRNAs. Since genetic variation in the FTO gene is associated with altered FTO transcript levels and body weight, it is likely that part of the phenotypic effect of this variation is mediated by deregulated miRNAs.

Conclusions: The epigenetic modification of an epigenetic modifier as described here adds a new layer to the complexity of the posttranscriptional regulation of gene expression.

W6-02

FAM134B-related hereditary sensory and autonomic neuropathy (HSAN2) is caused by impaired endoplasmic reticulum (ER) and Golgi architecture

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Degeneration of sensory neurons is characteristic for hereditary sensory and autonomic neuropathies (HSAN). Subsequently, pain loss in affected individuals leads to severe injuries and mutilations. We have previously shown that homozygous loss-of-function mutations in FAM134B cause HSAN type 2 (HSAN2). Here we addressed the pathophysiology of HSAN2 by a combination of biochemical and cell biological approaches and the analysis of Fam134b-knockout mice. Our studies on subcellular fractionations suggest that FAM134B is enriched in membranes of the ER and Golgi. In agreement with membrane shaping properties recombinant FAM134B increased the curvature of artificial liposomes in vitro. In vivo Fam134b-knockout mice develop a progressive sensory neuropathy similar to the human phenotype. Sensory nerve conduction studies showed smaller and delayed responses in KO-mice, whereas motoneuron function was unaffected. The ultrastructural analysis of sensory ganglion neurons revealed morphological changes of the endoplasmic reticulum (ER) and the Golgi cisternae in aged KO-mice. In conclusion, we propose to add FAM134B into the growing list of proteins with membrane-shaping properties required for long-term survival of neurons with complex neurites.

W6-03

In vivo evidence for incomplete neuronal autophagy in spastic paraplegia

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Recessive loss-of-function mutations in either SPG11, SPG15, or AP5Z1 cause complicated forms of hereditary spastic paraplegia (HSP) with a very similar clinical presentation. While AP5Z1 encodes the ζ-subunit of the adaptor protein complex 5 (AP-5), Spatacsin and Spastizin, the protein products of SPG11 and SPG15, associate with AP-5. The functions of the complex and of the respective proteins are largely unknown. A recent in vitro study suggests that Spatacsin and Spastizin are needed for autophagic lysosome reformation (ALR). The relevance of these observations for HSP, however, remained unclear. In order to unravel the functions of Spastizin and to understand the cellular basis of the loss of axon integrity observed in AP-5 related disorders, we generated a constitutive Spastizin knockout mouse model. Homozygous animals develop a severe gait disorder, muscle weakness, and ataxia. Morphological analysis reveals loss of corticospinal axons, cortical neurons, and cerebellar Purkinje cells. Degenerating neurons accumulate autofluorescent material in autolysosome-related structures. Increased levels of lysosomal enzymes in brains of aged knockout mice further support an alteration of the lysosomal compartment. We propose that impaired autophagic clearance results in accumulation of un-degraded material, and finally causes death of susceptible neurons. We thus provide evidence for the occurrence of ALR in vivo and for its relevance in HSP.

W6-04

Transcriptional regulator PRDM12 is essential for human pain perception

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Pain perception has evolved as a warning mechanism to detect tissue damage and dangerous environments. In humans, however, undesirable, excessive or chronic pain is a common and major societal burden for which medical treatments are currently suboptimal. New therapeutic options have recently emerged from the study of individuals with "congenital insensitivity to pain" (CIP). We report CIP in 11 families in whom we identified 10 different homozygous mutations in the PRDM12 gene (encoding PRDI-BF1 and RIZ homology domain-containing protein 12). PRDM proteins are a family of epigenetic regulators that control neural specification and neurogenesis. We determined that PRDM12 is expressed in nociceptors and their progenitors and participates in sensory neuron development in Xenopus embryos. Moreover, CIP-associated mutants abrogate the histone modification potential associated with wild type PRDM12. PRDM12 emerges as a key factor for orchestrating sensory neurogenesis and may hold promise as a novel pain therapeutics target.

W6-05

Olesoxime ameliorates Huntington disease-related pathology in the BACHD rat by suppressing calpain overactivation

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Huntington disease (HD) is a fatal human neurodegenerative disorder caused by a CAG repeat expansion in the HTT gene. A key event in the molecular pathogenesis of HD is the proteolytic cleavage of the mutant huntingtin protein (mHTT), which leads to the accumulation of toxic mHTT fragments. Several proteases including calpains and caspases are reported to play a critical role in this process. Here, we demonstrate that the mitochondria-targeting, neuroprotective compound, olesoxime, was able suppress to calpain hyperactivation, thereby drastically decreasing the amount of mHTT fragments as well as mHTT nuclear accumulation and cytoplasmic aggregates in BACHD rats, presumably by improving mitochondrial function. In this first long-term in vivo study, Olesoxime further prevented cortical thinning and ameliorated behavioral abnormalities. Thus, olesoxime constitutes a novel compound to decrease pathological forms of mHTT, encouraging further studies on its use as a therapeutic for HD.

W6-06

PRDM16 - a novel key player in cardiomyopathy and personalized medicine

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We recently identified a nonsense mutation in the transcriptional co-factor PRDM16 (PR domain containing 16) resulting in left ventricular non-compaction (LVNC) and dilated cardiomyopathy in human patients. To establish a personalized disease model for the latter, we faithfully recapitulated the LVNC by cardiomyocyte-specific overexpression of both mutant and wild-type (WT) PRDM16 in zebrafish. We observed an impaired cardiomyocyte proliferation with associated physiologic defects in cardiac contractility and cell-cell coupling during development in mutant, but not WT PRDM16 zebrafish and these defects persisted throughout adulthood. Moreover, mutant PRDM16 zebrafish displayed increased mitochondrial membrane potential and elevated levels of oxidative stress in the heart. In vitro, we identified differential binding partners between WT and mutant PRDM16, potentially underlying the defects reported in vivo. Furthermore, using both in vivo and in vitro gene expression analysis and WB, we identified multiple gene sets exclusively regulated by WT or mutant PRDM16. Intriguingly; pathway analysis revealed that several of these genes were associated with mitochondrial metabolism and cardiac contractility, again reflecting the cardiac phenotype presented by MUT PRDM16 zebrafish.

In a next step, using a phenotype-driven screening approach in the fish, we identified a melanocortin 4 receptor (MC4R) antagonist that rescued the physiologic defects associated with mutant PRDM16 during development. In addition, this compound also attenuated the contractile- and electrical defects observed PRDM16 mutants. Interestingly, MC4R expression was markedly upregulated in adult hearts of MUT PRDM16 zebrafish. Hence, increased MC4R expression is likely to be involved in mutant PRDM16-mediated LVNC cardiomyopathy. Given the fact that PRDM16 and MC4R are both highly metabolic genes of utmost importance for whole body physiology, the exploration of the link between both genes offers an exciting new scientific avenue to embark on.

Of note, MC4R antagonists could also rescue the cardiac defects seen in zebrafish models for arrythmogenic right ventricular compaction (ARVC), raising the intriguing question whether Prdm16 is also dysregulated and/or mutated in ARVC patients.

While future studies to further elucidate the exact mechanism of mutant PRDM16-related cardiomyopathy are warranted, our current findings underline the importance of personalized disease models. Indeed, such an

approach to investigate the aberrant activation of specific pathways, would greatly accelerate the exploration of disease biology and facilitate the development of innovative tailor-made therapies.

W7 CANCER GENETICS

W7-01

Constitutional Mismatch Repair Deficiency (CMMRD) Syndrome: What did we learn from the first 150 patients?

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Constitutional mismatch repair deficiency (CMMRD) resulting from biallelic germline mutations in one of the four MMR causes a distinct childhood cancer predisposition syndrome that overlaps with the recessive from of Turcot syndrome. The newly established European consortium Care for CMMRD (C4CMMRD) systematically collected and evaluated the clinical, pathological and genetic data from 146 molecularely confirmed CMMRD patients. Based on this analysis a 3-points scoring system for the suspected diagnosis CMMRD in a pediatric/young adult cancer patient was developed. Tumours highly specific for CMMRD are assigned 3 points, malignancies overrepresented in CMMRD 2 points and all other malignancies 1 point. To reach the diagnostic 3 points, pediatric or young adult patients who have only 1 or 2 points by their tumour need to show one or more additional (non-neoplastic) features which are weighted with 1 to 2 points according to their specificity for CMMRD and their frequency in the general population. Strategies to definitely confirm or refute the suspected clinical diagnosis were developed and evaluated in different laboratories of the consortium. They include refined mutation analysis protocols for the notoriously difficult PMS2 gene which is affected by biallelic mutations in >50% of CMMRD patients, a simple germline microsatellite instability (gMSI) assay which can be used as pre-test to substantiate the suspected clinical diagnosis or as a screening tool in large cohorts of patients, and ex vivo MSI and methylation tolerance assays that allow for a reliable diagnosis also in patients with equivocal mutation analysis results. With the broader application of the diagnostic criteria and subsequent assays to confirm/refute the diagnosis it will be possible to unequivocally identify most CMMRD patients at the time when they develop their first tumor. This will allow for adequate counseling of the family and adjustment of treatment modalities to the underlying defect in MMR capacity and the high risk of a second malignancy. C4CMMRD also proposed a protocol for surveillance that aims to detect the most common cancers in CMMRD, i.e. large and small bowel cancer, brain tumors and hematological malignancies. Currently, the optimal treatment modalities for CMMRD-associated tumors are unknown and it is not clear whether surveillance can improve the prognosis for CMMRD. Therefore, a registry established by C4CMMRD aims at collecting clinical and genetic data on larger cohorts of patients. Prospective systematic application of the outlined diagnostic strategies will help to recruit a presumably less biased spectrum of patients with this syndrome that will be analyzed to establish true cancer risks as well as adjusted treatment and surveillance modalities.

W7-02

Identification of ERCC2 as a cancer susceptibility gene for hereditary 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/OC in young patients, has been associated with a number of high-, moderate-, and low-penetrance susceptibility genes. Despite comprehensive testing by next-generation sequencing (NGS) there

is still a large portion of high risk cases where no mutation can be found in any of the known susceptibility loci. Therefore it is essential to extend the diagnostic spectrum by screening novel candidate genes. Here we report on the results of testing 94 genes in 717 patients from two different populations: German and Lithuanian.

Method: Inclusion criteria for the patients in this study were defined by the German Consortium for Breast and Ovarian Cancer and are based on the number of cases within the family, age of onset and occurrence of ovarian cancer. 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 19.7 % of the patients, BRCA1 or BRCA2 variations have been found. These were either clearly pathogenic loss-of-function mutations (43 %) or rare, unclassified missense variations with high probability of a deleterious effect (57 %). In 17.9 % of the patients we found null-mutations and rare, unclassified missense variants in the acknowledged BC/OC susceptibility genes ATM, CDH1, CHEK2, NBN, PALB2, RAD51C/D and TP53. Analysis of the non-BC/OC genes on the NGS panel identified the "excision repair cross-complementing rodent repair deficiency, complementation group 2" gene (ERCC2 or XPD) as a promising BC/OC predisposition candidate: we found 3 frame-shift mutations and 1 splice-site mutation in four independent BC/OC families. In all individuals tested so far, the mutations co-segregate with the occurrence of BC and/ or OC. Additionally we found 20 rare, unclassified sequence variations in ERCC2. These variants have a cumulative allele frequency of 2.9 % in our BC/OC cohort, which is 14.5-fold overrepresented compared to the "exome aggregation consortium" (ExAC) cohort (61486 exomes). Functional assays testing the ERCC2 variants are ongoing. Preliminary results show that at least some of the protein variants (e.g. NM_000400.3:p.Asp513Tyr) have lost their DNA repair ability.

Conclusion: Deleterious mutations and probably pathogenic missense variations in ERCC2, which are significantly overrepresented in our BC/OC cohort and co-segregate with the affected individuals, define ERCC2 clearly as a susceptibility gene for BC/OC predisposition. As part of ongoing research, affected individuals with excluded mutations in the known BC/OC predisposition genes should be tested for mutations in ERCC2 in order to determine clinical recommendations according to the phenotype.

W7-03

Multiplex Cap-Seq Analysis Detects Low-Dose Mosaic Mutations of RB1 in DNA from Blood

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Retinoblastoma is a rare malignant intraocular childhood tumor. In most children with sporadic unilateral retinoblastoma (>90%), the oncogenic variants occurred de novo in somatic cells. These oncogenic variants are readily detectable in tumor samples with conventional methods. In some patients with sporadic unilateral retinoblastoma, however, the first mutation occurred during embryogenesis resulting in mutational mosaicism. Conventional methods can only detect these mosaic mutations if the proportion of the mutant allele is large enough to give a clear signal. Detecting a mosaic mutation in DNA from blood becomes an even greater challenge when no tumor sample is available. Improving the detection of low-dose mutational mosaics in DNA from blood would increase the proportion of patients with sporadic unilateral retinoblastoma for whom the causative oncogenic variant can be determined if no tumor sample is available. We used custom DNA capture of a 200 kb region containing RB1 to perform 24-plex captures of blood DNA from retinoblastoma patients followed by sequencing on an Illumina HiSeq (>3,000x average coverage).

In a first step the characteristics of the method (including technical and DNA quality-related miscalls) were assessed using 56 blood DNA samples from unilateral retinoblastoma patients with known mutational status in the tumor. These included 17 patients for which the oncogenic variants had also been found in DNA from blood using conventional methods. Over 83% of the capture region had on average > 1000x coverage. However, the CpG island at the 5'-end of RB1 was one of the regions not successfully captured in most of the samples. All (17) samples containing oncogenic variants in blood DNA that were previously identified by other methods were confirmed, with mutant proportions ranging from 1.7% to 49.8% (median 40.7%). Furthermore, we identified two patients with mosaic mutations of RB1 (1.7% and 3.5%).

Next, we analyzed 96 blood DNA samples from patients where no tumor sample was available and no oncogenic variant had been found using conventional methods. Heterozygous oncogenic variants were identified in five samples. After applying a threshold of 1% and filtering out positions with recurrent miscalls as well as synonymous amino acid exchanges, we identified candidates for mosaic mutations of RB1 in 12 samples (8 base substitutions and 4 small length alterations), with mutant proportions ranging from 1.3% to 19.2% (median 5.9%). We are currently validating these candidates using highly sensitive, variant-specific detection methods.

W7-04

DNMT3A is a Powerful Follow-up Marker in NPM1 Mutated AML

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Background: NPM1 mutated (mut) FLT3-ITD negative acute myeloid leukemia (AML) is a distinct prognostically favorable AML subtype of. Robust data is available demonstrating that monitoring therapy response using NPM1mut-specific real time PCR is an important tool to early detect relapses and provides important information to guide therapy. Since next generation sequencing techniques have become available further gene mutations were detected that accompany NPM1mut in AML. Of these DNMT3Amut were the most frequent and stable ones (Krönke et al., Blood, 2013). Aim: 1) Analyse the stability of DNMT3Amut in paired diagnostic and relapsed samples. 2) Evaluate whether monitoring of DNMT3Amut provides additional information to monitoring of NPM1mut. Patients and Methods: Samples were selected from 359 NPM1mut de novo AML cases with an available DNMT3Amut status. First, to evaluate the stability of DNMT3Amut paired diagnostic and relapse samples of 103 patients were analyzed. NPM1mut status was assessed at diagnosis with a LightCycler melting curve analysis assay. Second, all diagnostic and follow-up samples (n=1,813) were quantified by real time PCR specific for the individual NPM1mut. Analysis for DNMT3Amut was performed using either the 454 technology (454 Life Sciences, Branford, CT) or the MiSeq instrument (Illumina, San Diego, CA). Deep DNMT3A sequencing of remission samples was performed using the 454 technology. Results: Out of 103 paired samples 61 (59.2%) carried a DNMT3Amut at diagnosis. 57/61 (93.4%) patients stably retained the mutation at relapse, in 4 (6.6%) the DNMT3Amut was lost. On the other hand 2 of 42 (4.8%) cases with DNMT3A wildtype at diagnosis gained the mutation at relapse. Thus, DNMT3Amut status was shown to be relatively stable (97/103; 94.1%) and thus qualifies as a promising target for follow-up controls. For comparison of DNMT3Amut and NPM1mut status during follow-up 54 patients that were NPM1/DNMT3A double mutated at diagnosis were selected according to the availability of at least one sample in first remission with an NPM1mut level <0.01%. These samples were reanalyzed by deep sequencing for the respective DNMT3A amplicons that had identified mutations at diagnosis. Of note, in 32/54 (59.3%) cases the DNMT3Amut persisted in the remission samples (NPM1mut low level <0.01% or negative) with high DNMT3Amut loads (median: 20%, range: 2-59%) that was only slightly below the load at diagnosis (median: 45%, range: 38-58%). Median overall survival and event free survival was worse for patients with persisting DNMT3Amut (n=32) compared to those who lost also the DNMT3Amut in remission (n=22) (69 vs 96 months; p=0.053 and 38 vs. 96 months; p=0.031, respectively). In conclusion, in 59.2 % of NPM1/DNMT3A double mutated AML the DNMT3Amut persists in remission and this was found to be prognostically adverse. Clones with DNMT3Amut as the sole mutation have a normal phenotype and thus DNMT3Amut may be regarded as premalignant mutation.

W7-05

Whole genome bisulfite sequencing (WGBS) in uveal melanoma identifies tumor class specific methylation signatures

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Uveal melanoma (UM) is the most frequent malignant tumor of the eye. Gene expression profiles (GEP), chromosome 3 copy number and gene mutation patterns divide uveal melanoma into two major classes, which are associated with metastatic progression. UM with monosomy 3 frequently metastasize whereas UM with disomy 3 are associated with a favorable prognosis. Therefore, tumor classification based on GEP or chromosome 3 copy number can be used to predict patients' prognosis. In order to find out if there are class specific methylation patterns and identify genes associated with altered DNA methylation we performed WGBS on DNA from 4 primary uveal melanoma samples, two of either class. To achieve even coverage of CpG island (CGI) and non CGI regions, two different libraries were generated from each sample. Both libraries, a bisulfite treated TruSeq DNA PCR-Free library and a post-bisulfite library prepared using EpiGnome Methyl-Seq-Kit, were sequenced on Illumina Hiseq2500 in a 2:1 ratio, respectively. In total 6 lanes per sample were sequenced resulting in an overall 38 to 42 fold coverage. For mapping and analysis of the data we use methylCtools, which can map bisulphite treated DNA with BWA. SAMtools is used for sorting BAM files. Duplicate reads are marked by PicardTools, which also yields the mapping statistics. Unsupervised cluster analysis by the 1000 most variant CpG sites grouped the 4 tumor samples in two classes in concordance with their chromosome 3 status. We next determined the regions differentially methylated (DMRS) between both tumor classes

(excluding DMRs on sex chromosomes and chromosome 3) using BSmooth pipeline and identified 1037 DMRs with a minimal length of 4 CG dinucleotides in a row and an average methylation difference > 0,4. The majority of these DMRs (72%) are hypermethylated in UMs with monosomy 3. Of the 1037 DMRs 134 overlap with a CGI, 504 are located in a CGI shore (0 - 2kb distance) and 69 are located in a CGI shelf (2-4kb distance). About 73% (761) of all DMRs map close (< 10kb) to the transcription start site (TSS) of a gene. Interestingly, 25 % of all DMRs are located between different TSSs of the same gene. To validate the class specific methylation pattern, we determined the methylation pattern of 11 of these DMRs in a set of another 25 UMs, 15 UM with disomy 3 and 10 UM with monosomy 3, and two DNA samples from cultured primary melanocytes, the presumed UM precursor cells. Methylation analysis was performed using Sanger sequencing of PCR products obtained from bisulfite treated genomic DNA. Cluster analysis performed on these methylation data grouped tumor samples in two groups congruent with their chromosome 3 status. Melanocyte samples formed a distinct but separate group, suggesting a distinct methylation pattern in these cells. Our study revealed tumor class specific methylation signatures that can be used for class assignment of UM samples.

W7-06

Integrated genomic, transcriptional and epigenomic analyses of germinal center derived B-cell lymphomas

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Germinal center-B-cell-derived (GCB) lymphomas are the most common lymphomas in children and adults. They include follicular (FL), diffuse large B-cell (DLBCL) and Burkitt lymphomas (BL) as well as intermediate subtypes. In the framework of the International Cancer Genome Consortium (ICGC) the BMBF-funded ICGC MMML-Seq network performs sequencing-based characterization of various subtypes of GCB-lymphomas. So far, more than 200 cases are in the sequencing pipeline with 141 finished whole genome sequence analysis of GCB-lymphomas and matched normal controls. In parallel, transcriptome and miRNAome sequencing from 165 tumors and 103 tumors, respectively, have been finished. DNA methylation has been determined in 178 lymphomas by 450K arrays as well as by whole genome bisulfite sequencing (WGBS) in a subset of 29 GCB-lymphomas and four controls. Findings are extended to the MMML-cohort containing more than 800 molecularly characterised B-cell lymphomas. Moreover, genomic, epigenomic and transcriptional patterns are intersected with reference epigenomes from representative lymphoma cell lines obtained by participation of the ICGC MMML-Seq in the IHEC project BLUEPRINT. With regard to somatic mutations the most frequently mutated genes in BL include MYC, TP53, ID3, CCND3, FBXO11 and SMARCA4. In FL, BCL2, CREBBP, MLL2 and TNFRSF14 are most frequently affected. In DLBCL, the landscape of mutated genes is more heterogeneous. To characterize the relationship between epigenomic features, genetic alterations and phenotype in these GCB-lymphomas we focused on the comparison between BL and FL. Combined analysis of WGBS and RNAseq data identified a total of 8,207 differentially methylated regions where the methylation state and expression of associated genes correlated (cDMRs). In BL and FL these cDMRs were significantly associated with GC dark zone and light zone B-cell gene expression, respectively, as well as with the deregulation of 58 Ingenuity pathways. Remarkably, these cDMRs were enriched in signaling pathways differentially activated in BL and FL and affected regulatory mechanisms altered by recurrent mutation in a large fraction of BL including cell cycle control, the ID3/TCF3 complex, the SWI/SNF remodeling complex and the Gαi signaling pathway. Furthermore, in BL we observed correlated loss of methylation and expression at SMARCA4-target genes along with overexpression of SMARCA4. SMARCA4 frequently carries missense mutations in the helicase domain leading to functional alterations of SMARCA4, imprinting the DNA-methylome of BL samples. Thus, integration of genomic, transcriptomic and epigenomic analysis deciphered multi-layer deregulation of pathways and complexes in GCB-lymphomas. Moreover, our global analyses of the epigenomic architecture of GCB-lymphomas connect specific epigenetic modifications and lymphoma entities and suggest that deregulation of the SWI/SNF complex by SMARCA4 mutation is of pathogenetic importance in a major subset of BLs.

W8 COMPLEX GENETICS

W8-01

Identification of common and low-frequency genetic variants associated with gyrification, a structural endophenotype of the brain correlated with higher cognitive functions

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One of the most prominent features of the human cerebral cortex is its pattern of tissue folding (gyrification) which occurs as a consequence of brain growth during fetal and early postnatal development. Recent heritability studies suggest that gyrification is influenced by genetic and environmental factors. There is accumulating evidence that the degree of folding, as measured by the gyrification index (GI), is a structural endophenotype which is correlated with higher cognitive functions, aging and neurological and psychiatric disorders. Despite these observations, not much is known about the genes and molecular pathways underlying gyrification. We therefore performed a genome-wide association study (GWAS) between the GI and a high-resolution set of single-nucleotide polymorphism (SNPs) in non-overlapping samples from 1000BRAINS, SHIP and SHIP-TREND, three large population-based cohort studies from Germany.

In total, data from 9,772,648 autosomal and X-chromosomal SNPs and 2,604 individuals were available. The most significant SNP surpasses the threshold for genome-wide significance (P=3.45E-8, beta=0.099, MAF=1.5%), explains 2.25% of the GI's total variance and increases the GI by 3.4% per effect allele. The SNP maps to a transcription factor gene located on chromosome 12 which is expressed in the developing cortex as well as in all four lobes of the adult cortex. Additional four loci were sub-genome-wide significant (P<5E-7). Overall, 40 loci provided evidence for strong-to-moderate association with the GI (P<5E-5). Of the loci's lead SNPs, 23 showed low-frequency (1≤MAF<5%) and 17 showed common MAF (≥5%).

This is the first systematic genetic study (GWAS) that investigates a folding trait in the human cortex. Our main finding highlights an influence of a transcription factor on cortical folding. Expression of this gene coincides with life periods critical for formation (development) and reduction of sulcal depth and gyral width during adulthood and aging. Strikingly, further high ranking SNPs implicate genes that have already been associated with brain disorders, such as schizophrenia (8p23, CSMD1, membrane-bound phosphatase) and Parkinson's disease (21q21, USP25, ubiquitin-specific phosphatase). To further strengthen our findings, we are currently genotyping the lead SNPs in 1,300 individuals from the SHIP study who are independent from the GWAS.

W8-02

Identification of shared risk loci and pathways between bipolar disorder and schizophrenia

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Bipolar disorder (BD) is a severe disorder of mood with a lifetime prevalence of about 1% and a high heritability of about 70%. The disease is characterized by recurrent episodes of mania and depression. BD shows substantial clinical and genetic overlap with other psychiatric disorders. Using genome-wide genotype data of the Psychiatric Genomics Consortium (PGC) the calculated genetic correlation was around 68% between BD and schizophrenia (SCZ), which was the highest correlation of BD with any of the psychiatric diseases investigated. However, research has not yet clarified what particular genes form the basis of this etiological overlap.

For both disorders a number of susceptibility genes have been identified, with the relevant biological pathways, however, being still largely unknown. In the case of SCZ, a PGC meta-analysis of data from more

than 36,000 patients and 113,000 controls identified a total of 128 independent genome-wide significant single nucleotide polymorphisms (SNPs) (Schizophrenia Working Group of the PGC, 2014).

The aim of the present study was to investigate whether these 128 SCZ-associated SNPs also contribute to the development of BD. For this purpose we conducted association testing of these SNPs in our large GWAS dataset of BD comprising of 9,747 patients and 14,278 controls (Mühleisen et al., 2014). In this dataset we combined our data obtained from four European countries, Canada, and Australia with the results of a recent large BD GWAS by the PGC (Sklar et al., 2011). As different reference panels were used for the imputation of the genotype data in both studies, we reimputed the summary statistics of the PGC BD GWAS (Sklar et al., 2011) using a recently proposed method for fast and accurate imputation of summary statistics (Pasaniuc et al., 2014). Then, a meta-analysis combining the PGC BD and our BD GWAS was performed using METAL (Willer et al., 2010). Overall, 107 SCZ-associated SNPs could be mapped to our reimputed BD GWAS data.

Our analysis revealed that 42 of the 107 SNPs showed nominally significant p values. The observed number of SNPs with a p value of < 0.05 was significantly higher than expected (p<2.2x10-16, Chi-Square test) providing further evidence that SCZ-associated loci contribute to the development of BD. The strongest associated SNP was located near the TRANK1 gene (p=8.8x10-8) which is a previously reported genome-wide risk gene for BD.

Pathway analysis for all 42 shared SCZ-BD SNPs was performed using INRICH (Lee et al. 2011) and Ingenuity pathway analysis. With both methods we identified a total of 27 nominally significant canonical pathways. These included calcium and glutamate signaling, axon guidance and synaptic long term potentiation, which is consistent with previous pathway analyses of BD.

Our analysis gives further insights into the disease-associated pathways shared between BD and SCZ. This may provide clues for new approaches to treatment and prevention of these major psychiatric disorders.

W8-03

Large GWAS in 16,900 individuals identifies numerous susceptibility loci including one genetic risk factor differentiating psoriatic arthritis from psoriasis vulgaris

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Psoriatic Arthritis (PsA) is a chronic inflammatory joint disease belonging to the complex diseases from the genetic point of view. It occurs in up to 30% of patients with the most common psoriatic skin manifestation, psoriasis vulgaris (PsV). Besides the well-known association of PsA to an HLA-C risk allele, genome-wide association studies (GWAS) enabled the identification of five further susceptibility loci at IL12B, TRAF3IP2, FBXL19, REL and RUNX3. All six susceptibility loci have been shown to overlap with those identified in PsV. The identification for genetic loci differentiating between both disease manifestations is a longstanding goal.

We now combined our GWAS in German individuals with that of a British PsA study and performed a joint GWAS encompassing a total of 1,402 patients and 5,749 controls. After imputation, association testing for 2,364,677 SNPs was performed using logistic regression under an additive model. We detected genome-wide significant association (p< 5.0E-08) to five loci, three previously identified ones (HLA-C, IL12B and TRAF3IP2) and two newly identified ones (DLEU1 and IL23R). While previous studies had revealed suggestive evidence for association to IL23R, this is the first time that genome-wide significant association (p= 2.19E-12) is shown for PsA.

For replication in an independent case-control cohort of 2,115 PsA patients and 7,634 controls we selected SNPs at these loci except for HLA-C. In addition, we explored further 19 candidate loci with 1) single p-values of less than 5.0E-05 (3 orders larger than genome-wide significance), and 2) five or more SNPs at a locus with p-values less than 5.0E-05 in relative neighborhood.

Association to four known loci IL12B, TRAF3IP2, REL and RUNX3 was replicated in the combined analysis with p-values in the combined study of 7.76E-24, 3.07E-19, 1.52E-10 and 3.25E-12, respectively. Of the two new loci reaching genome-wide significance in the GWAS, only IL23R could be replicated (poverall =2.48E-19). Furthermore, two of the candidate loci showed genome wide significance in the entire cohort. For IL23A it is the first time that genome-wide significant association in PsA (p =7.33E-09) is reported. We also identified genome-wide significant association (poverall =3.83E-08) to an intronic SNP at a completely new susceptibility locus for PsA comprising two different candidate genes, RAB5C and KCNH4. This locus has not been described in PsV and neither have the pathways of the two new potential susceptibility genes been implicated in PsA or PsV before. Further genetic and functional studies at this susceptibility locus are necessary to replicate this finding, identify the disease-causing variant, to dissect the relevant gene in PsA as well as to characterize the affected pathway.

W8-04

Meta-analysis of genome-wide association studies in alopecia areata resolves HLA associations and reveals two new susceptibility loci

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Background: Alopecia areata (AA) is one of the most prevalent autoimmune diseases, with ten known susceptibility loci so far. This disorder is characterized by a sudden onset of patchy areas of hair loss on the scalp and/or body.

Methods: To provide further insight into the genetic basis and the role of immunity in AA we performed a first meta-analysis by combining data from two genome-wide association studies (GWAS), and replication with supplemented ImmunoChip data for a total of 3,253 cases and 7,543 controls.

Results: The strongest region of association was the MHC, where we fine-mapped 4 independent effects, all implicating HLA-DR as a key etiologic driver. Outside the MHC, we identified two novel loci that exceed statistical significance, containing ACOXL/BCL2L11 (BIM) (2q13); LRRC32 (GARP) (11q13.5), as well as a third region that achieved nominal significance SH2B3 (LNK)/ATXN2 (12q24.12). Expression analysis of candidate susceptibility genes in these three regions demonstrates expression in relevant immune cells, as well as in the hair follicle. Finally, we performed a cross phenotype meta-analysis integrating our meta-analysis with data from seven other autoimmune diseases, providing insight into the molecular taxonomy of autoimmune diseases and the alignment of AA within this class of disorders.

Conclusions: Our findings have uncovered novel functional pathways that are disrupted in AA, including autophagy/apoptosis, TGFß/Tregs and JAK kinase signaling, lending further support the causal role of aberrant immune processes in AA.

W8-05

Role of miRNAs in the etiology of alopecia areata: A genome-wide miRNA association analysis

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Background: Alopecia areata (AA) is a common hair loss disorder characterized by a sudden onset of patchy areas of hair loss, which can occur on the scalp or elsewhere on the body. Immunological and genetic association studies support the hypothesis that AA is autoimmune in nature. Recent evidence points to a significant role of miRNAs in autoimmune diseases, however, their role in AA is not yet known.

Methods: We performed a systematic analysis to investigate whether common variants among known microRNA loci contribute to AA development. Gene-based analyses were performed by VEGAS for all miRNAs listed in miRBase and their flanking sequences using the largest GWAS data set of 3,253 patients and 7,543 controls.

Results: 78 of 615 miRNAs were nominally associated with disease. Seven of them remained significant after correction for multiple testing. After excluding miRNAs in the MHC region, we extracted target gene information for four miRNAs yielding a total of 2,072 target genes. Gene based p-values were calculated for the target genes revealing 24 of them to be significantly associated with AA after correction for multiple testing. One of these genes included IL2RA, a known AA locus, which further support our initial findings.

Conclusions: Our study is the first to suggest that miRNAs may play an important role in the pathogenesis of AA which could be of interest for development of therapies in the future.

W8-06

Nonsyndromic cleft lip with or without cleft palate: Genome-wide imputation identifies four novel risk loci and an enrichment of association signals in enhancer datasets relevant to craniofacial development

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Nonsyndromic cleft lip with or without cleft palate (nsCL/P) is one of the most common congenital malformations. It is considered to be multifactorial, with both genetic and environmental factors contributing to its etiology. Genome-wide studies and replication approaches have led to the identification of 15 nsCL/P susceptibility loci, with additional factors awaiting elucidation. Some of the yet unidentified genetic risk factors might be detected by increasing the marker density in genome-wide datasets using imputation.

We used individual genotype data from a published genome-wide meta-analysis including 399 cases, 1.318 controls and 666 trios, all of European origin (Ludwig et al. 2012, Nature Genetics). Based on about 500.000 genotyped markers, imputation was performed using 2184 alleles of the 1kGP data. Variants were statistically analyzed if they had a minor allele frequency ≥1% and an info score >0.4, yielding a high-density dataset of about 8.26 million variants.

At single variant level, one previously unreported SNP reached genome-wide significance (rs6740960 at 2p21, P=1.6x10-08). Additional 398 SNPs at 64 novel loci had P-values below 0.001 in the meta-analysis, and showed P<0.05 in both case-control and trio analyses, respectively. In a first replication assay, 32 SNPs (29 loci) were analyzed in an independent nsCL/P sample of European, Mesoamerican and Arab populations

(total: 609 cases, 1.745 controls). Six SNPs showed nominally significant P-values, and after combined analysis, four SNPs/loci reached genome-wide significance: 2p24.2, 2p21, 14q22.1 and 15q13.3. Three of these harbor candidate genes relevant to craniofacial development. Most interestingly, rs6740960 at 2p21 maps 5bp adjacent to a predicted Myc binding site about 100 kb upstream of the PKDCC gene. Of note, PKDCC is downregulated in mice that carry a deletion of the region homologous to the 8q24 nsCL/P major risk locus (Uslu et al. 2014), suggesting a new pathway underlying nsCL/P.

At genome-wide level, we investigated whether association results are enriched in functional datasets relevant to craniofacial development. In previously published enhancer datasets from human neural crest cells (Rada-Iglesias et al. 2012) and murine embryonic craniofacial tissue (Attanasio et al. 2013) we observed a significant overrepresentation of association signals ($P<0.05$). Notably, the same analysis in a non-craniofacial murine dataset (Visel et al. 2009) did not show enrichment, suggesting that the identified enrichment is specific to craniofacial development and that regulatory effects play a crucial role in nsCL/P development.

Our study identified four novel risk loci for nsCL/P and revealed an overrepresentation of association signals in functionally relevant datasets. Future analyses will include the assessment of heritability estimates using GCTA, and pathway analyses using IPA and the candidate genes at all risk loci established for nsCL/P.

W9 MONOGENIC DISEASE II

W9-01

A rare form of achromatopsia is caused by mutations in the gene for the Unfolded Protein Response regulator ATF6

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Achromatopsia (Total Colourblindness) is an autosomal recessive eye disorder characterized by low vision, lack of colour discrimination, photophobia and nystagmus. Electroretinographic recordings show absence or severely reduced function of cone photoreceptors in these patients. To date mutations in five different genes – all of them encoding for essential components of the cone phototransduction cascade - have been shown to cause the disorder and account for >75% of cases in our patient cohort of >1,000 patients.

In order to search for additional genes associated with this rare disorder we employed autozygosity mapping and whole exome sequencing in a family of Irish descent with three affected siblings. We identified a homozygous missense mutation in ATF6 that segregated with the disease in this family. Subsequent Sanger sequencing of the ATF6 gene in a cohort of unsolved patients with achromatopsia resulted in the identification of nine further families with either homozygous or compound-heterozygous mutations including missense variants, frameshift causing indel mutations and splice site mutations verified by cDNA analysis. Patients with ATF6 mutations presented with visual and ocular function deficits typical for achromatopsia. Retinal imaging revealed foveal hypoplasia with an essentially absent foveal pit and a variable degree of disruption of the cone photoreceptor layer at the macula. There was no evidence for extraocular manifestation of the disease. Other than the known achromatopsia genes, ATF6 has no specific or exclusive function in phototransduction but encodes the ubiquitously expressed Activating Transcription Factor 6 that is known as a key regulator of the Unfolded Protein Response (UPR) and cellular endoplasmic reticulum (ER) homeostasis. Functionally, we found that disease-associated ATF6 variants lead to attenuated ATF6 transcriptional activity in response to ER stress in patient fibroblasts. Atf6-/- knockout mice present with normal retinal morphology and function in young, but both rod and cone dysfunction at older ages. Our study demonstrates that mutations in ATF6 are a rare cause of achromatopsia (ca.1% in our patient cohort) and suggests crucial and unexpected role of ATF6 in human foveal development and/or cone photoreceptor function.

W9-02

Mutations in NBAS Cause Autosomal-Recessive Recurrent Acute Liver Failure with Onset in Infancy

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Acute liver failure (ALF) in infancy and childhood is a life-threatening emergency. Few conditions are known to cause recurrent acute liver failure (RALF) and in about 50% of cases, the underlying molecular cause remains unresolved. Exome sequencing in five unrelated individuals with fever-dependent RALF revealed biallelic mutations in NBAS. Subsequent Sanger sequencing of NBAS in fifteen further unrelated individuals with RALF or ALF identified compound heterozygous mutations in an additional six individuals from five families. Western blot analysis of mutant fibroblasts showed reduced protein levels of NBAS and its proposed interaction partner p31, both involved in retrograde transport between endoplasmic reticulum and Golgi. We suggest NBAS analysis in individuals with paediatric acute liver failure, especially if triggered by fever.

W9-03

Intragenic truncation of Laf4 causes Nievergelt-like Syndrome

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Nievergelt Syndrome (NS) is a congenital malformation characterized by mesomelic dwarfism caused by dysplastic leg bones with rhomboid shape. Occasionally, forearms are also affected. Typically NS-patients show normal intelligence and facial appearance. While Kurt Nievergelt himself already cleared the inheritance mode in 1944 to be autosomal dominant, the genetic cause for Nievergelt-Syndrome remained unknown until today.

In the present work we are describing a Caucasian patient with Nievergelt-like Syndrome. Besides presenting the typical skeletal malformation with triangular tibia and absence of fibula, the patient also showed ambiguous genitalia and brain dysfunction accompanied with myoclonic jerks and phase of apnea. The patient died at the age of three months.

In 2008, we mapped in this patient a deletion of the LAF4 gene (Lymphoid nuclear protein related to AF4, also named AFF3). LAF4 is a transcription factor normally expressed in lymphocytes and postnatal cortex where it plays a role in cell migration during brain development. To recapitulate in animal model the mapped genetic perturbation we first produced a Laf4KO mice which did not phenocopy the human malformations. We thus re-mapped the deletion breakpoints using a higher resolution 1M-Array-CGH and found an in-frame deletion in LAF4 that is predicted to produce a truncated protein. Additionally, we observed duplication of the promoter region of LAF4. By using the CRISPR/Cas9-technology we generated and homologous truncated Laf4 allele in mice. In these animals we could observe, in heterozygous condition, a phenocopy of the patient's skeletal malformation.

In summary, we suggest LAF4 as candidate gen for Nievergelt- and Nievergelt-like Syndromes.

W9-04

Mutations in KCNH1 and ATP6V1B2 cause Zimmermann-Laband syndrome

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Zimmermann-Laband syndrome (ZLS, MIM 135500) is a rare developmental disorder characterized by facial dysmorphism with early onset gingival enlargement, intellectual disability with/without epilepsy, hypo/aplasia of nails and terminal phalanges of hands and feet, and hypertrichosis. Most cases are sporadic, suggesting autosomal dominant de novo mutations. We performed whole exome sequencing (WES) on four unrelated parent-child trios and one single individual. WES data were processed to identify the disease gene(s) shared among unrelated patients. Although no gene was shared by all samples, KCNH1 had de novo variants occurring in three subjects (c.1399A>G, p.I467V; c.1405G>A, p.G469R; c.1054C>G, p.L352V). Two subjects shared the de novo missense variant c.1454G>C (p.R485P) in ATP6V1B2. The KCNH1 and ATP6V1B2 nonsynonymous variants were validated by Sanger sequencing, and parental genotyping confirmed their de novo origin. We next Sanger-sequenced DNAs of 19 additional individuals exhibiting clinical features fitting ZLS for mutations in KCNH1 and ATP6V1B2. The KCNH1 missense changes c.1399A>G (p.I467V) and c.1042G>A (p.G348R) were found to occur as de novo mutations in two subjects. Two heterozygous variants (c.974C>A, p.S325Y; c.1066G>C, p.V356L) were documented in one subject; both were de novo and in cis. Mutation-positive individuals had the major clinical features of ZLS. Seizures/epilepsy was present in all KCNH1-mutation positive subjects but absent in the individuals with ATP6B1V2 mutation. Recently, KCNH1 missense mutations have been reported in patients with Temple-Baraitser syndrome, a multisystem developmental disorder showing clinical overlap with ZLS.

ATP6V1B2 encodes a subunit of the vacuolar H+-ATPase, a multisubunit enzyme that mediates acidification in organelles by pumping protons against an electrochemical gradient. Structural analysis indicated a disruptive effect of the introduced residue on complex assembly. KCNH1 codes for Eag1/Kv10.1, a member of the ether-à-go-go family of voltage-gated K+ channels. Patch-clamp recordings in CHO cells transfected with wild-type or mutant KCNH1 cDNA demonstrated functional outward-rectifying K+ channels for four of the five mutants (except G469R) with activation thresholds at strikingly more negative potentials compared to wild-type. Accelerated activation and slower deactivation kinetics contributed to the gain-of-function effect of the four mutant channels. Co-expression experiments revealed a dominant-negative action of the G469R mutant over the wild-type channel regarding the current amplitude at more depolarized potentials, however, the heteromultimeric G469R/wild-type channels induced a pronounced K+ conductance at negative potentials similar to the other four ZLS-associated KCNH1 mutants. Taken together, our findings provide evidence that disturbances in voltage-dependent K+ currents and acidification of intracellular compartments cause the clinically recognizable ZLS phenotype.

W9-05

Mutations in SEC24D, Encoding a Component of the COPII Machinery, Cause a Syndromic Form of Osteogenesis Imperfecta

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As a result of a whole-exome sequencing study, we report three mutant alleles in SEC24D, a gene encoding a component of the COPII complex involved in protein export from the ER: The truncating mutation c.613C>T (p.Gln205*), and the missense mutations c.3044C>T (p.Ser1015Phe, located in a cargo-binding pocket) and c.2933A>C (p.Gln978Pro, located in the gelsolin-like domain). Three individuals from two families affected by a similar skeletal phenotype were each compound-heterozygous for two of these mutant alleles, with c.3044C>T being embedded in a 14 Mb founder-haplotype shared by all three. The affected individuals were a seven-year-old boy with a phenotype most closely resembling Cole-Carpenter syndrome, and two fetuses initially suspected to have a severe type of Osteogenesis imperfecta. All three displayed a severely disturbed ossification of the skull and multiple fractures with prenatal onset. The seven-year-old boy had short stature and craniofacial malformations including macrocephaly, midface hypoplasia, micrognathia, frontal bossing, and down-slanting palpebral fissures. Electron and immunofluorescence microscopy of skin fibroblasts of this individual revealed that ER export of procollagen was inefficient and that ER tubules were dilated, faithfully reproducing the cellular phenotype of individuals with Cranio-lentico-sutural dysplasia (CLSD). CLSD is caused by SEC23A mutations and displays a largely overlapping craniofacial phenotype, but it is not characterized by generalized bone fragility and presented with cataracts in the original family described. The cellular and morphological phenotypes we report are in concordance with the phenotypes described for the Sec24d-deficient fish mutants vbi (medaka) and bulldog (zebrafish). Our report assigns the fourth human phenotype to germline mutations in components of the COPII machinery.

W9-06

XRCC4, a novel key player in the molecular pathogenesis of Seckel syndrome

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DNA double strand breaks (DSBs) are highly toxic lesions, which, if not properly repaired, can give rise to genomic instability. Non-homologous end-joining (NHEJ), a well-orchestrated, multistep process involving numerous proteins essential for cell viability, represents one major pathway to repair DSBs in mammalian cells, and mutations in different NHEJ components have been described in microcephalic syndromes associated e.g. with short stature, facial dysmorphism, and immune dysfunction. By using whole-exome sequencing (WES) we now identified a homozygous mutation, c.482G>A, in the XRCC4 gene encoding a crucial component of the NHEJ pathway, in three affected brothers of a consanguineous Turkish family. Affected individuals showed typical clinical feature of Seckel syndrome, which is characterized by facial dysmorphism, severe microcephaly, intellectual disability, short stature, but no recognizable immunological phenotype. We showed that the XRCC4 c.482G>A mutation, which affects the last nucleotide of exon 4, induces defective splicing of XRCC4 mRNA mainly resulting in premature protein truncation and most likely loss of XRCC4 function. Moreover, we observed on cellular level that XRCC4-deficiency leads to hypersensitivity to DSB-inducing agent and defective DSB repair, which results in increased cell death after exposure to genotoxic agents. Taken together, our data provide evidence that autosomal recessive mutations

in XRCC4 induce increased genomic instability and cause Seckel syndrome defined by typical facial dysmorphism, primary microcephaly, and short stature.

POSTER

P-BASIC MOLECULAR MECHANISMS

P-Basic-001

Pelota mediates gonocyte maturation and maintenance of spermatogonial stem cells in mouse testes

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Pelota (Pelo) is an evolutionally conserved gene, and its deficiency in Drosophila affects both male and female fertility. In mice, genetic ablation of Pelo leads to embryonic lethality at the early implantation stage as a result of the impaired development of extra-embryonic endoderm (ExEn). To define the consequences of Pelo deletion on male germ cells, we temporally deleted the gene at both embryonic and postnatal stages. Deletion of Pelo in adult mice resulted in a complete loss of whole germ cell lineages after 45 days of deletion. The absence of newly emerging spermatogenic cycles in mutants confirmed that spermatogonial stem cells (SSCs) were unable to maintain spermatogenesis in the absence of PELO protein. However, germ cells beyond the undifferentiated SSC stage were capable of completing spermatogenesis and producing spermatozoa, even in the absence of PELO. Following the deletion of Pelo during embryonic development, we found that although PELO is dispensable for maintaining gonocytes, it is necessary for the transition of gonocytes to SSCs. Immunohistological and protein analyses revealed the attenuation of FOXO1 transcriptional activity, which induces the expression of many SSC self-renewal genes. The decreased transcriptional activity of FOXO1 in mutant testes was due to enhanced activity of the PI3K/Akt signaling pathway, which led to phosphorylation and cytoplasmic sequestration of FOXO1. These results suggest that PELO negatively regulates the PI3K/Akt pathway and that the enhanced activity of PI3K/Akt and subsequent FOXO1 inhibition are responsible for the impaired development of SSCs in mutant testes.

P-Basic-002

The first mouse model for ATP6V0A2 related cutis laxa enables closer insight into the pathomechanism

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Beyond the pathognomonic lax and wrinkled skin ATP6V0A2-related cutis laxa (MIM #219200, #278250) affects several organ systems. The patients suffer from a reduced bone density and a general connective tissue weakness. A cobblestone malformation of the frontal cortex present in the majority of cases is often associated with severe seizures and a mental retardation. Patients also show a glycosylation defect on the level of the Golgi apparatus and the disorder is therefore a congenital disorder of glycosylation type 2. The ATP6V0A2 gene codes for the a2 subunit of the V-type H+-ATPase. This subunit facilitates the assembly of the V-ATPase complex and is necessary for the proton transport.

We created an Atp6v0a2 knock-out mouse model. Homozygous mutants are viable, but show a growth retardation of around 20 percent. Histological investigations revealed a reduction of bone density and an abnormal layering of the frontal cortex. Lectin blots pointed to an aberrant glycosylation of serum proteins in these mice.

In parallel we are investigating an Atp6v0a2R755Q knock-in mouse model with an exchange of an amino acid essential for proton transport. In contrast to the knock-out the a2 protein is stable, but specifically lost the function to regulate the pH.

The reflection of the human phenotype in the knock-out mouse model shows the universality of the pathomechanism in different species. Further investigations of neurons of this mouse model and the analysis of brain specific proteins will enable us to better understand ATP6V0A2-related cutis laxa and possible find novel options for intervention. Together with the knock-in mouse model we will get further insights into the function of the a2 subunit and elucidate the link between pH-regulation and glycosylation.

P-Basic-003

The role of TorsinA in developing neurons

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Objective:

The aim of this study was to further analyze the role of TorsinA during neurogenesis. To this aim we analyzed whether overexpression of TorsinA in PC6-3 cells alters length and quantity of neurites. In addition analyzed whether the disease-related mutation of TorsinA (ΔGAG) had effects on neurite outgrowth in PC6-3 cells. These experiments were complemented by analysis of neurite outgrowth in in murine primary neuron cultures from TorsinA-knockout and -knockin mouse models.

Background:

The 3-basepair mutation of TorsinA (ΔGAG) leads to the early onset autosomal dominant inherited movement disorder DYT1 Dystonia, which leads to involuntary muscle contractions, causing twisting and repetitive movements or abnormal postures,. Due to the fact that loss of TorsinA leads to early death in the affected mouse models and shows high expression levels in early brain development we conclude that TorsinA plays an important role during neurogenesis and our aim was to further investigate the role of TorsinA in the developing central nervous system.

Methods:

We used Doxycyclin-induceable PC6-3 cells expressing TorsinA in wildtype and ΔGAG variants, differentiated using NGF in orderto measure the length and amount of neurites and the size of the growth cone. Analysis was performed by means of immunofluorescence microscopy and computational analysis was done using NeuronJ. Additionally, hetero-and homozygous TorsinA knockout- and knockin (ΔGAG]) mice were sacrificed at embryonic day 19 to obtain primary neuron cultures of the hippocampus.

Results:

Our results indicate that overexpression of TorsinA leads to a significant increase in growth cone size and elongation of neurites in PC6-3 cells, even when the mutant form of TorsinA is expressed. In contrast, loss of TorsinA leads to a significant reduction in the length of dendrites in hippocampal primary neurons.

Conclusions:

As seen in our experiments, TorsinA seems to play an important role during the neurogenesis, controlling the regulation of dendrite length. This may be causative for DYT1-Dystonia, since a reduction of dendrite length may lead to unexpected consequences for the whole brain structure which have to be elucidated in further experiments, as does the mechanism behind the ability of TorsinA to control the growth of dendrites.

P-Basic-004

Identification of genome-wide ionizing radiation-induced DNA alterations in human fibroblasts

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Medical and other applications of ionizing radiation have been considerably increasing over the last decades, as has the number of radiation accidents. The latter often involve exposition to one single acute dose of ionizing radiation (IR) that can either induce acute radiation sickness, localised injuries and/or stochastic consequences. While deterministic damages relate to IR-induced cell death and nekrosis, lower doses induce stochastic effects like mutations that may alter a cell's fate and contribute to tumorigenesis years after exposure. Specific mutations and altered gene expression patterns have been studied in different systems. However, there is only limited knowledge on the molecular impact of IR-exposure on a genome wide level To address questions concerning a potential dose response in the IR-induced mutation signatures, we analysed the dose response of primary human gingiva fibroblasts exposed to increasing doses of X-rays. After harvesting exposed and non-exposed cells at different time-points after irradiation, we extracted DNA as well as RNA and subjected these to Next-Generation-Sequencing analyses, in order to identify radiation-induced DNA-sequence alterations and possible effects at the gene expression and transcript level. This approach revealed a number of transcriptional alterations including the occurrence of novel fusion transcripts. The latter likely resulted from IR-induced gene fusions as a consequence of erroneous repair of DNA double strand breaks. These findings disclose IR-induced molecular lesions at a genome wide level. Further tests will have to reveal whether such profiles are tissue specific and will have the potential of serving as diagnostic indicators for IR-exposition.

P-Basic-005

Transcriptome changes in response to early life stress in the mouse

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Stressful experiences lead to an adaptive response that involves rapid and long-term behavioral as well as physiological and molecular processes. These processes are coordinated by the limbic–hypothalamic–pituitary–adrenal axis. In particular, adverse early-life experiences are known to result in cognitive deficits and behavioral alterations that can translate to various psychiatric disorders.

We used a mouse model of early life stress (ELS) that can be described as follows: after postnatal day 2, the dam was given reduced bedding placed on a raised fine-gauge steel mesh plate. As a result, dams from the ELS group more frequently left the nest, which ultimately led to fragmented maternal care. By applying electrophysiology, immunohistochemistry and behavioral tests, we determined marked alterations of synaptic function in the corticotropin-releasing hormone-expressing neurons of the hypothalamus. We further performed RNA Sequencing experiments of micro-punched hypothalamus to determine ELS-induced transcriptome changes in adult mice. Among the top 10 differentially expressed genes identified, several genes with possible stress-related functional roles were included such as (i) Efhd1 that has been already described as a survival factor for neuronal cells, (ii) Oprd1 that belongs to the Delta opioid receptors that are involved in neuronal protection against hypoxic and ischemic stress and (iii) Ankfn that was previously identified as a candidate gene in a genomic study of general vulnerability to substance abuse disorders in humans. In addition, we observed an ELS-induced decrease of mRNA levels of the Nrc3c1 gene which has been already shown to display decreased mRNA and increased methylation levels in several brain areas following chronic stress in mice.

Our data will provide novel targets for subsequent in-depth analysis of the role of the identified genes and their epigenetic regulation in stress response and resilience. Thus, we may open new avenues for the development of potential pharmacological interventions and epigenetic analysis in human cohorts in future projects.

P-Basic-006

Drosophila melanogaster as a model organism to follow up candidate genes for intellectual disability

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Intellectual disability (ID) is clinically and genetically extremely heterogeneous, and the number of known ID genes is still incomplete. Currently, the underlying genetic cause can be identified in only about half of the patients. Next Generation Sequencing (NGS) has been established as a powerful tool to identify mutations in both known ID genes and novel candidate genes. This is particularly true for detecting de novo mutations in sporadic cases of ID by using trio-exome-sequencing. However, the pathogenic relevance of newly identified de novo variants in so far unknown genes has to be further confirmed by i) identifying other patients with mutations in the same gene and/or by ii) characterisation of the candidate gene's role in the nervous system and by investigating the mutational consequences in cellular and animal models.

In order to follow up new candidate genes from NGS, we use Drosophila melanogaster as a model organism, as its nervous system is relatively easy to manipulate and to investigate. Ubiquitous and tissue specific knockdown of fly orthologues of ID candidate genes can be rapidly induced using the UAS/Gal4 system. Subsequent assays include among others the analysis of negative geotaxis behaviour assessing basic locomotor abilities as a marker for gross neurological function and the morphological evaluation of larval neuromuscular junctions as a model for the development of excitatory synapses. Here, we present several examples for functional follow-up of new candidate genes for ID (e.g. BBX, SETDB1) in the fruit fly using these assays for behaviour and synapse development. Significantly impaired geotactic performance was observed for the Drosophila orthologue of SETDB1 upon pan-neuronal knockdown and knockdown in motoneurons, but not upon knockdown in muscles. Flies with a pan-neuronal knockdown of the SETDB1 orthologue showed a significant increase in NMJ area and length, whereas pan-neuronal knockdown of the BBX orthologue resulted in an increased density of active zones.

Our findings of neurological phenotypes upon dosage reduction of these genes might support a causal relevance of the identified variants for the cognitive phenotypes in the patients.

P-CANCER GENETICS

P-CancG-007

Leupaxin regulates the expression of integrins and the response to radiation in prostate cancer cells

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The focal adhesion protein leupaxin (LPXN) is overexpressed in approximately 20% of prostate carcinomas (PCa) and supports the progression of PCa. In PC-3 and DU145 PCa cell lines downregulation of LPXN expression results in a reduced cell adhesion to different extracellular matrix substrates. Cell adhesion has recently been identified as a key determinant for cancer cell resistance to therapeutic interventions. Integrins and focal adhesion proteins play essential roles in these processes called cell adhesion-mediated drug (CAMDR) and radioresistance (CAMRR). In the present report, analysis of integrin expression in PCa cells after leupaxin knockdown showed reduced expression of the integrin β1 on RNA and protein level. Furthermore, LPXN as well as β1 integrin expression were inducible by ionizing irradiation. Knockdown of LPXN prevented this irradiation-induced upregulation of β1 integrin and resulted in a radio-sensitization of PCa cells. Likewise, inhibition of β1 integrin using an inhibitory antibody leads to a radio-sensitization of PCa cells. However, simultaneous inhibition of β1 integrin and downregulation of LPXN expression did not result in a synergistic effect, suggesting that LPXN and β1 integrin might mediate resistance to ionizing irradiation through the same signaling pathway. To find a possible explanation for the LPXN-dependent expression of integrin β1 the activity of the specificity protein 1 (SP1), which was shown to drive the expression of several integrins, was examined in PCa cells after overexpression of LPXN. This assay revealed that overexpression of LPXN was activating Sp1. This hypothesis is further supported by the fact that LPXN knockdown affects the activity of ERK1/2, which is regulating the DNA-binding activity of SP1. Taken together, these results show that leupaxin is involved in the resistance of PCa cells to radiation by regulating the expression of the integrin β1 through SP1.

P-CancG-008

Molecular cytogenetic analysis of Burkitt lymphoma of the ovary

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Burkitt lymphoma (BL) of the ovary is a rare manifestation of BL which represents ≈1.5% of all primary ovarian tumors. The biologic hallmark of BL is the IG-MYC translocation involving the MYC locus and the immunoglobulin heavy chain locus (IGH) or one of its light chain variants (IGL or IGK). Differentiation of BL from solid ovarian tumors (e.g. carcinomas) as well as other aggressive B-cell lymphomas including diffuse large B-cell lymphoma (DLBCL) is of utmost importance as different treatment strategies are required. However, in very few of reported cases in literature the diagnosis of BL has been supported by (molecular) cytogenetic investigations.

To investigate the characteristics of BL of the ovary we performed a comprehensive molecular cytogenetic and histopathologic analysis of 13 formalin-fixed paraffin-embedded ovarian biopsies in which between 1993 and 2014 a (histopathological) diagnosis of Burkitt (or a related) lymphoma was made. Fluorescence in situ hybridization was performed with probe sets for the detection of the Burkitt translocations t(8;14)(q24;q32)/MYC-IGH, t(8;22)(q24;q11)/MYC-IGL or t(2;8)(p12;q24)/IGK-MYC as well as break-a-part probes for BCL2, BCL6, MALT1 and IGH. In addition, a specific probe set to detect aberrations of the ETS1 region (Salaverria et al., 2014) was applied. Cases with MYC breaks without additional BCL2 and/or BCL6 breaks were classified as MYC single hit and those with additional BCL2 and/or BCL6 breaks as double hit (DH) lymphoma.

A total of 8/13 cases were MYC-break positive (all IGH-MYC). 6/8 of these IGH-MYC positive cases had no additional breaks in BCL2, BCL6 or MALT and were classified as IG-MYC single-hit while 2/8 cases were classified as double hit lymphomas (one MYC-BCL2 DH and one MYC-BCL6 DH). Of the 5/13 cases lacking MYC translocations, 1/5 carried an IGH break while all five were negative for BCL2, BCL6, and MALT1 breaks. Moreover, none of them displayed aberrations involving the ETS1 region and hence do not represent so-called “high-grade B-cell lymphoma with features of BL, MYC-, with 11q-gain/loss pattern” (Salaverria et al., 2014).

With pathology re-review of available material finally only 5/13 cases (all IG-MYC single-hit) were classified as BL while the others (including the DH lymphomas) were re-classified as DLBCL, intermediate or unclassifiable B-cell lymphomas. Genome wide analyses of copy number abnormalities is ongoing to evaluate if site-specific copy number abnormalities are associated with ovarian involvement in BL.

In conclusion, molecular cytogenetic investigations should be included in the diagnostic work-up of aggressive B-cell lymphomas of the ovary, as a substantial part of presumed BL could represent other subsets of aggressive lymphomas e.g. DLBCL and/or double-hit lymphoma. This is of particular importance as the treatment of BL differs from other lymphomas and therefore proper diagnosis of ovarian BL is mandatory.

P-CancG-009

Complexity of CEBPA Mutated Acute Myeloid Leukemia

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CEBPA mutations (mut) occur in 5-14% of patients with acute myeloid leukemia (AML). A difference in prognosis between CEBPA single- (sm) and CEBPA double-mutated (dm) AML has been reported with longer overall and event-free survival in dm cases.

Aim of the study was to elucidate the cause of the clinical difference between CEBPAsm and dm cases and to evaluate the clonal architecture of CEBPAmut AML.

The study included 229 CEBPAmut AML cases (129 sm, 100 dm) comprising 113 females and 116 males with a median age of 67.1 y (range: 15.7-87.6 y). 218 cases had intermediate risk (n=162 normal, n=56 aberrant karyotype), 11 cases adverse risk cytogenetics.

CEBPAdm were younger compared to sm (p=0.001) and occurred more often in females than in males (p=0.002). Concomitant mutations were present in more CEBPAsm cases compared to CEBPAdm cases (p=0.003). Furthermore, the amount of additional mutations was higher in CEBPAsm (mean: 2.1 mut) compared to CEBPAdm (mean: 0.3 mut). We additionally analyzed concomitant mutations according to functional pathways. No differences between CEBPAsm and dm were detected in myeloid transcription factors (GATA2, RUNX1) and oncogenes (KRAS, NRAS). Significantly higher frequencies were found in CEBPAsm for the following pathways: mutations affecting DNA-methylation (DNMT3A, IDH1/2, TET2; p=0.001), nucleophosmin (p<0.001), signaling (FLT3-ITD, FLT3-TKD; p=0.006) and chromatin modifiers (ASXL1, MLL-PTD; p=0.009). In contrast, the tumor suppressor gene WT1 was less frequently mutated in CEBPAsm (p=0.023). Regarding outcome, CEBPAdm cases with DNA methylation affecting mutations as compared to those without had shorter EFS (p=0.052) and OS (p=0.009). Furthermore, mutations in chromatin modifiers negatively influenced EFS (p=0.002) and OS (p=0.006) in CEBPAdm cases.

We further compared clonal architecture between CEBPAsm and dm cases. We considered cases with uniform mutation loads in all genes as monoclonal, while cases with heterogeneous mutation loads (range of loads for different mutations spanning >30%) were considered clonally diverse. CEBPAsm were more often clonally diverse compared to dm (p=0.002). In 63.3% of diverse CEBPAdm cases (n=19/30), the dominant clone comprised CEBPAmut compared to only 32.3% (n=22/68) of diverse CEBPAsm (p=0.009). In 20.0% (n=6/30) of CEBPAdm with subclonal CEBPAmut, the dominant clone was TET2mut and in 10.0% (n=3/30) WT1mut. In 2 cases (7.6%), the dominant clone had mutations in different genes. In 26.5% (n=18/68) of CEBPAsm with subclonal CEBPAmut, the dominant clone carried TET2mut, 8.8% (n=6/68) DNMT3Amut, 4.4% (n=3/68) ASXL1mut. In 27.9% (n=19/68) the dominant clone had mutations in different genes.

In conclusion, CEBPAdm occur preferentially in female patients and are correlated with younger age. Prognosis of CEBPAdm cases is adversely influenced by mutations affecting DNA-methylation and chromatin modifiers. Finally, clonal architecture is more complex in CEBPAsm compared to dm cases.

P-CancG-010

Hereditary Breast/Ovarian Cancer: A systematic screening of DNA repair genes in 300 consecutive patients.

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Introduction:

In about 25% of cases, hereditary breast and ovarian cancer (HBOC) is caused by mutations in the BRCA1 or BRCA2, both components of DNA repair pathways. In recent years, additional genes of the DNA repair system such as CHEK2, ATM, BRIP1, PALB2, RAD51C and others have been implicated in HBOC. Interestingly, many of these genes had been identified first (with biallelic mutations) in Fanconi Anemia

patients. We set out to study the contribution to HBOC of 25 DNA repair genes contained within the TruSight Cancer panel (IlluminaTM).

Methods:

All 300 patients fulfilled the inclusion criteria defined by the German Consortium for Breast and Ovarian cancer. Target enrichment was performed with the Illumina TruSight cancer panel which includes 94 genes associated with a predisposition towards cancer. Next generation sequencing data were generated on a MiSeq (Illumina). Variants were identified and analysed by GensearchNGS software (PhenoSystems). CNV analysis was carried out with the NextGENe CNV detection tool (Softgenetics).

Results:

In all samples, data analysis showed an even coverage of at least 50-fold across 98,5% of all coding regions investigated. We focused on protein truncating mutations since functional data on other variants in these genes are scarce. In addition to BRCA1 and BRCA2, we detected truncating mutations in the following genes: ATM, CHEK2, FANCA, FANCI, FANCL, FANCM and ERCC2. CNV analysis revealed deletions or duplications only in BRCA1 and not in any of the other genes studied.

Conclusions:

Mutation screening by NGS was able to identify monoallelic, likely pathogenic mutations in DNA repair genes other than BRCA1/2 in a significant number of HBOC cases. However, the causative association to HBOC and the prospective tumor risks for many of these mutations and genes have yet to be determined. Of note, extending the analysis to a larger number of genes proportionally increases the number of unclassified variants and the workload to survey and classify them.

P-CancG-011

Two different hereditary tumor disposition syndromes in a family with pancreatic cancer

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Introduction

Pancreatic cancer is the fifth leading cause of cancer mortality both in the USA and Europe and still has a poor prognosis (mean survival after diagnosis: < 6 months, 5-year survival rate: about 6%). However, in patients with early-stage tumors, 5-year survival rates reach up to 40%. Hence, the identification of patients at risk is crucial to reduce mortality. Pancreatic cancer is associated with numerous environmental and lifestyle risk factors and preexisting diseases. About 5-10% of patients have a genetic predisposition for this tumor. About 10 autosomal dominant tumor disposition syndromes are associated with pancreatic cancer, and predispose to a characteristic tumor spectrum. In many cases, specific management guidelines are available.

We present a family with 3 siblings affected by pancreatic tumors, all due to different causes.

Clinical Report and Genetic Findings

The index patient was diagnosed with familial adenomatous polyposis (FAP) at the age of 35, confirmed by a frameshift mutation c.3103dupC [c.3103_3104insC], p.Gln1035Profs*13 affecting codon 1035 in the APC gene which likely occurred de novo. He developed an intraductal papillary mucinous neoplasm of the pancreas (IPMN). He had 5 siblings, all tested negative for the APC mutation.

He came back for genetic counseling after a sister had died from colorectal cancer and 2 brothers had developed pancreatic cancer . Family history going back to great-grandparents also revealed 2 cases of esophageal cancer, 5 cases of breast cancer and prostate cancer in the paternal family. Differential diagnosis comprised Lynch syndrome, BRCA2 and PALB2 related hereditary tumors. A heterozygous indel mutation c.516_516+1delGGinsT, p.Lys172Asnfs of exon6/intron 6 in BRCA2 was identified both in the index and in one brother with pancreatic cancer but not in the third individual with pancreatic cancer that was supposed to be due to multiple risk factors.

Discussion

In the present family different causes of pancreatic tumors could be identified, including two different tumor disposition syndromes allowing predictive testing and specific surveillance in family members. Predictive testing revealed some of the family members to be carriers of the familial BRCA2 mutation. One brother most likely developed pancreatic cancer due to multiple risk factors (alcohol abuse, obesity, type 2 diabetes). The family illustrates the need for a thorough family history and multidisciplinary workup of the underlying genetic causes in families with several pancreatic tumors in order to offer adequate screening and providing individual management.

P-CancG-012

Complementing NGS panel sequencing by high resolution custom array CGH increases the mutation detection rate in hereditary breast and ovarian cancer

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As a diagnostic routine we perform next generation sequencing (NGS) and apply Illumina's TruSight Cancer panel to identify mutations that are causative for familial cancer predisposition in hereditary breast and ovarian cancers (HBOC). In order to also conduct a thorough detection of deletions and duplications we designed a customized array that covers all 94 genes that are present on the TruSight Cancer panel. In 11 families and 15 persons out of 232 individuals we detected copy number variants that were causative for tumor predisposition and affected the 10 genes that are recommended for diagnostic analysis by the German Consortium for Hereditary Breast and Ovarian Cancer . In addition to previously reported variants we found unreported deletions in ATM, RAD51C and an intragenic duplication in BRCA2. Besides adding about 5% to the total detection rate in HBOC cases, precise knowledge of the breakpoint positions makes it possible to carry out PCR for subsequent predictive testing of family members or segregation analysis. Generally, we can recapitulate copy number changes from NGS data to a certain degree. But in genomic areas with low read coverage we are not confident that NGS coverage analysis is sufficiently reliable. This may change with an increasing number of cases and appropriate software.

P-CancG-013

Functional studies on the role of TERT promoter genetic variation in breast carcinogenesis

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Altered telomerase activity is closely linked to cancer development but the molecular mechanisms controlling its expression remain unclear. A key regulatory step of telomerase activity is the transcriptional modulation of its catalytic subunit, human telomerase reverse transcriptase (hTERT).

Genome-wide association studies (GWAS) have identified the TERT-CLPTM1L region at 5p15.33 as a breast cancer susceptibility locus. In the Breast Cancer Association Consortium, we have identified several breast cancer risk-associated single nucleotide polymorphisms (SNPs) in the TERT promoter by large-scale fine-mapping. The six candidate SNPs, rs2736107, rs2736108, rs145544133, rs2736109, rs3215401 and rs2853669, reside in the same linkage disequilibrium block and form a risk-associated haplotype. This haplotype significantly decreases TERT promoter activity in luciferase reporter assays in normal breast epithelial and breast cancer cell lines, with rs145544133, rs3215401 and rs2853669 inhibiting promoter activity most effectively. This suggests that the causal variant may be one or more of these three SNPs. The SNP rs2853669 is located within an ETS transcription factor binding side and adjacent to an E-box motif, the consensus sequence for binding of the oncogenic transcription factor MYC. siRNA mediated knock-down of MYC showed the expected TERT repression but was neither affected by rs145544133, rs3215401 and rs2853669, nor the risk-associated haplotype. Results from ETS1 knock-down studies will be presented at the meeting. We also used chromatin conformation capture (3C) assays to explore interactions of the TERT promoter and the CLPTM1L promoter with a 100kb region in the TERT-CLPTM1L locus in normal breast epithelial and breast cancer cell lines as well as normal ovarian epithelial and ovarian cancer cell lines. No significant DNA interactions were found in the TERT-CLPTM1L locus, suggesting that the tightly controlled promoter activity of TERT may not significantly be modulated by interactions within this region.

Gaining insights into the molecular mechanisms of telomerase activation will increase our understanding of tumorigenesis. Furthermore, as TERT is a key regulator of telomerase activity, a hallmark of most cancers, it has great potential as a drug target for breast cancer prevention and treatment.

P-CancG-014

Comprehensive Genomic Characterization of Intrahepatic Cholangiocarcinoma

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Intrahepatic cholangiocarcinoma (ICC) is a cancer arising from the epithelium of the biliary tracts located within the liver. After hepatocellular carcinoma ICC represents the second most common primary liver malignancy with significant rise in incidence over the past three decades. To date surgical resection represents the only effective therapy, but is still associated with a poor prognosis. The molecular mechanisms underlying ICC tumorigenesis are not well understood, although recently the first sequencing studies have been performed on tumor samples derived from patients with Asian descent.

In order to identify common genomic alterations driving ICC development, we conducted the first sequencing study on patients of Central European origin. For this purpose we collected tumor and matching control tissue samples from 38 patients and performed whole-exome sequencing (WES) as well as microarray genotype and gene expression analyses. The generated data were used to obtain comprehensive genomic profiles comprising somatic mutations, copy number aberrations (CNAs) and gene expression patterns.

In total, more than 1,800 somatic mutations were identified by WES. CNA analysis revealed four frequently amplified and 11 deleted genomic regions across the cohort. A subset of variants affecting 37 established cancer driver genes were validated using Sanger sequencing implying a high heterogeneity in ICC genomic landscapes. In line with previous studies on ICC, mutations with a predicted functional impact were identified in tumor suppressor genes (TSGs) implicated in chromatin remodeling including ARID1A (14%), BAP1 (14%) and PBRM1 (11%). The p53 pathway was frequently affected by somatic mutations in the genes TP53 (11%) and ATM (8%) and by CNAs affecting CDKN2A (23%) and MDM2 (23%). Moreover, somatic alterations could be validated in genes implicated in the MAPK/ERK signaling pathway including GNAS (8%), KRAS (8%), NRAS (8%) and NF1 (5%). The frequently mutated oncogenes IDH1 (8%) and IDH2 (5%), as well as the TSG SMAD4 (8%) may also play an important role in the development of ICC.

Continuative work on integrating the WES-, CNA- and expression data will help to prioritize and identify further driver genes that are going to be validated in a larger sample collective which is currently recruited. However, the results of the present study already confirm and additionally expand the known genomic landscape of ICC. Finally, this will help to find new therapeutic approaches for the treatment of this orphan cancer.

P-CancG-015

Using cell free tumor DNA (ctDNA) to monitor disease courses in ovarian cancer, peritoneal cancer, and head and neck cancer

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With the technical progress in the field of molecular diagnosis, it is possible to monitor changes in the genetic constitution of a tumor by “liquid-biopsy”. It can be a new tool in guiding the therapeutic decision of physicians in cancer therapy. Low amounts of cell free tumor DNA (ctDNA) is circulating in the blood stream of a cancer patient. The ctDNA represents a non-invasive proxy of the tumor heterogeneity. Here we report our approach to get to the genetic information of the tumor by next-generation sequencing on a MiSeq Illumina device. We were able to isolate and quantify ctDNA in 3 different tumor entities (Ovarian Cancer, Peritoneal cancer and Head and Neck cancer). The amount of ctDNA in the plasma ranged from 1.6 to 34.01 ng/ml before treatment. We will further collect plasma samples over the course of treatment for each patient. Beside a quantitative statement, the genetic composition of the tumor and hence the ctDNA was of much interest. To comply with the standard NGS enrichment requirements we had to amplify the isolated DNA. At first a widely used method in paleogenetics was transferred, were the purification and amplification of the isolated ctDNA is without length bias.

As a result out of 6.9 million reads 99.6% of reads were mapped with a duplicate rate of 1.5% and an on-target read percentage of 18%. The average insert size was 164 bases with an on-target coverage depth of 47X. The 20, 30 and 100X coverage for the target region was 58, 52 and 16%. In comparison a commercially available kit (KAPA Hyper Prep Kit, Illumina® platforms) from Peqlab was used. Which should led to an overall better performance based on manufacture data. The results are currently under analysis and will be available until the poster presentation.

In conclusion, besides monitoring disease burden by cfDNA quantification, multi gene panel sequencing of cfDNA samples is possible and will provide individual tumor signatures enabling targeted therapies. Since on-target coverage does not achieve more than 50% of the target genes, more sensitive protocols are needed to extract a comprehensive signature map of individual tumors.

P-CancG-016

Low frequency of the two hotspot mutations POLE Leu424Val and POLD1 Ser478Asn in a large cohort of patients with suspected Lynch syndrome

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Background: Two specific and highly penetrant germline missense mutations in the polymerase genes POLE (c.1270C>G;p.Leu424Val) and POLD1 (c.1433G>A;p.Ser478Asn) have recently been identified as rare cause of mismatch repair proficient multiple colorectal adenomas and carcinomas, a condition termed Polymerase proofreading-associated polyposis (PPAP). The phenotypic pattern ranged from multiple adenomas resembling AFAP or MAP to a clustering or early-onset manifestation of colorectal cancer (CRC) compatible with an HNPCC or Lynch syndrome-like phenotype.

Around 75% and 30% of patients meeting the Bethesda or Amsterdam criteria for HNPCC, respectively, have microsatellite stable tumors. The aim of the present study was to further evaluate the clinical relevance and phenotypic spectrum of the two polymerase gene hotspot mutations in these patients using a large (n = 376) and well characterized cohort.

Methods: By a targeted next-generation sequencing approach (Illumina platform) or Sanger sequencing, we screened exon 13 of the POLE and exon 12 of the POLD1 gene for the presence of the two mutations. The patient cohort consisted of 47 unrelated familial CRC patients meeting the Amsterdam I or II criteria, and 329 unrelated patients fulfilling the Bethesda criteria, all of whom had microsatellite stable tumors.

Results: The POLD1 mutation c.1433G>A was not identified in the whole cohort. The POLE mutation c.1270C>G was found in 2/376 unrelated cases (0.5%). The index patient of one family, formally meeting the Amsterdam I criteria, and his mutation carrying mother had less than 10 colorectal polyps; however, the mutation carrying cousin had more than 20 polyps at 45 years of age suggesting an attenuated polyposis. Beside three CRC's the cousin also had an ovarian cancer diagnosed at age 33. The index patient of the other family formally met the Bethesda criteria, however, a subsequent careful review of the medical records demonstrated the presence of more than 50 colorectal adenomas at 38 years of age. Detailed information about the family was not available however several members of the maternal family died due to cancer at a rather young age.

Conclusions: According to our data, the two PPAP hotspot mutations are very rare in patients with suspected Lynch syndrome. The two unrelated cases with the POLE mutation had an attenuated polyposis rather than an HNPCC phenotype. Therefore our results support the theory, that POLE mutations are causative for an adenomatous polyposis. Nonetheless, since misclassification and a phenotypic overlap with polyposis syndromes is common in patients with suspected Lynch syndrome, screening for the POLE mutation might be considered, in particular in unexplained familial cases and patients with multiple colorectal adenomas.

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P-CancG-017

Monosomy 14 in a subgroup of primary uterine leiomyosarcomas.

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Recent results of molecular investigations suggest that smooth muscle tumors of the uterus constitute a pathogenetically heterogeneous group of tumors not only characterized by benign and malignant lesions and rare group of tumors with uncertain malignant potential (STUMP). Even the group of benign tumors, i.e. the leiomyomas consists of two larger subgroups characterized by either of two apparently mutually exclusive driver mutations i.e. rearrangements of high mobility group protein AT-hook 2 (HMGA2) usually accompanied by cytogenetically visible chromosomal alterations of the HMGA2 locus and mutations of mediator subcomplex 12 (MED12). Other mutations like e.g. rearrangements of HMGA1 and deletions of the long arm of chromosome 7 may either occur as secondary genomic alterations or as rare driver mutations. Histologically variant leiomyomas seem to display often unorthodox changes that were not described before in UL like extended uniparental disomies or “firestorm” or “chromothripsis” phenomena not affecting either of the typical chromosomal regions involved in common clonal chromosomal deviations in UL. Vice versa, clearly malignant lesions as well do not constitute a unique entity when investigated bv molecular methods. E.g. MED12 mutations not distinguishable from those found in leiomyomas can be found in a considerable percentage of leiomyosarcomas as revealed by a couple of independent studies.

To analyze a possible origin of leiomyosarcomas from ordinary leiomyomas likely as a result of additional genetic alterations occurring as secondary changes in MED12 mutated UL, we performed a molecular inversion probe array analysis of nine smooth muscle tumors. Samples were formalin-fixed and paraffin-embedded for routine histologic diagnosis prior to the subsequent molecular investigations. In all cases, histologic examination had revealed a smooth muscle origin of the lesions different from ordinary leiomyomas. Based on the current WHO classification, five tumors were leiomyosarcomas and four were smooth muscle tumors of uncertain malignant potential (STUMP). All but one tumor displayed gains and/or losses detectable by the copy number array analyses and in seven tumors more than three imbalances were noted resulting in an “unquiet” signal pattern. Even at a first glance, a comparison of the CNV array patterns with the results of histologic examination reveals that gross genetic alterations do not unequivocally indicate malignancy according to the WHO criteria recently proposed. A proposed working classification could be based on the number of breakpoints that have occurred with an increasing risk of malignancy associated with a growing number of breakpoints.

P-CancG-018

Copy number variation analysis in 85 suspected Lynch syndrome families reveals novel potential causative candidate genes

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Introduction: Lynch syndrome (LS) or hereditary non-polyposis colorectal cancer (HNPCC) is a tumour predisposition syndrome characterized by a high risk of colorectal cancer, endometrial cancer and a variety of additional malignancies. It is caused by germline mutations in one of four DNA-Mismatch-repair-(MMR) genes (MLH1, MSH2, MSH6 and PMS2) or deletions of the EPCAM gene upstream of MSH2. In up to 50% of suspected Lynch syndrome (LS) families, no germline mutation in the MMR genes or EPCAM deletion can be detected. Loss-of-function copy number variants (CNVs) contribute significantly to the mutation spectrum of hereditary tumor syndromes and might also contain yet unidentified genes responsible for Lynch syndrome.

Methods: Genomic DNA from 81 unrelated mutation negative patients from the German HNPCC Consortium and four patients from the University Medical Center in Leiden, Netherlands was genotyped using

Illumina´s HumanOmniExpress Bead Array. All but two patients showed loss of MSH2 in their tumor tissue, most of them were also MSI-H. Putative CNVs were identified by QuantiSNP v.2.2 and filtered according to empirically established criteria to select rare, non-polymorphic deletions and duplications ≥ 10kb in protein-coding genes and the regulatory regions of MSH2 which were present in not more than 0.2% of 1,320 population-based controls. CNVs that passed the filter criteria were validated by qPCR, further selected on gene level, and subsequently prioritized by gene functions and pathways.

Results: In total, 30 unique deletions (size 13-387kb) and 18 unique duplications (size 15-788kb) were found in 25 (21%) and 17 (15%) patients, respectively. Those 48 CNVs together encompass 71 protein coding genes. 33 genes were completely or partly deleted, 38 affected by duplications. None of the genes was affected in more than one patient. Five of these genes are promising candidates that are highly expressed in normal colorectal tissue. Three of these genes are involved in different cellular processes, such as cell adhesion, cell development and transformation, cell cycle checkpoint regulation, and cell volume or polarity control. One gene is known for double strand break repair and recombination and the last one possesses DNA helicase activity and is essential for the initiation of eukaryotic genome replication.

Conclusion: By applying stringent filter criteria we identified a group of rare, non-recurrent loss-of-function CNVs which might contain novel predisposing genes for LS. The ongoing further work-up of the most promising candidates includes the detection of germline point mutations by a targeted NGS approach, segregation analysis in families where further affected relatives are available, screening for somatic second-hits in tumor tissue, and pathway/network analysis.

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Analysis of circulating cell free DNA (ccfDNA): A promising tool for personalized medicine and cancer therapy

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Although significant progress has been made in the development of new therapy approaches, cancer remains one of the leading causes of death worldwide. In most cases cancer remains undetected until its advanced stages because up to now efficient screening techniques for early detection are not still available.

However recently published data indicate that circulating cell-free DNA (ccfDNA) could become a promising biomarker in cancer diagnosis, therapy and prognosis. The use of ccfDNA presents several conceptual advantages compared to classic genetic analysis via tumor-tissue sampling. CcfDNA analysis is non-invasive and enables day-to-day patient follow-up and monitoring of treatment response. Analysis of ccfDNA also allows detection of genetic and epigenetic alterations within the tumor. Careful analysis of these alterations could provide valuable information to tailor the clinician's choice of treatment.

Despite the fact that ccfDNA is known since long time and despite urgent need of secure biomarker in cancer therapy analysis of ccfDNA in the clinics is far from reality. This is mainly due to reported discrepancies and contradictory data on the analysis certainly caused by lack of normalization of the experimental conditions. We therefore started a pilot study with patients suffering from colorectal cancer in order to establish analysis of ccfDNA in our routine laboratory. Optimization and normalization of the Workflow of the pilot study Covers all aspects of the complete procedure: starting with blood sampling, isolation of the ccfDNA, determination of its concentration and determination of tumor-derived ccfDNA part and its fragmentation. Prior to analysis tumor derived DNA-fragments are enriched by cold-PCR and presence of sequence variants are either shown by next generation sequencing (NGS) or - if the mutation is known - by quantitative PCR, digital PCR and by NGS. To validate results DNA is isolated from respective tumor specimen and genes which are known to be frequently mutated in colon Cancer are sequenced by use of appropriate gene panels on Ion Torrent Personal Genome Machine (PJM) or Ion Proton. In our presentation we will show First data concerning the feasibility of the approach.

P-CancG-020

Identification of GPRC5A as a disease modifier in BRCA1 and BRCA2 mutation carriers

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The GPRC5A gene encodes an orphan G-protein coupled receptor highly expressed in lung tissue. Barta et al. (2012) and Fujimoto et al. (2010) reported a high susceptibility to carcinogen- and inflammation- induced lung adenocarcinomas in Gprc5a knockout mice suggesting a role in cancer predisposition. Within a consanguineous breast cancer (BC) family of Turkish origin, we identified a homozygous frameshift mutation p.Arg61fs in the first coding exon of the GPRC5A gene in two affected sisters (disease onset 26y and 33y, respectively) via linkage analysis and subsequent exome sequencing, suggestive of an autosomal recessive trait. Interestingly, Sokolenko et al. (2014) reported a ten-fold increase of the heterozygous GPRC5A p.Arg61fs mutation in BRCA1 c.5382insC BC patients (5.1%; 6/117) compared to BRCA1-negative BC patients (0.5%; 8/1.578). Thus, we hypothesized that GPRC5A could act as recessive BC gene and a disease modifier when heterozygously inactivated. While the search for homozygous or compound heterozygous GPRC5A mutation carriers is ongoing (international SEARCH study), we genotyped the p.Arg61fs mutation in a large series of BRCA1/2-positive (n=1.563), BRCA1/2-negative (n=2.352) and control individuals (n=2.979). A weak association was found in BRCA1/2 mutation carriers (16/1.563, CF=1.02%) and BRCA1/2-negative cases (22/2352, CF=0.94%) versus control individuals (23/2979, CF=0.77%). Remarkably, the detailed analysis of the BRCA1/2 mutation carrier cohort, consisting of 1099 affected and 464 unaffected individuals, revealed that the 16 GRPC5A mutation carriers solely coincide with the affected subgroup, resulting in an estimated relative risk for BRCA1/2-positive individuals carrying the GPRC5A frameshift mutation of 1.424 (95%CI=1.086-1.429; p=0.005). Nine of them were detected in the cohort of 814 BRCA1-positive BC patients (CF=1.11%) and seven were identified in the group of 285 BRCA2-positive BC patients (CF=2.46%). In both cohorts, the carriers of the GPRC5A frameshift show a tendency to an earlier age at diagnosis. The mean age at diagnosis among BRCA1 mutation carriers is 39.67 (30.39-48.95) and 38.64 (31.8-45.48) in presence of GPRC5A p.Arg61fs. In the group of BRCA2 mutation carriers the mean age at diagnosis is 42.86 (32.91-52.81) and 39.71 (33.01-46.41) in presence of the GPRC5A frameshift mutation. In summary, we provide evidence for GPRC5A p.Arg61fs as a disease modifier in BRCA1/2 mutation carriers. Based on this data, further studies have been initiated to elucidate the role of GPRC5A p.Arg61fs in BC pathogenesis by analyzing 30.000 BRCA1/2 mutation carriers and 80.000 mostly sporadic BC cases (OncoArray GWAS).

P-CancG-021

Increased diagnostic yield of Gorlin-Goltz syndrome using next generation sequencing based panel testing

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Gorlin-Goltz syndrome, also known as basal cell nevus syndrome (BCNS) is an autosomal dominant disorder that is characterized by a broad spectrum of developmental abnormalities and a predisposition to neoplasia, such as basal cell carcinoma. BCNS is primarily caused by mutations in the PTCH1 gene, which encodes the receptor for the hedgehog signalling pathway. Furthermore, mutations in other genes involved in the same pathway might also be associated with BCNS. For many years only the high penetrance PTCH1 gene has been part of routine molecular diagnostics of a BCNS mainly due to time and cost limitations incurred by Sanger sequencing. However, the recent implementation of next generation sequencing now provides increased capacity and speed at reduced costs.

Therefore, the aim of this study was an advanced molecular diagnostics of BCNS patients using a next generation sequencing based approach that included a panel of genes that might be associated with BCNS, i.e. PTCH1, PTCH2, SHH, SUFU, HHIP, SMO, CYLD, and BAP1. DNA was isolated from blood from a total of

24 BCNS patients. The respective genes were enriched using a custom-designed Haloplex assay (Agilent) and sequenced on a MiSeq (Illumina).

In 13 patients (54.2%) disease-causing or disease-associated variants were identified. Of those, seven patients had heterozygous mutations in the PTCH1 gene and one had a germline mosaic that could not be detected with Sanger sequencing. In another patient a disease-causing mutation in the SUFU gene was identified, that segregated with other affected family members. Moreover, in one patient with an unclassified PTCH1 variant, an additional loss of function mutation in the BAP1 gene - that was previously associated with mesothelioma - was identified.

These results indicate that the benefit of the implementation of NGS into molecular diagnostics is not limited to increased throughput and lower cost, but also contributes to an increased diagnostic yield and facilitates the detection and characterization of genetic diseases.

P-CancG-022

Next-generation sequencing (NGS) for the diagnosis of hereditary breast and ovarian cancer using the TruSight Cancer Panel: The Erlangen Experience

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Breast and ovarian cancer (BC/OC) predisposition has been associated with a number of high- and moderate to low-penetrance susceptibility genes. With the advent of NGS-based panel sequencing, simultaneous testing all these genes has become feasible. Here we report on our results of a panel-based screening of 15 genes (BRCA1, BRCA2, RAD51C, RAD51D, CHEK2, PALB2, ATM, NBN, CDH1, TP53, MLH1, MSH2, MSH6, PMS1 and PMS2) that have been associated with breast and/or ovarian cancer predisposition. We sequenced 169 patients seen in our interdisciplinary outpatient clinic who fulfilled the diagnostic criteria for BRCA1 and BRCA2 testing established by the German Consortium for Breast and Ovarian Cancer including 40 high risk patients with previously excluded mutations in BRCA1 and BRCA2 mutations. 37 out of the 169 patients had a triple-negative tumor. Altogether we identified 35 (21%) deleterious mutations including one genomic CHEK2 deletion as well as 28 (17%) variants of unknown significance (VUS), 7 in BRCA1/2 and 21 in the other genes. Of the clearly pathogenic mutations, 21 (60%) were identified in BRCA1 and BRCA2. The remainder was 2 splice site and 1 nonsense mutation in RAD51D, 1 splice site mutation in RAD51C, 2 frame shift, 1 splice site mutation and 1 genomic deletion in CHEK2, 2 frame shift mutations in PALB2, 1 known missense mutation in TP53, 1 frame shift and 1 splice site mutation in NBN und 1 frame shift mutation in MSH6. Of the 37 patients with the triple-negative tumor 8 (22%) carried a deleterious germline mutation. Interestingly, only 7 harbored a deleterious BRCA1 mutation whereas in one patient a RAD51D splice site mutation (c.577-2A>G) was identified. One patient with breast cancer and a positive family history was found to carry two deleterious mutations, in PALB2 (c.1046delA) and NBN (c.1397+1delG). The NGS based sequencing of this 15 gene-panel revealed disease-causing mutations in further high- and moderate penetrance genes other than BRCA1 and BRCA2, like RAD51C, RAD51D, CHEK2, PALB2 and TP53, as well as mutations in the low-penetrance susceptibility gene NBN. The downside of this approach is an increase of VUS as well as the detection of mutations in so far “non-breast-cancer” genes. The interpretation of these variants can be challenging, as our knowledge of the genotype/phenotype correlation is still limited. Analysis of familial segregation and tumor DNA as well as data sharing in large repositories will eventual help improve interpretation of these VUS. The identification of further mutation carriers will also allow better assessing their penetrance and thus improve clinical management and genetic counseling of these patients.

P-CancG-023

Reported MSH2 inversion and intron 1 mutation are no recurrent events in 84 mutation-negative German patients suspected of Lynch syndrome

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Introduction

Lynch syndrome (hereditary non-polyposis colorectal cancer / HNPCC) is an autosomal-dominantly inherited tumor predisposition syndrome, characterized by a high risk of colorectal cancer, endometrial cancer and a broad spectrum of additional malignancies. Causative are germline mutations in one of four DNA-Mismatch-repair (MMR) genes (MLH1, MSH2, MSH6 and PMS2) or deletions of the EPCAM gene upstream

of MSH2. However, a pathogenic mutation in one of these genes can only be found in about half of the patients suspected of Lynch syndrome.

It is likely that some mutations are still undetected by the current diagnostic routine. For example, a pathogenic mutation in MSH2 intron 1 (c.212-478T>G) causing a splice defect by pseudo-exon inclusion was found in one family.1 Recently, a large genomic inversion including exons 1–7 of the MSH2 gene was reported in 6 of 10 unexplained Lynch syndrome patients.2,3

To investigate if these two changes in MSH2 are recurrent events undetected by our diagnostic approach, we screened 84 mutation-negative German patients with MSH2-deficient tumors for these mutations. In addition, we sequenced the MSH2 promoter region and the last exon of EPCAM (including the stopp codon).

Methods

All patients fulfilled the revised Bethesda guidelines and showed MSH2-MSH6-defects in their tumors. No pathogenic germline mutation/deletion was found in MSH2, MSH6 or EPCAM. To test for the MSH2 inversion, we performed PCR analyses for the two inversion breakpoints and a control fragment as described2,3. The MSH2 intron 1 mutation locus was amplified and sequenced as described.1 Furthermore, we sequenced the MSH2 promoter region and EPCAM exon 9.

Results

None of our patients was tested positive for the inversion in MSH2 or harboured the intronic MSH2 mutation. Mutations in the promoter region of MSH2 or exon 9 of the EPCAM gene were also not found in our patient cohort.

Conclusion

The MSH2 inversion and MSH2 intron mutation described by other groups could not be found in our mutation-negative patients and are therefore no recurrent events or founder mutations in German patients with MSH2-deficient tumors. Even though somatic mutations in MSH2 have been described as a frequent cause in germline mutation-negative patients, we still expect other pathomechanisms such as rearrangements/inversions, regulatory defects or intronic mutations in MSH2 causing Lynch syndrome in cases with positive family history. Therefore further investigations including quantitative cDNA-analyses as well as deep intronic sequencing of the genes MSH2 and MSH6 by NGS are planned.

Literature

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P-CancG-024

Identification of a highly prevalent BRCA1 stop mutation c.4183C>T in the Tyrolean Lower Inn Valley region enables cost-effective testing for inherited breast and ovarian cancer

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Background: Despite technical advances, germline BRCA testing poses significant financial burden on healthcare systems. Selective screening for founder mutations in BRCA1 and BRCA2 may be used as a cost-effective approach for mutation analysis. However, in most European regions this is not feasible because of genetic heterogeneity.

Methods: Diagnostic testing for hereditary breast and ovarian cancer (HBOC) was performed in 238 seemingly unrelated high-risk families from Western Austria. Subsequently we investigated the prevalence of the BRCA1 mutation c.4183C>T (p.Gln1395Ter) in tumour specimens from unselected patients with breast (n=471) and ovarian (n=186) cancer, using the TaqMan allelic discrimination technique. Haplotype analysis was performed and clinicopathological features of mutation carriers were compared to non carriers. Cancer registry data were evaluated for a possible impact of this mutation on regional cancer incidence.

Results: The BRCA1 mutation c.4183C>T accounts for 20% of BRCA1 or BRCA2 mutant alleles in our cohort of HBOC patients. Most patient families could be traced to the Tyrolean Lower Inn Valley (LIV) region where c.4183C>T was found in 75% of HBOC families. The mutation was subsequently detected in 4.1% of

unselected breast (26% of triple negative breast cancer) and 6.8% of ovarian cancer patients from that region but in none of the patients from other regions of the Tyrol. A common haplotype is shared by all Tyrolean mutation carriers, as expected for a regional founder effect. Cancer incidences showed a region-specific increase in age-stratified breast and ovarian cancer risk with a SIR for 1.23 and 2.13, respectively.

Conclusion: High frequency of a BRCA1 founder mutation in the Tyrolian LIV region is associated with increased breast and ovarian cancer risks. Based on the high mutation prevalence we suggest targeted mutation analysis for all women with breast or ovarian cancer and ancestry from the LIV region.

P-CancG-025

Low prevalence of truncating FANCM mutations in German breast cancer families

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Monoallelic mutations within several Fanconi anemia (FA) complementation group genes including FANCD1/BRCA2, FANCJ/BRIP1, FANCN/RAD51C and FANCO/PALB2 confer moderate to high risk for breast and/or ovarian cancer (BC/OC), while the role of other FA-associated genes in BC/OC pathogenesis remains elusive. Gracia-Aznarez et al. identified a heterozygous nonsense variant within the FANCM gene (p.Arg1931Ter) in a large BC only pedigree. A subsequent case-control study including a large series of BRCA1/2-negative familial BC cases and geographically matched controls derived from Spain, Italy, Netherlands and Australia revealed an association with the BC phenotype, with a carrier frequency (CF) of 0.293% (10/3,409) in cases, 0.128% (5/3,896) in controls and an estimated odds ratio (OR) of 2.29 (95%CI=0.71–8.54). Albeit the association did not achieve levels of significance due to the low mutation frequencies in these cohorts, FANCM represents a plausible candidate gene since FANCM is essentially required to anchor the multi-subunit FA core complex to chromatin after DNA damage. Recently, Kiiski et al. reported a significant association between another truncating FANCM mutation (p.Gln1701Ter) and BC risk in the Finish population (OR 1.86, 95% CI = 1.26–2.75; P =0.0018). By analysing the exomes of 24 BRCA1/2-negative index cases from high-risk BC/OC families of German origin, we also found the p.Gln1701Ter mutation. Similar to the data presented by Kiiski et al., the heterozygous FANCM p.Gln1701Ter mutation was present 16 times in a large cohort of familial BRCA1/2-negative BC index cases of German origin (16/5,190, CF=0.308%) and 6 times in 4,532 geographically matched control individuals (6/4,136, CF=0.132%), with an OR of 2.33 (95%CI=0.86–6.67). Mutational analysis of FANCM by NGS in 396 controls, 241 unselected triple-negative and 513 familial BC cases identified further truncating alterations (p.Glu1300Ter, p.Gln1327ValfsTer16) and missense mutations (p.Pro90Leu, p.Ala216Val, p.Tyr413His, p.Arg579Cys, p.Leu733Pro, p.Val1857Met) predicted to be probably damaging/disease causing (PolyPhen/MutationTaster). Despite a subsequent cases-control study of these variants in another 1,600 cases and 1,000 controls, the association of FANCM mutations with the BC phenotype did not reach levels of significance most probably due to their very low mutation frequencies in the German population. The mean age at first BC diagnosis of

individuals carrying truncating FANCM alterations was 49 years with predominantly ER+, PR+, HER2- tumours of grade 2. Noteworthy, several non-BC/OC tumour entities have been reported in first degree relatives of affected mutation carriers. Due to the apparently low FANCM mutation frequency, however, large collaborative studies are required to quantify the risk for BC and possibly other cancer entities associated with deleterious FANCM alterations.

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Ovarian cancer family with a pathogenic mutation in the RAD51C gene

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Causative germline mutations in the RAD51C gene are detected in 1-1.5 % of high-risk families with breast and ovarian cancer. Predominantly, RAD51C mutations are found in families with a history of ovarian cancer and rarely in families with a history of breast cancer alone. For carriers of a pathogenic mutation, the lifetime risk of ovarian cancer is estimated to be increased from 1.5% (general population) up to 10-20%. The risk of breast cancer seems to be increased as well, although the value in routine clinical management is still unclear.

At the Regensburg Center for Hereditary Breast and Ovarian Cancer, a 47 years old healthy woman presented for genetic counseling. Her mother and her mother's sister developed ovarian cancer and died at the age of 60 and 69, respectively. The family fulfilled the criteria for testing in the BRCA1 and BRCA2 genes. Because both affected relatives with ovarian cancer were deceased, molecular testing was offered to the healthy counselee with a calculated heterozygote risk of 21.9 % according to Cyrilic2.1.3 algorithms. No pathogenic mutation was detected in the BRCA1 or BRCA2 gene after full sequence analysis with the Sanger dideoxy terminator sequencing method. Also, the 43 year old healthy sister of the counselee requested consultation and testing of the BRCA genes but again no pathogenic mutations were identified. Subsequently, the RAD51C gene was analyzed in the sisters. Both shared a heterozygous c.1026+5delGTA mutation not yet reported in the literature and absent in the 1000Genome as well as the ESP Database. In silico analyses predicted a pathogenic effect on RNA splicing, thus mRNA analysis was performed in lymphocyte RNA. RT-PCR amplification and sequencing of the obtained product demonstrated skipping of exon 8 of the RAD51C gene leading to a frameshift and premature stop codon (p. Arg322SerfsX22). This mutation is thus classified as pathogenic and predictive DNA testing can now be offered to further relatives of the pedigree.

P-CancG-027

BRAF V600E is frequently found in papillary thyroid cancer

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Objectives: PTC (papillary thyroid cancer), especially when associated with Hashimoto's thyroiditis, exhibits activating point mutations of the BRAF–gene in most cases (70%), among which BRAF V600E is predominant (90%). In cases of advanced disease, mutation carriers may profit from targeted therapy by BRAF inhibitors, e.g. Vemurafenib. As the BRAF V600E mutation is not a feature of other thyroid neoplasia than PTC, mutation detection can be used as a diagnostic tool to define PTC on a molecular basis. Thyroid nodules with equivocal iodine uptake and ultrasound morphology are usually subjected to fine needle aspiration cytology (FNAC), which may encounter unresolved differentials, as well. To address this diagnostic dilemma with respect to PTC, we subjected stained smears or cell blocks to microdissection and allele-specific PCR for the detection of the BRAF V600E mutation.

Methods: We performed 220 FNAC in 2013 in patients with hypofunctional or indifferent thyroid nodules with malignant aspects by thyroid image reporting and data system (TIRADS). One patient with known PTC was subjected to FNAC of a suspicious lymph node (LN). Among these 221 aspirates, cytology detected 38 samples with malignant or equivocal morphology. Of these, 16 samples with enough cell counts were subjected to molecular analysis using microdissection and PCR.

Results: The BRAF V600E mutation was detected in 5/16 samples (31 %). On a cytologic basis four mutation positive samples were suspicious for PTC (confirmed by surgery), one suspicious for a follicular neoplasia. The remainder 11 mutation-negative samples consisted of one PTC, four samples suspicious of follicular neoplasia, three samples suspicious of undetermined thyroid proliferation, accompanied by regressive changes in one sample. There was no BRAF V600E mutation in the LN aspirate with a suspicious cytomorphology.

Conclusion: The BRAF V600E mutation was detected in 5 of 6 PTC cytology samples. This high detection rate is probably due to the use of microdissection prior to PCR. This preliminary data shows that the BRAF V600E PCR approach may therefore be helpful in the diagnostic workup of FNAC aspirates with questionable PTC cytomorphology. This method may also help to stratify PTC patients for a putative BRAF inhibitor therapy.

P-CancG-028

Is the radiosensitivity of BRCA1 mutation carriers increased?

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The BRCA1 gene is involved in the repair of DNA double strand breaks. Our question was whether BRCA1 mutation carriers accumulate chromosomal damages after mammography and radiotherapy, respectively, due to a reduced repair capacity for DNA double strand breaks.

To measure the radiosensitivity of BRCA1 mutation carriers we analyzed PHA-stimulated lymphocytes of 10 healthy BRCA1 mutation carriers after ex vivo exposure to mammography or 4 Gy Cobalt60 (Theratron). We performed fluorescence in situ hybridization with an all-human-centromer-probe to enumerate modal chromosome number, acentric fragments and dicentric chromosomes by two observers. Contradictory results were validated by an experienced cytogeneticist. We determined the percentage of aberrant metaphases and the number of aberrations per aberrant metaphase in at least 24 metaphases per person and exposure. The results were compared to identically treated lymphocytes of unblinded age-matched controls (nine healthy women without family history of breast-/ovarian cancer but without known BRCA-status).

After radiation with 4 Gy the rate of aberrant metaphases (median: 74.0% in BRCA1 carriers versus 59.2% in healthy controls; p=0.011) as well as the number of aberrations per aberrant metaphase (median: 3.6 versus 2.4; p=0.027) were significantly higher in the BRCA1 mutation carriers than in controls. The increasing number of aberrations was mostly caused by acentric fragments (median 2.7 versus 1.8; p=0.028) and dicentric chromosomes (median 0.5 versus 0.2; p=0.017). In contrast, after mammography (comparable to 0,005 Gy)BRCA1 mutation carriers did not differ significantly from healthy controls (median rate of aberrant metaphases: 24.0% versus 16.0%; p=0.617; median number of aberrations per aberrant metaphase: 1.3 versus 1.1; p=0.651).

In summary, lymphocytes of BRCA1 mutation carriers seem to show an increased radiosensitivity after radiation with 4 Gy but not after mammography.

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P-CancG-029

Aberrant patterns of DNA methylation in lung cancer

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Lung cancer is one of the most common causes of cancer related death in Germany. Non-small cell lung cancer (NSCLC) is associated with poor prognosis and an overall 10-year survival rate of 8–10%.

DNA methylation, which is catalyzed by DNA methyltransferases, is an epigenetic mechanism commonly aberrant in lung carcinomas and therefore investigated in the frame work of the German Center for Lung Research (DZL). This reversible covalent DNA modification allows the cell to modify its genetic activity during

differentiation. Moreover, it can be affected by environmental factors (e.g. exposure to tobacco smoke, asbestos or air pollution).

Using a BeadChip approach we investigated DNA methylation patterns in different surgical specimen of tumor samples, their corresponding controls as well as lung cancer cell lines (n=90). Paired biopsies, which were collected during bronchoscopy, and bisulfite pyrosequencing were used for data validation. We identified ~900 loci corresponding to ~370 genes aberrantly methylated in lung carcinomas as compared to controls (FDR < 1 x 10^(-23), σ/σmax > 0.4). Furthermore, we identified entity specific DNA methylation patterns, differentiating between squamous carcinoma and adenocarcinoma of the lung, the predominant histological NSCLC subtypes. Currently we test the suitability of epigenetic modifications for new diagnostical tools in lung cancer.

This study aims at identifying alterations in the DNA methylome of lung cancer entities to improve our understanding of how epigenetics contributes to lung cancer development.

P-CancG-030

Diagnostic yield of a comprehensive gene panel for hereditary tumor syndromes

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Background: In a considerable number of patients with a suspected hereditary tumor syndrome, no underlying germline mutation can be identified in the genes that are most likely affected, given the tumor spectrum of the patient and his family. NGS-based gene panels are a promising tool to improve correct classification and patient care in these families. To analyse the diagnostic yield and clinical utility of a more comprehensive approach, we established and validated an extensive panel of hereditary cancer-associated genes.

Methods: The Illumina TruSight™Cancer Sequencing Panel of 94 known genes for hereditary tumor syndromes was extended by adding further 54 relevant genes. All samples were sequenced on an Illumina MiSeq sequencer and analysed using Cartagenia BENCHlab NGS platform, Variant Studio (Illumina), and the SeqPilot software (JSI Medical Systems). For technical validation, 54 patients with a broad spectrum of known germline mutations in 16 different genes were included. In addition, 89 patients with a suspected hereditary tumor syndrome were analysed. This cohort consisted of cases with multiple primary tumors, a striking familial clustering of various tumors, and patients with distinct but unexplained syndromes such as HNPCC or colorectal polyposis.

Results: All variants except for 2 deletions and 1 duplication (size 24-65 bp) were confirmed. Few exons did not reach our quality standards and were analysed by Sanger sequencing. According to the preliminary data, we newly identified 1 predicted high-penetrant loss-of-function mutation in SDHA and 4 likely pathogenic missense mutations in 5/89 patients (6 %) with suspected tumor syndrome including a PTEN missense mutation in a patient with features compatible with Cowden syndrome and a POLD1 missense mutation in the proofreading domain in a patient with adenomatous polyposis and renal cancer. In 2/54 patients (4 %) with known mutations, we found two additional variants including a NF1 nonsense mutation in a case with a known TP53 frameshift mutation. Heterozygous variants in putative low penetrant risk genes (e.g. CHEK2, ATM) were identified in 12 patients (5 with and 7 without a known causal mutation, respectively).

Conclusions: We demonstrate that the application of a comprehensive gene panel can identify the etiology in some patients with unexplained disease. In addition to the diagnostic benefit, the extended analysis will broaden our knowledge about the tumor spectra of established genes. As expected, low penetrant risk alleles occur in both patient groups and likely act as modifiers. However, our findings also show that some patients harbor predicted pathogenic mutations in >1 established cancer gene which makes the interpretation of the phenotypic contribution of those alterations even more challenging and clearly indicate that the classification of a variant as pathogenic should be done with caution. The analysis of 95 additional cases with a suspected tumor syndrome is ongoing.

P-CancG-031

Detection of new point mutation of brca1 in breast cancer patient

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BACKGROUND/ METHODS:

Breast cancer is the most common malignant cancer in women worldwide. BRCA1 is a tumor suppressor gene linked to breast and ovarian cancer risk. [1] Since the identification of BRCA1 twenty years ago more than 500 different point mutations in BRCA1 gene have been described. Mutations in this gene can increase the risk of developing breast and/or ovarian cancer and a number of other cancers as well [2]. Searching for mutations in the BRCA1 gene by Sanger sequencing, the gold standard for mutation detection, is expensive. However, it is often difficult to distinguish a neutral polymorphism from a disease-causing mutation using available databases.

RESULTS:

Here we report a woman, diagnosed with inflammatory breast cancer at the age of 46. She has been shown to be a carrier of a new BRCA1 mutation. Inflammatory breast cancer is uncommon and very unusual aggressive type of breast cancer that accounts 1 to 5% of all diagnosed breast cancers. The symptoms that may occur in this type of cancer include skin redness, swelling, tenderness, warmth in the breast and an orange peel like texture of the skin. Our patient has a strong family history of breast cancer. She came for genetic counseling only because of a hormone-receptor negative result. Molecular analysis of BRCA1 gene in this woman revealed a single nucleotide change of exon 13 (c.4333C>A, p.Pro1445Thr), with unknown significance. This novel point mutation causes proline to threonine substitution at amino acid 1445. To the best of our knowledge, this mutation has not been described before.

CONCLUSIONS:

This novel mutation may have an important influence on the predisposition and development of familial breast cancer. Exact pathogenicity of this point mutation should be confirmed by family history, detection of the mutation in affected family members with illness and exclusion in healthy family members.

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P-CancG-032

Exome sequencing identified potential causative candidate genes for hyperplastic polyposis syndrome

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Purpose: Hyperplastic polyposis syndrome (HPS), also known as serrated polyposis syndrome (SPS), is a yet poorly defined colorectal cancer (CRC) predisposition characterized by the occurrence of multiple and/or large serrated lesions throughout the colon. A serrated polyp-CRC sequence (serrated pathway) has been postulated, however, to date, only few molecular signatures of serrated neoplasia (BRAF, KRAS mutations, CpG Island Methylation, microsatellite instability) were described in a subset of HPS patients and neither the etiology of the syndrome nor the distinct genetic alterations during tumorigenesis have been identified.

Methodology: To uncover predisposing causative genes, the exomes of 11 unrelated and clinically well characterized HPS patients with sporadic appearance were sequenced (Illumina HiSeq platform) using leukocyte DNA. The variants were filtered for rare truncating germline mutations (nonsense, frameshift, highly conserved splice sites) assuming a monogenic disease model. For data analysis and variant filtering the GATK software and in-house tools (VARBANK pipeline) were applied.

Results: Altogether, 260 rare truncating germline variants were identified. After stringent filtering steps including quality scores, the comparison with large datasets from population-based controls, detailed manual investigations of the variants and data mining according to functions and pathways, 135 unique variants in 132 genes remained. Each patient harboured several variants (range: 9-16). Six genes were affected by biallelic variants (recessive model) in at least one patient and 19 genes by heterozygous variants (dominant model) in at least two patients. The majority of these genes is supposed to be associated with cancer or is involved in molecular and cellular functions related to tumorigenesis such as DNA repair or apoptosis. Another 53 genes, which are affected by heterozygous variants in only one of the patients, are regarded as interesting candidates according to functional scores and known somatic mutations in colorectal tumours. In a validation cohort of 20 unrelated HPS patients, three of the candidate genes were affected by additional truncating point mutations.

Conclusions: Using exome sequencing we identified new potentially causative genes for HPS, some of them are recurrently mutated. However, the number of variants per patient is also in line with a more oligogenic etiology of polyp predisposition. The current work-up includes the validation of all variants by Sanger sequencing, testing of relatives to determine the phase of assumed biallelic variants and segregation with the phenotype where applicable. All validated variants are included in a pathway and network analysis.

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P-CancG-033

The same RAD51C mutation is associated with familial breast cancer in two families

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Germline mutations in DNA repair genes BRCA1 and BRCA2 contribute to up to 40% of all hereditary breast and ovarian cancer cases. Many of the familial gynecological cancers, however, cannot be explained by mutations in these genes. Some of the remaining heritability may be attributed to other susceptibility genes of varying penetrance. Germline alterations in RAD51C are predominantly present in 1.3-2.9% of the familial ovarian cancer with or without breast cancer cases, but are rare in families with a history of breast cancer alone. Only one RAD51C deletion and one missense mutation, classified as pathogenic, have been detected so far in breast cancer-only families. The lifetime risk for ovarian cancer in carriers of a RAD51C mutation was estimated to be more than 9%, whereas the underlying risk of developing breast cancer remains unknown.

Here we describe a heterozygous RAD51C splice donor mutation, c.1026+5_1026+7delGTA in intron 8, in peripheral blood lymphocytes from two unrelated women with breast cancer and positive family history. This mutation has been already reported in two families with hereditary breast and ovarian cancer from France and the UK, but not so far in breast cancer-only families. Our first patient was diagnosed with invasive ductal breast cancer with triple negative hormone receptor status at the age 51. The pedigree of the patient included two second degree female relatives with breast cancer and lower abdominal cancer of unknown primary origin, respectively. Mutations in other susceptibility genes were excluded using a Multigene Cancer Panel. The identified mutation affected an evolutionarily conserved position of a splice donor site in Exon 8 of RAD51C. Further analysis by RT-PCR from RNA extracted from the patients' blood confirmed the skipping of exon 8 and loss of 61 base pairs, which was predicted to cause a frameshift resulting in a premature translational termination (p.Arg322Thrfs*22). Sequencing of DNA from the patient derived tumor sample revealed loss of the wild type RAD51C allele. Additionally, this variant was identified in DNA isolated from a tumor sample of the second woman affected by breast cancer in this family, showing a co-segregation with the malignancies in this family.

The same mutation was also detected in a second unrelated patient who developed breast cancer at the age 43 and a non-Hodgkin lymphoma 20 years later. The mother of this patient was also diagnosed with breast cancer, whereas there was no evidence of other cancer entities in this family.

Although RAD51C has been described as primarily an ovarian cancer susceptibility gene, our findings suggest that genetic testing of RAD51C in routine diagnostics in hereditary breast cancer-only families is warranted.

P-CLINICAL GENETICS

P-ClinG-034

Cerebral white matter abnormalities in 6p25 deletion syndrome

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Cerebral white matter abnormalities in children are generally considered to be suggestive of an underlying metabolic defect. According to van der Knaap, multifocal white matter lesions are most commonly seen in static, nonmetabolic encephalopathies with congenital CMV infection high in the differential diagnosis (van der Knaap et al. 2006).

We report the phenotype and genotype of a 4 years and 11 months old boy who was transferred to hospital after an accident. CT-scan and cMRI showed a cranial fracture as a result of the accident and two incidental findings: 1) large bifronto-temporal arachnoidal cysts and 2) multifocal white-matter-lesions involving the periventricular, deep, and subcortical cerebral white matter. The clinical phenotype of the boy included mild developmental delay, strabism, slightly dysmorphic features and very mild hearing impairment.

The molecular karyotype obtained from a blood sample of the patient using a CytoScan HD (Affymetrix) SNP-Array detected a ~ 3,9 Mb heterozygous microdeletion in 6p25.3 - 6p25.2 deleting 34 genes, 18 of which were OMIM annotated including the transcription factor FOXC1. Metabolic work up and PCR for CMV-DNA from a Guthrie card sample were negative.

Microdeletions in 6p25 are thought to be associated with a distinctive clinical phenotype – the 6p subtelomeric deletion syndrome – that includes sensorineural hearing loss, anterior chamber eye defects, cardiac defects, developmental delay and other developmental and behavioral abnormalities, hypotonia, hip dysplasia, cerebellar abnormalities and a characteristic facial appearance. Few reports exist that describe multifocal T2-weighted and FLAIR abnormalities involving the periventricular, deep, and subcortical cerebral white matter associated with the 6p25 deletion in young children and in adults.

From our case and from the review of the literature we conclude that a microdeletion in 6p25 should be considered as the cause for unclear multifocal cerebral white matter abnormalities in patients who in addition have dysmorphic features and multiple congenital anomalies even when intellectual abilities are within the normal range (Vernon et al. 2013).

P-ClinG-035

A Novel Mutation in RIPK4 Identified by Exome Sequencing Causes Bartsocas-Papas Syndrome without Cleft Lip/Palate

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Three children from a consanguineous Kuwaiti family presented with ankyloblepharon, sparse and curly hair and hypoplastic nails, suggesting a possible diagnosis of CHAND syndrome. Sequencing of TP63 did not reveal any pathogenic mutation. We performed homozygosity mapping in the family followed by exome sequencing of the index patient. In total, we identified 3 homozygous mutations in the linked region, located in the RIPK4, MX2 and PWP2 genes. Recently, several mutations had been reported for Bartsocas-Papas syndrome in the kinase domain of RIPK4. Since the symptoms observed in our patients are a subset of the Bartsocas-Papas syndrome symptoms, we considered the homozygous RIPK4 mutation (c.850G>A, p.Glu284Lys) identified in our patients and co-segregating with disease as the true pathogenic mutation. Most of the recently reported Bartsocas-Papas patients experienced a severe phenotype and only a single sporadic patient showed a milder phenotype including bilateral cleft lip/palate. The affected children of the here reported family present with even a milder phenotype lacking any orofacial clefting. Of interest, our family is the first demonstrating a mutation just outside the active side of the enzyme which might explain the milder phenotype. Accordingly, by three-dimensional homology modelling of RIPK4, we showed that this mutation might have effects on protein dimerization which is probably a less severe mode of action. Our findings show that the clinical spectrum of Bartsocas-Papas syndrome can extend from a very severe lethal phenotype to a much milder form which lacks many of the symptoms that are considered to be characteristic for this syndrome. Especially the lack of a cleft lip and palate and the overall mild phenotype is an important information for the clinicians suggesting that in patients with ankyloblepharon and ectodermal features, the diagnosis of an attenuated form of Bartsocas-Papas syndrome has to be considered accompanied by screening for mutations in RIPK4.

P-ClinG-036

Therapeutic consequences of genetic family screening in inherited primary arrhythmia syndromes – 15 years in review

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The three most common inherited primary arrhythmia syndromes are the long QT syndrome (LQTS), catecholaminergic polymorphic ventricular tachycardia (CPVT) and the Brugada syndrome (BrS). Whenever a distinct disease causing mutation is found, those findings facilitate screening for relatives at risk. In the following we present the therapeutic consequences for genetically affected family members.

Between 1/2000 and 11/2014, n=501 relatives of 178 index patients with one of the arrhythmia syndromes mentioned above were screened for the respective familial mutation (targeted genetic screening on KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2 or RYR2). n=235 (46%) of the relatives tested were carriers of the familial mutation. In 192 (82%) of the carriers, therapy was indicated and initiated. n=36 (15%) of the relatives carrying

the familial mutation already had suffered from (likely) cardiac symptoms (syncope, seizure, aborted sudden cardiac death, sudden death). After initiation of therapy, resp. in compliance with known precautionary measures, only 2 patients continued to be symptomatic and no one died.

Genetic testing of potentially affected relatives of an index patient with a disease causing mutation frequently leads to therapeutic consequences. Even when a specific therapy is not always mandatory, life style recommendations (e.g. early antipyretic medication, avoidance of certain drugs) are indicated for every affected relative. With adequate, mostly not very restricting therapy, resp. some precautionary measures, the prognosis in most cases is good once the disease is detected.

P-ClinG-037

(Epi)genetic aberrations of Temple syndrome/upd(14)mat syndrome are detectable in patients with the referral diagnosis of Silver-Russell syndrome

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Maternal uniparental disomy [upd(14)mat] and related (epi)genetic mutations of the imprinted region 14q32 are associated with Temple syndrome [TS14, formerly upd(14)mat syndrome]. The phenotype of TS14 comprises pre- and postnatal growth retardation, failure to thrive in early childhood, a prominent forehead, micrognathia, small hands and feet, scoliosis, recurrent otitis media, muscular hypotonia and motor delay, and precocious puberty. As there is evidence of impaired intellectual disability and obesity in some patients, TS14 has been suggested as a differential diagnosis of Prader-Willi syndrome (PWS) and methylation as well as gene dosage analysis of the chromosomal region 14q32 is recommended after exclusion of the molecular diagnosis of PWS. Corresponding to other imprinting disorders, different types of molecular alterations can occur in TS14: upd(14)mat, microdeletions, and hypomethylation (epimutation) of the paternally methylated alleles of the IG- and/or MEG3-DMRs (differentially methylated regions) in 14q32. With the increasing application of multilocus testing in the diagnostic workup of imprinting disorders it turns out that also patients referred with clinical features compatible with Silver-Russell syndrome (SRS) carry one of the TS14 specific aberrations. We screened our diagnostic cohort of more than 600 patients with features of SRS by tests for multiple imprinted loci (MS-MLPA, MS-SNuPE), and thereby we identified 6 patients with TS14: 3 with upd(14)mat, 2 with submicroscopic deletions affecting imprinted regions in 14q32 and 1 with an epimutation. Two of these patients currently visit high school. In summary, our data show that TS14 can be a differential diagnosis of SRS, we therefore recommend to include 14q32 testing in patients referred with the diagnosis of SRS after exclusion of the mutations/epimutations in 11p15.5 and upd(7)mat. In case of the molecular diagnosis of TS14, the families should be counseled that the TS14 phenotype can be variable, and is associated with a PWS-like phenotype only in some cases. However, more follow-up data are needed to further precise the prognosis and the long-term features of this imprinting disorder (scoliosis, metabolic disorders). Finally, TS14 is probably underdiagnosed as it is tested so far only in specific subpopulations with growth retardation (PWS, SRS). Our study is supported by the BMBF (01GM1114C) and COST (BM1208).

P-ClinG-038

Autosomal-recessive osteogenesis imperfecta due to COL1A2- and PPIB-gene mutations. Infantile and fetal phenotypes and diagnostic pitfalls.

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Osteogenesis imperfecta (OI) is a heterogeneous group of connective tissue disorders characterized by a predisposition to fractures and variable extraskeletal symptoms. OI can result from mutations of at least 12 various genes, 9 of them, PPIB-gene including, causing very rare recessive OI. Most of the OI cases (~90%) are caused by mutations in COL1A1- or COL1A2-gene. The mutations in these two genes affect collagen type 1 synthesis (chains α1 and α2) and the vast majority of them follows AD pattern of inheritance.

We refer on a 4,5 year-old boy from a consanguineous family with clinical findings of OI type III-IV (a very short stature, femurs and arms fractures, only mild skeletal deformities, inability to stand or walk without support) and molecular finding of homozygote mutation in COL1A2-gene: p.Gly322Ser (c.964G>A). This mutation had been described so-far only in a heterozygote form, causing a mild form of OI - type I. The boy

had also slight blue scleras and dentinogenesis imperfecta and his mental status was age-appropriate. The parents initially denied any health problems (both were 160 cm tall), after repeated questioning they admitted a history of possible femur and arm fractures in young age. The analysis confirmed heterozygosity for p.Gly322Ser mutation in both of them.

Further we refer on a consanguineous family with a 6-years old daughter with clinical symptoms of OI type IV (COL1A1-, COL1A2-gene analysis negative) and with 2 following gestations interrupted in the 23rd/16th GW; the first of them due to fetal ultrasound pathology. The autopsy findings of the first fetus had been consistent with Pena-Shokeir Typ 1 Phenotype (or FADS - fetal akinesis/hypokinesis deformation sequence): marked signs of arthrogryposis multiplex (both large and small joints), myodystrophy and excessive lungs hypoplasia. In addition there had been found also bone fractures (radius, ulna, humerus, femur bilat.) and craniofacial dysmorphy. NGS-analysis confirmed in the fetus a homozygous mutation c.374A>T (p.Asp125Val) in PPIB-gene, which indicates recessive OI type IX. Both healthy parents are heterozygotes, the sister with OI (meanwhile 12 years old) is a homozygote for this mutation. CVS analysis of the following pregnancy confirmed homozygozity of the PPIB-gene mutation and in the aborted fetus could be demonstrated increased fragility of soft tissues, loose large joints and deformities (lower limbs), pterygia (upper limbs), camptodactyly and craniofacial dysmorphy. The bone-structure was pathologic, rarefying, with high fragility, however, without any primary fractures.

P-ClinG-039

Novel DCK1 mutation in a patient with Hoyeraal-Hreidarsson syndrome

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X-linked Hoyeraal-Hreidarsson syndrome (HHS) is a rare disorder, characterized by intrauterine growth retardation, microcephaly, cerebellar hypoplasia, bone marrow failure with pancytopenia and developmental delay, and is caused by mutations in DKC1. HHS is allelic to dyskeratosis congenita (DKC), and albeit clinically distinct, premature shortening of the telomeres is a hallmark of both disorders.

We report on a boy who was born in the 33rd gestational week as the first child of healthy and nonconsanguineous Vietnamese parents. Clinical problems included intrauterine growth retardation, ponto-cerebellar hypoplasia, progressive aplastic anemia, recurrent infections, tongue leukoplakia and severe developmental delay. He died at the age of 8 months of pneumonia and bone marrow failure. The clinical diagnosis HHS was confirmed by the detection of a novel and maternally inherited missense mutation (c.1072T>C, p.C358G) in DKC1.

The patient expands the spectrum of HHS-associated DKC1 mutations and will thus contribute to establish detailed genotype-phenotype correlations.

P-ClinG-040

Third case of Leri Pleonosteosis due to microduplication at 8q22.1

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Here we report on the third case of a patient with Leri Pleonosteosis, an autosomal dominant inherited rheumatoid disease with flexion contractures of fingers, short and broad hands, decreased joint mobility, joint pain, and mild facial features, where microduplication at 8q22.1 could be shown to be causative.

The 5½ years old girl was born to non-consanguineous healthy parents with birth weight of 3.900g, length of 53cm and OFC of 36cm. She was able to sit at age 7-8 months and could walk at age 14-15 months but still broad based and tiptoeing. Because of joint pain at thumbs, the initial diagnosis of rheumatism/juvenile polyarthritis was made, however, the diagnosis could not be confirmed by laboratory findings and failure of MTX treatment. At examination, the girl had short and broad fingers and toes, clinodactyly of 5th fingers, flexion contractures of fingers, incomplete supination of forearms, mild knee contracture, marked knock-knees, and mild facial anomalies with slightly up-slanting palpebral fissures, mild epicanthus, hypertelorism, everted lower lip, and dysplastic external ears. Her length was 116cm (P50), weight 25kg (P90), and OFC 52cm (P50-75). Because of her developmental delay, physical and speech therapy was prescribed. The family history was uneventful.

Based on radiographs with short and broad metacarpals and proximal phalangeal bones, the preliminary diagnosis of an acromicric dysplasia was made.

Because of the combination of slightly developmental delay and dysmorphic features an array-CGH analyses was performed in this patient and revealed an approximately 1.167Mb de novo duplication of the chromosomal region 8q22.1. The duplication, encompassing 7 genes, was confirmed by qPCR analyses and FISH using BAC clone RP11-413N8. As in the recently published 2 cases with Leri pleonosteose (Banka et al., Ann Rheum Dis., 2014), also in our patient GDF6 and SDC2 genes are located within the duplicated region which gives further support that overexpression of these genes is disease causing.

P-ClinG-041

Menkes disease with discordant phenotype in female monozygotic twins

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Menkes disease (MD) is a rare X-linked recessive disorder caused by mutations in the ATP7A gene. This neurodegenerative disorder typically affects males and is characterized by impaired copper distribution and the malfunction of several copper-dependent enzymes. Classical MD is characterized by sparse and kinky hair (pili torti), distinctive facial features, failure to thrive, hypotonia, and deterioration of the nervous system with seizures, developmental delay and intellectual disability. Children with Menkes syndrome typically begin to develop symptoms during infancy, and death usually occurs by age of three years. Early subcutaneous copper histidine supplementation may modify disease progression. Initial diagnosis is based on clinical features and reduced levels of serum copper and ceruloplasmin, definitive diagnosis is based on molecular genetic testing.

Here, we report female monozygotic twins with a heterozygous ATP7A mutation. One twin girl is healthy at the current age of 3.5 years (showing only one abnormal strand of hair), whereas the other twin girl developed MD and died at the age of 3 years. The diagnosis MD was suspected at first neuropediatric examination at the age of 10 months due to characteristic features including severe developmental delay with regression, hypotonia, ataxia, poor feeding, hypopigmented kinky hair, pudgy cheeks, cutis laxa, and recurrent bronchitis. Serum copper and ceruloplasmin concentrations and excretion of copper in 24-h urine collection were decreased. Pili torti were confirmed by light microscopy. Bladder diverticulae and cranial vascular tortuosity were revealed by sonography and MRI, respectively. The disease course of the affected twin girl was rather stable under treatment with subcutaneous copper histidine until she incurred a fatal febrile infection.

Molecular genetic testing confirmed the diagnosis of MD: a known pathogenic deletion of exon 6 of the ATP7A gene was detected. The deletion was also demonstrated in the healthy twin sister, and excluded in the mother. Presumably, the affected girl developed MD due to skewed X-inactivation, although X-inactivation studies couldn't prove this theory. Both girls showed normal X-inactivation in blood and in buccal mucosa. X-inactivation studies in fibroblasts of the affected girl failed, unfortunately, due to lack of growth.

This case not only is a rare example of an affected girl with MD, but also demonstrates the possibility of a discordant phenotype in monozygotic twin girls. As speculated in other X-linked diseases, the process of monozygotic twinning may in itself be a trigger for skewed X-inactivation leading to a discordant phenotype.

P-ClinG-042

„Angelman-like syndromes": 10-year-old boy with 9q34.3 microdeletion including the EHMT1 gene (Kleefstra syndrome)

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Some 10% of children with a clinical diagnosis of Angelman syndrome (AS) do not have an identifiable molecular defect and of these, most have an AS-like syndrome that is clinically and molecularly distinct from AS. AS-like conditions comprise deletions of 22q13.33 (SHANK3), 2q23.1 (MBD5), 17q21.31 (KANSL1), and 9q34 (Kleefstra syndrome, EHMT1), and Pitt–Hopkins (TCF4), Christianson (SLC9A6), Mowat–Wilson (ZEB2) and Rett (MECP2) syndromes (Tan et al. 2014).

The propositus, a boy, was first seen by us at age 3 years for evaluation of a syndromal mild-to-moderate sensorineural hearing loss. He had atactic gait, developmental delay, and facial abnormalities including mild microcephaly, synophrys, arched eye brows, midface retrusion, and proganthism. Mucopolysaccharidosis, Cornelia de Lange and Angelman syndromes were considered. However, biochemical and genetic studies (GJB2/GJB6 genes, karyotyping, Fragile-X and Angelman syndromes) were all normal and no diagnosis could be established at that time. Upon re-evaluation at age 10 years, he was of normal height and weight (P25-P50), and his occipitofrontal circumference had normalized (P10-25). He could speak phrases of 3-4 sentences (first words at 4 1/2 years) and could read simple words and numbers. His intellectual disability (ID) was mild-

to-moderate (IQ 51, Snijders-Oomen non-verbal intelligence test-revised) and motor development remained impaired. A heterozygous 9q34.3 microdeletion was identified using MLPA (kit P245, MRC Holland) and array analysis (Affymetrix CytoScan HD); it spanned 432 kb and 12 genes (including EHMT1) and had originated de novo.

Since 2004, submicroscopic subtelomeric deletions of 9q34 have been known to cause a recognizable mental retardation syndrome, and later haploinsufficiency for the EHMT1 gene (euchromatic histone-lysine N-methyltransferase) was found to be responsible for the main phenotypic features (Kleefstra et al. 2009). Some 113 patients with 9q34 deletions or EHMT1 gene mutations have been described (Orphanet 2015). Clinical features shared by the Angelman and Kleefstra syndromes include moderate-to-severe ID with minimal speech, hypotonia in childhood, seizures, sleep disturbances, midface retrusion, and prognathism. Other hallmarks of AS include very late walking and ataxia, and can be also present in patients with larger 9q34 deletions. Differences discriminating between the AS and Kleefstra syndromes include childhood-onset obesity (absent in the propositus), conotruncal and septal heart defects (absent in the propositus), and male genital abnormalities (cryptorchism in the propositus). Based on this and further observations we suggest that individuals with an AS-like condition and normal AS testing should be further studied using (1) microarray testing and, if normal, (2) a comprehensive gene panel (such as the MPIMG1/Kingsmore test).

Reference: Tan et al. If not Angelman, what is it? A review of Angelman-like syndromes. AJMG-A 2014; 164A(4):975-92.

P-ClinG-043

Spacious molecular cytogenetic analysis of a de novo mosaic microduplication in 5q35.3 excluding NSD1 in a boy with microcephaly and no speech

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We report the case of a 4-year-old boy presenting with microcephaly, lack of expressive speech and behavioural problems starting at the age of 2 years. Physical development is appropriate. Milestones at the childhood were unremarkable. Audiometry and EEG displayed normal results. Clinical examination of the heart and other internal organs showed normal results. At age of 4 years his behaviour improved due to his continuous attendance for a kindergarten.

Conventional chromosome analysis showed no numerical or structural aberration. But array-CGH revealed a 2,96 Mb duplication in 5q35.3 to be under suspicion for mosaicism. Furthermore, we characterised the pure partial trisomy with a band specific probe localised in 5q35.3 (BlueGnome). FISH results showed an interstitial duplication and could prove the mosaic. In 32% of examined cells we could see an enhanced signal in the duplicated region. 68% of the evaluated cells showed a normal signal pattern.

In our patient the duplication segment contains 42 genes, whereas for 6 genes OMIM diseases are known (PROP1, NHP2, GRM6, ADAMTS2, LTC4S, SQSTM1, FLT4). Surprisingly, NSD1 located outside.

Dikow et al. 2013 proposed a reversed Sotos syndrome for duplication patients including NSD1 with microcephaly, short stature, learning disability or mild to moderate intellectual disability, mild facial dysmorphisms, motor delay and may be behavioural problems. Some features of our patient are overlapping for possible reversed Sotos syndrome, for example microcephaly (-1,1cm<3. Centile) and aggressive behaviour in early childhood. But he had normal length (67. Centile) and weight (81. Centile) and no motor retardation.

In conclusion, due to 30% mosaic duplication 5q35.3 without NSD1 gene and no internal malformation, the prospective development of our patient should be positive. Phenotype is not really predictable depending on the percentage and tissues distribution of unbalanced cells. It is likely that mosaic duplications are underestimated because they are not associated with obvious clinical effects in some individuals.

P-ClinG-044

Urocanase deficiency detected by clinical-exome-sequencing in a girl with mental retardation, secondary microcephaly, cerebellar hypoplasia and gait ataxia

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Urocanase deficiency is an extremely rare autosomal recessive metabolic disorder affecting the enzyme Urocanase in the histidine pathway. Three metabolic defects in the catabolism of histidine have been described so far: Histidinemia (OMIM 235800), Formiminotransferase deficiency (OMIM 229100) and Urocanase

deficiency (OMIM 276880). Urocanase deficiency was first described in 1971 in a male patient with mental retardation by Yoshida et al., and to date, only three additional cases from two further families have been described in the literature.

We report on a 6 year old girl, the first child of consanguineous Turkish parents, who presented with pronounced psychomotor and speech delay. Early motor development was severely delayed, with crawling from an age of 1½ years and independent walking further delayed by severe ataxia. At the age of 5 ½ years she could walk independently but required occasional support due to the ataxia. MRI of the brain at age 2½ years revealed cerebellar hypoplasia, and secondary microcephaly was noted. At the age of two years the girl spoke two words; at the age of 6 she used simple word-combinations. Apart from epicanthic folds, a broad nasal bridge and full cheeks, no further dysmorphic features were noted.

After normal results from Array-CGH analysis as well as sequencing of the genes CASK2 and MECP2, clinical-exome-analysis was performed using a trio-analysis approach, comparing the healthy parents with the affected child (TruSightOne Panel, Agilent). A homozygous missense mutation in Exon 7 of the Urocanase-1 gene (UROC1) was detected: c.655G>A; p.Val219Ile. The parents are heterozygous carriers of this mutation which was validated by Sanger Sequencing. The mutation was predicted to be pathogenic by the software tool Alamut. Further metabolic validation for the patient is currently ongoing and will be presented together with the clinical and molecular-genetic data.

P-ClinG-045

De novo deletion of proximal 8q11.21q12.1, a rare condition of dwarfism

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We report on a 7 years old girl with growth retardation and dystrophy (length 104 cm, weight 11 kg, head circumference 45, 9 cm), all parameters until 3th centile. She is the second child born to non-consanguineous parents. Delivery was at term, the girl showed prenatal growth retardation with birth weight 1700 g, length 42 cm, head circumference 30 cm.

The child is a friendly girl showing minor dysmorphic signs: small mandible, epicanthic folds, clinodactyly on both sides, hallux valgus on both sides. Radiological examination showed a retarded bone age of about 2 years. She had myopia, hypochromic microcytic anemia and obstipation. Milestones of motor development were normal, but speech development was delayed. She expressed single words at the age of 3 years. An enrollment in regular schools was not possible due to speech- and concentrations problems.

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 8q11.21q12.1 (ViiA 7 Real-Time PCR System) were used for investigation of healthy family members.

Cytogenetic analysis revealed a normal female karyotype (46,XX). However, array analysis showed a heterozygous deletion of proximal 8q11.21q12.1 by a copy loss of 490 oligonucleotide probes. The deletion spans approximately 9.02-9.06 Mb and consists of 24 genes. In our family both parents status were examined and revealed normal results. Therefore the deletion is de novo.

A case with similar deletion of proximal 8q (q11-q13) in a girl has been described in the literature. Schinzel reported a patient as a patient with a Silver-Russel Syndrome like feature. Silver-Russell syndrome is characterized by severe pre- and postnatal growth retardation. Mental development is normal in the majority of cases. There are a variety of less consistent findings in patient with Silver-Russell syndrome or Silver-Russell syndrome-like features (Schinzel et al, 1994). Common features and differences between both syndromes will be outlined.

In conclusion, patients with pre- and postnatal growth retardation, dysmorphic facial and body signs and speech problems should be investigated for this deletion of proximal 18q.

P-ClinG-046

A novel patient with compound heterozygous mutations in SUCLA2 and a mild mitochondrial encephalomyopathy

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In a 15 year old girl with suspected mitochondriopathy of unknown nature we performed Next-Generation-Sequencing with an Illumina TruSightOne panel containing about 4,800 known disease genes. This revealed two compound heterozygous mutations, p.Arg284His and p.Asp333Gly, in the SUCLA2 gene. Parents were

confirmed to be heterozygous carriers of one of the mutations each. SUCLA2 encodes the beta subunit of the succinate-CoA ligase, a Krebs-cycle enzyme that catalyzes the reversible formation of succinate and ATP from succinyl-CoA and ADP. Homozgyous or compound heterozygous mutations in SUCLA2 are causative for a mitochondrial DNA depletion syndrome with (Leigh like) encephalomyopathy and mild methylmalonic aciduria. Onset of disease is usually in the first months with severe hypotonia. Later, severe muscular atrophy, progressive scoliosis, dystonia and hyperkinesia follow. Some children have epilepsy, postnatal growth retardation and severe sensorineural hearing impairment. Prognosis is usually poor with early lethality.

Strikingly, the phenotype in our patient is relatively mild. She was born after uneventful pregnancy, and development was reported to be normal during the first year of life. After an infection she was reported to show developmental regression with loss of sitting abilities and muscular hypotonia between 15 and 19 months of age. She developed hyperkinesia and dystonia. At the current age of 15 years she cannot walk and needs support for sitting. Her active speech is limited to a single word, but her reception is better, and she can communicate by signs and with a computer. She can calculate up to 100. Her hearing is normal, and no seizures occurred. Conventional karyotyping, testing of MECP2, GALC and DYT1 and metabolic screening in blood revealed no abnormalities. Due to increased lactate levels in liquor and periventricular signal alterations in brain MRI, a mitochondrial disorder was suspected. Interestingly, testing for mitochondrial DNA depletion was normal which would have been a typical marker for SUCLA2 associated mitochondriopathies.

The maternal mutation p.Arg284His has not been reported so far; however, a missense mutation at the same, highly conserved position was reported in a homozygous state in two patients with typical disease manifestations. The paternal mutation p.Asp333Gly has been reported in two Finnish patients, homozygous in one and compound heterozygous with a larger deletion in the other. This mutation was suspected to be hypomorphic, reducing but not completely abolishing its enzymatic activity. Interestingly, both patients also had a comparatively mild disease course and no mitochondrial DNA depletion was detected.

Our findings expand the clinical spectrum of SUCLA2-associated mitochondrial encephalopathies to the mild end of the spectrum and confirm the hypomorphic effect of the p.Asp333Gly mutation. The patient's father originating from Finland confirms this as being a Finnish founder mutation.

P-ClinG-047

Recurrent inverted 8p duplication deletions share common breakpoints which predispose to structural imbalances

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Inverted 8p duplication deletions are recurrent chromosomal rearrangements with an occurrence of about 1:20.000 newborns. It is thought to be caused by non-allelic homologous recombination between duplications of the olfactory receptor gene cluster at 8p23.1. The presence of a paracentric inversion polymorphism at 8p23.1 in ~26% of the European population also seems to contribute to the formation of inverted 8p duplication deletions. Most cases of 8p duplication deletions were described cytogenetically whereas there are only few cases analyzed using array-CGH up to now.

We report here four new cases: Two prenatal cases and two children aged 9 and 10 years with 8p duplication deletion which were analyzed using array-CGH. The size of the terminal deletion is constantly 6.9 Mb, whereas the size of the duplication is ranging from 3.4 to 30.8 Mb. Breakpoints were found to be located both in p23.1 (6.9 and 12.8 Mb), with a copy number normal region of 5.9 Mb between deletion and duplication. The clinical symptoms include cardiac anomaly, developmental delay (especially speech), severe hypotonia and dysmorphisms.

As one of our reported cases with only a small duplication would cytogenetically not have been identified as an inverted 8p duplication deletion, our analysis is underlining the importance of array-CGH analysis.

P-ClinG-048

New case of biallelic TRMT10A deficiency identified by exome sequencing confirms the associated phenotype of primary microcephaly with intellectual disability and short stature

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Recently a new syndrome of young onset diabetes, short stature and microcephaly with intellectual disability has been described in a large consanguineous family of Moroccan origin. By linkage analysis and exome sequencing a homozygous nonsense mutation in the TRMT10A gene was identified in all three

affected siblings. The protein encoded by TRMT10A (also RG9MTD2), which was proposed to have tRNA methyltransferase activity, was shown to be ubiquitously expressed with enriched levels in the affected tissues brain and pancreatic islets and to be absent in lymphoblasts from the affected siblings. We now report a new case of biallelic TRMT10A deficiency in a girl born to apparently non-consanguineous parents of Kosovo origin. By exome sequencing in our patient we identified a homozygous nonsense mutation (c.379C>T) in the TRMT10A gene. Of note, this is the same mutation as recently reported, introducing a premature stop codon at position 127 of the protein. Our patient presented with primary microcephaly, intrauterine onset borderline growth, mild intellectual disability and fine motor problems, a high palate with uvula bifida and minor facial features such as long narrow face with narrow palpebral fissures, long thin nose and small mouth. At age 4 years a seizure disorder started. Notably, at age 8 years, our patient did not yet manifest diabetes, which was of adolescent onset in the previously described family. In conclusion, our report of a novel patient confirms together with a recently published missense mutation in another pedigree the phenotype of the novel syndrome associated with biallelic TRMT10A deficiency including short stature and microcephaly with intellectual disability.

P-ClinG-049

Mutation Spectrum in German Patients with Familial Hypercholesterolemia

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Autosomal dominant familial hypercholesterolemia (ADH) is characterized by elevated plasma levels of low-density lipoprotein cholesterol (LDL-C) and a dramatically increased risk to develop cardiovascular disease (CVD). The prevalence of ADH is about 1:500, with a higher frequency because of founder effects in some populations. Based on this frequency ADH is clearly underdiagnosed in most countries. Mutations in three major genes have been associated with ADH: LDL receptor gene (LDLR), apolipoprotein B gene (APOB) and proproteinconvertase subtilisin/kexin 9 gene (PCSK9).

In our study we investigated the mutation spectrum in 120 patients (51% females) clinically diagnosed in a specialized lipid clinic (Charité/ Berlin) with possible or probable ADH, based on the Dutch Lipid Clinic Network Criteria. In a three step mutation screening procedure (direct DNA sequencing) we sequenced the coding region of the LDLR gene followed by sequencing the site of the major disease causing mutation in the APOB gene, c.10580G>A (p.Arg3527Gln) and, finally, sequencing of the PCSK9 coding region.

We found pathogenic mutations in 53 patients (44%) in one of the analysed genes. As expected, most of the mutations were identified within the LDLR gene (about 90%). Heterozygous missense mutations in the APOB gene were detected five patients (about 9%) and no mutation in the PCSK9 gene was found.

In 48 patients we detected a total of 51 LDLR mutations. 33 of the patients showed missense mutations. In addition we identified 6 small deletions (≤20 nucleotides), 5 slice-site mutations and 7 nonsense mutations. Three of the 48 patients were probable compound heterozygous. Six of the mutations identified have not been described before: c.340_344delTTTCG (p.Phe114Leu+12X); c.1880C>A (p.Ala627Asp); c.1277delT (p.Met450X); c.1072T>A (p.Cys358Ser); c.1561G>A+c.1562C>T (codon: GCC>ATC, p.Ala521Ile); c.1649_1668del20 (p.Val550Aspfs*2).

The results of the mutation screening will be presented together with phenotypic data and will be discussed with respect to previous data on German ADH mutations and phenotype-genotype correlations.

P-ClinG-050

Detection of disease-causing mutations in cases of unexplained early-onset ataxia by using panel based mid-throughput sequencing

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Spinocerebellar ataxia (SCA) is a large heterogeneous disease group mainly characterized by ataxia and progressive neurodegeneration. SCAs with an early onset age under 30 years (early-onset ataxias, EOAs) are mostly caused in autosomal recessive genes and show a wide range of clinical symptoms. Due to the large

variability and overlap of phenotype and the rapidly increasing amount of (often very rare) ataxia related genes, it is challenging to find a molecular diagnosis in these patients.

In order to identify underlying genetic mutations in unexplained patients we compiled a study cohort of 131 cases with EOA. All patients were screened negative for SCA1, 2, 3, 6, 7, 17 and FRDA. Patients with a genetic mutation sufficient to explain the ataxia or with neurodegenerative disease in more than one generation were excluded beforehand. For screening our cohort, we widened our already existing ataxia specific HaloPlex panel (Agilent) with recently published ataxia genes to comprise an overall number of 132 genes. Samples were processed for the target region of 474237bp and sequenced with paired-end runs (2x 150bp) on a MiSeq (Illumina) followed by bioinformatic analyses using our in-house pipeline. Received variant lists were filtered with different criteria: non-synonymous, exonic/splicing, MAF ≤1%, in-house database frequency ≤5x.

By this approach, on average, >95% of the target region was covered ≥20x with a mean coverage of 254 ± 68 reads. The analysis of filtered variants identified 17 patients (13%) with clear pathogenic biallelic mutations in 8 genes (1x POLR3B, 7x SACS, 2x ATM, 2x NPC1, 1x PNPLA6, 2x SYNE1, 1x CLN5, 1x GBA2) which could be phenotypically confirmed by review of patient symptoms. Probably pathogenic mutations in 6 genes (2x SETX, 1x SYNE1, 1x ITPR1, 1x KIF5C, 1x SACS, 1x TTPA) could be identified in 7 patients (5,3%) which still need to be checked for plausibility by functional assays or corresponding abnormalities of brain morphology established for each of these genetic diseases. We additionally found 8 patients (6,1%) with a potential de-novo mutation in a dominant ataxia gene (4x ITPR1, 2x PRKCG, 2x SPTBN2, 1x CACNA1A) which still needs to be validated. For 35 patients the obtained putative mutations still need to be reanalyzed for plausibility by checking clinical and functional changes.

By using an ataxia specific gene panel in combination with mid-throughput sequencing we solved a definite share of 13% of unexplained cases with EOA by identifying homozygous or compound-heterozygous recessive ataxia mutations. Moreover, for at least 15 additional patients (11%) the identified mutation is very likely to be disease-causing, thus yielding an overall number of 24% with likely pathogenic mutations. Disease specific panel sequencing thus enables a highly effective, cost-efficient and fast detection of underlying mutation which could facilitate therapeutic treatment of symptoms or precise prediction of the course of the disease.

P-ClinG-051

Deciphering the genetic basis of idiopathic short stature

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Shortness of stature is a common medical concern in childhood and has an incidence of 3 % in the general population. After excluding already known defects the underlying cause remains unknown in approximately 80 % of patients. 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 10% of patients with short stature. Based on these results we proposed that rare single nucleotide variants (SNV) might explain a majority of the remaining unknown genetic basis.

Recently, we and others confirmed whole exome sequencing as suitable method to identify the genetic causes in syndromic short stature patients. These results implied a heterogeneity with more than 200 genes involved in short stature by power analysis.

To address this hypothesis we thoroughly built a study group of more than 500 families with idiopathic short stature. We selected 64 individuals on account of the height standard deviation score and performed whole exome sequencing. We confirmed mutations in known short stature genes in 7 patients and identified variants located in 7 genes associated with human height in the general population. Furthermore, we identified potential recessive, dominant and x-linked inherited pathogenic variants in genes involved in epigenetic modification, cell cycle regulation, ubiquitination and protein synthesis.

In conclusion, we were able to identify novel candidate genes for short stature. Analysis of further individuals with short stature will lead to a more elaborate and detailed view on mechanisms involved in growth regulation.

P-ClinG-052

Characterization of NAHR-mediated type-1 NF1 deletions

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Neurofibromatosis type 1 (NF1) occurs with an incidence of 1 in 3000 and is caused by mutations in the NF1 gene. 5% of all NF1 patients exhibit large deletions encompassing the entire NF1 gene and its flanking regions. Different types of large NF1 deletion have been identified which are distinguishable in terms of their size and breakpoint position. The majority of large NF1 deletions (70-80%) span 1.4-Mb and are characterized by breakpoints located within low-copy-repeats, termed NF1-REPa and NF1-REPc, which exhibit 97.5% sequence homology within 51-kb. These 1.4-Mb deletions have been designated "type-1 NF1 deletions" and they are caused by non-allelic homologous recombination (NAHR). Two NAHR hotspots located within NF1-REPa and REPc have been identified; these are termed PRS1 and PRS2, and are separated by 20-kb. These NAHR hotspots encompass only 3-kb and 2-kb respectively, and exhibit sequence identity between the NF1-REPs. It is important to note that the breakpoints of the NAHR-mediated type-1 NF1 deletions represent the sites of strand exchange as identified in the recombination products but do not necessarily represent the sites of the recombination-initiating DNA double strand breaks (DSBs). Previous studies have suggested that most type-1 NF1 deletion breakpoints cluster within the PRS1 and PRS2 hotspots, but a methodical analysis of breakpoint position in a large number of type-1 NF1 deletions has not so far been performed. It therefore remains unclear whether NF1 deletions harbouring breakpoints in either PRS1 or PRS2 are mediated by DSBs occurring at the same genomic position within the NF1 REPs. Differential processing of the recombination products could account for the different sites of strand exchange observed in PRS1- and PRS2-positive NF1 deletions. The aim of our present study is to determine the precise breakpoint positions in 67 patients with type-1 NF1 deletions initially identified by MLPA and to gather additional information about the relationship between PRS1 and PRS2. In order to identify the breakpoints (sites of strand exchange) at the highest possible resolution, we performed SNP genotyping of long-range paralog-specific PCR products amplified from NF1-REPa or NF1-REPc, respectively. We also performed breakpoint-spanning PCR and sequence analysis of the respective PCR products. Our analysis indicates that 52 of the 67 type-1 NF1 deletions investigated harboured breakpoints within the 2-kb PRS2 hotspot. Only 9 deletions (13%) had breakpoints located within PRS1 whereas 6 deletions exhibited breakpoints that were located outwith PRS1 and PRS2. Our findings clearly indicate that PRS2 is a very strong NAHR hotspot since it harbours 78% of all type-1 deletion breakpoints. The sequence analysis of the regions of strand exchange in all type-1 NF1 deletions promises to provide further information about recombination initiation sites and the processing of the recombination intermediates of type-1 NF1 deletions.

P-ClinG-053

Out of frame deletion in the dystrophin gene leads to an in frame transcript in a boy with Becker muscular dystrophy

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We report on a boy with high levels of creatine phosphokinase (CPK) between 5.000 and 15.000 U/l that were determined due to reported pain in the knees at the age of 5 years. At this time only fine motoric deficits were reported by the parents; statomotoric development was normal. The patient was further investigated in our neuromuscular outpatient clinic. At the age of 10 years he was still able to run long distances; climbing stairs was performed without any problems. Gowers sign was still negative; the boy reported only calf pain under efforts and fatigue. Clinical examination showed only decent lumbar hyperlordosis and minimally reduced proximal muscle strength. For further clarification DNA analysis with MLPA (multiplex ligation-dependent probe amplification) was performed and revealed a hemizygous deletion of exons 12 to 29 of the dystrophin gene. The adjacent exons 11 and 30 are present which was also confirmed by PCR (ex12ex29del / c.(1332-?)_(4071+?del)). The deletion is predicted to disturb the reading frame which is associated with Duchenne muscular dystrophy (DMD) according to the reading frame shift hypothesis. This result does not fit to the clinical presentation of the patient. A similar deletion is described in the LOVD database (http://www.dmd.nl/) for one patient with typical picture of DMD but no further clinical follow-up nor molecular genetic characterization. Additional molecular genetic analysis of our patient on cDNA level revealed a

transcript missing exons 9 to 29 (r.832_4071del) meaning exons 10 to 12 existing on DNA level are spliced out in the mRNA. This leads to an in frame transcript which matches the milder clinical course of our patient.

Our finding is in line with the fact that only approximately 92% of cases are consistent with the reading frame hypothesis. In conclusion, we suggest performing RNA analysis in patients harboring a deletion on DNA level not suitable to their clinical presentation.

P-ClinG-054

A novel de novo mutation in the gene CEACAM16 in a German family with hearing impairment

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At least 50% of hearing defects are presumed as having a genetic basis, which can be assigned nonsyndromic, as well as autosomal recessive (DFNB) in the majority (75%) of cases, autosomal dominant (DFNA) in approximately 20% of cases, in addition to rare prevalence of X-linked (1-4%) and mitochondrial (< 1%) inheritance. To date, approximately 30 genes are implicated in dominantly inherited hearing loss (HL). However, the present assignment of two DFNA genes is based on the identification of a single mutation in one family (e.g. DIABLO and CEACAM16) along with supporting experimental data in animal models inferring significance of these genes in normal hearing. Therefore, detection of a second novel mutation for these genes is vital to confirm that the mutation is indeed associated with HL and to help delineate the mutational spectrum of the disease causing gene.

In this study, we investigate a proband with moderate, bilateral HL with adolescent onset and without a family history of HL. Next generation sequencing using the TruSight One panel (Illumina) encompassing approximately 100 HL genes was performed. For improved analysis, the parents and the proband were sequenced together using the MiSeq. During analysis, pathogenic mutation was not detected in all the inherited variants, but interestingly a heterozygous c.1094T>G (p.Leu365Arg) de novo mutation in CEACAM16 (carcinoembryonic antigen-related cell adhesion molecule 16, MIM 614591) (DFNA4B, MIM 614614) was discovered in the proband. Verification of the de novo mutation and paternity confirmation was performed using Sanger sequencing and PowerPlex analysis, respectively. This novel mutation is classified as disease causing by pathogenicity prediction programs (SIFT, MutationTaster). Additionally, this mutation is positioned in a highly conserved amino acid and a moderately conserved nucleotide considering 19 species (phyloP). In the first and only family with a heterozygous CEACAM16 mutation, mutation in the MYH14 (MIM 608568) gene in the adjacent DFNA4A locus (MIM 600652) was excluded; however, similar to the proband we present, HL started in adolescence and progressed with age to approximately 50 dB showing a flat audiogram profile. Based on similar phenotypic expression, it is suggested that the de novo mutation described in CEACAM16 may be the cause of HL in our proband, especially as MYH14 and other HL genes lacked pathogenic mutation. In summary, this study highlights screening of trios with hearing impairment using the TruSight One panel as a powerful strategy for finding mutations in HL genes and describes the second mutation in CEACAM16 in a family with HL, therefore serving as the validation of CEACAM16 for DFNA4B.

P-ClinG-055

A Novel Oculo-skeletal Syndrome with Intellectual Disability caused by a Particular MAB21L2 Mutation

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Recently mutations in the single-exon gene MAB21L2 were identified by exome sequencing in individuals from five unrelated families affected with bilateral eye malformations. Two unrelated individuals of this cohort carry the identical heterozygous de novo missense mutation (c.151C>T; p.Arg51Cys) in MAB21L2 and exhibit a recognizable phenotype defined by bilateral anophthalmia, intellectual disability, and a distinctive humero-femoral dysplasia. Specific radiographic anomalies comprise severe rhizomelic shortness of the limbs and abnormal joint formation. Their novel oculo-skeletal phenotype is sufficiently characteristic to differentiate it from other conditions with rhizomelic shortness of the limbs. The other phenotypes associated with different heterozygous MAB21L2 mutations include autosomal dominant bilateral colobomatous microphthalmia, and

bilateral colobomata with non-ophthalmologic manifestations. Our studies delineate a novel oculo-skeletal syndrome caused by a particular MAB21L2 mutation and show that the characteristics of these two patients are part of the phenotypic spectrum of MAB21L2 gene mutations which cause a range of structural eye malformations such as microphthalmia / anophthalmia and ocular coloboma.

P-ClinG-056

Expanding the Clinical and Genetic Spectrum of Epidermolytic Ichthyosis, Congenital Reticular Ichthyosiform Erythroderma and Superficial Epidermolytic Ichthyosis

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Intermediate filaments (IF) are a primary component of the cytoskeleton and essential for normal tissue structure and function. Type I and type II keratins are the largest subgroups of the intermediate filament family, and mutations in keratin genes are associated with many rare skin disorders.

Epidermolytic ichthyosis (EI, MIM#113800) is characterized by diffuse erythroderma and blistering at birth and progressive hyperkeratosis later in life. EI is usually inherited in an autosomal dominant manner and caused by heterozygous mutations in the keratin genes KRT1 (MIM*139350) or KRT10 (MIM*148080). Heterozygous mutations in KRT10 can also cause congenital reticular ichthyosiform erythroderma (CRIE, MIM#609165), also known as ichthyosis with confetti (IWC), which is characterized by small islands of normal skin surrounded by erythroderma. These confetti-like spots appear in childhood and increase in size and number with time. Heterozygous mutations in KRT2 (MIM*600194) can cause superficial epidermolytic ichthyosis (SEI, MIM#146800), also known as ichthyosis bullosa of Siemens. Patients with SEI show generalized erythroderma and blistering at birth, but the symptoms usually improve with age. The phenotype is generally milder than in patients with EI.

Here we describe 23 families diagnosed with EI, CRIE and SEI in which we found heterozygous mutations in the genes KRT1, KRT10 and KRT2. One mutation in KRT1 in a family with EI, one mutation in KRT2 in a family with SEI and five mutations in KRT10 in families with CRIE have not been reported yet. Our finding expands the phenotypical and genetic spectrum of EI, CRIE and SEI and may contribute to a better understanding of these diseases.

P-ClinG-057

Pathogenic significance of deletions distal to Wolf-Hirschhorn syndrome critical region on 4p16.3

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Wolf-Hirschhorn syndrome (WHS) is a contiguous gene deletion syndrome, since haploinsufficiency of several neighboring genes was inferred to cause the core clinical findings including the characteristic facial appearance, growth delay, intellectual disability and seizures.

Patients with chromosome 4p deletions are quite rare but important to define Wolf-Hirschhorn syndrome critical regions (WHSCR) by determining the smallest region of overlap in patients with and without the Wolf-Hirschhorn Syndrome (WHS) phenotype. The WHSCR has been mapped to chromosome 4p16.3 between 1.4 and 1.9 Mb from the 4p terminus. WHSC1 is considered the candidate gene for growth and developmental delay as well as facial features while LETM1 is the favoured gene for the manifestation of seizures. WHSC2 has been proposed for more global aspects. Many affected individuals (55%) have a deletion with no other cytogenetic abnormalities whereas about 40-45% have an unbalanced translocation.

We report on a 10-year-old girl with a small deletion of chromosome 4p16.3 and intellectual disability (ID) (global IQ 62), aggressive behavior and attention deficit. In the second year of life a myoclonic-astatic epilepsy was diagnosed. Chromosomal microarray analysis (CMA) revealed a 1.4 Mb deletion of chromosome 4p16.3 sparing all three candidate genes, LETM1, WHSC1 and WHSC2. The patient has in addition a small deletion (172 kb) of chromosome 6q26 containing two exons of the PARK2- gene and a small duplication (211 kb) of chromosome 3q29. This duplication lies within a larger recurrent microaberration syndrome but affects neither of the proposed ID candidate genes PAK2 and DLG1. Maternal inheritance could be excluded for all three aberrations but the girl's father in whom seizures and learning difficulties are known was not available for testing.

Our patient displays characteristic features of WHS including intellectual disability and seizures and thus may represent a mild form of WHS. In the past, LETM 1 was considered the major gene for seizures. Nevertheless, both LETM1 deletion patients without seizures as well as patient with seizures and 4p16.3 deletions preserving LETM1 have been described. These findings suggest that additional genes for seizures reside distal to WHSCR. CTBP1, PIGG and CPLX1 have already been proposed as candidate genes and are deleted in our patient as well. Moreover, ID is recurrently described in patients with deletions distal to WHSCR proposing one or more genes in this region to be causal ID genes. This supports the advisement of expanding the critical region from 0.4 Mb to 1.9 Mb from the 4p terminus.

P-ClinG-058

Combination of IL36RN and AP1S3 mutations in generalized pustular psoriasis and further evidence for genetic overlap of pustular psoriatic manifestations

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Recent research in psoriasis has identified pustular psoriatic manifestations as either Mendelian traits or as major genetic risk factors in contrast to the numerous associated SNPs in classical plaque psoriasis vulgaris. Autosomal recessive mutations in IL36RN have been primarily identified in generalized pustular psoriasis (GPP), a severe pustular variant of psoriasis. The same mutations have also been associated to both, a severe adverse drug reaction following antibiotic treatment - acute generalized exanthematous pustulosis (AGEP) - and to a localized pustular manifestation - palmoplantar pustular psoriasis (PPP). Heterozygous missense variants in AP1S3 have been implicated in a very specific psoriatic disease, acrodermatitis continua of Hallopeau (ACH), accompanied by pustular disease (GPP or PPP). Therefore, genetic overlap between the different psoriatic manifestations can be expected for both genes.

We screened our cohorts of 45 GPP patients, 2 AGEP patients, and 3 ACH patients for coding variants in IL36RN and AP1S3 and for genomic aberrations in IL36RN with MLPA; an analysis of IL36RN was also performed in 251 PPP patients. We identified homozygous or compound heterozygous mutations in IL36RN in 13 of 45 GPP patients (29%). The most common mutations were p.Ser113Leu and p.Pro76Leu, present on 46%/ 27% of mutated alleles, respectively. We also identified p.Ser113X as a new mutation. Genotype phenotype correlation revealed that carriers of two mutations in IL36RN were significantly younger at first manifestation of GPP than non-carriers. The frequency of concomitant psoriatic manifestations (psoriasis vulgaris and psoriatic arthritis) was lower (23%) in carriers than in the whole group (31%).

Analysis of IL36RN in ACH revealed one heterozygous carrier (33%), while mutations could be excluded in AGEP. Of note, we could not confirm the previous association of PPP to IL36RN, although our cohort was larger and of sufficient power to detect even smaller effect sizes than in the previous study. Our results suggest that IL36RN's involvement has been over-estimated in initial studies of GPP and PPP, and that carrying two mutations is associated to younger manifestational age, more severe and more specific manifestations.

Analysis of AP1S3 revealed no carrier of a missense variant in ACH, indicating genetic heterogeneity, while one AGEP and one GPP patient carried previously described variants in heterozygous state, respectively. Another GPP patient was homozygous for a rare missense variant previously undescribed. Molecular modelling suggested a lost hydrogen bond to the protein's binding partners within the complex. In GPP, risk variants were observed exclusively in patients carrying two IL36RN mutations.

Our studies point to combination of IL36RN and AP1S3 mutations in GPP and to an involvement of AP1S3 in AGEP. Overall, our studies indicate even larger genetic overlap between the pustular manifestations than previously thought.

P-ClinG-059

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

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McCune-Albright Syndrome (MAS) is a sporadic disorder characterized by the classic triad of polyostotic fibrous dysplasia, café-au-lait skin pigmentation, and peripheral precocious puberty. 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 three patients with clinical signs of McCune-Albright syndrome (MAS). No mutation could be identified by Sanger sequencing of GNAS in DNA extracted from either whole blood or bone tissue. We subsequently reanalyzed the samples by deep next-generation sequencing (NGS). With a coverage of >100 000 we identified causative mosaic missense mutations in GNAS in DNA isolated from peripheral blood, saliva and bone tissue. 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. Deep sequencing allowed the detection of the disease causing mutation in a primarily not affected tissue (blood and saliva). Thus, the need of invasive tissue biopsies for molecular testing in MAS is dispensable in the cases reported.

P-ClinG-060

Finding mutations in GJB2 non-coding region reveals its importance in Hearing Loss genetic testing in Iranian population

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Objective: Hereditary hearing loss is the most common neurosensory disorder in humans. Half of the cases have genetic etiology with extraordinary genetic heterogeneity. Mutations in one gene, GJB2, are the most common cause for autosomal recessive non-syndromic hearing loss (ARNSHL) in many different populations. GJB2 encodes a gap junction channel protein (connexin 26), and is located on DFNB1 locus on chromosome 13q12.11 which also involve another connexin gene, GJB6. Mutation screening of GJB2 revealed that a high number of patients with deaf phenotype have heterozygous genotype and carry only one mutant allele. As the first comprehensive study in Iran, we have targeted GJB2-related Iranian heterozygotes, looking for second mutant allele which leads to hearing impairment. They bear first mutation in their coding exon of GJB2.

Method: Using PCR-based direct sequencing, we assessed 103 patients with ARNSHL for variants in non-coding exon and promoter region of this gene, for the first time in Iran.

Result: We have identified the second mutant allele in splice site of exon-1 of GJB2 which is known as IVS1+1G>A in 17 probands. We found no mutation in promoter region of GJB2.

Conclusion: Our findings reveal that IVS1+1G>A mutation in non-coding exon of GJB2 is the most common mutation after 35delG within multi ethnical Iranian heterozygote samples. It emphasizes to approach exon1 of GJB2 in case of ARNSHL genetic diagnosis.

P-ClinG-061

Clinical and molecular characterisation of a patient with a deletion in 16q22 involving CTCF

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In the last years the number of genes which encode for proteins involved in chromatin structure, epigenetic modification, and regulation of gene expression and which are also causal for intellectual disability has risen. Among these genes are NSD1, EHMT1, CREBBP, and MECP2. Recently, Gregor et al. (2013) added the CTCF gene to this list These authors described three patients with mutations in CTCF and a fourth patient with a deletion in 16q22.1 involving CTCF.

Herein we present another patient with a de novo 0,6 Mb deletion in 16q22.1 diagnosed by array-CGH using the 105K Agilent array. She is the first of two children. Development in her younger brother is normal. In

the proband global developmental delay was noted early in life. Despite physiotherapy she learned to walk at 23 months and spoke first words at the age of 18 months. Height and weight are in the normal range, head circumference is on the 3rd centile. At the age of 6 5/12 years she was diagnosed with developmental delay especially affecting speech, gross and fine motor skills, and visuo-spatial skills. Mild intellectual disability was suspected but has not been formally tested. A cranial MRI scan was normal. Except for a left-sided epicanthal fold no obvious dysmorphism is present. Strabism is treated with glasses and occlusion. Diagnostic work-up not only included array-CGH but also methylation analysis.

The findings in the patient presented herein especially overlap with those of the patient described by Gregor et al. who was diagnosed with a 0,3 Mb deletion in 16q22.1 involving CTCF. From the report in the literature and our experience haploinsufficiency for CTCF is associated with intellectual disability but no obvious other symptoms. However, the number of known patients is small. We also suggest that patients are carefully followed up because mice which are haploinsufficient for CTCF are at an increased tumour risk (Kemp et al., 2014).

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P-ClinG-062

Next Generation Sequencing in patients with hypertrophic cardiomyopathy identifies carrier of double and compound heterozygous mutations

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Hypertrophic cardiomyopathy (HCM) is characterized by unexplained left ventricular hypertrophy. The disorder occurs with a frequency of 1:500 and follows an autosomal dominant mode of inheritance. HCM shows large genetic heterogeneity and a highly variable penetrance, ranging from asymptomatic patients to sudden cardiac death. Currently, mutations in more than 50 genes are reported to be associated with HCM. Most mutations are found in the genes MYBPC3 and MYH7 (40-60% of all cases). However, in about 5% of the patients more than one pathogenic mutation in a single gene (compound heterozygous or homozygous) or in different genes (digenic) are found, which most likely leads to a more severe form of the disease. Therefore, the detection of all pathogenic mutations in the index patient is essential for excluding the disease in other family members and it would be reasonable to sequence many genes simultaneously, as done with Next Generation Sequencing.

We analyzed 75 HCM patients from Germany and Spain using a customized HaloPlex panel from Agilent with 24 genes associated with HCM. Sequencing was performed with the Illumina MiSeq.

In 23 patients no pathogenic or potentially pathogenic mutation was found, 45 patients had one mutation and in 7 patients more than one mutation was detected. In the 52 patients with detected mutations we identified 41 different sequence variations in 11 genes. The majority of the patients (79%) had mutations in the genes MYBPC3 and MYH7. All 7 patients with more than one mutation had at least one pathogenic mutation in one of these two genes. The other affected genes in these patients were ACTN2, LDB3, PRKAG2, TNNT2 and VCL. Four of the patients carried 2 mutations in two different genes, one patient was compound heterozygous and 2 patients were compound heterozygous and in addition were carrier of a third mutation in a second gene.

In conclusion, we detected in 7 out of 75 HCM patients more than one mutation in a single or in different genes. This ratio (9%) is slightly higher than it is in the literature (~5%) but shows the importance of the simultaneous sequencing of many genes associated with HCM.

P-ClinG-063

Brain-thyroid-lung syndrome in a child caused by a deletion on chromosome 14 proximal of the NKX2.1 gene locus

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Brain-lung-thyroid syndrome (BLTS) is a rare disorder characterized by congenital hypothyroidism (CH), infant respiratory distress syndrome (IRDS) and benign hereditary chorea (BHC). BLTS is caused by mutations in the NKX2.1 gene.

NKX2.1, also known as, TITF-1, TTF-1 or T/ebp, is a member of the homeodomain-containing NK-2 gene family. As transcription factor, NKX2.1 is involved in the development of thyroid, lung and central nervous system formation. Considering this expression pattern, a NKX2.1 deficiency can lead to benign hereditary chorea (BHC), congenital hypothyroidism (CH) and respiratory distress syndrome (IRDS). The presence of this phenotype is known as brain-lung-thyroid syndrome. Homozygous NKX2.1 knock out mice, die shortly after birth due to respiratory failure and in addition they have no thyroid tissue. Heterozygous NKX2.1+/- mice appear to be normal. However, the coordination skills of NKX2.1+/- mice were poorer than comparable wild-type mice and additionally, these mice possessed higher TSH values. So far, in humans only heterozygous mutations in the NKX2.1 gene have been described. In previously documented and published cases of patient with brain-thyroid-lung syndrome a mutation or deletion of NKX2.1 is always present and therefore NKX2.1 is associated with brain, thyroid and lung diseases. Here we present one patient with the clinical features of brain-thyroid-lung syndrome but who does not carry any mutation in the NKX2.1 gene locus. Using array comparative genomic hybridization, we identified a heterozygous 1 Mb deletion on chromosome 14, which is approximately 195,000 bp proximal to NKX2.1. The deleted region encompasses 3 genes: MBIP, BRMS1L and RALGAPA1. Therefore these genes may have an impact on the normal function of NKX2.1. Currently, we analyse whether NKX2.1 directly interacts with one of these 3 proteins or whether one of the three proteins directly binds to the NKX2.1 gene promoter. Recently, a heterozygous deletion 200 kb proximal to the NKX2.1 gene has been identified in a patient with a choreiform movement disorder. The smallest common deleted region of our patients and the described patient encompasses just the MBIP gene (MUK-binding inhibitory protein). MBIP is expressed in the thyroid anlage, lung and forebrain. The protein contains a leucine zipper motif and has been described as an inhibitor of MAPK upstream kinase (MUK). MBIP can bind to MUK-dual leucine zipper-bearing kinase (DLK) and leucine-zipper protein kinase (ZPK) and inhibit the kinase activity to induce the JNK / SAPK signaling pathway. Our current aim is to clarify whether MBIP is involved directly or indirectly in the NKX2.1 gene expression or regulation. This finding will help to identify completely new mechanisms of NKX2.1 regulation and a deeper understanding of the thyroid development and the possible cause of thyroid disorders.

P-ClinG-064

Eight novel mutations within the extended patatin domain of PNPLA1 causing autosomal recessive congenital ichthyosis

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Autosomal recessive congenital ichthyosis (ARCI; OMIM #615023) is characterized by a generalized scaling of the skin resulting from differentiation and cornification defects of keratinocytes.

Eight genes are known to cause ARCI: TGM1, NIPAL4/ICHTHYIN, ALOX12B , CYP4F22, ALOXE3, ABCA12, PNPLA1 and CERS3. PNPLA1, patatin-like phospholipase domain containing 1, is characterized by the presence of a highly conserved patatin domain which displays lipolytic and acyltransferase activities. About its exact function not much is known to date. The causative association of mutations in PNPLA1 with ichthyosis in human and dog has been shown by our group in 2012.

Here we present eight novel mutations in PNPLA1 found in six ARCI patients.

The eight novel mutations presented in this work confirm the causal relationship of PNPLA1 mutations and ichthyosis as previously reported by our group. Their strict localization within the extended patatin domain (position 1-288) of the PNPLA1 protein emphasizes the importance of the extended patatin domain for PNPLA1 function in the skin. Mutations in positions later than 288 very likely might represent polymorphisms.

Notably all six patients and all three previously reported cases of PNPLA1 mutations to date presented as collodion babies at birth.

P-ClinG-065

Camurati-Engelmann disease: differential diagnosis for neuromuscular disorders in childhood

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The Camurati-Engelmann disease (CED) or progressive diaphyseal dysplasia is an autosomal dominant bone disorder. Hallmarks of this disorder are bilateral symmetric thickenings of the diaphyses of the long bones. The hyperostosis and sclerosis affect initially the cortical diaphysis of the femora and tibiae, subsequently the short tubular bones and the posterior part of the vertebral bodies and arches. Sometimes pelvis and skull bone plates are involved. Most common symptoms are muscle weakness, bone pain, easy fatigability, a wide based waddling gait, and joint contractures.

As a result of bone thickenings a variety of symptoms is described: atrophy of visual or auditory nerves, acral blood circulation disorders, diseases of the hematopoietic system (anemia, leukopenia) or a delayed puberty. Further symptoms are asthenic habitus, scoliosis, and premature tooth decay. The onset of the disease is usually in childhood. The severity of the symptoms is very variable, even within a family.

The CED is caused by heterozygous mutations in the TGFB1 gene (transforming growth factor-beta 1) located on chromosome 19q13. The multifunctional peptide controls e. g. bone formation and degradation, cell proliferation and cell differentiation in different cell types and influences apoptosis. More than 90% of patients with CED have identifiable mutations in the TGFB1 gene.

Here we report a 32-year-old man who showed normal development up to age 2 and then suffered from increased fatigability and pain in the legs. Increasing muscle weakness in the legs gives rise to a wide based waddling gait, a restriction to climb stairs and to get up from the squat (positive Gower sign).

Electromyography (EMG) investigation in childhood revealed evidence of a neuromuscular disorder. However, electroneurography (ENG) and muscle biopsy showed non-specific changes. Hereditary motor and sensory neuropathy type I (Charcot–Marie–Tooth disease), myotonic dystrophy type I (DM1) and spinal muscular atrophy (SMA) could not be verified by molecular analysis. In the context of the disease of the mother, who suffers from fibromyalgia, by re-rating of the son`s anamnesis and X-ray of the right hip and leg, a CED was suggested, because he showed non-progression of the disease and improvement of the symptoms.

This suspicion was confirmed by analysis of the TGFB1 gene: it revealed the heterozygous mutation p.Arg218His in this patient. This mutation is highly conserved, up to Xenopus tropicalis, and one of the most common alterations in exon 4 of the TGFB1 gene (p.Arg218Cys, p.Arg218His and p.Cys225Arg) located in the region of the TGF-ß1 latency-associated peptide (LAP). These mutations lead to structural changes of the peptide and thus interrupt the dimerization of LAP and the binding to active TGF-ß1, which leads to an increased presence of active TGF-ß1 in the cell.

P-ClinG-066

A diagnostic approach that facilitates the identification of deep intronic and disease-causing variants in patients with GPI deficiencies

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The glycosylphosphatidylinositol (GPI)-anchor is a glycolipid that links more than 300 different proteins (GPI-AP) via the C-terminus to the cell membrane in all tissues. Defects in synthesis process of the GPI-anchor represent a new subclass of glycosylation disorders with a phenotypically diverse disease entity including intellectual disability and epilepsies as its cardinal features. Distinct facial features, organ malformations and abnormalities in routine laboratory parameters such as the alkaline phosphatase activity, allow a further delineation of syndromic forms, such as Mabry syndrome.

Currently, about 30 genes are known to participate in the synthesis of the GPI-anchor. In several of these genes coding pathogenic mutations have been identified that cause GPI-anchor deficiencies. However, the interpretation and workup of non-coding and inconclusive sequence variants in patients affected by GPI-anchor deficiencies requires the necessity of complementary techniques.

We present a stepwise diagnostic procedure that enables an efficient workup for patients with a suspected GPI-anchor deficiency. First, we perform NGS based sequencing of a gene panel comprising all known genes

of the GPI-anchor synthesis pathway to screen for potentially pathogenic mutations. To assess the GPI-deficiency on a functional level, we perform flow cytometry analysis on blood or fibroblasts. Gene expression profiling via qPCR or MLPA and NGS compliments the workup. With these orthogonal techniques we were able to identify missense mutations and a deep intronic founder mutation in PGAP3 in three unrelated patients that lead in compound heterozygosity to a GPI-deficiency.

P-ClinG-067

Gain of function mutation in STIM1 (p.R304W) causes Stormorken Syndrome

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Stormorken Syndrome was first reported as an autosomal dominant disorder in a Norwegian family in 1985. It is characterized by congenital miosis, thrombocytopenia and thrombocytopathy, asplenia, tubular aggregate myopathy, migraine, ichthyosis and dyslexia/mental retardation. In our patient the diagnosis was made at the age of 17 years when she gave birth to an also affected girl (Morin et al., Hum Mutat. 2014 Oct;35(10):1221-32). Exome sequencing in mother and daughter was performed to identify the underlying genetic defect in the patients. An intensive analysis mainly focusing on truncating changes of the index patient did initially not lead to a promising candidate gene. As an activating mutation (p.D84G) in the Ca sensing protein stromal interaction protein 1 (STIM1) was known to cause premature platelet activation, thrombocytopenia and bleeding in mice and after constitutive activation of STIM1 was described to cause tubular-aggregate myopathy in 2013, among the 428 heterozygous missense variants the change c.910C>T (p.R304W) in STIM1 was classified as pathogenetically relevant. The same missense mutation was identified in a 32-year old French patient and his son, both affected by Stormorken syndrome (Morin et al., Hum Mutat. 2014 Oct;35(10):1221-32). The mutation was absent in unaffected family members, was not found in 100 unrelated control persons and was not annotated in human variation databases such as dbSNP. The function of the mutation c.910C>T (p.R304W) in STIM1 was explored by calcium imaging experiments using STIMR304W transfected HEK 293T cells and fibroblast from one patient and obtained results are in agreement with impairment of calcium homeostasis. The p.R304W mutation causes a gain of function effect associated with increase of both resting Ca levels and store operated calcium entry. A link between the mutation c.910C>T (p.R304W) in STIM1 was simultaneously described by two other research groups and until now 13 patients with Stormorken Syndrome and the p.R304W mutation in STIM1 have been described.

P-ClinG-068

Two patients with proximal deletion 20q syndrome

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Few descriptions of constitutional proximal deletions of chromosome 20 involving 20q11.2-20q12 have been published. Recurrent clinical features are intellectual disability, severe feeding problems with failure to thrive, short stature, microcephaly, dysmorphic facies, and birth defects such as cleft palate, heart defect, and polydactyly.

We describe two unrelated female patients with proximal deletion 20q syndrome.

Patient 1 is the second child born to healthy parents of German origin with an unremarkable family history. She was born at full term with normal measurements. The patient was hospitalized for the first six weeks of life due to neonatal pneumonia, central apnea, and hypoglycemia. Bilateral hip contracture, knee flexion contracture, ulnar deviation of fingers, and adducted thumb were attributed to oligohydramnios. Uncoordinated oral movements led to feeding problems with failure to thrive and dystrophy. At 24 months of age her growth curves crossed the percentile curve for height(81cm; P4) and dropped below the 3rd percentile for weight (8kg) and OFC (46cm). Delayed milestones were consistent with global developmental delay. At ten years of age she shows an understanding of spoken language, but speaks only a few words. She attends a school for children with intellectual disability. She has behavioral abnormalities (refusal, provocative behavior).

Patient 2 is the third child born to healthy parents of German origin with an unremarkable family history. She was born at GA 37 weeks with weight 2840g (P38), length 48cm (P26) and OFC 33cm (P26). A median cleft palate was diagnosed after birth. Feeding problems due to initial poor suck, regurgitation, and uncoordinated oral movements led to failure to thrive. At 2½ years of age, all measurements were below the 3rd percentile. Delayed milestones were with consistent with global developmental delay. She has behavioral problems (violent temper, anxiety, jealousy).

Array CGH analyses revealed proximal interstitial deletion of 20q in both patients. Patient 1 has a 5.4 Mb deletion between bands 20q11.22 and 20q12. The array CGH formula (ISCN 2013/hg19) is arr 20q11.22q12(32.469.776-37.882.268). The deleted region contains 104 HGNC genes, including 46 OMIM annotated genes. Patient 2 has a 7.6 Mb deletion spanning the bands 20q11.23 to 20q13.2; the array CGH formula is arr 20q11.23q13.12(34.754.101-42.401.901). This region contains 69 HGNC genes, including 30 OMIM annotated genes. The two deletions overlap in a 3.1 MB region in band 20q11.23, which contains 36 HGNC genes, 21 of which are annotated in OMIM.

The results for Patient 1 were confirmed by metaphase FISH using BACs RP11-774C15 and RP11-652M11. The Abbott FISH probe for 20q12 (D20S108) was used for Patient 2. The deletions were de novo in both patients.

The presence of the recurrent clinical findings intellectual disability, failure to thrive, and birth defects in our two patients and patients with deletions in similar regions are suggestive of a proximal deletion 20q syndrome.

P-ClinG-069

Partial de novo deletion of AP1S2 in a boy with global developmental delay, secondary microcephaly, and muscular hypotonia

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Background: Ten to 12 % of male intellectual disability follows an X-linked pattern of inheritance, with more than 80 different forms of X-linked mental retardation (XLMR) currently known.

The gene AP1S2 maps to Xp22.2 and encodes the sigma 2 subunit of clathrin-associated adaptor protein complex 1 (AP1). Because AP1 is involved in the transport of proteins between the trans-Golgi compartment and endosomes, as well as in the interaction with synaptic vesicle proteins, it plays an important role in neurotransmitter release.

Seven families with mutations in AP1S2 causing XLMR type 59 (OMIM 300629) have been reported. In addition, one child with a similar phenotype and a maternally inherited deletion of the entire AP1S2 gene has been published.

Case Report: The patient is the only child of non-consanguineous German parents with an unremarkable family history. Ventriculomegaly was diagnosed on prenatal ultrasound at GA 17 weeks. Amniocentesis revealed a normal male karyotype. The boy was delivered at GA 39 weeks with normal measurements (weight 2925g [P10], length 51cm [P35], OFC 33cm [P4]). He presented to medical genetics at age 23 months with global developmental delay, secondary microcephaly, muscular hypotonia, a 4 cm x 1 cm nevus on chest right, two café-au-lait spots on the abdomen and right lower limb, and dysmorphic facies (narrow face, thick vermillion border, uplifted earlobes). His measurements were weight 9 kg (<P3), length 86 cm (P17), and OFC 47 cm (<P3). He could crawl, sit unsupported, and walk with assistance. Speech was delayed, with only the single word “Papa” in active use.

Methods: We performed conventional peripheral blood chromosome analysis using G-banding, array CGH on peripheral blood DNA (Cytochip Oligo 4x180K v1.0/Fa. Illumina/Bluegnome), and quantitative PCR (qPCR).

Results: Peripheral blood chromosome analysis revealed a normal male karyotype 46,XY. Array CGH revealed a complex hemizygotic 116 kb deletion of Xp22.2, including exons 1 and 2 of AP1S2 located at the distal part of the deleted region. However, two consecutive oligos spanning approximately 20 kb of non-coding sequences within the deleted region proximal to the AP1S2 deletion were not indicative of a deletion. This non-deleted segment was assumed to be an artefact and was therefore initially interpreted as deleted. However, quantitative PCR (qPCR) confirmed the partial deletion of exons 1 and 2 of AP1S2 gene as well as the non-deleted, „bridge-like“, small segment within the 116 kb deleted region. Simultaneous qPCR of maternal DNA revealed a normal result for this region. Consequently, the deletion seen in our patient can be interpreted as de novo.

This case is, to the best of our knowledge, the first description of a de novo partial deletion pf the AP1S2 gene.

P-ClinG-070

How to find the second patient of its kind

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In the recent years disease causing genes could be identified for many monogenic disorders in high throughput sequencing data sets such as exomes and whole genomes. Usually such approaches were successful, if a case group with several phenotypically similar patients was available. The analysis of such cohorts was relatively simple from a statistical point of view and is often referred to as intersection filtering.

However, for all the cases that did not yield conclusive results more sophisticated approaches are now required. We designed an online expert system that assists in matching patients on the phenotype as well as on the genotype level. For such case groups the burden of rare variants is tested for statistically significant associations per gene. In addition users provide their knowledge about the expected pathogenicity of mutations, based on their human experitse. By this means the status of variants of unknown clinical significance can be effectively resolved by a community driven effort.

Currently more than 1000 expert users are registered on the platform and participate in solving Mendelian disorders.

P-ClinG-071

The 2q23.1 microdeletion syndrome – an unexpected case

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The 2q23.1 microdeletion syndrome is characterized by severe intellectual disability, pronounced speech impairment, short stature, microcephaly and epilepsy. Moreover, the majority of the patients display hypotonia, stereotypic repetitive behavior, sleeping disturbances and ataxia. The clinical features show similarities to Angelman, Rett and Smith-Magenis syndrome. In 2q23.1 microdeletion syndrome the distinct phenotype is associated with haploinsufficiency of the methyl-CpG binding domain protein 5 (MBD5) gene.

Here we report a female patient with a 200 kb microdeletion on chromosome 2q23.1 detected by array CGH analysis. The microdeletion was confirmed by quantitative PCR and involves the non-coding region of the MBD5 gene. The girl now aged 20 months presents with some clinical features of the 2q23.1 microdeletion syndrome including infantile hypotonia, microcephaly, craniofacial dysmorphism, feeding difficulties and bruxism. However, her length is at the 3rd centile, and her motor and speech development is comparably normal, as she walked freely at 18 months, spoke first words at 16 months of age and shows good verbal comprehension.

This case points out the variability of the clinical phenotype in 2q23.1 microdeletion syndrome and its challenge for genetic counseling.

P-ClinG-072

Targeted next-generation sequencing identifies a novel SYNGAP1 mutation in a patient with intellectual disability and epilepsy

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Syndromic and non-syndromic intellectual disability with epilepsy is clinically and genetically a very hetergeneous disorder. In this context all forms of mendelian inheritance with phenotypic variability has been reported and causative mutations in over 200 genes have been identified. The SYNGAP1 gene encodes the Synaptic Ras GTPase-activating protein 1 that is involved in correct spine synapse maturation. Mutations in this gene are responsible for a non-syndromic form of intellectual disability and cases associated with both autism spectrum disorder and epilepsy have also been found.

We report on a 9-year-old female patient with a moderate intellectual disability and impaired linguistic abilities. She showed no remarkable dysmorphic features. Antiepileptic treatment started at the age of 5 years because of absences.

Molecular testing of Fragile X syndrome was normal. Array CGH showed a heterozygous deletion (125kb) inherited from the mother effecting only a noncoding area on chromosome 7q35. Because of an unlikely pathogenic significance of this finding, we performed targeted next-generation sequencing (NGS) with a panel including 63 genes which have already been described as a cause of syndromic and non-syndromic intellectual disability mostly including epilepsy. We identified a heterozygous novel mutation c.509+1G>T in the SYNGAP1

gene effecting a highly conserved donor splice site and leading most probably to an aberrant splicing. Various splice site mutations in SYNGAP1 associated with developmental disorders have been reported before. Segregation analysis revealed that this mutation occurs de novo like most of the described SYNGAP1 mutations.

Gene-panel diagnostics with next-generation sequencing is an appropriate method to detect causative mutations in case of a disease with genetic heterogeneity or an unrewarding clinical phenotype.

P-ClinG-073

Familial DICER1 tumor syndrome; A new familial case presenting various typical and so far not reported childhood tumors

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DICER1 syndrome is a rare genetic tumor disposition caused by heterozygous germline mutations in the DICER1 gene. DICER1 is a member of the RNase III family and is involved in the generation of microRNAs. First in 2009 somatic and germline mutations in DICER1 were identified in pleuropulmonary blastoma (PPB). Starting out from familial PPB within recent years from a yet limited number of cases several other DICER1 associated tumors were added giving rise to the still evolving spectrum of DICER1 tumor syndrome. The major manifestations of DICER1 syndrome besides PPB are cystic nephroma, ovarian sex chord stromal tumors and thyroid nodes. Here we present a new familial case of DICER1 syndrome affecting three family members with various tumor manifestations including typical manifestations like PPB, cystic nephroma and nodular thyroid changes, unusual manifestations like embryonal germ cell tumor in the brain and ovarian teratoma, and as well childhood basalioma and pilomatrixoma, so far not reported in DICER1 syndrome. Hereby we contribute to the expanding clinical phenotype of the DICER1 tumor syndrome.

P-ClinG-074

A new familial case of microdeletion syndrome 10p15.3. Clinical findings in two affected siblings

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In 2012 a small terminal microdeletion in the short arm of chromosome 10 in the region 10p15.3 was first reported as microdeletion syndrome. By now 22 cases – only one familial- have been reported. Characteristic findings comprised variable cognitive impairment or developmental delay, a disorder of speech development, as well as uncharacterized dysmorphic signs. We here report on a new case of microdeletion syndrome 10p15.3 in an eight year old girl, a foster child, with intellectual deficits, disorder of speech development, behavioural problems, congenital heart defect, and several dysmorphic signs. The same microdeletion was subsequently identified in the six year old maternal half sister, showing very similar developmental and cognitive problems, thereby in particular a major disorder of speech development. The mother has not reached graduation from school and is reported as dissocial person with severe alcohol abuse. Thus inheritance of the microdeletion from a probably symptomatic mother can be assumed. The presented cases add up to the as yet small number of reported cases of microdeletion 10p15.3 and thereby might help to establish the full clinical spectrum of this rather newly-discovered syndrome.

P-ClinG-075

High phenotypic variability in six new patients with a FOXG1 mutation

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The FOXG1 syndrome is described with a specific core phenotype including severe developmental delay, absent speech, stereotypies and dyskinesias, epilepsy, poor sleep pattern, severe postnatal microcephaly and characteristic brain anomalies including simplified gyral pattern, white matter abnormalities and corpus callosum hypogenesis. Intragenic mutations and deletions of the FOXG1 gene have been detected in more

than 35 patients up to date. The spectrum of sequence variants includes nonsense, frameshift and missense mutations. Here we present the clinical and molecular findings of six new patients at the age of 4 to 16 years. Next generation sequencing revealed the heterozygous FOXG1 mutations p.S185C, p.E154Gfs*301, p.A381Pfs*4, p.P259R, p.L325Ffs*130, and p.N187K. While all patients had normal birth measurements, at time of last examination head circumference varies between normal (3/6) and -4 SD microcephalic (2/6), length between normal (3/6) and -3 SD short stature (2/6). The patient with the frameshift mutation p.E154Gfs*301 and the patient with the missense mutation p.N187K showed the most severe psychomotor delay; both were not able to sit or walk unassisted, and did not speak any words at the age of 4 and 5 years, respectively. Among the other five patients four were able to sit unassisted and three patients even learned to walk. Hand or head stereotypies were noted in 3/6 patients, as well as dyskinesias in 3/6 patients. In 2/6 patients motor regression was observed. Interestingly, 3/6 were able to speak up to 20 words in contrast to most other patients with a FOXG1 related disorder reported in the literature. 4/6 patients developed epileptic seizures between 4 months and 6 years with low response to antiepileptic treatment in two of them. Poor sleep pattern was reported in 3/6 patients, a serious problem for families. MRI scan of the brain showed characteristic dysgenesis of corpus callosum and white matter signal abnormalities in the patients carrying the p.E154Gfs*301 and the p.N187K mutation. Patients carrying the p.S185C and the p.L325Ffs*130 mutation had normal MRI. Comparison of the clinical data of our patients with the previously published cases revealed a higher phenotypic variability than expected. Further genotype-phenotype correlations revealed the most severe developmental delay associated with the recurrent reported frameshift mutation p.E154Gfs*301 and the new missense mutation p.N187K. There was no correlation between head circumference and developmental or epilepsy outcome. These data may have further implications regarding genetic counselling of families on results of genomic sequencing and phenotypic outcome.

P-ClinG-076

FVII deficiency in children

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Factor VII (FVII) deficiency (OMIM 227500) is an autosomal recessive bleeding disorder. There is a clear demarcation in FVII levels between homozygotes / compound heterozygotes and heterozygotes and an overlap between heterozygous individuals and normal depending on FVII reducing haplotypes which can influence the individual FVII level. Clinical phenotypes of FVII deficiency are highly variable. Homozygous and compound heterozygous individuals may develop severe bleeding symptoms such as gastrointestinal bleeding or intracranial hemorrhage. Heterozygous carriers of pathogenic mutations are usually asymptomatic and their detection often occurs incidentally for instance prior to a planned surgery.

We here present two pediatric cases of severe FVII deficiency. In addition, we report on a heterozygous carrier of a pathogenic deletion encompassing both F7 and F10 genes.

Case 1 is a 6-month-old baby boy, first child of non-consanguineous Chinese parents, who was diagnosed to have severe FVII deficiency with FVII level <1,7% shortly after birth. At that time, he presented with intracranial bleedings and excessive haematoma. Direct sequencing of the F7 gene revealed two compound heterozygous point mutations, c.[64+1G>A];[379T>C], both not found in FVII databases so far. Each parent was heterozygous for one of the mutations.

Case 2 is a 6-month-old baby boy who presented with bloody stool during infectious disease. Clotting tests showed a clearly reduced FVII activity (<4%). The infant was the first child of a consanguineous Syrian couple. Both parents had borderline low FVII levels. Direct sequencing and MLPA analysis revealed a homozygous deletion of exon 2 of the F7 gene in the boy. Both parents are heterozygous carriers of this deletion. Long-range PCR analysis could fine-map the breakpoints identifying a 6-bp-insertion in-between (c.64+430_131-6delinsTCGTAA). The 4,35 kb deletion changes the acceptor splice site of intron 3 likely resulting in inefficient splicing of exon 3.

Case 3 is an 8-year-old German girl with combined FVII and FX deficiency diagnosed as part of presurgical work-up in preparation for a planned tonsillectomy. Her FVII and FX levels were 35% and 51%, respectively. The tonsillectomy was postponed and molecular diagnostics were initiated. Sequencing analysis of the F7 gene only detected FVII reducing polymorphisms in homozygous state. No point mutation could be found in her F10 gene. MLPA analysis revealed a complete heterozygous deletion of both F7 and F10 genes combined with FVII reducing polymorphisms on the second allele likely resulting in further reduction of the FVII level in the girl.

P-ClinG-077

A TRPV4 mutation identified by exome sequencing causing a clinical phenotype with myopathy and skeletal dysplasia

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Mutations in TRPV4 have been described in neuropathies (HMSN2C) as well as myopathies (distal spinal muscular atrophy) and skeletal dysplasias. Whereas the phenotypes have formerly been discussed as separate entities (although caused by mutations in the identical gene) recent case reports have suggested an overlap of the skeletal, muscular and neurological features in patients with TRPV4 mutations.

We report on a family (mother and son) with a complex clinical phenotype consisting of contractures (bilateral pes cavus, knee and hip contractures, scoliosis) and muscle weakness. Due to the contractures, distal arthrogryposis (DA) was suspected. Analysis of the frequently mutated genes in DA was negative (TPM2, MYH3, TNNI2, TNNT3). For further evaluation of this obviously autosomal dominant phenotype, clinical exome analysis was chosen (TruSight Exome, Illumina MiSeq). A heterozygous pathogenic mutation in TRPV4 (c.806G>A; p.Arg269His) was identified in the mother and confirmed in both mother and son by Sanger sequencing.

As previous case reports suggested, mutations in TRPV4 do not only cause specific, clinically well delineated phenotypes but can cause a highly variable complex syndromal phenotypes with neuropathy, myopathy, skeletal dysplasias and associated complications, which can complicate establishing a diagnosis. Especially in cases like this, exome sequencing is an important diagnostic tool.

P-ClinG-078

BRWD3 gene mutations cause a distinct macrocephaly phenotype.

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Macrocephaly is defined as a head circumference above the 97th percentile. Often it is associated with developmental delay or intellectual disability of varying degree, behavioural disturbances, autism and seizures. Due to the enormous genetic heterogeneity of syndromic macrocephaly standard techniques so far reveal disease-causing mutations only in a minority of patients. Thus, there is clearly a need of novel approaches to elucidate the underlying molecular defects in a greater portion of macrocephaly patients.

Here, we report on a self-developped NGS macrocephaly gene panel comprising 46 genes (MySeq platform, Illumina). We identified a novel BRWD3 nonsense mutation in a boy with macrocephaly and intellectual disability. The BRWD3 (Bromodomain and WD repeat-containing protein 3) gene is located on chromosome Xq13 and constitutes a member of the JAK/STAT pathway. In addition, three further boys with macrocephaly and developmental delay and BRWD3 mutation are reported. Their BRWD3 mutation was detected via the X-linked ID gene screening project in Berlin. Comparing the four affected boys with BRWD3 mutation, we hypothesize that BRWD3 mutations cause a recognizable macrocephaly phenotype with variable degree of intellectual disability.

P-ClinG-079

Deletions in 14q24.1q24.3 are associated with congenital heart disease, brachydactyly and mild intellectual disability

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Interstitial deletions of chromosome bands 14q24.1q24.3 are rare. We report on three unrelated patients with overlapping de novo deletions of sizes 5.4 Mb, 2.8 Mb and 2.3 Mb in this region. While some clinical problems such as intestinal malrotation, cryptorchidism and ectopic kidney were only ob¬served in single patients, all three patients had mild intellectual disability, congenital heart disease, brachydactyly,

hypertelorism, broad nasal bridge, and thin upper lips. It appears likely that haploinsufficiency of one or seve¬ral of the 19 genes in the common deleted interval (ACTN1, DCAF5, EXD2, GALNTL1, ERH, SLC39A9, PLEKHD1, CCDC177, KIAA0247, LOC100289511, SRSF5, SLC10A1, SMOC1, SLC8A3, ADAM21P1, COX16, SYNJ2BP, SYNJ2BP-COX16, ADAM21) was responsible for these problems, but apart from SMOC1, mutations in which cause autosomal recessive Waardenburg an¬ophthalmia syndrome, no disease associations have so far been reported for the other genes. Functional studies and a systematic search for mutations or chromosome aberrations in this region will elucidate the role of individual genes in the clinical manifestations and will provide insight into the under¬lying biological mechanisms.

P-ClinG-080

Evaluation of the PKHD1 mutation database for autosomal recessive polycystic kidney disease (ARPKD) entries

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The main clinical features of ARPKD are bilaterally enlarged polycystic kidneys and a congenital hepatic fibrosis. The disease occurs in 1 of 20,000 live births and often manifests prenatally. Mutations of the PKHD1 gene are the only known cause so far. The longest ORF contains 66 exons and the detailed protein function is still unknown. The PKHD1 mutation database (http://www.humgen.rwth-aachen.de) has been initiated in 2003 to collect changes of the PKHD1 gene in a locus specific database. About 750 PKHD1 changes (pathogenic mutations, variants of unknown significance and polymorphisms) extracted from the literature and detected in our own patient cohort have been included so far. Latest updates encompass the inclusion of about 150 variants. To facilitate the accuracy of predictions regarding the pathogenicity of variants, frequency data from the literature and allele frequencies from dbSNP are provided. Additionally segregation data, the evolutionary conservation and a bioinformatic validation are allocated. The inclusion of mutations documented in the international ARegPKD patient registry (www.aregpkd.org) is arranged.

To further elucidate the characteristics of PKHD1 mutations we analyzed the current database entries regarding the occurring types of mutations. We found that 58% of the pathogenic mutations are missense changes, 20% are frameshifting, 13% are nonsense, 8% are intronic and 1% are other mutations (e.g. in-frame deletions). It is known that mutations are mainly distributed throughout the whole coding region and adjacent intronic bases. However, to further investigate the detailed distribution of PKHD1 mutations we conducted additional analyses. We plotted the pathogenic exonic database entries against the predicted functional protein domains. Interestingly we found that only 41% (n=269) of the mutations lie within these protein domains. The obtained results are presented. Most pathogenic PKHD1 mutations are private variants. We determined the mutation frequencies in the literature and our own patient cohort and calculated that 53% of the known pathogenic mutations are probably private changes. However, there exist as well several recurrent mutations. In detail c.107C>T (p.Thr36Met) accounts for about 15% (12,5% in the own cohort) of pathogenic changes, c.1486C>T (p.Arg496X) for 5 %, c.9689delA (p.Asp3230ValfsX34) and c.5895dupA (p.Leu1966ThrfsX4) each for about 4%. In summary 19 more frequent recurrent mutations were recognized and are presented. Furthermore we used missense database entries (n=334) to evaluate the sensitivity and specificity of pathogenicity predictions with web-based tools as PolyPhen2 and MutationTaster referring to PKHD1. In case of known pathogenic missense mutations a bioinformatics validation predicted the mutations to be pathogenic in 58-76%. Detailed results are presented. We are convinced that these data will improve the molecular genetic diagnostics of ARPKD.

P-ClinG-081

Compound Heterozygosity for Variants in the GDF5 Proregion is associated with Brachydactyly Type C

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Brachydactyly Type C (BDC) is a skeletal disorder, which is caused by mutations in GDF5 and typically follows an autosomal dominant mode of inheritance. Here we describe an affected girl with characteristic clinical features of BDC including proximally-set thumbs, hyperphalangy of the index finger, shortening of index and middle fingers, and clinodactyly of the 5th finger as well as broad big toes and brachydactyly of the toes. Molecular genetics analyses revealed compound heterozygosity for two GDF5 variants that were both predicted in silico to be disease-causing. The first variant, variant 1, is listed in dbSNP without frequency data and has been observed once in the NHLBI Exome Sequencing Project in a heterozygous state. This variant was also present in the clinically inconspicuous mother. The second variant, variant 2, has to our knowledge never been reported so far. To further delineate which of these GDF5 variants is associated with reduced biological activity we performed in vitro analyses. Variant 2 induced SMAD dependent signaling and chondrogenic differentiation to a degree comparable with GDF5WT. In contrast, variant 1 had considerably attenuated capacity to activate SMAD signaling and chondrogenesis. Western Blot analyses revealed a reduced GDF5 amount after expression of variant 1, whereas protein amounts upon expression of GDF5WT and variant 2 were comparable. We suggest that variant 1 is a mutation that is most likely to underlie the patient's phenotype and should not be regarded as a SNP, even though it showed clinical non-penetrance in the mother. However, modulation of BDC penetrance by variant 2 cannot be excluded.

P-ClinG-082

A microdeletion in 15q13.3 unmasks a hemizygous recessive mutation in TRPM1 in two siblings with developmental delay, reduced vision and symptoms of night blindness

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In patients with developmental delay or intellectual disability, array comparative genomic hybridization (aCGH) is now a standard diagnostic procedure to elucidate a microdeletion or microduplication as the underlying genetic cause. Microdeletions in 15q13.3 are associated with a variable phenotype consisting of intellectual disability, cardiac malformations, seizures, autism and schizophrenia. Penetrance is not 100% as the deletion is also present in persons that have no obvious clinical findings.

We report on two siblings, a four year-old boy and three year-old girl with developmental delay and a low visual acuity in the range of 0,1 – 0,25 with symptoms of night blindness. Through aCGH we identified the typical 15q13.3 deletion of 1.8-Mb in size in both siblings and their mother. The mother had no history of developmental delay and vision was normal. As reduced vision and symptoms of night blindness are not typically associated with the 15q13.3 microdeletion, we suspected and confirmed a hemizygous mutation in the TRPM1-Gene, which lies within the deleted region. Homozygous or compound heterozygous mutations in TRPM1 are known to cause congenital stationary night blindness-1C. The father of the siblings was unavailable for testing in TRPM1. This family further illustrates that chromosomal microdeletions can potentially unmask hemizygous recessive mutations on the other allele.

P-ClinG-083

Ten years apart: the second family with non-syndromic autosomal recessive intellectual disability due to a CRBN gene mutation

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In 2004 CRBN (cereblon) was only the second gene ever to be implicated in non-syndromic autosomal recessive intellectual disability (ARID). CRBN is part of the Cullin 4-Ring ubiquitin ligase complex CUL4 and functions as a substrate receptor which recruits proteins for ubiquitination. Extensive functional analyses of the nonsense mutation identified in single kindred with mild ARID showed altered CRBN degradation as the likely pathomechanism. Nevertheless, despite the broad application of whole-exome sequencing in ARID families no second mutation was reported to date, which would have confirmed its relevance beyond the initial family reported. Now, 10 years later we report this second CRBN mutation.

We performed homozygosity mapping and exome sequencing in a family with two affected siblings of a consanguineous family with mild non-syndromic intellectual disability. Both affected individuals show a similar phenotype to the previously described cases with an estimated IQ of 50 to 70 and without dysmorphic or autistic features. We identified a homozygous splice-donor mutation in intron 8 of CRBN (c.835+1G>A) co-segregating with the phenotype in the family. In silico analysis using multiple splice site programs predicted disruption of the splice donor. Through cDNA analysis we could exclude the existence of full length CRBN transcript and characterize three aberrant transcripts in the affected individuals. One transcript leads to a frame-shift and a premature stop-codon before the last exon and thus is most likely subject to degradation through NMD. Two of these transcripts lead to an in-frame deletion and to an abruption of a C-terminal ubiquitination site. Thus this mutation likely interferes with the normal regulation of CRBN and leads to an altered formation or activity of the complex. The identification of this new mutation confirms the implication of the CRBN gene in autosomal recessive non-syndromic intellectual disability, possibly due to CRBN deficiency caused by altered autoubiquitination.

The rareness of CRBN mutations underlines the heterogeneity in ARID and emphasizes that, although a second mutation is still needed to confirm pathogenicity, a gene implicated in ARID may not be discarded only because of pending replication studies. Pathway enrichment methods along with large scale collaborations may offer an opportunity to accelerate this confirmation process.

P-ClinG-084

A boy with partial trisomy 12p due to a maternally inherited unbalanced translocation t(12;22)

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Patients with partial or complete trisomy 12p are rare.

We present an 8 months-old male patient with a partial trisomy 12p due to a maternally inherited unbalanced translocation t(12;22)(p12.1;p11).

The boy is the first child of healthy, non-consanguineous parents. He was born at 37 weeks after an uneventful pregnancy with normal birth length, weight and OFC. From the early beginning he showed complex developmental delay, muscular hypotonia and secondary microcephaly (OFC after 6 month -2 SD). The facial features include a round face with prominent forehead, full cheeks, short palpebral fissures, low-set ears, a long philtrum with thin upper lip and slightly everted lower lip. Apart from a small penis and cryptorchidism no other major malformations or functional abnormalities of the inner organs are known as yet.

G-banded chromosomal studies (resolution 550 bands) and FISH analysis with a chromosome 12p-specific subtelomer probe (Abbott) revealed the trisomy of 12p, which was further characterized by array-CGH analysis using a 180k-chip (Agilent Technologies). The affected chromosomal region 12pter-12p12.1 has a size of about 25,2 Mb and contains 260 OMIM genes:

arr[hg19] 12p13.33p12.1(194,249-25,346,677)x3.

The patients mother carries a balanced reciprocal translocation t(12;22). His father has a normal karyotype.

Finally, we review the small number of reported cases with pure partial trisomy 12p in comparison to the clinical and molecular data of our patient. On studies by Izumi et al. (2012) a minimal critical genomic region within 12p13.31 containing 26 genes was defined as causative for the Pallister-Killian syndrome with tetrasomy 12p as well as for the very similar, but less severe (partial) trisomy 12p phenotype. To understand the pathogenesis and phenotypic differences between Pallister-Killian syndrome and trisomy 12p, further patients and molecular studies are needed.

P-ClinG-085

Warburg micro syndrome in two siblings with cataract detected by prenatal ultrasound investigation

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The case report presents the family with autosomal recessive Warburg micro syndrome. Bilateral cataracts was detected by routine pregnancy ultrasound screening in the second trimester in two pregnancies (the first one was terminated) of unrelated parents.

Next-generation sequencing revealed in both siblings two not described mutations c.538G>T; p.(Glu180) and c.943C>T; p.(Arg315) in RAB3GAP1 gene, which were assessed as pathogenic.

Mutations in RAB3GAP1 gene are associated with Warburg micro syndrome (cataracta cong., microphthalmia, microcephaly, hypotonia, mental retardation, hypogenitalism, stigmatization). All these symptoms were present in the male infant from the second pregnancy.

Both parents are confirmed as carriers with the recurrence risk 25% for their offspring . Preimplantation and prenatal diagnosis was applied. The fetus of next pregnancy was healthy carrier of the maternal mutation.

P-ClinG-086

Mutations in the steroid-thyroid hormone and retinoid receptor NR4A2 are associated with unspecific mild intellectual disability with language impairment

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The most frequent causes of sporadic intellectual disability are chromosomal abnormalities, de novo point mutations and small insertions/deletions. In our ongoing study of the genetics of intellectual disability, chromosomal microarray analysis (CMA) in a patient with mild intellectual disability and pronounced delay in language development revealed an 89kb-deletion on chromosome 2q24.1 (157,120,975-157,210,126), affecting only a single gene, NR4A2. Analysis of both parents confirmed that the deletion occurred de novo.

NR4A2 belongs to the steroid-thyroid hormone and retinoid receptor superfamily. It is a transcription factor with strong expression in brain, which is essential for the generation and maintenance of dopaminergic neurons. Mice expressing reduced levels of NR4A2 displayed hyperactivity in a novel environment and were deficient in the retention of emotional memory, and have therefore been suggested as an animal model for schizophrenia. Three patients with genetic defects in NR4A2 have been described in the literature, so far: 1) A de novo missense mutation in NR4A2 in a patient with autism spectrum disorder, mild intellectual disability (IQ 60), and a delay in language development, 2) a de novo deletion affecting NR4A2 and a second gene (GPD2) in a patient with mild to moderate intellectual disability and speech delay, and 3) a deletion of NR4A2 and 6 further genes in a patient with severe ID, expressive vocabulary of a few simple words and short phrases, epilepsy and adult-onset motor regression. Consistent clinical features in all four patients were mild to severe intellectual disability and a marked delay in language development. Apart from that there were no common abnormalities or malformations. These genetic findings together with the biological function of the encoded protein suggest that mutations in NR4A2 are associated with an unspecific form of intellectual disability with language impairment. Identification of further patients will allow refining the clinical spectrum of this syndrome. Mutation screening in patients with language impairment and mild intellectual impairments seems warranted. Our findings add NR4A2 to the ever increasing list of genes associated with intellectual disability.

P-ClinG-087

A novel splice site mutation in TRPS1 gene in a family with trichorhinophalangeal syndrome type 1

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The trichorhinophalangeal syndrome type 1 (TRPS I) is a rare, autosomal dominant genetic disease caused by haploinsufficiency of the TRPS1 gene. TRPS I is characterized by craniofacial and skeletal

malformations. Patients have sparse, thin scalp hair, pear–shaped nose, long flat philtrum and a thin upper vermillion border. Skeletal abnormalities include cone-shaped epiphyses, short stature and hip dysplasia.

We report a 12 year old girl and her father, both with the clinical diagnosis of a trichorhinophalangeal syndrome type 1. The copy number analysis of the chromosomal region 8q24 via MLPA, to exclude a deletion or duplication, yielded no variations. The sequencing analysis of the TRPS1 gene revealed the novel mutation c.2096+1G>A in the consensus splice site in intron 4. Our index patient as well as its father carry this mutation. The two clinical unaffected brothers of the index patient show no sequence variation. Actually, only one splice site mutation is described in the specialist literature. This mutation provokes an in-frame skipping of exon 6 and causes a typical trichorhinophalangeal syndrome type 1.

Bioinformatics calculation indicates, that our new splice site mutation c.2096+1G>A causes the loss of the splice donor site in intron 4. Regarding this evaluation and the segregation analysis the mutation is expected to be disease causing. To estimate the consequence of the possible alternative splicing (e.q. exon skipping or elongation of open reading frame), we will investigate the aberrant mRNA variants.

P-ClinG-088

Noonan-like versus 12q24 duplication syndrome: a novel case of 14-year-old male patient with a 12q23.3q24.3 duplication encompassing PTPN11

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We report on the identification of a partial duplication of 12q23q24 in a 14-year-old male patient with short stature, dysmorphic features with hypertelorism, upslanting palpebral fissures, bilateral iris coloboma, and dysplastic ears. Prenatally, bilateral hydronephrosis was diagnosed and led to an elective labour induction at 36 gestational weeks. Over time, the patient developed compensated chronic renal failure due to renal dysplasia and urinary tract malformation. He displayed cryptorchism with dysplastic testes and developed a prominent kyphoscoliosis with chest deformity and a moderate mental retardation with partially (auto)aggressive behaviour. By 12 years of age, symmetric swellings of the proximal interphalangeal joints of hands and feet with episodes of tenderness, redness, and hyperthermia were reported. While initial cytogenetic investigations displayed a normal male karyotype, screening by aCGH displayed a partial duplication of 12q23q24 of approximately 13 Mb: arr 12q23.3q24.3(107,588,085-120,903,074)x3. Further examination by FISH confirmed the result (ish dup12(RP11-17I17++,RP11-357K6++) and illustrated a tandem duplication. There was no evidence of parental origin of the duplication.

The patient reported herein is affected by a de-novo partial tandem duplication of 12q23q24 encompassing PTPN11 but also approximately 100 further genes whose expression may be affected by their gene dosage, e.g. UBE3B and TBX5.

Noonan syndrome (NS) is caused by activating mutations in protein tyrosine phosphatase, non-receptor type 11 (PTPN11) located in 12q24. It is characterized by distinct facial features, postnatally reduced growth, heart defects, developmental delay, bleeding diathesis, and susceptibility to certain cancers, particularly juvenile myelomonocytic leukaemia. PTPN11 encodes a central molecule of the RAS-MAPK pathway. Besides PTPN11, causative mutations of other genes encoding proteins involved in this pathway were also reported. In addition, there were reports on patients with suspected NS carrying a duplication in 12q encompassing PTPN11 leading to a Noonan-like syndrome (NLS) caused by gene dosage effects of duplicated PTPN11.

Even though there are overlapping features, NS was not suspected in our patient. This is in contrast to formerly reported patients that carry partially overlapping duplications of similar genomic size and were reported as NLS. Regarding our patient, the dozens of genes affected by the duplication and the varying extent of the duplications up and downstream of PTPN11 reported so far, the term NLS might be a misleading oversimplification biased by the clinically suspected NS in the patients previously reported. In contrast, the descriptive term of 12q24 duplication syndrome might be a more precise term. In view of the known cancer susceptibility in classical NS, it will be of crucial interest for the reported patients whether individuals with a 12q24 duplication syndrome also face an increased cancer risk.

P-ClinG-089

Whole-exome sequencing in monozygotic discordant twins

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By definition monozygotic (MZ) twins carry an identical set of genetic information. Observation of early post-twinning mutational events has been found to cause phenotypic discordance among MZ twin pairs. These mutational events comprise genomic alterations at different scales, ranging from changes affecting only single nucleotides to larger copy-number variations of varying sizes. Here we performed whole-exome sequencing (WES) in nine discordant MZ twins to identify smaller mutational events in the affected twin. Five of the MZ twins were discordant for congenital heart defects (CHD), two for endocrine disorders, one for omphalocele, one for esophageal atresia (EA), and one for congenital diaphragmatic hernia (CDH). We hypothesized that an underlying heterozygous mutational event in the affected twin has a dominant effect. WES was performed using a 100bp paired-end read protocol as per the manufacture's recommendations on an Illumina HiSeq2000 sequencer. Data analysis was done by standard protocols using the VARBANK pipeline (CCG, Cologne) including filtering against in-house controls (not present in in-house controls). Only one change was found affecting the coding region of TMPRSS13 in the affected twin with CHD of twin pair 4 when using the de novo probability tool DeNovoGear.

Screening all nine MZ twins by using standard filter criteria without DeNovoGear lead to 4672 variants in which both twins differed. After filtering for variants only present in the affected twins and absent in in-house controls, 586 variants remained. All of these remaining 586 variants were then visually inspected for their read quality, decreasing the number of variants to 70 present only in the affected twin. These remaining 70 variants are currently investigated by Sanger sequencing for validation purpose.

P-ClinG-090

Novel compound heterozygous mutations in PLEC1 causing epidermolysis bullosa simplex with muscular dystrophy

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Epidermolysis bullosa simplex with muscular dystrophy (EBS-MD) is an autosomal recessive disorder caused by mutations in the plectin 1 gene (PLEC1). EBS-MD is characterized by early childhood onset of progressive muscular dystrophy and blistering skin changes (OMIM #226670).

Here, we report on a 28-year-old man who has been suffering from chronic skin disease with blistering and yellow nails since his birth. In addition, he developed a reduction of his muscular strength over at least the past five years. The patient showed generalized muscular atrophy, particularly affecting proximal muscles, and a moderate proximal but also distal tetraparesis. Finger extension was severely impaired on both sides while finger flexion showed normal strength. No deep tendon reflexes could be elicited. Neurological examination further revealed mild bilateral ptosis, moderate bilateral ophthalmoparesis into all directions and a moderate generalized facial weakness. A biopsy of the left deltoid muscle showed signs of muscular dystrophy and mitochondrial disease.

Analysis of the large PLEC1 gene was performed by partial exome enrichment using the TruSight One panel consisting of 4813 disease-associated genes followed by next generation sequencing (NGS) on a MiSeq desktop sequencer (Illumina). Data analysis was done by the software GensearchNGS (PhenoSystems), pathogenicity and splice predictions were carried out using the algorithms embedded in Alamut (Interactive Biosoftware). More than 98 % of the coding region of PLEC1 was covered at least 20x. We identified two heterozygous mutations in PLEC1. The first one was a small duplication of 4 bp (c.1106_1109dup) in exon 11 leading to a frame-shift and a preliminary stop codon at amino acid position 417 (p.Gln371Leufs*47). The second variant was a synonymous mutation (c.1482C>T, p.Gly494Gly) which was predicted to create a novel 5'-splice site 19 bp upstream of the original 5'-splice site by high scores of five different splice prediction tools. The new splice site would result in a loss of the last 19 bp of exon 14 in the PLEC1 transcript and also in a frame-shift leading to a preliminary stop codon at position 503 (p.Glu495Cysfs*9). The effect of the synonymous potential splice mutation was checked by mRNA analysis in the patient. Both parents were sequenced for the PLEC1 mutations detected in their son in order to confirm the autosomal recessive inheritance of the disease: the small duplication was inherited from the mother and the synonymous mutation

from the father. Both PLEC1 mutations have not been described before in any mutation or polymorphism database and could be classified as pathogenic because of their truncating effect.

In summary, we could identify the molecular cause of the EBS-MD disease in our patient by “clinical exome” sequencing. The NGS approach applied for this case seems to be an efficient method for analysis of large genes for which Sanger sequencing is not established in routine diagnostics.

P-ClinG-091

Trisomy 18. New case of a survival child: cytogenetical and phenotype data

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Trisomy 18 (T18) is characterized by multiply malformations, high frequency of heart defects (HD), severe retardation, high incidence of mortality (from published data 5-10% patients survival up age 1-year). Patients with mosaic forms displayed significant variability of phenotype’s expression, longer survival and need for medical management.

In Belarus 98% of pregnant women undergoing to conventional screening 1-2-st trimesters. T18 was detected in 159 fetuses during 2009-2014, 95% pregnancies were terminated. 8 children with T18 were born because the pregnant refused from invasive procedure or decided to continue the pregnancy with affected fetus. We presented the 1,5 years old boy with mosaic T18: phenotype, cytogenetics, genetic counseling, management. Case report. 41 years old pregnant, counseled due to age-related genetic risk, refused from invasive diagnostics. Results of combined screening (US and biochemical markers) were normal (risk<1:360). Pregnancy complicated by intrauterine growth retardation; labor was at 33 weeks by cesarean section. Newborn male underwent for genetic examinations due to prenatal hypoplasia (weight 915g, length 37cm, OFC 26cm), dysmorphisms, neurological disturbances, HD (perimembranouse ventricular septal defect, trikuspid valve’s insufficiency, foramen ovale, pulmonary vein dilatation, pulmonary hypertension). Renal, brain abnormalities were not found by US, MRI studies. The infant suffered from bronchopulmonary dysplasia, pulmonary hypertension, feeding difficulties, anemia. Severe growth, motor, mental retardation was detected by follow-up. Karyotype: 47,XY,+18[9]/46,XY[10]. Patient’s management: symptomatic treatment; cardiac surgery at 10 months old, regular cardiovascular evaluations; nutrition; growth, mental development follow-up.

Conclusion. Prenatal screening plays the main role for prevention outcome with chromosomal disorders. But, children with mosaic 18T may present a longer lifespan and need for complex medical care. So, information collection concerning phenotype of survival patients may be important for management strategies improving at each life stage.

P-ClinG-092

Characterization of a novel KCNJ2 mutation detected in Andersen-Tawil syndrome patients

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The Andersen-Tawil syndrome (ATS) represents a rare hereditary disorder characterized by a triad of symptoms including cardiac arrhythmias, periodic paralysis and physical dysmorphologies. However, most patients suffering from the syndrome do not manifest all three phenotypic features mentioned. The clinical phenotype and also the severity of the various symptoms can differ even in the same family.

ATS is inheritable in an autosomal dominant fashion, but it also occurs sporadically. About 60-70 % of the patients exhibiting clinical ATS symptoms show genetic abnormalities in KCNJ2. This gene encodes the inwardly rectifying potassium channel Kir2.1 and it is predominantly expressed in excitable tissues as heart, brain and skeletal muscle. Mutations in KCNJ2, mostly resulting in complete loss-of-function when expressed alone, constitute the majority of ATS cases.

A molecular genetic screening performed in a family that is affected by the Andersen-Tawil syndrome, revealed a novel mutation in KCNJ2 (c.434A>G, p.Y145C). All individuals carrying the mutation exhibit phenotypes associated with this disorder. It should be analyzed whether the detected variant may constitute the patients’ phenotypes.

Mutant as well as wild type Kir2.1 channels were expressed in HEK293 cells. In order to investigate the effect of variant p.Y145C on ion channel functionality, electrophysiological measurements were performed using the patch clamp technique. The results obtained indicate that the expression of mutant Kir2.1 channels results in impaired conductivity. Moreover, confocal laser scanning microscopy was utilized to examine the

cellular localization of the mutant in comparison to the wild type ion channel. No differences in cellular localization were detected. The clinical background and the results obtained from this study will be presented.

P-ClinG-093

Werner syndrome in a 51-year-old patient diagnosed at the Rare Diseases Centre Hannover

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A 51-year-old woman was referred to our rare disease centre by her doctor because of complicated diabetes mellitus and cushingoid habitus. First symptoms were detected at the age of 34 years, when, just after she had given birth to her third daughter, diabetes mellitus of unknown type was diagnosed. When we saw her she presented with multiple features of early aging, multiple metabolic problems and missing adipose tissue primarily at the extremities. Because of these symptoms we hypothesised some kind of familial lipodystrophy, however we failed to identify any mutations in the genes LMNA and ZMPSTE24.

Because of some facial features including prominent, beaked nose and micrognathia as well as ankle ulcerations we assumed that this could be a case of Werner syndrome, which was subsequently proven by compound heterozygosity for two formerly described mutations (c.1105C>T and c.1165delA) in the WRN gene.

Werner syndrome is an extremely rare disease with a prevalence of around 1/200,000 (highest prevalence in the Japanese population) (ORPHA902). Werner syndrome is characterized by post-pubertal onset (of premature ageing including greying hair, atherosclerosis, osteoporosis, muscle loss, diabetes mellitus and skin changes, including the very typical ankle ulcerations. Patients with Werner syndrome are at high risk for developing malignancies of sarcoma type and also skin cancers, meningioma, thyroid cancer and others. WRN encodes a RecQ helicase. Mutations therein lead to genome instability. Our patient had not developed any malignancy so far but is now included in several suitable early detection programs.

This case illustrates one of the most important shortcomings of our health care system in relation to patients with rare diseases: the enormous diagnostic delay. Additionally our case demonstrates the importance of both careful clinical assessment of patients in interdisciplinary consultation hours and the availability of genomic assessment tools at rare disease centres.

P-ClinG-094

A new gene-specific approach to predict mutations based on protein sequences and functional information.

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Nowadays next generation sequencing has become an indispensable method in clinical diagnostics for genetic disorders. The ability to sequence several candidate genes in a single run with gene panels or even with whole exome sequencing increase the chance to find the causative mutations in more heterogeneous diseases, like epilepsy or retinal dystrophy. However, the challenge remains to discover possible pathogenic mutations without an animal model or functional research approaches. So far, several prediction tools such as SIFT, PolyPhen or CADD are preferred to distinguish between pathogenic and benign mutations. These methods make use of a genome or exome wide approach but still remain on the sequence level. For clinical diagnostics this generality can be a drawback, because prediction methods do not work optimal for some genes or gene families related to a genetic disorder.

We take the advantage that functions, protein domains and known disease causing mutations are well described in literature and various databases for clinical relevant genes. Therefore we developed a gene specific variant prediction method that combines standard prediction tools with gene specific features for a machine learning approach. This method uses features such as locations of known disease mutations and polymorphisms, protein domains, protein structures, amino acid modifications, motifs, and functional sites.

This new approach should help to evaluate the pathogenicity of new single nucleotide variants in known disease causing genes. This comprehensive and gene specific evaluation of possible pathogenic mutations will improve diagnostics for genetic disorders in the field of clinical genetics.

P-ClinG-095

Genetic Disorders in Arab Societies of Israel and the Palestinian Authority

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Background: Rare inherited diseases often show diverse and complicated phenotypes and thus impose a relevant challenge not only from the medical point of view but also for the families and their social live. In Middle Eastern societies consanguinity is a deeply rooted cultural trait. Recent studies estimated the prevalence of consanguineous marriages among the Palestinian Arab and Israeli Arab population to 44,3 % and 25,9 %. This predisposes these societies for the occurrence of rare genetic diseases with autosomal recessive traits. The present study is an ongoing collaboration between physicians and scientists from Israel, the Palestinian Authority, and Germany aiming to investigate the molecular background of diverse rare genetic diseases combining up to date microarray and next generation sequencing technology.

Methods : Patients were carefully selected for inherited forms of neurological disorders including mental retardation or ophthalmological diseases in 79 consanguineous families with more than one affected. Informed written consent was signed by all participants. Genotyping on family level was performed using Agilent 250 K SNP arrays when applicable. Whole exon sequencing (WES) was done for one or sometimes more affected per family mainly using Agilent's Sure Select All Exon V5 enrichment system on an Illumina HiSeq2500 sequencer (2 X 100 bp). Data analysis was accomplished using an in house bioinformatics pipeline based on ANNOVAR .

Results : Massive parallel sequencing usually results in a large number of variants (> 25.000), filtering for rare variants (in our own NGS database and in 1000g, ESP6500) and for functional relevance (ns,ss,indel) reduced this count to < 1.200. Due to the consanguineous background of the families, this relatively large number could be further reduced by targeting those regions revealed by homozygosity mapping or focusing on homozygous variants in general. A statistical evaluation of the WES performance showed high coverage (~ 80 % cov 20X) and target enrichment (~ 80X mapping depth on target) parameters. Several disease causing mutations could be identified in genes like SLC25A15, APTX, ACO2, ADAT3, CRB1, DNAJC6, RLBP1 and several more. The molecular background could be solved in 23 (~ 29 %) families and for 16 additional families (~ 20 %) promising candidate genes are currently under investigation.

Conclusion: A next generation sequencing approach offers an affordable and fast possibility to screen large patient cohorts for rare disease causing variants. Focusing on patients with a consanguineous family background allows the discovery of rare mutations for neurodegenerative and related diseases in a relatively high proportion of the families.

P-ClinG-096

Are genetic variants of C-reactive protein prognostic markers for further cardiovascular events in patients with coronary heart disease?

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Background: Periodontitis and coronary heart disease are both triggered by inflammatory reaction which is influenced by the genetic predisposition. C-reactive protein (CRP) plays an important role in inflammation and its expression is, among others, genetically controlled. Two CRP SNPs (rs1800947, rs1417938) are shown to be associated with different gene expression. One aim of this longitudinal cohort study (ClinicalTrials.gov Identifier: NCT01045070) was the evaluation of the prognostic importance of these CRP variants for further cardiovascular events in in-patients with coronary heart disease (CHD).

Patients and methods: At baseline a total of 940 consecutive patients with angio¬graphically proven CHD of the Martin-Luther-University Halle-Wittenberg (Germany), Department of Medicine III, were prospectively included in the study. The one-year cardiovascular outcome of the patients was evaluated considering the

predefined, combined, primary endpoint (cardiovascular death, myocardial infarction, and stroke/TIA (transient ischemic attack)).

Results: All 940 CHD patients completed the one-year follow up. 7.2% of the patients achieved the primary endpoint (myocardial infarction: 2.0%, stroke/TIA: 1.7%, cardiovascular death: 3.5%). In Kaplan-Meier survival curves and the log-rank tests, the genotype-, allele- and haplotype constellation of the CRP-polymorphisms rs1800947 and rs1417938 could not be proven as significant predictors for adverse cardiovascular events regarding the one-year outcome. Diabetes mellitus (Hazard ratio 1.79) and low body mass index (Hazard ratio 0.94) were confirmed to be prognostic markers for the predefined cardiovascular endpoint in cox regression analyses.

Conclusions: Genetic variants of CRP gene, rs1800947, rs1417938, could not be regarded as prognostic markers for further cardiovascular events among in-patients with CHD.

P-ClinG-097

Axonal Charcot-Marie-Tooth neuropathy caused by mutation in the gene encoding histidyl-tRNA synthetase (HARS)

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Charcot-Marie-Tooth (CMT) neuropathies are genetically heterogeneous and can be caused by mutations in several different genes. In this study, we determined the causative mutation in an extended German pedigree affected by CMT. Seven individuals from three generations had clinical symptoms and signs of a predominantly motor neuropathy. Neurophysiological examinations revealed normal nerve conduction velocities and severely reduced amplitudes of motor action potentials. Mutations in twelve known CMT2 genes were excluded by Sanger sequencing. Genome-wide linkage studies revealed a single region of interest on chromosome 5q. Sanger sequencing of positional candidate genes identified a missense mutation c.395C>T, p.Thr132Ile in the HARS gene encoding histidyl-tRNA synthetase. The mutation segregated with the phenotype in the family, affected a highly conserved amino-acid residue and was absent from public databases covering harmless variants in the human genome. Functional tests in yeast showed that p.Thr132Ile renders the HARS protein functionless. A single family with a HARS mutation has been reported earlier suggesting that HARS mutations might be associated with CMT (Vester et al., 2013). Our study presenting a second, unrelated family with a different HARS mutation confirms the role of HARS in the pathogenesis of hereditary peripheral neuropathies.

P-ClinG-098

Polyneuropathy, Hearing loss, Ataxia, Retinitis pigmentosa and Cataract (PHARC): A rare differential diagnosis in deaf-blind individuals.

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We report on a 52-year old German woman with hearing loss and blindness clinically diagnosed as Usher syndrome type 2. The rationale for this clinical diagnosis was the combination of a postlingual sensorineural hearing loss and a profound visual impairment with pigmentary abnormalities of the retina. She is the only affected person in the family and although not knowingly consanguineous, the parents origin from the same small village in a remote rural area. A detailed review of the development of the visual impairment revealed a more complex eye involvement which appeared untypical for Usher syndrome. The patient suffered from a childhood cataract with removal of the lenses at the age of 5 years. During school age she experienced a deterioration of visual acuity with the increasing need for optical aids. An ophthalmic examination at the age of 32 years states a visual acuity of less than 0.05 on both eyes, a central scotoma, a secondary glaucoma and a cystic edema of the macula. Moreover, our patient suffered from peripheral sensory polyneuropathy which was attributed to a diabetes mellitus diagnosed at the age of 39 years. A mild ataxia was also present although not very pronounced and partially explainable by the orientation problems due to blindness. The combination of symptoms, especially the congenital cataract and the central scotoma, prompted us to think of a PHARC disease. Molecular analysis of the ABHD12-gene revealed a homozygous 2bp-deletion-3bp-insertion (c.337_338delGAinsTTT) in exon 3 resulting in a frame shift with a premature stop codon (p.Asp113Phefs*15). This mutation has previously been described in other patients with the disease. A verification of homozygosity was not possible since both parents already deceased. PHARC (MIM 604489) due to ABDH12-mutations has initially been identified as a phenocopy of Refsum disease in a Norwegian family (Fiskerstrand et al 2009). Our patient demonstrates that polyneuropathy and ataxia might be clinically

distinct which led to the initial diagnosis of Usher syndrome. Our study also demonstrates that the initial symptoms and the time course of the ocular disease are extremely important for the correct diagnosis, since secondary effects like pigment abnormalities of the retina in late stages are unspecific.

P-ClinG-099

Novel mutations of the fukutin gene in a patient with a childhood onset of a limb-girdle muscular dystrophy-dystroglycanopathy type C4

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Limb-girdle muscular dystrophy-dystroglycanopathy type C4 (MDDGC4; LGMD2M) is a rare autosomal recessive disorder caused by mutations in the fukutin (FKTN) gene. First symptoms start in infancy or early childhood and are progressive with a variable severity. Patients can show a hypotonia, delayed motor development and proximal muscle weakness. The MDDGC4 is a part of a heterogeneous group of muscular dystrophies with defects in glycosylation of alpha-dystroglycan. Mutations in FKTN were first described in patients with Fukuyama congenital muscular dystrophy occurring predominantly in Japan. Affected children present generalized hypotonia in infancy, are bedridden before reaching adolescence and show severe mental retardation with CNS involvement. Only a few cases outside Japan with mutations in FKTN are known to date.

Here, we report a case of a patient with a compound heterozygosity of two novel mutations c.895A>C; p.S299R in Exon 7 and c.1325A>G; p.N442S in Exon 10 of the FKTN gene. These mutations are predicted to be pathogenic based on the fact that they both disrupt a highly conserved amino acid residue. Parental studies revealed that one mutation was inherited from the patient´s father. The mother was already deceased bevor genetic counseling of the daughter took place. The patient´s first symptom, starting at the age of 7 years, was muscle pain especially in the thighs. A slowly progressive muscle weakness started in childhood affecting predominantly the proximal muscles of lower extremities whereas the calf muscles are hypertrophic. At the beginning of her 30s, she started to use a wheelchair, developed a cardiomyopathy and complained about a dysphagia as well as respiratory difficulties in lying position. Intelligence is normal. The patient's CK is elevated, which improved upon corticosteroid treatment in the first years after diagnosis.

To our knowledge, we present here the first German patient with two novel mutations in FKTN gene leading to MDDGC4. This provides a further insight in heterogeneity of phenotypes caused by mutations in FKTN gene.

P-ClinG-100

Maternal Uniparental Disomy 20 (upd(20)mat): a new imprinting disorder?

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Maternal uniparental disomy of chromosome 20 (upd(20)mat) is a rare molecular finding which has been reported in only 9 patients. Six of them had an isolated upd(20)mat in lymphocyte, whereas three also had mosaicism for complete or partial trisomy of chromosome 20. However, all these patients showed similar clinical features, including intra-uterine growth retardation, short stature, and intractable feeding difficulties with failure to thrive often requiring gastric-tube feeds. We report on a further patient with isolated upd(20)mat, referred because of intra-uterine and postnatal growth retardation and feeding difficulties in early childhood. At the age of two years the proband had mild muscular hypotonia with hyperlordosis, but no further dysmorphic signs, and psychomotor development was normal. Partial GH deficiency was diagnosed by stimulation tests at the age of nearly five years. Since that time he has continuously taken GH therapy, which has led to catch-up growth to a height between the 75th to 90th percentile. He has not developed any further medical problems and is in a regular classroom setting. These findings are in accordance with others from the literature. Therefore we suggest that upd(20)mat is a new imprinting disorder, and its testing should be included in the diagnostic workup of children with idiopathic growth retardation and without further considerable clinical (dysmorphic) features.

P-ClinG-101

New criteria facilitate the differential diagnosis of ichthyosis with confetti

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Ichthyosis with confetti (IWC) is a genodermatosis caused by dominant negative mutations in the keratin 10 gene (KRT10). Small heterozygous deletions, insertions, or duplications within KRT10 lead to a frameshift and consequently to an arginine-rich C-terminus. Patients with IWC are born as collodion babies with prominent hyperkeratosis or with a generalized extensive erythema. During childhood numerous confetti-like patches of healthy skin begin to form. These spots are the first indication for the diagnosis of IWC and may be recognizable during the second year of life yet. The adult cutaneous manifestation of IWC is a generalized scaly erythroderma interspersed with hundreds to thousands of confetti-like patches of healthy skin, where the pathologic mutation appears to be replaced by the wild type sequence through copy neutral LOH.

The aim of the presented study was to investigate clinical and genetic details of a substantial number of ichthyosis with confetti (IWC) patients in order to define major and minor criteria for diagnosis of this rare disease.

Systematic clinical investigation of patients with IWC revealed a novel spectrum of phenotypes. Several features qualified for major criteria for diagnosis. Most importantly, they include malformation of ears, hypoplasia of mammillae, and dorsal acral hypertrichosis. Direct sequencing revealed different frameshift mutations in intron 6 or exon 7 of KRT10 in each patient. Here we present the phenotypic and genetic spectrum of IWC in six patients. Major and minor criteria deduced from these observations will improve diagnosis of IWC. Ectodermal malformations, which are present in all patients, suggest novel classification of IWC as a syndrome.

P-ClinG-102

Microdeletion on chromosome 3p14.1 including partial deletion of FOXP1 gene: Case report with 5 year follow-up: 5 ½ year old boy with macrosomy (>97P), muscular hypotonia and mixed specific developmental disorder

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We report a now 5 ½ year old male patient with a microdeletion on chromosome 3p14.1 (171 kb) including a partial deletion of the FOXP1 gene and describe the disease progress over a 5-year period. Clinical features present in this patient are macrosomy (above 97 percentile) including macrocephaly (above 97 percentile), muscular hypotonia, strabismus convergens and megaureter with hydronephrosis. The patient has a mixed specific developmental disorder with decreased statomotor abilities. The patient has developed no speech.

The patient was born in the 40+5 WOP as a second child to non-consanguine parents of Russian descent. Pregnancy and birth were uneventful. In the first days after birth a slight muscular hypotonia and hypertelorism was noticed. By the age of 6 months the patient presented with muscular hypotonia, statomotoric retardation and strabismus convergens. At the age of 8 months several dysmorphic features were present. (Frontal forehead protrusion, almond-shaped eyes, down-slanting palpebral fissures, epicanthus inversus, short philtrum, orofacial hypotonia, retrognathic mandible, tapering of fingertips as well as plump fingers.)

The child was born with normal weight (P50-75), normal length (P75) and macrocephaly (P>97). At the age of one month the length and head circumference were above 97th percentile and have remained there for the last 5 years, body weight remained normal. Interestingly macrosomy has not been previously reported in patients with deletion in FOXP1 gene. Macrosomy may be a new feature in the clinical spectrum of small deletions in the 3p14.1 region.

P-ClinG-103

Phenotypic variability of Phosphoglucomutase 1 Deficiency in Siblings with Pierre Robin Sequence Identification of PGM1-mutation by whole-exome sequencing

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Congenital disorders of glycosylation are genetically heterogeneous syndromes and result in impaired glycoprotein production. During N- glycosylation, glycan precursors are assembled from monosaccharide units and then covalently attached to the nascent peptide chain.

Genetic defects in this process cause CDG type I. PGM1 deficiency is a genetically distinct subtype of CDG-1. Phosphoglucomutase 1 catalyzes the interconversion of glucose 6-phosphate and glucose 1-phosphate, which is important for nucleoside production and can be used for synthesis of glycogen and protein glycosylation.

Usually patients present with a highly variable multisystem phenotype including hepatopathy, exercise induced myopathy, dilative cardiomyopathy, malignant hyperthermia, rhabdomyolysis and risk of hypoglycemia. Abnormal glycosylation of coagulation factors may lead to bleeding events. Bifid uvula (with or without cleft palate) is a key feature and an important diagnostic sign.

We report on a pair of female siblings from consanguineous parents originating from Armenia. The girls were born at term with a severe presentation of Pierre Robin sequence (PRS) . The neonatal period was complicated with frequent obstructive apneas and feeding problems. The girls achieved normal milestones and a normal cognitive development. However growth was retarded and followed the 3rd centile. Slightly intermittent CK elevation was noticed, without muscle weakness. The soft palate was surgically repaired without complications, and orthodontic treatment followed to improve retrognathia. The second child had more severe obstructive apneas and major complications at surgery of the cleft palate at the age of one year. Excessive CK elevation and rhabdomyolysis were life-threatening. In order to stabilize the respiratory tract a tracheostomy was necessary. The next pregnancy ended with a stillbirth at 6 months of gestation due to a major intracranial bleeding. The fetus was affected with PRS. Two further healthy siblings were born.

Studies have shown that supplementation with galactose leads to improvement of glycosylation in cells of patients and complex carbohydrates stabilize blood glucose.

With the approach of whole-exome sequencing a homozygous frame shift mutation in the PGM1 gene was found in both affected siblings and the fetus: Chr1:64100586_CT>C. The mutation was heterozygous in the healthy parents, indicating recessive inheritance.

Conclusion:

Bifid uvula is a frequent malformation in over 80% of patients with PGM1 deficiency. PRS was reported previously only once with a confirmed PGM1-mutation, while one other case was described as having “first branchial arch syndrome”. Our family demonstrates the interfamilial variability in severity, ranging from early lethality and stillbirth following a prenatal bleeding event to mild exercise related myopathy.

P-ClinG-104

Juvenile Myoclonic Epilepsy in Velocardiofacial Syndrome

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The Velocardiofacial Syndrome (VCFS) due to a microdeletion 22q11.2 is associated with a broad spectrum of phenotypes, comprising variable degrees of heart defects, palatal anomalies, psychiatric disorders, distinct dysmorphism, intellectual disability and others. 21 % of VCFS patients are reported to have epileptic seizures that can be attributed to hypocalcaemia in 2/3 of cases. In the remainder, fever, recurrent infections and structural brain anomalies, such as polymicrogyria or lesions due to haemorrhage may predispose to VCFS-associated epilepsy, often with a focal origin in the electroencephalogram (EEG).

By contrast, we report on 3 VCFS patients suffering from generalized epilepsy (GE) that could not be attributed to hypocalcemia or focal EEG anomalies. Interestingly, all 3 individuals presented with the phenotype of juvenile myoclonic epilepsy (JME).

Little is known about the genetic background of JME but there is evidence that disturbed GABA receptor functioning contributes to this disorder.

A comprehensive review of the literature as well as screening of a cohort of 174 VCFS cases revealed an accumulation of GE (especially JME) among microdeletion 22q11.2 carriers.

We conclude that GE and JME in particular, is a recurrent feature of VCFS. We discuss possible underlying pathomechanisms triggered by the microdeletion and putatively resulting in disturbed interaction with GABA receptors.

P-ClinG-105

Alpha-Thalassemia/Mental Retardation Syndrome (ATRX) – A novel maternally inherited splicing mutation identified by Clinical Exome Sequencing

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Alpha-Thalassemia/-Mental Retardation Syndrome (ATRX): Wilkie et al. (1990) reported 5 unrelated patients, 2 of whom were previously reported by Weatherall et al. (1981), presenting with mental retardation and alpha-thalassemia without molecular abnormalities of the alpha-globin gene complex on chromosome 16p. The patients showed a strikingly uniform phenotype comprising severe mental handicap, characteristic dysmorphic facies, genital abnormalities, and an unusual, mild form of hemoglobin H disease. Gibbons et al. (1995) could show, that ATRX syndrome is caused by diverse mutations in the ATRX gene.

Clinical presentation of our index patient: The boy was born prematurely in the 37th week of gestation as the first child of healthy non-consanguineous parents. He presented with early onset hypotonia, periodic breathing abnormalities, feeding difficulties and craniofacial dysmorphism. In addition, hypospadias, hypoplasia of the corpus callosum and patent foramen ovale were noted. Pronounced motoric and mental retardation, as well as lack of speech became increasingly apparent. Hypertonic musculature including opisthotonus developed along with autoagressive tendencies. The head growth decreased and secondary microcephaly with synostosis of the metopic suture developed. Weight gain was also inadequate.

Genetic investigations: Chromosomal analysis, Array-CGH and sequencing of the SHH gene and the CHDR7 gene all yielded normal results. In cooperation with the patient's parents, clinical exome analysis was subsequently performed. A trio-analysis approach, comparing the healthy parents to the affected child was chosen, using the TruSight One Panel by Illumina, which contains 4813 genes associated with known clinical phenotypes. The sequence variant c.4558-3T>G in intron 15 of the ATRX gene, which has not been described in the literature to date, was detected in a hemizygous state in the affected boy and in a heterozygous state in the healthy mother. The sequence variant was verified by Sanger sequencing in both mother and child.

With respect to the overlapping phenotype of the patient to other reported ATRX cases, additional diagnostic steps were performed in order to further establish the significance of this sequence variant. X-Inactivation studies in the mother revealed a severely skewed X-inactivation and mRNA studies from peripheral blood samples demonstrated, that this intronic mutation does indeed affect correct splicing of the ATRX mRNA.

The clarification and verification of the diagnosis ATRX-Syndrome, even despite the severe burden and the 50% recurrence risk for further boys with ARTX-Syndrome, was enlightening for the family and of great importance for future family planning.

P-ClinG-106

Multiple exostoses, mental retardation, cutis laxa in a boy with a de novo 8q24.11-q24.12 interstitial deletion- a new contiguous gene syndrome

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Multiple exostoses is a genetically heterogeneous condition. It may occur isolated or as part of a complex contiguous gene syndrome of the long arm of chromosome 8 have been associated previously with several clinical entities; e.g. 8q23.3-q24.13 deletions in tricho-rhino-phalangeal syndrome type I (Langer- Giedion-syndrome; TRPS [MIM 190350]. A genomic rearrangement of the band 8q24.11-q24.12 is not described in the literature.

Here we described an 18 –year- old boy with mental retardation, epilepsy, multiple exostoses, cutis laxa, facial dysmorphism, delayed psychomotor development, psychiatric problems, hearing defect and heart defect and a characteristic behaviour profile with hyperactivity and impulsivenes.

This boy was the second child of healthy nonconsanguineous parents. The family history revealed no stillbirths, intellectual disability or birth defects. The karyotype was normal (46,XY). Array CGH analysis (Agilent 244K) revealed a heterozygous deletion of ~2,29 located at 8q24.11-8q24.12 (chr8: 118920136□121210447)

(UCSC Genome Browser hg18). Parental testing confirmed this was de novo deletion. There are ten Refseq genes (EXT1, SMAD12, TNFRSF11B, COLEC10, MAL2, NOV, ENPP2, TAF2, DSCC1, DEPDC6) in this region and nine genes reported in OMIM. The microdeletion syndrome is not contained in the database of Genomic variants. The microdeletion 8q24.11-q24.12 contains seven genes without known human phenotype. The heterozygous loss of EXT1- gene is cause of typical multiple exostoses. TNFRSF11B-gene associated with Paget disease (MIM 239000) and TAF2-gene with mental retardation (both-autosomal recessive disorders). Referring to our patient we report in detail clinical and molecular genetics aspects of the 8q24.11-q24.12 microdeletion syndrome.

P-ClinG-107

Whole exome sequencing (WES) in fetuses and patients with congenital central nervous system malformations

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Introduction

The birth prevalence of central nervous system (CNS) malformations ranges between 0.5-2 in 1.000 live births. In the majority of cases the etiology remains unkown. In the present study we aimed to identify disease causing mutations using whole exome sequencing (WES) in 2 fetuses and 3 patients with non-isolated CNS malformations.

Methods

WES was performed using Illumina Genome Analyzers IIx and Agilent Enrichment Kit (Illumina HiSeq platform). Data analysis was done by standard protocols using the VARBANK pipeline (CCG, Cologne). Families were filtered for both disease causing autosomal dominant de novo as well as disease causing autosomal recessive mutations. We filtered for SNPs, deletions and insertions in coding regions that affect the protein structure and the 5' and 3' splicing sites. Identification of mutations was followed by Sanger sequencing validation.

Results

In one fetus, we identified a splice site mutation (G268R) in the ACTB gene, known to cause Baraitser-Winter-syndrome (BWS). Comparison of the phenotypic spectrum of BWS shows large phenotypic overlap with the fetus investigated here. Sanger sequencing of the identified mutation in further 148 patients and fetuses with CNS malformations did not show further mutation carriers. Currently, "Exon trap" is being performed to further characterise the identified splice site mutation. We further identified two compound heterozygous mutations (V594L and S1492T) in the COL16A1 gene in a patient with CNS and anorectal malformations. Expression of COL16A1 during early embryogenesis in murine uro-rectal structures prompted us to Sanger sequence COL16A1 in 149 patients with ARM. Sequence analysis of COL16A1 and analysis of exome data of the other fetus and two patients is ongoing.

Conclusion

Preliminary analysis provides evidence, that a certain proportion of patients with unclear CNS malformations will be revealed by future WES studies.

P-ClinG-108

A novel heterozygous FLNB mutation in a patient with Larsen syndrome

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We describe a 42-year-old German male with multiple congenital skeletal deformities. The patient is the second son of unaffected and non-consanguineous parents. He was born, followed by unremarkable pregnancy, at 40 weeks of gestation with a birth weight of 3,580 grams (60th centile), length of 52 cm (56th centile) and a head circumference of 37 cm (95th centile). At birth, he presented with dislocation of the knee joints, anterior dislocation of the tibia, bilateral pronounced clubfeet, and distinct dislocation of both hips. Further, he had broad distal phalanges of the hands, supernumerary carpal bones, and 13 pairs of ribs. In addition, facial dysmorphism with a flat face and nose, prominent forehead, hypertelorism and myopia was noted. During the neonatal period, a position correction of the legs using an extension treatment for reduction

of the knee and redressing plaster of Paris casts were performed. Later, clubfeet were corrected by an osteotomy of the metatarsal bones and extension of the Achilles tendon. By the age of 14, the patient was wearing orthoses. Due to chronic pain and astasis, an arthrodesis of the right knee joint was performed. He later developed a cervical scoliosis with an occipito-craniocervical instability of the upper cervical spine. Therefore, a stiffening of the cervical spine (C0-C2) and a return displacement of the soft palate were conducted. Abnormalities of the cardiovascular system were observed. At the age of 37 years, he developed a chronic myeloid leukemia.

Based on his medical history and clinical course, the clinical diagnosis of Larsen syndrome was suggested and molecular genetic analysis of the FLNB gene was performed. We detected the heterozygous mutation c.2055G>C in exon 13 predicting the amino acid substitution p.Q685H, located within the immunoglobin-like filamin repeat 5 of the FLNB protein. This variant was not present in dbSNP135, 1000 Genomes data, HGMD and ExAC browser precluding that it represents a rare nonsynonymous polymorphism. Parental genotyping revealed this alteration to be de novo in the patient. Glutamine at position 685 is evolutionarily conserved. In silico analysis predicted the amino acid substitution Q685H to be a likely damaging alteration. As the mutated guanine at position 2055 in the FLNB coding region is the last nucleotide of exon 13, we performed in silico splice site prediction analysis using three programs. Two programs still identified the mutated splice donor site in intron 14, but with a lower score, while one program failed to detect the altered splice site. We speculate that the c.2055G>C mutation could cause aberrant splicing leading to a shortened FLNB transcript. However, the mutation may only weaken the splice donor leading to a full-length transcript and producing a FLNB protein with the p.Q685H change. To the best of our knowledge, this is the first report of a novel mutation in the FLNB gene within the filamin repeat 5 associated with Larsen syndrome.

P-ClinG-109

Distal hereditary motor neuropathy due to BSCL2 mutation in a two generation family

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Distal hereditary motor neuropathy (dHMN) is a group of diverse neuromuscular disorders ranging from dHMN-I to dHMN-VIIB, which are characterized by distal muscular atrophy and progressive motor weakness without sensory impairment. Clinically, dHMN can cause heterogeneous phenotypes even within a family and is sometimes misdiagnosed as amyotrophic lateral sclerosis (ALS).

We report on a family suffering from motor neuropathy of variable severities. The index patient was a 50-year-old man who first noticed fasciculation at age 48. First examination showed bilateral first interosseus (IOD I) atrophy and distal muscle weakness of the upper and lower extremities. After the electrophysiological work-up he fulfilled the criteria of a clinically probable ALS. Early in the disease course nerve conduction studies (NCS) also revealed an axonal neuropathy. Clinical symptoms were rapidly progressive. His sister showed at age 51 a unilateral atrophy of the IOD I and an axonal neuropathy of the peroneal motor nerve. Needle EMG only denoted denervation in the affected IOD I. Another 45-year-old sister did not notice any clinical symptoms and the examination was normal. However, the NCS and needle EMG displayed a pure motor axonal neuropathy. Their mother was diagnosed at age 62 with a multifocal motor neuropathy (MMN) due to a unilateral atrophy and weakness in the IOD I and a motor neuropathy with conduction block in the ulnar and peroneal nerve.

We analyzed 68 genes associated to dHMN, ALS and CMT from the TruSight Exome panel (Illumina) in the index patient, his mother and one sister following sequencing on a MiSeq (Illumina). Data analysis was performed by GensearchNGS (PhenoSystems) and pathogenicity predictions were made by Alamut (Interactive Biosoftware). We filtered for variants common to all three family members and found a heterozygous missense mutation in the BSCL2 gene (c.455A>G) creating a known amino acid change from asparagine to serine (rs137852972; p.N88S; OMIM # 600794) which was predicted to be pathogen by Alamut. This mutation could be confirmed by Sanger sequencing in one additional affected family member and was not present in family members without neuromuscular symptoms. The phenotype of BSCL2 mutations also comprises a complicated type of hereditary spastic paraplegia (SPG17; Silver Syndrome). It is most likely that modifier mutations exist explaining the variable phenotypes and the known reduced penetrance. However, no additional mutation in BSCL2 or in other genes could be detected which could explain the differences within the presented family.

In summary, by using clinical exome sequencing we could identify a known BSCL2 mutation in a family suffering from a broad phenotypic variation from dHMN-V to ALS. It was convenient to apply next generation sequencing (NGS) technologies, since the disease could not be definitively categorized clinically and the large number of genes associated with dHMN precluded successive Sanger sequencing.

P-ClinG-110

Two novel families with speech and language defects due to a nonsense mutation or intragenic deletion in FOXP2

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Since the identification of a missense mutation in the forkhead transcription factor FOXP2 in a large multigenerational family with autosomal dominant language and speech disorder in 2001, only few cases have been described. To date, disruptions of this gene are still the only known monogenic cause of speech and language impairment, often termed Childhood Apraxia of Speech (CAS). So far, mainly chromosomal rearrangements such as translocations or larger deletions affecting FOXP2 have been reported. Intragenic deletions or convincingly pathogenic point mutations in FOXP2 have up to date only been reported in three families.

By chromosomal microarray testing with an Affymetrix CytoScan HD-Array we identified a de novo, heterozygous deletion of six exons of FOXP2 in a pair of monozygotic twins. Both girls were referred because of mildly delayed motor development (walking age 18 months) and severely delayed speech development (first words at age 4 years, first sentences at age 8 years). Their non-verbal IQ was tested to the low normal range, and they attended a school for children with special needs. At age 11 years they presented as friendly girls with no other anomalies or major dysmorphism. They spoke in short sentences with slurred speech.

We subsequently selected 15 patients with mild intellectual disability or developmental delay and more severe language or speech impairment from our study group of individuals with intellectual disability. Mutational screening of FOXP2 revealed a stop mutation (p.Arg345Term) in a 13 year old boy with normal motor development (sitting at 8 months and walking at 14 months). His speech development was delayed with speaking first single words at age 4 years. At age 8 years his non-verbal IQ was tested to the low normal range. He attended a school for children with special needs and presented as a friendly boy. He spoke in short, simple sentences, but often slurred and dyspraxic. The mutation was also found in his mother who reported to have been mildly developmentally delayed and stuttering in childhood. In her, dyspraxia and slurred speech could be noted. The younger brother of the index patient was also referred because of developmental delay, but at the age of 7 years he spoke fluently in complex sentences. He did not carry the familial mutation in FOXP2.

Our findings further expand the genotypic spectrum of aberrations in FOXP2 and show that the associated phenotypes may not only include significant speech and language defects but also mild cognitive deficits. Identifying a mutation in our small series of 15 tested patients with mild ID and more severe speech impairment indicates that FOXP2 aberrations may not be as rare as previously assumed.

P-COMPLEX GENETICS / COMPLEX DISEASES

P-Compl-111

Achalasia associated HLA-DQβ1 insertion shows a north-south gradient among European populations

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Idiopathic achalasia represents a motility disorder of the esophagus with a lifetime prevalence of 1:10,000. It is characterized by a failure of the lower esophageal sphincter to relax due to a loss of neurons in the myenteric plexus. Although the cause of this neuronal degeneration is mainly unknown, autoimmune processes seem to be involved in individuals with genetic susceptibility. Most recently, we identified an insertion of 8 amino acids in the cytoplasmic tail of HLA-DQβ1 as strong achalasia risk factor (Gockel et al., Nat Genet 2014). Here, the insertion showed disease association with P = 7.72 x 10-26 in 1,480 patients and 5,262 controls from Central Europe (Belgium, Germany, The Netherlands), Italy, and Spain.

The aim of the present study was to assess whether the HLA-DQβ1 insertion also confers risk in the Polish population. For this purpose, we analyzed rs28688207 – causing the HLA-DQβ1 insertion – in 106 achalasia patients and 402 controls from Poland. Furthermore, we assessed the frequency and epidemiological consequences of the insertion on the population level among all studied populations.

Analysis of rs28688207 in the Polish sample revealed a highly significant association with idiopathic achalasia (P = 1.84 x 10-04). The insertion was present in 7.1% of patients compared to 2.1% of controls. After combining all four European data sets (1,586 patients; 5,664 controls) the insertion was achalasia associated with P = 9.58 x 10-28. However, the frequency of the insertion was substantially lower in the Polish sample compared to other European populations. For instance, the insertion is present in 8.7% of patients and 3.3% of controls in the Central European population as well as 10.1% and 5.0% in Spanish patients and controls. The highest frequency was observed for the Italian sample with a frequency of 16.1% in patients and 8.0% in controls, which is four times more common compared to the Polish sample. Accordingly, the insertion shows a stronger attributable risk (AR) in the Italian population compared to the Polish population (AR_Italy = 16.8%; AR_Poland = 9.9%). Based on our data, we estimate that 8.4% more patients with idiopathic achalasia exist in the Italian compared to the Polish population.

In conclusion, we show that the 8 amino acid insertion in HLA-DQβ1 also confers disease risk in the Polish population. Furthermore, we found that the frequency of the insertion substantially differs among European populations with a geospatial north-south gradient. Although our study provides further insights into the genetic disease architecture, the data implicate that much more work is necessary to elucidate the etiological complexity of idiopathic achalasia.

P-Compl-112

Meta-analysis of genome-wide imputed GWAS confirms known significant loci and identifies new suggestive loci in patients with Dupuytren´s disease

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Dupuytren´s disease is a complex disease with a strong genetic basis. To unravel this genetic basis we performed a genome-wide association study (GWAS) comparing cases and controls for single nucleotide polymorphisms (SNPs) to identify genomic regions that are associated with this aging-associated disease.

Here we have included 1580 cases and 4491 controls in a meta-analysis of three genome-wide imputed GWAS datasets: dataset 1: 186 cases and 447 controls (KORA, Helmholtz Center Munich) genotyped for 904440 SNPs on the Affymetrix Genome-Wide Human SNP Array 6.0; dataset 2: 538 cases and 1208 controls (Popgen, University of Kiel) genotyped for 587352 markers on the Affymetrix Axiom Genome-Wide CEU 1 Array; replication dataset: 856 cases (University Medical Center Groningen) and 2836 controls (LifeLines) genotyped for 234939 markers on the Illumina HumanCytoSNP-12. Using linkage information from large reference cohorts (1000 Genomes or HapMap) allowed the imputation of many more SNPs than were genotyped on the array. The same quality control criteria were applied to each GWAS dataset and each dataset was imputed separately with IMPUTE2 and the 1000 Genomes reference set. GWAMA was used for the genome-wide association meta-analysis. We used a fixed effects model adjusting for population stratification.

Our meta-analysis revealed significant association with six of the nine loci identified before in the first GWAS for Dupuytren´s disease. The strongest association signal was again observed on chromosome 7p14.1, for SNP rs17171229, P=1.11E-28; OR: 2.02. This imputated SNP gave a slightly stronger signal than the previous top SNP, rs16879765, P=1.52E-27; OR: 2.02. Moreover, we could identify three new suggestive loci on chromosomes 1q32.1, 2q24.3 and 8p21.2 with P<1E-05 that need to be replicated. We did not observe suggestive association for two previous genome-wide significant loci on chromosomes 1p36.12 and 7q31.2. Thus we were able to replicate for the first time previously identified susceptibility loci for Dupuytren´s disease in a meta-analysis of genome-wide imputated GWAS datasets. Our findings further corroborate the strong and robust genetic basis observed for Dupuytren´s disease. Furthermore, the results demonstrate that genome-wide imputation increases the association power for finding new suggestive risk loci in Dupuytren´s disease. These need to be replicated in an independent dataset. Genomic sequencing of the GWAS loci is the next step to identify underlying causative genetic variants.

P-Compl-113

Identification and characterization of functional genetic variants in Dupuytren's Disease

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Dupuytren's Disease (DD) is a progressive, aging-associated fibromatosis disorder of the palm and fingers, leading to progressive flexion contractures. DD is the most frequent genetic disorder of connective tissue and current treatment of DD consists largely of surgical removal of the contracted connective tissue, which is, however, associated with risk of neurovascular injury and recurrence. Hence, unraveling the molecular etiology of DD is needed to provide insight into potential therapeutic targets for treatment of DD. Here, we aim to identify causative variants, including both coding and noncoding variants, which directly predispose to DD.

We have previously identified nine disease-associated chromosomal regions using genome-wide association studies (GWAS). A 500kb GWAS-identified candidate region on chr7p14.1 encompassing the top SNP is being further analyzed for variants that could explain the strongest disease-association signal by targeted next-generation genomic sequencing of 96 patient samples. After validation of identified variants, the potentially functional variants will be tested for their roles in gene expression, functional properties of myofibroblasts derived from DD tissue and regulatory pathways involved in DD pathogenesis. We expect our experiments to pinpoint genetic variants that underlie the manifestation of DD, which peaks around 60 years of age, and gene networks to unravel pathomechanisms leading to the complex and disfiguring disorder.

P-Compl-114

Novel Loci for Sporadic and Familial Coarctation of the Aorta

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Coarctation of the aorta (CoA) is a relatively common condition that accounts for 5–8% of all congenital heart defects. It can be detected in up to 20% of patients with Ullrich-Turner syndrome, in which a part or all of one of the X chromosomes is absent. The etiology of non-syndromic CoA is poorly understood. In the present work, we test the hypothesis that rare copy number variants (CNV) contribute to the etiology of non-syndromic CoA.

We performed high-resolution genome-wide CNV analysis in 70 individuals with sporadic CoA and 3 families with CoA (n=13), including 605 controls, using the Affimetrix SNP-6.0 microarray platform. In addition, we performed a GWAS with CNVs, performed a CNV burden test and a linkage analysis. Results of the CNV analysis were validated by multiplex ligation-dependent probe amplification.

We identified a significant abundance for large CNV burden (>100kb) on the X chromosome in approximately 22% of males with CoA (11 out of 51; p=0.01). Association analysis in the sporadic cohort revealed 14 novel loci for CoA. One CNV locus on chromosome 21q22.3 in the sporadic CoA cohort overlapped with a locus identified in all familial CoA cases covering a suitable candidate gene. We identified one CNV locus within a familial linkage locus with a further candidate gene; another locus overlapped with a region implicated in Kabuki syndrome, which features several cardiac manifestation including CoA. In the familial cases, we identified a total of 7 CNV loci that were exclusively present in cases but not in unaffected family members. Loci identified by linkage analyses matched with 22 candidate loci in the CHDWiki database, a collaborative knowledge base and gene prioritization portal aimed at mapping genes and genomic regions with corresponding congenital heart defects (CHDs).

Our study provides important new insight into the genetic causes of CoA by allocating components of a complex and heterogeneous genetic etiology. The results of our analysis strongly point several potentially causal genetic variants. A systematic screening for point mutation in genes involved therein is ongoing in our cohort.

P-Compl-115

Delineation of the mutational spectrum in two susceptibility genes for bipolar disorder, neurocan (NCAN) and adenylate cyclase 2 (ADCY2)

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Bipolar Disorder (BD) is a common, genetically complex neuropsychiatric disorder with a significant impact on the global burden of disease. Patients suffer from recurrent episodes of strongly elevated (mania) and depressed mood. The life-time prevalence ranges between 0.5-1.5% in all populations world-wide. Heritability estimates between 60-80% indicate that genetic factors play an important role in the development of this disorder.

We have recently published two large Genome Wide Association Studies (GWAS) that provide strong evidence for an involvement of genetic variants in neurocan (NCAN, Cichon et al. 2011) and adenylate cyclase 2 (ADCY2; Mühleisen et al. 2014) in BD. NCAN encodes a glycoprotein that plays a role in neuronal development. It is expressed in the extracellular matrix and involved in neuronal cell adhesion as well as cell migration. We could show that NCAN-/- mice display a behavioural phenotype that resembles mania and that their behaviour can be normalized following the administration of lithium, an effective drug given to the majority of patients with BD (Miró et al. 2012). ADCY2 is a key enzyme in cAMP regulated GPCR signalling pathways. The association finding of genetic variants in ADCY2 with BD suggests that disturbances in neurosignalling are involved in BD.

For GWAS association signals, it is normally unclear whether they represent the functionally relevant variants or just proxies in linkage disequilibrium (LD). The disease-associated locus may also contain rare, deleterious susceptibility alleles which escape detection through the common SNP arrays used in GWAS.

Therefore, to uncover the whole mutational spectrum in NCAN and ADCY2 and to detect the putative functional variant underlying the GWAS association signal, we conducted a Next-Generation-Sequencing (NGS) based re-sequencing in 960 German BD patients. We designed a custom amplicon panel using Illuminas TruSeq Custom Amplicon©-Kit and performed the sequencing on a MiSeq© System from Illumina©. Alignment and variant calling was done using the MiSeq reporter tool v2.4 and variant annotation according to HGVS was conducted in Illumina VariantStudio©. Three exons could not be sequenced by NGS and were separately sequenced using Sanger Sequencing.

Our preliminary analysis with stringent quality criteria (coverage > 20, min alternative allele frequency 15%) detected 178 unique variants, 78 of them being missense variants. We did not find any splice or nonsense variants. We are currently focussing on the in silico functional characterization of the missense variants using different tools as SIFT and PolyPhen-2 and databases like ENCODE and Fantom5 with the results being presented on the poster.

P-Compl-116

Role of PLD3 rare variants in European non-familial Alzheimer's disease

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Interest in the role of rare genetic variants in the etiology of complex diseases such as Alzheimer's disease (AD) is increasing. In January 2014, Cruchaga et al. provided evidence supporting the role of rare variants in the phospholipase D3 (PLD3) gene in both familial late onset AD (LOAD, age-at-onset [AAO] >65 years) and in non-familial AD (nfAD). To confirm the latter finding, we investigated the PLD3-variants reported by Cruchaga et al. in 3,568 nfAD cases and 3,867 controls of German or Spanish descent. Four coding PLD3-variants reported by Cruchaga et al. with P<0.15 (p.M6R, p.P76A, p.V232M, p.A442A) were genotyped in three independent nfAD case-control samples using Sequenom's iPLEX-assay. The two Spanish samples comprised: (i) 2,166 cases and 2,754 controls (Fundació ACE); and (ii) 461 cases and 180 controls (St Pau Hospital). The German sample comprised 941 cases and 933 controls from three multicenter studies. All nfAD cases fulfilled NINCDS/ADRDA criteria, and all participants provided written informed consent. Association was tested using Armitage's test for allelic trend (INTERSNP). In addition, a burden analysis was performed with the collapsing test COLL. In the Spanish and German samples, the variant p.M6R was found to be monomorphic. No evidence for an association between nfAD and any of the three polymorphic PLD3-variants was found in the analyses of the three individual study samples or in the analysis of the combined sample: p.P76A (p=0.65, OR=1.45), p.V232M (p=0.97, OR=1.01), and p.A442A (p=0.55, OR=1.09). Interestingly, the power of our case-control study was sufficiently large to detect an OR of 2 or larger, as reported by Cruchaga et al. Since the association reported by Cruchaga et al. was mainly for LOAD and was stronger in cases with a positive family history, we stratified our samples according to: (i) AAO (i.e. ≤65 or >65 years); and (ii) family history (defined as the presence of at least one self-reported dementia case in the family). However, neither analysis generated evidence for association (P>0.05). Similarly, the burden analysis revealed no significant

differences between nfAD cases and controls in terms of the occurrence of any of the four PLD3-variants (P>0.05). In conclusion, our analyses in two European populations did not implicate rare PLD3 coding variants in nfAD susceptibility. This calls into question both their genetic-epidemiological relevance in the investigated populations, and their importance in the pathogenesis of nfAD. However, we cannot rule out the role of PLD3 in samples enriched in AD cases with familial history of AD. The results presented in this work are currently in press in Nature.

P-Compl-117

Analysis of a potential interaction between the WNT10A- and the EBF1-risk loci for androgenetic alopecia

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Androgenetic alopecia (AGA) is the most common form of hair loss in men and is characterized by a progressive loss of hair from the scalp. The pathogenesis of AGA is driven by androgens based on a genetic predisposition and key pathophysiological signs include changes in hair cycle dynamics and a miniaturization of affected hair follicles. However, the underlying biological mechanisms are still elusive. In recent years, GWAS have identified twelve genetic risk loci that contribute to AGA etiology (e.g. Heilmann et al. 2013). Among them a locus on chromosome (chr) 2q35, located intronically in WNT10A. Here, allele-specific expression analyses showed a regulatory effect of the most strongly associated SNP at this locus (rs7349332) on the expression of WNT10A. Subsequent data base research for non-coding functional elements at this locus revealed that rs3856551, a variant in strong linkage disequilibrium (r2=0.96) with the GWAS variant rs7349332, is located within a transcription factor binding site for EBF1. Remarkably, the gene encoding for EBF1 is spanned by another AGA risk loci on chr 5q33.3, suggesting a potential functional interaction between these risk loci. We therefore hypothesized that EBF1 is a regulator of WNT10A expression in human hair follicle and that allele-specific differences in binding affinity of EBF1 to its target site at 2q35 may lead to differences in WNT10A mRNA expression.

Indeed, bioinformatic analyses predicted a reduced EBF1 binding affinity to its target site on 2q35 in dependence of the C-allele of rs3856551. To functionally confirm that a reduction in EBF1 binding influences WNT10A expression, we performed an in vitro luciferase assay by co-transfecting murine fibroblasts with (i) a luciferase vector construct that contained a computationally predicted WNT10A promoter sequence and the 2q35-EBF1 binding site with either the C- or the T-allele for rs3856551 and (ii) an EBF1-expression vector. Confirming the results from the in-silico prediction, our first experiments indicate that EBF1 is able to activate the WNT10A promoter and that the activation is significantly weaker for the rs3856551 C-allele. Thus, our analyses suggests that there may be a functional interaction between the AGA risk loci on chr 2q35 (WNT10A) and 5q33.3 (EBF1) and that AGA risk variants at 2q35 have an influence on the binding affinity of EBF1, which may subsequently lead to allele-specific differences in WNT10A expression. Additional experiments are currently ongoing to confirm this interaction between EBF1 and WNT10A expression at mRNA- and protein-level in mouse fibroblasts and human keratinocytes. It is hoped that these analyses will further elucidate the underlying molecular mechanisms for the AGA-associated risk variants on chr 2q35 (WNT10A) and chr 5q33.3 (EBF1) and thereby contribute to a deeper understanding of the yet unknown biological causes of AGA.

P-Compl-118

Identification of a potential new candidate gene region for Bardet-Biedl-syndrome at chromosome 14 by homozygosity mapping in an affected offspring of a couple of Austrian extraction.

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In a 23 year old female patient, 1 month after birth a bilateral hexadactyly of her feet was surgically corrected. Birth weight was 3.2 kg, length 50 cm, OFC 36.0 cm. She has 2 older healthy brothers and her parents are healthy too. At 2 years an altered vision with strabismus divergens and horizontal nystagmus was recognized. Besides myopia alta, right -9.5, left -8.75, an impaired vision of 0.25, right and 0.3, left was found. With 12 years a fundus tabulatus and suspected fundus albipunctatus o.u. was seen. At 17 years of age a bilateral cataract surgery was performed. A photoptic ERG examination with 18 years revealed no signal and

electrophysiological findings supported diagnosis of retinitis pigmentosa (RP). Visus was reduced to 0.15 (right) and 0.1 (left) at this time and was 0.1 bilateral with 20 years of age. An increased blood pressure was found, but renal function and morphology according to sonographic examinations did not show any obvious anomalies or polycystic malformations with 14 years of age. A gynecological examination performed under general anesthesia at this time did not allow determining if ovaries are present and hypogenitalism could not be ruled out. However later puberty occurred. The patient now is 163 cm with 86 kg consistent with obesity and a moderate mental impairment was diagnosed by neuropediatric examinations. Cytogenetic and array CGH analysis revealed normal results. Bardet-Biedl-syndrome (BBS), a ciliopathy is a rare disorder (< 1/100.000) that shows multisystem involvement with a wide clinical spectrum and variable onset of symptoms. Since BBS is highly heterogeneous with 19 causative genes identified thus far, following mainly an autosomal recessive mode of inheritance, in many cases no other affected patients are present in a particular family. A relatively distant relationship (consanguinity) of the parents of our patient, who as their relatives originate from a small village from Lower Austria, is possible. Based on these considerations we performed a 250 k Affymetrixs SNP array analysis using DNA of the patient for homozygosity mapping. Only a single segment larger than 1 Mb of homozygosity in the whole genome was identified. This segment of more than 8 Mb in size is flanked by the markers RS17524152 and RS10138542 on chromosome 14. The segment 14q13.1-q21.1 contains more than 30 genes. However since the only known BBS gene (BBS8) on chromosome 14, as well as the 2 known RP genes RP27 and RP51 and the sometimes resembling Leber congenital amaurosis gene (LCA13) on chromosome 14 are located outside of the homozygous segment, a new genetic locus for the disorder of our patient can be considered. To avoid missing potential causative BBS or RP gene mutations mapped outside of the identified segment, we currently apply panel diagnostics first and in case that this approach does not explain the cause of the disorder, then to focus on NGS analysis of the candidate genes in the homozygous segment identified.

P-Compl-119

Exome sequencing of two large Cuban families densely affected with bipolar disorder

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Bipolar disorder (BD) is a major psychiatric disorder affecting more than 1% of the world's population. The disease is characterized by recurrent episodes of manic and depressive symptoms and shows a high heritability of about 70%.

Molecular genetic candidate and lately genome-wide association studies have identified several susceptibility genes contributing to the etiology of bipolar disorder. However, the disease driving pathways and regulatory networks remain largely unknown. Models of illness are most consistent with a polygenic contribution of common and rare variants to disease susceptibility. As the cumulative impact of common alleles with small effect may only explain around 38% of the phenotypic variance for BD (Lee et al., 2011), rare variants of high penetrance have been suggested to contribute to BD susceptibility. One way to evaluate this hypothesis is to investigate large pedigrees densely affected with BD, in which the existence of a genetic variant of strong effect inherited from a common ancestor may be more likely (Collins et al., 2013).

In the present study we aim to investigate the role of rare nonsynonymous variants in individuals of two multiply affected families from Cuba. Both pedigrees were examined by trained psychiatrists using clinical interviews. The first six-generation pedigree was composed of 60 individuals (38% males) including 21 individuals with a known psychiatric disorder. DNA was available for a total of 13 individuals. These included four cases with BD and two with a major depressive disorder. The second family comprised of 76 individuals (50% males, four generations, 31 individuals with known psychiatric disorder). DNA was available for 21 individuals including four cases with BD type I, six with BD type II and two with a recurrent major depressive disorder. The exceptional lifetime prevalence of BD in these two large pedigrees makes them promising candidates for identifying genetic risk factors of Mendelian-like effects. For this purpose we selected 16 affected individuals from both families (eight females, eight males) and performed exome sequencing using the Agilent SureSelect post-capture target enrichment system for Illumina paired-end sequencing library on

the HiSeq platform. For data analysis, the VARBANK pipeline of the Cologne Center of Genomics was used. In addition, all family members with available DNA (n=34) were genotyped on the Illumina Psych Chip to create polygenic risk-score analyses in order to evaluate the contribution of common variants in the two pedigrees.

Exome sequencing of the selected 16 individuals has recently been completed. Preliminary results show the existence of rare exonic variants in the investigated individuals. Further evaluation and interpretation of these results is currently underway and will be presented.

P-Compl-120

GRHL3 mutation screening identifies de novo splice site mutations in patients with apparently nonsyndromic cleft palate

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Van der Woude syndrome (VWS) is a common syndromic form of cleft lip and palate. Mutations in the interferon regulatory factor 6 (IRF6) gene account for ~70% of cases. Recently, grainy head-like 3 (GRHL3) was identified as a novel gene underlying VWS. Phenotype analyses suggested that individuals with a GRHL3 mutation are more likely to have cleft palate and less likely to have cleft lip or lip pits than individuals with an IRF6 mutation.

Notably, common variants in the regulatory region of IRF6 have been shown to confer risk for nonsyndromic cleft lip with or without cleft palate (nsCL/P), which has a genetically complex background. We therefore investigated whether also common variants in the GRHL3 region are associated with nsCL/P. Based on genome-wide imputed data from our previously published meta-analyses of GWAS data we tested ~1300 SNPs in the GRHL3 coding region +/- 200kb for an association in patients. None of the association P-values in that region withstood correction for multiple testing (lowest P-value observed: rs10903069, P=0.013), suggesting that single common variants at the GRHL3 locus do not contribute to nsCL/P. To identify whether common variants within the GRHL3 gene region (coding region, introns, UTR) show evidence for association in aggregate, we performed a gene-based analysis for nsCL/P using VEGAS. No evidence for gene-based association was identified.

Of note, VWS patients have lip pits as sole symptom in addition to clefting. Lip pits can be subtle and sometimes difficult to recognize, especially after surgical treatment. Insofar VWS might sometimes appear as nonsyndromic clefting. We therefore evaluated whether mutations in the GRHL3 coding region account for clefting in apparently nonsyndromic cleft patients. We sequenced the complete coding region, including all four transcript isoforms and adjacent splice sites, of GRHL3 in 576 patients with nsCL/P and 96 patients with nonsyndromic cleft palate only (nsCPO). Observed rare variants were investigated with respect to de novo occurrence. Fourteen rare single-base variants were found, two of which were splice site mutations that occurred de novo in the affected families. All three splice site mutation carriers had an apparently nonsyndromic CPO. Two missense mutations were identified which were classified as “potentially damaging” by prediction programs. One of them was found in a CPO patient who had inherited the mutation from an unaffected parent, the second potentially damaging missense mutation was identified in an nsCL/P patient (no parental DNA available for segregation testing).

In summary, we did not find evidence for common variants in GRHL3 being causative for nsCL/P. However, we demonstrated that rare mutations in the coding region of GRHL3, known as a gene underlying a frequent syndromic form of clefting, can mimic nonsyndromic clefting.

P-Compl-121

Re-sequencing of ISL-1 in 208 bladder exstrophy patients and functional characterization of Isl1 in zebrafish

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The bladder exstrophy-epispadias complex (BEEC) represents the severe end of the uro-rectal malformation spectrum, and is thought to result from aberrant embryonic morphogenesis of the cloacal membrane and the urorectal septum. The most common form of BEEC is isolated classic bladder exstrophy (CBE). Several cell lineages including the cloacal region have been suggested to contribute to bladder and

external genitalia development. Such lineages include Isl1 type developmental gene-positive cell lineage. Recently the gene was shown as expressed in genital tubercle. Our own group found association on a genome-wide level between CBE and a region on chromosome 5q11.1 harboring human ILS-1 using a genome-wide association study (GWAS) and meta-analysis. In order to follow up these findings we re-sequenced ISL-1 in all 208 patients included in our previous GWAS and meta-analysis including the promoter and possible enhancers. In order to establish the zebrafish (danio rerio) as a suitable vertebrate model organism for BEEC we analysed the localisation and termination of the expression of Isl1 in zebrafish larvae using mRNA-in-situ-Hybridisation. If possible a BEEC model will be induced either by over-expression of Isl1 using mRNA-injections or by knock-down using anti-sense morpholinos.

P-Compl-122

Enhancer variant enrichment analysis in bipolar disorder

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Bipolar disorder (BD) is a common and highly heritable mental illness and genome-wide association studies (GWAS) have robustly identified genetic variants implicated in disease susceptibility. However, the majority of loci are located in non-coding regions of the genome, thus requiring further functional annotation/refinement.

Enhancers are distal regulatory elements that control the activation of tissue- and cell-type specific gene expression. Currently, large-scale identification of actively transcribed enhancers is performed by different techniques such as chromatin immunoprecipitation (ChIP) or cap analysis gene expression (CAGE) coupled with next-generation sequencing (NGS) across various tissues and developmental stages. The Fantom5 enhancer atlas (Andersson et al. 2014) represents a systematic resource for active enhancers in tissues including human adult and fetal brain as well as neurons and glia cells. The integration of GWAS signals and these functional genomic regions provides novel opportunities to elucidate biological mechanisms underlying BD. Therefore, we investigated in this study whether brain or neuron cell related enhancers are enriched among top BD GWAS hits.

Results from the largest BP meta-analyses comprising a sample of 24,025 patients and controls (Mühleisen et al. 2014) provided the basis for this analysis. We mapped GWAS loci with a nominal significant p-value to enhancer regions defined by the Fantom5 study. Investigated tissues included different brain regions, neurons, glia cells as well as tissues not related to BD as controls. The enrichment of associated SNPs in enhancers was assessed by permutation analysis. Briefly, the observed number of SNPs in specific enhancers was compared to a randomized set of enhancer regions with the same structure as the specific enhancer.

In total, 20 SNPs with a nominal significant p-value were observed in neuron, 14 in brain and none in skin enhancers. Preliminary results suggest that this observed enrichment in brain-related enhancers is significant when compared to randomized enhancer sets (neurons $p=0.015$, skin $p>0.99$). A detailed analysis of several regions is ongoing and will be presented.

Overall, our results provide the first systematic integration of brain-related enhancers in the largest BP GWAS to date and subsequently enable the discovery of relevant biological processes suitable for further functional studies.

P-Compl-123

Resequencing study of five candidate microRNAs in bipolar disorder

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Bipolar disorder (BD) is a severe neuropsychiatric disorder with an estimated lifetime prevalence of 1%. The disease is characterized by recurrent episodes of mania and depression and shows heritability estimates

ranging between 60 and 80%. Molecular genetic studies have identified the first susceptibility loci contributing to BD, but the disease relevant pathways remain largely unknown.

MicroRNAs (miRNAs) are a class of 21-25-nucleotide small non-coding RNAs that control the expression of their target genes by binding to target sites in messenger RNAs. Accumulating evidence suggests that miRNAs contribute to basic mechanisms underlying brain development and plasticity. This in turn suggests their possible involvement in the pathogenesis of various psychiatric disorders, including BD. This hypothesis is supported by the results of the largest genome-wide association study (GWAS) of BD to date (Mühleisen et al., 2014). In this study, a single-nucleotide polymorphism in an intergenic region flanking MIR2113 was among the strongest findings. Using the summary statistics of this BD GWAS (Mühleisen et al., 2014), we and others have recently performed a genome-wide analysis of miRNA coding genes. We identified a total of nine BD-associated miRNAs including the brain-expressed MIR499, MIR708 and MIR1908 as the most promising candidates for further analyses (Forstner et al., GfH 2014). Furthermore, a recent study by Strazisar and colleagues (2014) identified two rare functional variants flanking miR-137 which represents a genome-wide significant risk locus for schizophrenia. Interestingly, both variants were also found to be associated with BD.

Therefore, the aim of the present study was to determine whether rare variants within these five candidate loci contribute to the development of BD. For this purpose we performed Sanger sequencing of 1,000 BD patients and 1,000 sex-matched healthy controls, all of German origin.

So far, we have generated preliminary data for MIR2113 and MIR137. In the first 96 patients and 288 controls we detected a total of five rare variants (minor allele frequency <3%) at the MIR2113 locus. These included two patient-specific variants directly flanking MIR2113. Regarding MIR137, we did not find any variant in the premature miRNA sequence per se. However, our preliminary results suggest that a higher number of a 15 bp sequence tandem repeat (STR) located six bases upstream of the premature miR-137 might be associated with BD. Notably, we found a number of 4-12 STR in about 32% of our patients and only in about 27% of our controls. This tendency is in line with previous observations by Strazisar et al. (2014).

We hypothesize that sequence variants at miRNA loci may contribute to the development of BD. The investigation of the other three miRNAs and the remaining samples for MIR2113 and MIR137 is currently underway and will be presented.

P-CompI-124

An isoform-specific WNK1-mutation together with a WNK1 null-allele cause Sensory Neuropathy type 2 (HSAN2)

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Hereditary sensory and autonomic neuropathies (HSANs) are characterized by progressive loss of function of peripheral sensory nerves. HSAN2A (OMIM 201300) represents a disease subtype where sensory dysfunction predominates and autonomic involvement is usually milder. The disorder starts in early infancy or childhood with numbness in the hands and feet leading quickly to loss of pain and temperature perception. Consequently, patients suffer from burn, injuries, and mutilations. HSAN2A is an autosomal-recessive disease and linked to mutations in exon 9 of the WNK1 gene. This exon was initially believed to be a single open reading frame (ORF), termed HSN2, within a large intron of the WNK1 gene. However, it was subsequently shown that HSN2 is an alternatively-spliced exon of a neuron-specific 28 exon isoform (NM_001184985) of the WNK1 gene. Routine HSAN2 diagnostics is limited to sequencing of only the WNK1/HSN2 exon since mutations are usually restricted to this exon.

Here, we present a patient with the clinical diagnosis of HSAN2, harbouring a “classical” WNK1/HSN2 mutation together with an unusual WNK1-mutation on the second allele that disrupts all isoforms of the WNK1 protein. Both parents are healthy carriers of one of the mutations. The mutations were identified by applying a custom-made next-generation sequencing panel (Haloplex, Agilent Technologies) including all known HSAN genes and genes causing congenital insensitivity to pain (CIP).

This case study illustrates the complexity of the HSAN2 genetics and suggests that screening of the entire coding region of WNK1 is important in cases suspicious for HSAN2A. Moreover, the disorder exemplifies the relevance of mutations in tissue-specific isoforms.

P-CYTOGENETICS / CNVS

P-CytoG-125

A case with MNX1-negative Currarino syndrome and de novo duplication of chromosome 3q26.32-q27.2

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Currarino syndrome (CS) is characterized by the triad of a sickle-shaped sacrum or total sacral agenesis below S2, hindgut anomaly, and presacral tumor. Additionally, spinal anomalies such as tethered cord are frequently associated. Nearly all familial cases and 30-50% of sporadic cases carry a mutation in MNX1. In 57% of cases a truncating mutation is detected, suggesting haploinsufficiency as the underlying mechanism.

Here we report a case with clinical diagnosis of CS and comorbid malformations of the brain (agenesis of corpus callosum, discontinuity of the pituitary stalk, ectopic posterior pituitary), and hypopituitarism.

Sanger sequencing showed no coding mutation of MNX1. Molecular karyotyping, utilizing a SNP-chip (single nucleotide polymorphisms), showed a duplication of chromosome 3q26.32-q27.2 encompassing 7.9 Mb, and 56 RefSeq genes. FISH analysis of the duplicated region showed a tandem orientation and quantitative PCR confirmed the de novo occurrence. The 5' breakpoint mapped in the gene IGF2BP2 (between exon 1 and 2). However, the transcription level of IGF2BP2 mRNA was not different compared to controls.

The observed de novo duplication of chromosome 3q26.32-q27.2 has been previously associated with central nervous system malformations, yet not with hypopituitarism. The causality between the present duplication and the expression of CS remains unclear; however, the finding of another patient with 3q duplication and a sacral tumor may be indicative of a rare association1.

[1] Dundar M, Uzak A, Erdogan M, et al. 2011. Partial trisomy 3q in a child with sacrococcygeal teratoma and Cornelia de Lange syndrome phenotype. Genet Couns. 22:199-205.

P-CytoG-126

Microdeletions on 6p22.3 3 are associated with Mesomelic Dysplasia Savarirayan type

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Mesomelic dysplasia is characterized by shortness of the middle limb segments. Here we report on two unrelated patients with mesomelic dysplasia Savarirayan type both of whom have a 2 Mb overlapping microdeletion on chromosome 6p22.3. The deletions encompass four known genes: MBOAT1, E2F3, CDKAL1, and SOX4. The first patient was reported with congenital deficiency of tibia and fibula with normal feet. The second patient was originally described as a type of mesomelic dysplasia distinct from Nievergelt Syndrome and was later classified as mesomelic dysplasia Savarirayan type. Here we show that microdeletions on chromosome 6p22.3 are associated with mesomelic dysplasia Savarirayan type. Since none of the knock-outs of the genes within the deletion shows mesomelic dysplasia phenotype in mice it is likely that the phenotype is due to a regulatory effect affecting a neighbouring gene. ID4, which is localized telomeric of the deletion, is expressed in the developing limb buds and plays a role in osteoblast differentiation. Therefore, we suggest that the deletion removes a regulatory boundary element and brings several limb enhancer elements into close proximity of ID4, causing misexpression and subsequently the limb defects.

P-CytoG-127

Clinical relevance of familial inheritance of 7q35 microdeletion and unbalanced translocation of chromosomes X and 21 detected by array analysis

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We report on a 21-month-old girl with mental retardation, developmental delay, campodactyly and dysmorphic features including broad bridge and epicanthus. She is the first child of a 23-years-old mother and a 35-years-old father. Both parents are healthy and non-consanguineous. The two sisters of the mother are mental retarded.

Standard cytogenetic analysis (GTG banding) revealed a translocation between chromosomes X und 21 in the patient. This translocation appears to be unbalanced with breakpoints p22.32 distal at the short arm of the X-chromosome and q21.1 proximal at the long arm of chromosome 21. This translocation leads to a partial monosomy of chromosome 21 and on the other hand to a partial trisomy of X-chromosome.

Standard cytogenetic analysis on the mother revealed a balanced translocation between chromosomes X und 21. Only the altered chromosome 21 from the mother was inherited to the child, while the chromosome X was the one not involved in the translocation process.

Array-based comparative genomic hybridization (aCGH) with the 180K oligonucleotide microarray (Agilent Technologies, Santa Clara, CA, USA) revealed a deletion of 0,73 Mb (position 146,016,832-146,748,865 on hg19 assembly) on 7q35, which comprises the CNTNAP2-Gen (OMIM #604569) in both mother and child in addition. The results were confirmed by FISH (Illumina/BlueGnome). The mother of the girl has the same microdeletion of 7q35. Patients with microdeletion 7q35 show mental retardation, developmental delay especially language delay, dysmophisms und behavioral disorder. CNTNAP2 encoded a contactin associated protein of the neurexin family. It is involved in neural-glia-exchange and neuronal exchange between potassium channels and myelinated axons.

Thus our index patient has three different types of chromosomal alterations. Clinical features could be identified fitting to all deletions/duplications detected. It canot be decided which alteration has the most important influence of the patient`s condition. It can be proposed that a combination of alterations may lead to stronger clinical abnormalities. The deletion of chromosome 21 comprises 12 genes. Databases acquire patients with mental retardation and facial dysmophisms and patients without any phenotype changes. 70% of women with trisomy X or partial trisomy X do usually not display strong physical features. Therefore we postulated that the clinical findings in the most severally affected index case in this family are do linked to the microdeletion 7q35.

P-CytoG-128

Interstitial Duplication of Chromosome Region 1q25.1q25.3: Report of a Patient with Mild Cognitive Deficits, Tall Stature and Facial Dysmorphisms

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Isolated interstitial duplications of chromosome band 1q25 are apparently very rare; no patients with detailed molecular and clinical characterization of duplications restricted to this region have been published to date. We report on a 9-year-old girl with mild cognitive deficits, tall stature, macrocephaly and discrete dysmorphic features in whom a de novo interstitial 7.5 Mb duplication of 1q25.1q25.3 was detected by SNP array analysis (arr[hg19] 1q25.1q25.3(173,925,505-181,381,242)x3 dn). The duplicated region was inversely inserted into chromosome band 1q42.2: 46,XX,der(1)(pter→q42.2::q25.3→q25.1::q42.2→qter). Overexpression of one or several of the 87 genes in the duplicated interval was presumably the major causative factor for the clinical manifestations. As this is the first patient with a comprehensively characterized isolated de novo duplication of chromosome band 1q25.1q25.3, reports of additional patients with overlapping duplications will be needed to establish detailed karyotype-phenotype correlations and to gain information on the contribution of individual genes, notably LHX4, for the clinical manifestations.

P-CytoG-129

Phenotype-driven prioritisation and interpretation of copy-number variations

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Clinical classification of CNVs identified via techniques such as array comparative genome hybridisation (aCGH) typically involves the inspection of lists of known and unknown duplications and deletions. This is done to distinguish pathogenic from benign CNVs. An important step is the comparison of the individual's phenotypic abnormalities with those associated with Mendelian disorders of the genes affected by the CNV.

Often there is not much known about these genes, such that model organism phenotype data is often used. Currently, almost 6000 genes in mouse and zebrafish are, when knocked out, associated with a phenotype in the model organism, but no disease is known to be caused by mutations in the human ortholog. However, searching model organism databases and comparing model organism phenotypes with patient phenotypes is hindered by the difficulty in integrating phenotype information across species and the lack of appropriate software.

We present an integrated CNV ranking scheme based on phenotypic matching to a patient's clinical findings, degree of overlap with known benign or pathogenic CNVs, and the haploinsufficiency score.

We show that this ranking scheme leads to a significant improvement compared with rankings that do not use phenotypic information. We provide a software tool called PhenogramViz, which supports phenotype-driven interpretation of aCGH findings based on multiple data sources, including the integrated cross-species phenotype ontology Uberpheno, in order to visualise gene-to-phenotype relations.

P-CytoG-130

Phenotypical variability in two siblings with terminal deletion 5p15.33 and duplication 5q35.3, likely caused by meiotic recombination in a pericentric inversion 5 carrier

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Deletions in the short arm of chromosome 5 are associated with cri-du-chat syndrome (OMIM 123450), a condition best known for its cat like cry. The vast majority of cases are sporadic, with less than 0.5% presumed to be due to a pericentric inversion of chromosome 5 in a healthy carrier parent. In contrast, terminal duplications of the short arm of chromosome 5 are associated with Hunter-McAlpine syndrome (OMIM 601379), a clinically distinct entity from cri-du-chat syndrome.

Patient 1 is a 16 9/12 year old boy with secondary microcephaly (OFC 52,5cm; <P3), atrioventricular septal defect, learning disability, behavioural abnormalities (introvert, shy), ADD, and long slim fingers. Delivery was at GA week 41, with weight 2620g (low, <P3), length 50cm (P9), and OFC 34cm (P7).

Patient 2 is a 13 4/12 year old girl with secondary microcephaly (OFC 51cm; <P3), low weight 35kg (<P3), height 152cm (P10), long slim fingers, brachydactyly type E with bilateral short metacarpal V of hands, bilateral short metacarpal IV and V (r>l) of feet, and joint hyperextensibility. Early developmental milestones were notable for delayed walking at 18 months of age. Abnormal behaviour (severe anxiety, not speaking with non-family members) was noted at age 3 years. She was treated for autism by pediatric psychiatry. Learning disabilities necessitated repeating Grade 6.

The siblings are the only two children of healthy non-consanguineous parents in their early 40s. The family history is notable for a maternal uncle with learning disabilities and a shy an introverted personality. His wife and son have schizophrenia. A different male maternal cousin has learning disabilities. A male paternal cousin has attention deficit disorder (ADD).

We performed conventional peripheral blood chromosome analysis using G-banding, array CGH on peripheral blood DNA (Cytochip Oligo 4x180K v1.0/Fa. Illumina/Bluegnome), and metaphase FISH (Telvysion FISH probes, Abbott).

Conventional cytogenetic analysis revealed a normal karyotypes for both patients. Array CGH revealed a terminal loss of 4.4 Mb in 5p15.33 and a terminal gain of 2.9 Mb in 5q35.3. Validation with telomere specific FISH probes showed a cryptic rearrangement with a single signal of 5qtel on the telomeric ends of the short and the long arm of one chromosome 5 (C84c11/T3-, D5S2907+) in both patients.

The findings of a terminal deletion and a terminal duplication are indicators of meiotic recombination in a parental carrier with pericentric inversion 5. The parents have declined cytogenetic analysis. Thus we are unable to confirm our suspicion. Because the presumed inverted segment comprises approximately 96% of chromosome 5, the likelihood of an interstitial crossover event would be very high. This sib pair is an example of phenotype variability in patients with identical chromosomal rearrangements.

P-CytoG-131

A boy with interstitial deletion of 11q14.1-q14.2, global developmental delay, and dysmorphic facies

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The literature reports only nine cases describing interstitial deletion of 11q. This is in contrast to the well described phenotypic spectrum of Jacobsen syndrome (OMIM 147791). Though the current data is limited and must therefore be interpreted with caution, it suggests that proximal deletions between 11q14.1 to 11q14.3 show a more severe phenotype that includes intellectual disability than deletions between 11q14.3 and 11q22.2.

Our patient is a 5-year-old boy with global developmental delay and dysmorphic facies. He is the older of two children born to non-consanguineous German parents with an unremarkable family history.

We performed conventional peripheral blood chromosome analysis using G-banding, array CGH on peripheral blood DNA (Cytochip Oligo 4x180K v1.0/Fa. Illumina/Bluegnome), quantitative PCR (qPCR), and metaphase fluorescence-in-situ hybridisation (FISH). The patient had an apparently normal male karyotype. Array CGH uncovered a cryptic 8.2 Mb deletion of 11q14.1 to 11q14.2. The deleted region contains 30 HGNC genes, which includes twelve genes with OMIM annotation. The deletion was confirmed by qPCR. Simultaneous qPCR of parental DNA showed a normal result for this region. This is consistent with a de novo deletion in our patient. Metaphase FISH was performed using the BACs RP11-46D24 and RP11-113K21 on patient and parental DNA to determine the likelihood for recurrence and to exclude a balanced parental chromosome rearrangement.

We present the detailed clinical and molecular cytogenetic data for our patient, a review of the literature on proximal interstitial deletion 11q, and suggestions for possible genotype-phenotype correlations.

P-CytoG-132

6q22.33 Microdeletion in a Family with Intellectual Disability, Variable Major Anomalies and Behavioral Abnormalities

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Interstitial deletions on the long arm of chromosome 6 have been described for several regions, such as 6q16, 6q22.1, and 6q21q22.1, and with variable phenotype such as intellectual disability/developmental delay, growth retardation, major and minor facial anomalies. An overview is given by Rosenfeld et al., reviewing twelve reported cases of the literature and proposing phenotype-genotype correlation [Rosenfeld et al. 2012]. However, an isolated microdeletion of the sub-band 6q22.33 has not been described so far and thus, no information is available concerning the specific phenotype associated with such a copy number variation.

Here we define the clinical picture of an isolated 6q22.33 microdeletion based on the phenotype of six members of one family with loss of app. 1 Mb in this region. Main clinical features included mild intellectual

disability and behavioral abnormalities as well as microcephaly, heart defect, and cleft lip and palate. The mother attended supportive school; however she did not require a legal guardian and lived independently with regular social support regarding the childcare.

P-CytoG-133

CNV Analysis in 108 patients with esophageal atresia with or without tracheoesophageal fistula

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In order to identify disease-causing copy number variations (CNVs) we performed array-based molecular karyotyping in a cohort of 107 patients with esophageal atresia with or without tracheoesophageal fistula (EA/TEF). Molecular karyotyping was performed using the HumanOmniExpress 12 v1.1 chip. Application of standard filter criteria, filtering against in-house controls (n=1307), DGV database (excluded if present in DGV > 3 times with 100% phyical overlap) and final visual inspection using Genome Studio yielded 25 putative disease causing CNVs (15 duplications and 11 deletions). Final validation of these CNVs in the patients and the patients' parents using qPCR confirmed 18 CNVs. Two of these were de novo duplications involving chromosomal regions Chr2:131349899-131991166 and Chr3:8519329-8551649, comprising POTEJ, CYP4F3OP, GPR148, AMER3, ARHGEF4, FAM168B and PLEKHB2 (CHR2) and LMCD1 (CHR3) respectively. Although the remaining 16 CNVs were inherited from a healthy parent, all of these were absent in our 1307 in-house controls (frequency < 0.0008) suggesting that these rare CNVs might be involved in the formation of EA/TEF by incomplete penetrance. Our study provides a certain line of evidence that structural genomic imbalances contribute to the development of EA/TEF.

P-CytoG-134

Possible dosage effect of CNTN4 and CNTN6 on psychomotoric development

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We report on a girl with an interstitial duplication of chromosomal region 3p26.3 detected by array CGH analysis. The girl was presented at the age of 24 months because of global developmental delay. The pregnancy, resulting from ICSI-therapy, was uneventful and the child was delivered spontaneously at term. Birth weight (3600g), head circumference (35.5 cm) and length (53 cm) were in the normal range. There were only unspecific minor facial dysmorphies and no obvious malformations. After birth reduced movements and feeding difficulties were striking and she developed a profound psychomotoric retardation (no speech, no walking, no sitting at the age of 2 years). Additionally, she showed stereotypic movements, nystagmus and bruxism.

Chromosomal analysis was apparently normal and the differential diagnoses of Angelman syndrome as well as untypical Rett syndrome were not supported by methylation analysis of the SNRPN locus or sequence analyses of the FOXG1 and CDKL5 genes.

Array CGH analysis uncovered an interstitial 1.05 Mb duplication of 3p26.3 with breakpoints within the genes CNTN6 and CNTN4 (arr[hg19] 3p26.3(1,287,405-2,335,472)x3 dn). The duplication was confirmed by FISH analysis and was excluded in both parents by quantitative PCR (qPCR).

The genes CNTN4 and CNTN6 encode neural adhesion molecules and participate in neurogenesis (growth of developing axons, formation of axon connection) and in the maintenance of the adult nervous system. It is suggested, that the heterozygous loss of CNTN4 is causative for the developmental delay in the 3p deletion syndrome (Dijkhuizen et al 2006). In addition, deletions and duplications of CNTN4 (de novo or paternally inherited) were described as associated with autism.

To the best of our knowledge, only one patient – a 7-year old boy – with a microduplication of CNTN4 and CNTN6 only was described so far. This boy showed moderate intellectual disability, autism and stereotypic behavior (Guo et al. 2014).

We report on a rare case of microduplication of 3p26.3 supporting that alteration of CNTN4 and/or CNTN6 gene could be causative for developmental delay and behavior abnormalities.

P-CytoG-135

Genomic CNVs can cause sudden infant death syndrome (SIDS)

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The contribution of monogenic disorders to in sudden infant death syndrome (SIDS) is established (most commonly LQTs and MCAD deficiency). In contrast the importance of complex genetic predispositions is less clear. Also copy number variations (CNV) involving critical genes may predispose to SIDS.

Patients: We performed genome-wide SNP and CNV genotyping of 368 SIDS cases using the Illumina HumanHap 660v3 quad array. 320 cases originated from the multi-center German study on sudden infant death (GeSID) and 48 cases were recruited from the Sheffield SIDS study in the U.K. As controls we used 823 population based individuals from the KORA cohort.

Methods: In a GWAS design we compared SIDS cases with population controls for genome-wide SNP association. As age matching is not feasible in SIDS we performed sex matching of controls and geographical adjustment by multidimensional scaling. We included Chr.X markers as SIDS exhibits 2:1 male:female sex bias indicating possible genetic risk factors on the sex-chromosomes. Markers were imputed to 4.8 Mio SNPs using the haplotype backbone provided by the 1000 Genomes project. Autosomal SNP-markers were analyzed using an additive model adjusted for sex. Markers on Chr. X were analyzed stratified by sex and then meta-analyzed. In another line of analysis we called genome-wide CNVs in the SIDS cases using the CRLMM algorithm.

Results: The GWAS analysis showed no significant association beyond genome-wide significance level ($p<5x10-8$) neither with autosomal markers nor with markers on Chr.X. CNV analysis revealed deletions in five cases: One case had a 5.2 Mb deletion in 5q3, another case had a 2.2 Mb deletion in 18p1 and a third case had a 370 kb deletion in 5q2. Two further unrelated cases had nearly identical 400 kb deletions in 4q1. None of these CNVs involved genes previously identified to be involved with SIDS or Mendelian diseases involved with premature death.

Conclusion: Our GWAS was powered to detect common SNP association signals with OR≥1.8 and MAF≥0.2 with ≥95% power. The failure to detect such associations in the human genome is well in accordance with the expectation of strong evolutionary selection against any common genetic SIDS risk factors. The detection of CNVs in 1,4% of cases (5/368) indicates a small but significant contribution of structural genomic mutations to SIDS etiology.

P-CytoG-136

Maternally inherited microduplication 7q11.23 in two brothers: variable clinical features and autistic behaviour

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Background. Several well-described microdeletion syndromes allow a clinical diagnosis mainly as a result of characteristic facial features. The reciprocal microduplication is less often recognized due to an only subtle or even absent dysmorphic appearance. Accordingly, microduplication of the Williams Syndrome critical region (WSCR) may be assumed rather for behaviour anomalies like speech delay and autistic features. Proper diagnosis may be further impeded by clinical variability.

Clinical report. We observed 2 brothers (aged 7 and 4 a), who were both mentally retarded, the younger boy presenting with tall stature (> Pc 97), completely lacking speech, autistic behaviour, high forehead, large protruding ears and pointed chin suggesting a fra(X) background; the older boy was less dysmorphic, showed unusual neatly placed straight eyebrows, short upper lips and broad forehead, short stature (Pc 3), and was able to speak 3 word phrases. The mother has low-normal intellectual ability, normal speech and normal facial features. .

Lab investigation and results. The more severely affected younger sibling was investigated first. Standard lymphocyte culture resulted in a normal karyotype. FMR-1 PCR amplification resulted in a product of normal length excluding abnormal CGG-repeat expansion. Microarray analysis ((SNP-Array CytoScan HD, Affymetrix) revealed a microduplication 7q11.23 encompassing the WSCR. MLPA-studies (SALSA P245-B1 vs 32, MRC Holland) including the mother and the older brother confirmed maternal transmission of the microduplication to both children.

Discussion. Expressive speech delay is the hallmark of 7q11.23 microduplication, often associated with autistic features. A high degree of variability of mental disability is noteworthy and was also seen in this family. Apart from cognitive and speech problems there was a clear difference in the facial appearance and growth in both boys. Although an apparent similarity to fra(X) syndrome patients has not been recorded so far, it now seems reasonable to consider also dup(7)(q11.23) in patients negative for fra(X). Parental transmission of dup(7) has been reported only in a few cases so far. In order not to overlook normal transmitting probands it is important to evaluate all members at risk in a family faced with this particular condition.

P-CytoG-137

Overlapping 7q36.1-q36.2 deletions in two patients with intellectual disability

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Terminal deletions of the long arm of chromosome 7 are well known and frequently associated with holoprosencephaly due to loss of the SHH gene in 7q36.3. In contrast, deletions affecting the proximal part of 7q36 are less common and only few patients were described so far.

Here we present clinical data of two patients with overlapping deletions in 7q36.1-q36.2 identified by array-CGH. The first patient is a 7 years old boy, who is the first child (of three) of non-consanguineous parents, born after uneventful pregnancy. After birth he had feeding problems and showed in the following time a delayed psychomotor development and seizures. The MRI of the brain detected enlarged ventricles. Furthermore, he had microcephaly, cataract, hearing impairment and hypotonia. Metabolic screening, GTG-banded chromosomes (550 bands) and FMR1 gene analysis were normal. Array-CGH revealed 12 polymorphic CNVs and an additional deletion of approximately 6.0 Mb in chromosomal region 7q36.1-q36.2. This loss was confirmed by FISH analysis using BAC clone RP11-148K1. Both parents demonstrated normal karyotypes and no deletion was observed using BAC clone RP11-148K1.

The second patient is a 11 years old girl of non-consanguineous parents with global development delay dysmorphic features and hypotonia. The girl demonstrated an apparently normal karyotype and FMR1 gene analysis was unremarkable. Array-CGH analysis identified two losses that were not found in the Database of genomic variants. One of these losses was a de novo deletion within chromosomal region 7q36.1-q36.2 of about 3.2 Mb. The other loss was observed in 5q32. This loss was also detected in DNA sample of her father. FISH using BAC clone RP11-148K1 confirmed the deletion in 7q36.1-q36.2. Both parents demonstrated normal karyotypes.

The deletion of the second patient is located within the 6 Mb deletion of the first patient. Within the 6 Mb deletion of the first patient 26 additional genes were found that were not deleted in the second patient. Both patients demonstrated the deletion of three genes that were associated with Mendelian diseases: KCNH2 (OMIM 152427: long QT syndrome 2 (LQTS), short QT syndrome 1), ASB10 (OMIM 615054: primary adult open-angle glaucoma), and PRKAG2 (OMIM 602743: cardiomyopathy, Wolff-Parkinson-White syndrome). Patient 1 shows two additional disease causing OMIM genes: EZH2 (OMIM 601573: Weaver syndrome) and DPP6 (OMIM 126141: ventricular fibrillation).

Only few patients with deletions in 7q36.1-q36.2 were described so far. The different clinical finding in these patients seems to demonstrate a phenotypic variability. Further analyses of cases with 7q36.1-q36.2 deletions are necessary to characterize the spectrum of clinical findings in these patients. Because of the deleted genes and the expected phenotypes, patients with 7q36.1-q36.2 deletions should be in continuous medical attendance.

P-CytoG-138

Cytogenetic stability in potential (stem) cell-based therapy using GTG, SKY, and locus-specific FISH

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With the development of (stem) cell-based therapies, the question on safety of the use of (stem) cell-based products in humans is becoming increasingly relevant. Consensus of the European Medicines Agencies is: “In

conclusion, on the basis of the state of art, conventional karyotyping can be considered a valuable and useful technique to analyze chromosomal stability during preclinical studies. " (Barkholdt et al., 2013)Therefore a potential tumorigenic risk due to genetic instability or altered cells should be analysed in advance of the cell therapy.

372 chondrocyte samples (78 adherent cultures and 294 spheroids) from six donors using Trypsin-Giemsa staining (GTG-banding), spectral karyotyping (SKY) and locus-specific fluorescence in situ hybridization (FISH) were analyzed in a preclinical study. Further analyses are in progress.

For at least 3 passages, our genetic analyses revealed no significant chromosomal abnormalities applying these techniques [e.g. fra(4)(q31)- only single event in passage 3 of PM 4]. We were able to identify clonal occurrence of polyploid metaphases and endomitoses with increasing cultivation time (passage 4-10). Furthermore, we could observe a loss of the Y-chromosome in the spheroids of the two male donors with prolonged cultivation times. Noteworthy are the following chromosomal aberrations: trisomy of the chromosomes 1,7,8,12, and translocation of the chromosomes 7 and 9, as it has been described in connection with extraskeletal myxoid chondrosarcoma (Sjögren et al., 2003).

Our results show that with increasing cultivation time numerical and/or structural chromosomal aberrations were detected. A combination of different (molecular) cytogenetic techniques is useful to increase the knowledge and experience of potential cell therapeutics and to shape the safety of cell therapy of ATMPs.

P-CytoG-139

Chromosomes in a genome-wise order change the landscape of genetics

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Although a non-random distribution of chromosomes was suggested already in the early days of human cytogenetics (Miller et al 1963) it is commonly accepted by the majority of cytogeneticists, that the chromosomes in a metaphase spread are generally arranged in a completely random way. Since the observation of the bilaterally symmetric distribution of DNA and chromosome specific fluorescence in situ hybridization (FISH) signals in leukocytes in 1990 (Burger et al. 1990), we demonstrated in a series of publications (e.g. Chaudhuri and Reith 1997; Chaudhuri and Walther 2013) the genome-wise organization of chromosomes in human and murine cells. In other words, the maternal chromosomes are tethered to one centriole while the paternal chromosomes are connected to the other. Earlier Gläss (1956/57) observed such segregation in the regenerating liver cells of rats and Pera (1970) presented a hexaploid metaphase spread of a vole, in which the six sets of chromosomes were apparently lying in their distinct haploid domains. In insects and plants there are proven examples of separation of the parental genomes in the nuclei (Brown and Nur 1964; Leitch et al. 1991).

As a rule, rather than an exception, we found this genome-wise haploid order of chromosomes in a variety of samples from different human tissues; in different species of macaque monkeys; in mice (Mus musculus); in aberrant human karyotypes with triploidy, tetraploidy, uniparental disomy (UPD); in human blood samples subjected to pod-FISH (parental origin determination FISH) (Weise et al. 2008) and samples with small supernumerary marker chromosomes (sSMC) (Tönnies et al. 2007). The detailed analysis of the 3 clinical cases with SMC and UPD shed light on the functional role of this more general genome-wise order. Our results show that there is not only a defined nuclear architecture in interphase but also in metaphase allowing bilateral organization of the two haploid sets of chromosomes. Moreover, evidence is provided for the parental origin of the haploid groupings. This genomic order may now substantially change the landscape of genetics, for example, by upgrading the tools of diagnosis (Chaudhuri et al. 2008) and the understanding of genetic mechanisms like epigenetics or codominance of the two alleles of a gene (Chaudhuri et al. 2005; Chaudhuri and Walther 2013). 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.
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P-GENETIC COUNSELLING / GENETIC EDUCATION / GENETIC SERVICES / PUBLIC POLICY

P-Counse-140

Genetic Counseling in infertility is a significant challenge which needs more attention

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OBJECTIVE
Acquiring information on consanguinity genetic history by responding to questions by infertile couples.
STUDY DESIGN
We surveyed 438 couples who were referred to Shiraz and Behbahan infertility centers (Iran)
RESULTS
97.02% of participants never referred for genetic counseling and 2.98% of reported that they had at least a genetic counseling meeting for consanguineous marriage. Consanguineous marriages were more common in rural areas. Moreover, most participants (96/33%) reported never have done any kind genetic test.
CONCLUSION
These data, the first middle east study (of which we are aware) to investigate genetic background of a sample population of infertile couples. Having more close collaboration with genetic centers in regard of genetic counseling and genetic tests by health providers such urologists and gynecologists at infertility centers is strongly recommended to inform couples about risks, benefits, and possible outcomes by using genetic testing through genetic counseling.

P-Counse-141

NGS-based clinical utility gene cards: Disease-specific guidelines for the diagnostic use of Next generation sequencing

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As part of the EuroGentest project, a Coordination Action funded by the European Commission for harmonizing genetic testing across Europe, a group of experts has authored guidelines on the diagnostic use of next generation sequencing (NGS). This document (http://www.eurogentest.org/fileadmin/templates/eugt/pdf/NGS_Guidelines/EuroGentest_NGS_guidelines_2014_-_final_draft_02-12-2014_v2.pdf) gives a detailed description and discussion of the most important aspects connected to diagnostic NGS: clinical utility, diagnostic yield and rating schemes, counselling issues, implications of different types of NGS-based tests and secondary findings. Also included is technical information on validation and quality parameters as well as reporting and the distinction between research and diagnostics.

In parallel, as part of the EuroGentest Clinical Utility Gene Card (CUGC) initiative, we have collected data on publicly or commercially available NGS panels regarding tested genes, associated diseases and cross-referencing to OMIM and Orphanet was put together and made available online (https://eurogentest.eshg.org/index.php?id=668.). By comparing tested genes between different providers, wide variation among available NGS panels for one and the same disease group was revealed. In order to guide evidence based decision making on essential and optional genes to be tested for, expert consensus is required. Due to their compact and concise format the clinical utility gene cards, disease- or disease group-specific guidelines, regularly published as a section of the European Journal of Human Genetics, were adapted in order to include NGS-based diagnostics. This novel CUGC format will be presented here.

All published CUGCs are annually revised, and a similar procedure is planned for the EuroGentest NGS-based CUGCs as well. So far we have identified 33 laboratories having launched a total of 1127 clinical NGS test panels covering 2399 genes. We continue to encourage experts and NGS providers to contact us regarding guideline establishment and entry in the database, and to contribute to expanding the encyclopedia of NGS-based CUGCs.

P-Counse-142

Etiological diagnosis in children with intellectual disability: Impact on quality of life of their families.

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Background: Intellectual disability (ID) or developmental delay (DD) occurs in 2-3% of all children. Every year, 200-250 children are evaluated for the cause of their ID/DD in our outpatient centers. It has been shown that parents of a child with ID/DD have increased stress levels combined with decreased quality of life. Uncertainty about etiology has been shown to be associated with psychoemotional burden for mothers of children with ID/DD. However, there is no prospective study on the impact of an etiological diagnosis on quality of life (QoL) of parents, so far.

Hypothesis: Etiological diagnosis of ID/DD is associated with improvement of QoL, and social/medical supply in the families.

Methods: In the context of an ongoing prospective study, questionnaire data were assessed from parents presenting their child with ID/DD for evaluation in the neuro-genetic consultation. Family-related QoL was assessed with the family-related life quality questionnaire FLQ. Mental and physical health were assessed by the German version of SF-12 health survey. A questionnaire on qualitative aspects was specifically designed for this study. The study includes three measurements: before the genetic evaluation (T1), immediately after receiving the genetic test results of the neuro-genetic evaluation (T2) and one year later (T3). The primary endpoint for the main question will be the change of QoL (FLQ) between T1 and T3. Descriptive data of participants from T1 are presented and compared with data of a normative sample via comparison of means by t-test analysis. P values < 0.05 were considered significant in descriptive manner.

Results: Valid data of N = 111 families with a child with ID/DD (59% males) were available. Age of children at T1 ranged from 4 to 241 months (median: 54 months). ID/DD was estimated "severe" by the parents in 51,4 %. 98 % of parents considered an etiological diagnosis of high relevance, 82% expected emotional relief, 32,4 % indicated that family planning depends considerably on the recurrence risk. Parents who estimated their child's development as "severely delayed" report lower QoL in all three scales and the total value of the FLQ compared to parents who estimated the delay less severe. Physical health (SF-12) of parents in our study showed no difference to the German normative sample but mental health (SF-12) was significantly lower than in the normative sample.

Conclusion: Etiological diagnosis is considered of high value for the large majority of the parents of children with ID/DD in this prospective study. In detail, parents seem to expect emotional relief and relevant information with regard to family planning.

Preliminary analyses of our prospective study underscore the emotional burden of parents who do not know the etiology of their child's ID/DD. Ongoing research will examine the questions whether QoL can be improved by a diagnosis and whether parents with an etiological explanation will report improvements of social/medical support.

P-Counse-143

First outcomes of the European Network of Imprinting Disorders – EUCID.net: Common nomenclatures and first steps towards harmonized diagnostic procedures

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Imprinting disorders (IDs) are a group of rare congenital diseases affecting growth, development and metabolism with a lifelong impact on patients´ quality of life. Efforts to elucidate the aetiology of IDs have been fragmented across Europe and standardisation of diagnostic and clinical management was lacking. In 2013, our Action, supported by the European COST programme (BM1208), has drawn together researchers, clinicians, SMEs and patients organisations of the known human IDs in an interdisciplinary pan-European network for Human Congenital IDs (EUCID.net), working to advance understanding of the pathophysiology with the major aim of translating this knowledge to improvement of diagnostic and clinical management for the benefit of the patients and their families. The ID network currently consists of 47 groups from 22 countries. After 1.5 years, we are able to report on the first outcomes of our cooperation: We suggest a common nomenclature of IDs (e.g. Temple syndrome for the formerly upd(14)mat syndrome) and overlapping molecular findings (e.g. multilocus imprinting disturbances). A standardised nomenclature for imprinted loci, CpGs and aberrant methylation patterns is currently in progress in cooperation with HGV. For a harmonized patient

recording, a common HPO based questionnaire has been developed. Our Action initiated a EMQN quality assessment scheme for Silver-Russell and Beckwith-Wiedemann syndrome, leading to the creation of standard operation procedures. The first consensus on diagnosis and therapy of Silver-Russell syndromes is expected for October 2015, consensus papers for Beckwith-Wiedemann syndrome and pseudohypoparathyroidism Ib will follow in 2016 and 2017. The network decided to use LOVD as a common database to make the data compatible and useful as a springboard for collective research initiatives. After more than a year, the active networking between the groups is documented by a growing number of joint publications, a mutual staff exchange by short term scientific missions, and stimulating training schools. All relevant outcomes are available on the website of EUCID.net (www.imprinting-disorders.eu), and we encourage all colleagues working on imprinting disorders to contact us and to contribute their knowledge and experiences to our Action.

P-Counse-144

se-atlas – a Project to improve the Search for Experts for Rare Diseases

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Introduction

The information platform se-atlas (www.se-atlas.de) provides help to patients, their relatives and doctors to find the right specialists for specific rare diseases. Most of the diseases have genetic origins. se-atlas includes information on expert centers for rare diseases, genetic counseling clinics, and patient organizations in a cartographic way or in a list format. The project is financed by the German Federal Ministry of Health and started in June 2013. The project's primary objectives are to steadily increase the data set, to ensure its quality and to make the search more efficient and user friendly. A web interface to the Orphanet information platform guarantees a continuous data synchronization. Patient organizations as well as expert centers can register themselves and update their data.

A challenging topic in the project is to develop a solution which allows the users to find the individually most relevant entries. For this the entries have to be classified with the corresponding rare diseases. For realizing this process in a user-friendly way, an assisting wizard has been designed and realized. It is based on the Orphanet classification.

Methods

Since July 2014 a weekly updated version of the se-atlas system is accessible for project partners, the consulting committee and dedicated test persons. Altogether over 70 login data have been sent out.

For enabling a target group-appropriate development, the feedback of stakeholders representing several domains has been gathered. In most cases the test users have been interviewed. To analyze the users' behavior, tasks have been assigned to test users and the execution has been monitored. To reconstruct the behavior, the users were asked to think aloud.

Results

The test system was very helpful during development. Received feedback was directly implemented. The test system was updated regularly. It has become evident that Orphanet classification was known to most experts in rare diseases, but there was a lack of knowledge about its structure and thus to its impact on the search functionality.

The test users often took advantage of the possibility to enter specific diseases including ICD-10-GM or Orphanet code before the selection of the top categories. Two different behaviors were observed. Some users tend to choose many diseases, others rather favored a general selection of disease groups.

Discussion

Testing has shown that se-atlas provides a good solution for the representation of expert centers for rare diseases, genetic counseling clinics, and patient organizations. The classification wizard allows the registering bodies and patient organizations to classify themselves and helps to get an overview of the Orphanet classification. So users can better understand when and why their searches were successful or not.

However, first experiences also show that the classification is considered by users to be very cumbersome. It is possible that this will affect the acceptance of the system.

P-Counse-145

Structure and utilization of genetic counselling services in Germany: outcomes of the GenBIn -Study BIn

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Background: Until recently, nationwide empirical data on the structure of genetic counselling services and utilization of these services were not available for Germany. This lack impacts an informed evaluation of changes and developments of counselling service structures and utilization patterns incurred by the enactment of the Gendiagnostikgesetz(GenDG)and the subsequent implementation of guidelines for the provision of genetic counselling services by the Gendiagnostikkommission(GEKO)in 2012. Objectives: To create a data bank that allows for:(i)comparing the status of counselling service structures and utilization before the implementation of the GEKO guidelines with structural developments and evolving utilization patterns of services after implementation and for

(ii)assessing structural changes and utilization patterns of counselling services in Germany since the implementation of the GEKO-guidelines.

Methods: A retrospective empirical study to describe genetic counselling service structures and utilization patterns in 2011 via a standardized documentation format developed and piloted in collaboration with geneticists/genetic counsellors. The format addresses organizational structures, referral pathways, case management, workload, timelines and initiated diagnostic procedures. 33 genetic counselling centres based at universities and 78 counselling centres in private practice (Niederlassung) were asked to participate. Each centre was to document up to 100 counselling cases seen in 2011, selected by defined criteria. Results: 55%(n=18)of the university based centres and 22%(n-17) of private practice centres submitted data in time, totalling a documentation of 2092 counselling cases. In 2011 significant differences were found between university based centres and private practice centres in regard to (i) referal of cases(83% of all cases in private practice were referred by obstetricians/ gynaecologists(OB&GYN) as compared to 32% of all cases counselled at university centres); (ii)indication for referral by OB/GYN(most cases referred to private practice asked for PND counselling, whereas most cases referred to university based centres asked for counselling for hereditary cancer risks),(iii) the total spectrum of cases counselled (university centres counsel more cases with counsellees or family members affected by diseases/disorders). This results in different reported workloads, waiting times and timelines for case management(higher/longer at university based centres). Discussion: The GenBIn-data base provides a baseline for assessing utilization patterns of genetic counselling services and evolving service structures in Germany after the implementation of the GEKO guidelines. The data will facilitate an informed assessment of the impact of the GenDG on the provision of genetic counselling services. This will be provided by the GenBIn follow-up study. (Supported by the German Federal Ministry of Health, grant number: IIA5-2513-FSB-203)

P-Counse-146

Continuity in the History of Human Genetics in Germany?

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„Human Genetics is both a fundamental and an applied science.“ Like F. Vogel and A. Motulsky stated in 1986, in the history of Human Genetics in Germany both aspects are seen. Since the 1960s there was ‘genetics of man’ at the faculty of natural sciences and ‘human genetics’ as a field at the medical faculty, also named ‘medical’ or ‘clinical genetics’. This forced to differentiate between Anthropology and Human Genetics.

“The knowledge of the conditions affected by heredity makes it possible to follow and control their development and fluctuation in the population and to ascertain the behaviour of hereditary diseases down through the ages.” So Tage Kemp at the opening of The 1st International Congress of Human Genetics.

A) In 1945 Human Genetics was not established at German universities and efforts were made to change this. First was established the institute at the university of Goettingen (F. Lenz), followed by Kiel (W. Lehmann), Muenster (O v. Verschuer), but also Berlin (H. Nachtsheim) and Munich (K. Saller). The Wissenschaftsrat (German Science council) stated in 1960 that a chair for Genetics is necessary at every medical faculty. Right afterwards there were set up Heidelberg (F. Vogel), Freiburg (H. Baitsch), Erlangen (G. Koch) and Hamburg (W. Lenz). This was the stimulus for the professionalization of Human Genetics in Germany. Most of the named scientist started working in human genetics (human heredity) before 1945.

B) The first handbooks were English ones translated into German, like those of L. Snyder, translated by W. Lehmann (1955) and C. Stern (1955). The first German monographs were written by O. von Verschuer (1959), F. Vogel (1961) and W. Lenz (1961). There was no difference in content.

C) The field of activity were first paternity tests and genetic counselling. At the beginning the question of payment was unsolved and lack of money was characteristic. The further development was according to that in other countries.

D) German scientists contributed to the International Congresses of Human Genetics and participated. They referred to aspects of human genetics and also of other medical fields like paediatrics, neurology and otolaryngology.

E) The nuclear bombs on Japan and the nuclear testing raised the question of the influence on the genetics of man. Therefore human genetics gained interest of public policy. The first time that results of genetic analysis were reasons for a decision was in the regulations of the abortion law (§ 218) in 1975. Other laws like the Genetic Diagnostic Law (Gendiagnostikgesetz) followed in 2009.

Conclusions:

1. The history before 1945 had nearly no influence, because one main aim of the occupying states was to rebuild the medical services and denazification was realised.
2. There was continuity in content like diagnosis and therapy of hereditary diseases and in person as founders of institutes after 1945.
3. The German scientists of Human Genetics were part of the international scientific community.

P-Counse-147

A retrospective view on External Quality Assessment of Charcot-Marie Tooth disease over 16 years

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Charcot-Marie-Tooth disease (CMT) is a clinically and genetically heterogeneous disorder of the peripheral nervous system. CMT1A is the most frequent autosomal dominantly inherited form caused by a 1.4-Mb tandem duplication in chromosome 17p12. In 1997 the first annual German external quality assessment (EQA) was performed for CMT1A. At this time the molecular genetic analysis was mainly based on 5 methods: RFLP Southern blotting, STR markers, PCR for junction fragments within the CMT1A-REP elements, PFGE and FISH. 10 laboratories in Germany participated. In the year 2000 the scheme was offered to a wider audience with 22 participating laboratories. Only one genotyping error occurred. Since 2008 the European Molecular Genetics Quality Network (EMQN) has organised the EQA scheme. In 2012 the number of participating laboratories increased to 66 from 22 countries (198 reports) representing countries from around the globe.

Methods like FISH, PFGE and Southern blotting have disapeared; PCR, qPCR and MLPA are now used. Next generation sequencing (NGS) allowing simultaneous sequence analysis as well as gene dosage determination is a future perspective.

Currently at least 60 genes are associated with disorders of the peripheral nervous system. Consequently the scheme scope has been extended to sequence analysis of GJB1 (CX32) (CMTX1). In 2012 again only 1 genotyping error occured. The efforts that have been made for an EQA in molecular genetic diagnosis for Charcot-Marie-Tooth disease demonstrate very good laboratory analytical performance. Nevertheless national laws affecting human genetics are different, thus harmonization in political terms (e. g. predictive testing) remains as important task.

P-Counse-148

The new Austrian Reproductive Medicine Act in comparison to Germany and Switzerland

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The new Reproductive Medicine federal law Act of Austria has caused much uproar in different parts of the society. While more conservative parts and the Catholic Church of Austria demand amendments, so do others and claim the new law would still not cover all aspects of modern life. The following abstract portrays the most important points that are likely to come into effect.

Main changes include

1. the legalization of in-vitro-fertilization for lesbian couples;
2. the legalization of in-vitro-fertilization with third-party semen donations;
3. the legalization of egg donation (donors > 18a, recipient <45a);
4. the legalization of semen donation for heterosexual couples (donors > 18a);
5. the legalization of preimplantation diagnostics under certain conditions.

Singles however may still not access these techniques.

Preimplantation diagnostics remains legitimate in case that after three or more tries no pregnancy occurred; three or more medically proven pregnancies resulted in miscarriages caused by a genetic disposition and said genetic disposition may cause a miscarriage or “genetic disorder”. This “genetic disorder” is not specified but has to cause massive brain damage or non-curable persistent pain or render the patient dependent on life support measures; furthermore there must not be a cure for this condition. Gender determination is legal only if the genetic disorder is gender-dependent.

It is obvious that these changes will have an impact on the current situation of reproductive medicine and genetics in Austria, making it one of the most progressive countries in this regard in Europe. The new Austrian Reproductive Medicine Act will come into effect on April 1st, 2015.

In Germany, the new „Embryonenschutzgesetzes“ (embryo protection law) passed Bundestag in 2011, allowing PID in case a parent suffering from a genetic disorder that may cause a miscarriage or stillbirth. A medical and psychosocial counselling and a written consent of the mother are mandatory; the PID may only be carried out in a special center and after an ethics commission approved.

In Switzerland, PID has been outlawed since a change of the Reproductive Medicine Act in 2001. As of 2014, parliament has met to discuss circumstances under which it could be allowed in the future.

All in all, the germanophone countries of Europe portray the changes of thinking that have occurred in the last twenty years and it is likely that the legal situation will eventually become more liberal in the years to come.

P-Counse-149

Human Genetics in Medical Education at the Medical University of Graz

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Medical Genetics is one of the fastest changing and expanding fields in modern biosciences, having changed drastically from its beginnings in the 1960s. After years of pettiness, training and education in human genetics is nowadays an integral part of every modern and advanced medical curriculum.

The new curriculum for human medicine of the Medical University of Graz was established in 2004 and revised in 2014. In its core, it is similar to German “Reformstudiengänge” and characterized through its combination of clinical and pre-clinical modules. While in the first 2 years mostly basics in the field of classic natural sciences are taught (e.g. physics, chemistry, anatomy, physiology, pathology), the following 3 years focus on the clinical training. After that, all students have to complete their practical year in order to graduate.

The implementation of this new curriculum also resulted in new learning objectives in the field of human genetics which are compulsory for all students to learn. At the Medical University of Graz, most of the teaching is conducted by the Institute of Human Genetics albeit some of the clinical lectures are taught by the Universities Children’s Hospital. This secures that the basic genetic principles of the first semester of the study are revised in the eighth.

Apart from the compulsory teaching a special training consisting of 90 teaching units of 45 minutes each is offered for students interested in molecular human genetics, its research and clinical use. Topics covered include NGS, linkage analysis, single cell analysis, cardio- and oncogenetics and genetic counselling.

Furthermore, general clinical traineeships and special traineeships for 6th-year-students are offered and sought-after at the Institute of Human Genetics and every year the number of diploma theses completed grows. Special electives also deal with questions of ethics in genetics and reproductive medicine and complement the teaching of Genetics at this university. In addition, doctoral schools and lectures allow both physicians and natural science graduates to deepen their knowledge of this field and obtain a PhD.

P-MONOGENIC DISEASE - FORM GENE IDENTIFICATION TO PATHOMECHANISM

P-MonoG-150

Two cases with acrodysostosis due to novel point mutations of the PDE4D gene

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The rare congenital disorder acrodysostosis (OMIM #614613) is a skeletal dysplasia with an autosomal dominant mode of inheritance, characterized by peripheral dysostosis, midface hypoplasia, short stature, accelerated bone age and global developmental delay. Recent work has shown that mutations in PDE4D and PRKAR1A, both of which play a role in the cAMP pathway, can cause acrodysostosis with or without hormone resistance.

We report on two unrelated cases of German origin with acrodysostosis and mutations in PDE4D gene. Patient 1 is a male with a missense mutation in PDE4D inherited from his affected mother. He is heterozygous for the mutation c.91A>G on exon 8 (p.Ile209Val), which has been identified by analyzing the data from our 2742 Mendelian disease causing Next Generation Sequencing (NGS) gene panel in the context of the phenotype using our web-based tool Phenotypic Interpretation of eXomes (PhenIX, http://compbio.charite.de/PhenIX/). Both affected individuals of this family show a phenotype of intellectual disabilitiy, midface hypoplasia and typical dysostosis of hand bones.

Patient 2 is a male with short metacarpals and phalanges, cone shaped epiphyses, midface hypoplasia and developmental delay and is heterozygous for the mutation c.2030A>G (p.Tyr667Cys) in exon 15. The mutation was identified as de novo by direct Sanger sequencing of PDE4D.

We compare the phenotypes of our two families carrying novel mutations affecting the PDE4D gene with the clinical data of currently available publications.

P-MonoG-151

Methyltransferases and Intellectual disability – an NGS-gene-panel approach

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We recently described the characterization of the Methyltransferase-like 23 gene METTL23 for mild non-syndromic autosomal recessive intellectual disability (ID) in two unrelated families, one from Austria, the other from Pakistan. In the Austrian family we identified a homozygous 5bp frameshifting deletion (c.281_285delAAGAT; p. (Gln94Hisfs*6)), whereas in the consanguineous Pakistani family the nonsense mutation c.397C>T; p.(Gln133*) was detected. Both changes lead to truncation of the METTL23 protein, which disrupts the predicted catalytic domain and alters the cellular localization. 3D-modelling of the protein indicates that METTL23 is strongly predicted to function as an S-adenosyl-methionine (SAM)-dependent methyltransferase and possibly to methylate heat shock proteins (strong association shown by expression analysis).

A number of methyltransferases have been described recently in association with intellectual disability, e.g. NSUN2 and SETD5. The biggest subgroup of the human methyltransferasome is the seven-β-strand subgroup. These enzymes methylate a great variety of different substrates like tRNAs, rRNA, mRNA caps and miRNA; they also represent a very interesting group of enzymes for possible candidate genes that affect brain development and function, as recent publications show. Kleefstra syndrome (EHMT1), Kabuki syndrome 2 (KMD6A) and cerebral creatine deficiency syndrome 2 (GAMT) are examples of syndromes with ID caused by alterations in genes involved in the methylation process.

To investigate this enzyme family further, we designed a HaloPlex ™ Target Enrichment System (Agilent) for analysis of the coding plus 10bp flanking regions of 145 methyltransferase genes. We included the following

genes in the panel: all methyltransferase-like METTL genes, those seven-β-strand methyltransferases with at least evidence at transcript level and enzymes with confirmed functionality out of the SET-motif-, membrane- and homocysteine-groups of methyltransferases. For sequencing we used Illumina MiSeq V3 reagent and the Illumina MiSeq platform. We analyzed 48 samples from patients with mild to severe ID where no genetic cause had been found. From the detected 5693 variations, those with allele frequencies higher than 10-5 were excluded (ExAC Browser, Broad Institute). The protein prediction programs PROVEAN and SIFT (J. Craig Venter Institute) were used to further narrow down the candidate variants. The 14 DNA variants that remain after this filtering procedure are found in 14 candidate genes in 12 patients. We are currently further investigating these variations to elucidate their possible pathogenic role in the etiology of ID. In sum, the fact that disruption of the methyltransferase METTL23 causes non-syndromic mild autosomal ID supports the importance of methylation processes for brain function and development and suggests the need for further investigations of methyltransferases using next-generation sequencing techniques.

P-MonoG-152

TALENs, fast and effective mutagenesis tool to study dominant de novo mutations: the WDR45 example

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Increasing Trio sequencing for intellectual disability delivers more and more potentially disease causing de novo DNA variants. This situation necessitates an effective validation tool. Here we present an effective genome editing approach based on embryo microinjection of TALENs. As a test case we chose to generate a mouse model for X-linked dominant neurodegeneration caused by de novo loss-of-function mutations in WDR45. In humans, the course of the disease is two-staged with developmental delay and intellectual disability in childhood and a second phase of rapid neurological deterioration characterized by parkinsonism and dementia occurring in adolescence or early adulthood. At this time, neuroimaging findings are characteristic with hypointense signals in substantia nigra and globus pallidus. After microinjection of TALEN mRNA surviving mouse embryos have been transferred in groups of 10 into the oviducts of 3 pseudopregnant females resulting in 19% mutated founders (5/26). Mutations are deletions between 10 and 57bp in length with a predicted frameshift in 80% of mutations. From the in situ design and delivery of the TALENs (~1month), over in vitro testing in cell culture to the in vivo injection into embryo's (~2months) until the genotyping of the founder mutants it only took 4 months. For further breeding we chose a Knock-Out mouse harboring a 20bp deletion in Exon 2 of Wdr45. Investigation of nine month old male brains (n=8) revealed numerous degenerated neurons and large axonal spheroids, clear signs of neurodegeneration in medulla oblongata, cerebral cortex and thalamus, exclusively found in Wdr45 mutant animals. Furthermore, a neurological behavioral screen of 12 month old animals hints towards motor impairment demonstrating the effectiveness of the TALEN approach to functionally validate de novo mutations.

P-MonoG-153

A C-terminal nonsense mutation exposes the DFNB84 gene, PTPRQ, as a candidate gene for autosomal dominant non-syndromic hearing loss

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Hearing loss is the most common sensory deficit and genetically extremely heterogeneous. We have conducted targeted next-generation sequencing of 66 known deafness genes in a patient from a 4-generation family with six family members affected by non-syndromic mild to severe sensorineural hearing loss. Onset of mid to high frequency hearing impairment ranged from early childhood to the 2nd decade of life. We identified a heterozygous nonsense mutation, c.6881G>A (p.Trp2294*), in the last coding exon of the PTPRQ gene (NM_001145026.1). Biallelic PTPRQ mutations are a known cause of autosomal recessive deafness with vestibular dysfunction (DFNB84), but mutations in this gene have so far not been linked to autosomal dominant non-syndromic hearing loss (ADNSHL). NGS and Sanger sequencing of all PTPRQ exons (including previously described alternatively spliced 5' exons) did not identify a second mutation. Furthermore, NGS of

all N-scan-predicted potential coding regions of a putative “extended” PTPRQ transcript did not detect another pathogenic aberration. The p.Trp2294* mutation co-segregated with hearing loss in this large family. It was also found in a 2-year-old family member in whom results of audiologic assessment at different points of time fluctuated between (borderline) normal hearing and mild hearing impairment. In view of the very variable age of onset of hearing loss in this family, this boy may develop hearing loss in the future. All previously reported truncating PTPRQ mutations are associated with autosomal recessive NSHL, and none of them localizes in the last exon. In contrast to these mutations, mRNA carrying the c.6881G>A (p.Trp2294*) mutation in the gene’s last exon could escape nonsense-mediated decay. The PTPRQ protein resulting from this allele would lack only the six C-terminal residues and could exert a dominant-negative effect, a possible explanation for an allelic form of deafness that is clinically and genetically distinct from DFNB84.

P-MonoG-154

HIVEP2: A New Causative Gene for Intellectual Disability?

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Intellectual disability (ID) has an estimated prevalence of 2-3%. Due to its extreme heterogeneity, the genetic basis of ID remains elusive in many cases. Recently, whole exome sequencing (WES) studies revealed that a large proportion of sporadic cases are caused by de novo gene mutations.

Applying WES to ID patients at different centers, we identified three patients carrying de novo mutations in HIVEP2 (human immunodeficiency virus type I enhancer binding protein 2). Two of the mutations are nonsense mutations and one a 1 bp deletion resulting in a preliminary stop codon. In silico prediction programs (SIFT/ Mutation Taster) predict nonsense mediated mRNA decay as the consequence of all three mutations, pointing to a loss of function and haploinsufficiency as the common disease-causing mechanism of the three HIVEP2 mutations. None of the three mutations was present in the Exome Variant Server or in 2500 in house exomes.

All three patients presented with moderate ID, muscular hypotonia, mild dysmorphic signs and variable mild structural brain anomalies. Growth parameters were in the normal range except for slight microcephaly at birth in one patient. Two of the patients presented with behavioral anomalies including hyperactivity and aggression.

HIVEP2, also known as Schnurri-2, belongs to a family of zinc finger-containing transcriptional proteins involved in growth and development. Many of the genes regulated by HIVEP2 are implicated in brain development, e.g. SSTR-2, c-Myc and several genes of the NF-□B pathway. HIVEP2- knockout mice have been shown to exhibit several behavioral features suggestive of schizophrenia such as working memory deficits, as well as increased anxiety and hyperactivity. Based on the genotype-phenotype correlation and the previously published functional data we propose HIVEP2 as a novel ID gene candidate gene.

P-MonoG-155

Recurrent ALDH18A1 de novo mutations cause a novel form of autosomal dominant cutis laxa with progeroid features

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Syndromes with cutis laxa are a heterogeneous group of diseases either inherited in an autosomal recessive, autosomal dominant or X-chromosomal fashion. The progeroid forms resembling De Barsy syndrome (autosomal recessive cutis laxa type 3; ARCL3), are due to biallelic mutations in the genes PYCR1 and ALDH18A1 encoding two functionally related mitochondrial proteins involved in proline de novo synthesis.

In the present study we report on five unrelated individuals from different countries who fulfilled the major diagnostic criteria for ARCL3. Using conventional and gene panel sequencing we identified in all affected individuals heterozygous mutations in the ALDH18A1 gene, encoding for pyrrolin-5-carboxylate synthase 1 (P5CS), affecting the highly conserved amino acid p.Arg138. Further sequencing and copy number investigations revealed no second mutation in this gene. In four families in which DNA from the parents was available we proved a de novo origin of these mutations that therefore have to be regarded as the cause of the disease. Using patient derived fibroblasts and transient overexpression we showed that the mutated protein is stable and able to bind P5CS-WT molecules. While the mutated protein still targeted to mitochondria we found a significant alteration of its distribution within the mitochondrial network. Furthermore, using native gel electrophoresis, we found that the protein complex containing P5CS-p.Arg138Trp was smaller in size in comparison to the complex obtained from control cells, indicating an alteration of its composition or modification.

In summary, we showed that recurrent de novo mutations in ALDH18A1 are causative for a novel autosomal dominant form of cutis laxa with progeroid features. Our results provide new insights into the etiology of cutis laxa diseases and have potential impact on the diagnostics and clinical management of patients suffering from cutis laxa syndromes.

P-MonoG-156

Chd7, the protein affected in CHARGE syndrome, regulates the neural crest cell guidance molecule Sema3a.

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Heterozygous CHD7 (chromodomain helicase DNA binding protein) mutations are the underlying cause of CHARGE syndrome (MIM 214800), a congenital multiple malformation disorder. Coloboma, heart defects, atresia of the choanae, retarded growth and development, genital hypoplasia, ear anomalies and deafness are features mostly observed, as well as hypoplasia of the semicircular canals, facial nerve palsy, cleft lip and palate and tracheoesophageal fistula. To further characterize the pathogenesis of CHARGE syndrome we performed a genome-wide microarray expression analysis on wild-type and Chd7 deficient (Chd7Whi/+ and Chd7Whi/Whi) mouse embryos at day 9.5 (onset of neural crest cell migration). We identified a misregulation of several genes involved in neural crest cell migration, guidance and ectoderm- to- neural crest cell interactions, including the neural crest cell guidance molecule Sema3A. Our data suggest an evolutionary conservation of a Chd7-Sema3a regulatory loop during mouse and Xenopus development, because loss of function of Chd7 in Xenopus leads also to a reduction of Sema3a expression. Furthermore, co-injection of

Sema3A RNA can partially rescue the Xenopus Chd7 knockout phenotype. Therefore, we hypothesize that Sema3a might be an important effector in the pathogenesis of CHARGE syndrome. In summary, we identified the neural crest cell guidance molecule Sema3a as a Chd7 target gene and our data provide evidence that Sema3a expression is positively regulated by Chd7. Future research will be directed at analyzing the importance of this regulatory loop in NC development as well as the pathogenesis of CHARGE syndrome.

P-MonoG-157

Whole Exome Sequencing (WES) in families with left ventricular outflow tract obstruction (LVOTO)

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Congenital heart disease (CHD) is one of the most common birth defects. In the left ventricular outflow tract obstruction (LVOTO) subgroup, encompassing bicuspid aortic valve, aortic stenosis, coarctation of the aorta and hypoplastic left heart syndrome or a combination, the disease burden is among the highest. Although heritability analysis has indicated substantial genetic causation, none of the published loci has been shown to explain a high proportion. To identify candidate genes, we performed whole exome sequencing (WES) in families with a LVOTO phenotype.

25 families have been sampled as part of a nation-wide study conducted by the German Competence Network for Congenital Heart Disease. We only examined families with a minimum number of two cases and at least one of the affected individuals presenting with a LVOTO phenotype. WES was performed in cases as well as two informative healthy relatives per family. To identify mutations in plausible candidate genes we developed a prioritization workflow based on rare SNVs, INDELs and CNVs, which was taking into account the relevant inheritance mode as well as CHD-specific gene prioritization information.

Although depending on the number of affected and sequenced samples, as well as family relatedness and population structure we obtained on average 1.4 rare autosomal recessive variants (MAF 0.1%) and observed 34.76 dominant inherited variants (MAF 0.1%), not taking reduced penetrance into account. After reevaluation of the family structure we identified several families with dominant and recessive putative variants. Among them was an already known cardiac candidate gene MYH11 discovered in a family with dominant inheritance. In addition, we identified a novel putative recessive candidate gene, CNTROB encoding a centrosomal protein that is required for centriole duplication and cytokinesis, which is inherited in compound heterozygote manner in affected family members and has thus far not been implicated in CHD.

WES in families with LVOTO provides evidence for the role or rare variants in disease pathogenesis. Therefore this study holds the potential to identify putative novel variants and might contribute to a better genotype-phenotype correlation in the future.

P-MonoG-158

DYNC2LI1 mutations broaden the clinical spectrum of dynein-2 defects

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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 (IFT), components of the cilium, the basal body, or the centrosome. Many of the associated phenotypes include brain malformations, polydactyly, kidney cysts, retinal degeneration, and skeletal abnormalities. Today, mutations in 14 different genes have been identified to be causative for skeletal ciliopathies. Because of the genetic heterogeneity of skeletal dysplasias, disrupted proteins affect either the dynein motor (DYNC2H1), or components of the IFT transport complexes (WDR34, WDR60 and others) or the basal body region (NEK1, EVC, EVC2).

The phenotypic spectrum of skeletal ciliopathies (e.g. short rib-polydactyly syndromes [SRPS type I-V], asphyxiating thoracic dystrophy [ATD/Jeune] and Ellis-van Crefeld syndrome [EVC]) includes short ribs,

narrow thorax, short stature, polydactyly and other skeletal abnormalities. In a patient of non-consanguineous parents with an intermediate phenotype between Jeune and EVC we performed whole exome sequencing. Variants were selected based on potential ciliary function as identified in a yeast two-hybrid screen with NEK1, a basal body protein involved in SRPSII. We identified compound heterozygous nonsense (p.R208X) and missense (p.T221I) mutations in DYNC2LI1 - dynein, cytoplasmic 2, light intermediate chain 1 - segregating in the family. DYNC2LI1 is ubiquitously expressed and is part of the dynein-2 complex important for retrograde IFT. Recently, mutations in other components of the dynein-2 complex (DYNC2H1, WDR34 and WDR60) have been identified in patients with a overlapping clinical phenotype. The hypothetical DYNC2LI1 protein caused by the nonsense mutation lacks the coiled-coil domain involved in protein interaction and dimerization. Modelling of the nucleoside triphosphate hydrolase domain revealed that p.T221I is close to the nucleoside binding site. The exchange to isoleucine at this position might cause steric clashes and decrease the site of the nucleoside binding pocket. Therefore, the mutation is expected to impair the hydrolase function of DYNC2LI1. As DYNC2LI1 is part of the dynein-2 complex and defects of other components of this complex have been associated with alterations of cilia length and morphology we performed further immunofluorescence analysis of DYNC2LI1 depleted cells. We identified a significantly reduced cilia length in DYNC2LI1 knockdown cells proposed to affect cilia function. In addition, depletion of DYNC2LI1 induced altered cilia morphology with broadened ciliary tip as reported in other retrograde IFT defects. Our results expand the clinical spectrum of ciliopathies caused by mutations in the light intermediate chain of the dynein-2 complex.

P-MonoG-159

Gene Panel Sequencing for Low Bone Mass Disorders Reveals High Detection Rate in Osteogenesis Imperfecta and Frequent Monogenic Causes in Early Onset Osteoporosis

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Disorders with low bone mass leading to increased fracture rates have their origin in skeletal development and/or homeostasis. While a large part of the infantile or juvenile onset cases can be readily diagnosed as osteogenesis imperfecta (OI) in patients with later onset often no genetic disease is suspected. In order to offer comprehensive genetic testing at an affordable cost we developed a next-generation sequencing-based gene panel comprising 72 genes relevant for disorders with abnormal bone mass. Technically the sequencing results reliably showed a coverage of >20X in 98% of the target region. Bioinformatic evaluation was done using the PhenIX and GeneTalk platforms. We here present exemplary genotyping results of 30 patients of Indian origin with different forms of OI and 50 German osteoporosis patients (21 women, 29 men) with age of onset below 50 in women and 60 in men. In all OI cases the genetic cause was identified with 49% mutations in COL1A1 and COL1A2, 10% mutations in WNT1, followed by other rare recessive forms. In our cohort of osteoporosis patients we unexpectedly identified bona fide mutations and highly pathogenic variants not found in databases in more than 25% of the cases, thus proving the relevance of the rare-among-the-common hypothesis. Here the most frequently mutated genes were LRP5, COL1A1, RUNX2, and WNT1. In these cases genetic diagnostics can radically change the interpretation of disease phenotype, prognosis and recurrence risk and may in the future provide the basis for individualized therapies. Besides the established OI genes and LRP5 our results demonstrate the high relevance of WNT1 for all forms of low bone mass disorders.

P-MonoG-160

Investigation of GRIN2A in common epilepsy phenotypes

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Recently, mutations and deletions in GRIN2A have been identified to predispose to benign and severe idiopathic focal epilepsies (IFE), revealing a higher incidence of GRIN2A alterations (up to 20%) among the more severe phenotypes. The present study aimed to explore the phenotypic boundaries of GRIN2A mutations by investigating patients with one of the two most common epilepsy syndromes: i) idiopathic generalized epilepsy (IGE) or ii)temporal lobe epilepsy (TLE). Whole exome sequencing data of 238 patients with IGE as well as Sanger sequencing data of 84 patients with TLE were evaluated for GRIN2A sequence alterations. Additionally, two independent cohorts comprising 1469 IGE and 330 TLE patients were screened for structural deletions (>40 kb) involving GRIN2A. Apart from a presumably benign, non-segregating variant in a patient with juvenile absence epilepsy, neither mutations nor deletions were detected in either cohort. These findings suggest that mutations in GRIN2A preferentially are involved in pediatric IFE and do not contribute significantly to other frequent epilepsy phenotypes, such as TLE or IGE.

P-MonoG-161

Targeted next generation sequencing for molecular diagnostics of hereditary hearing loss

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Hereditary hearing loss (HL) is the most common sensory disorder, affecting approximately 1:700 newborns worldwide. Genetic etiology of hereditary HL is estimated in up to 60% of the cases occurring in developed countries. More than 120 causative genes have been identified up to now. We present the results of the first year of targeted next-generation sequencing diagnostics in HL patients at our Institute. We used one of two gene-panels covering either 119 or 152 deafness-associated genes to analyze a mixed cohort of 48 individuals with syndromic (N = 19) or nonsyndromic (N = 29) hearing loss. We identified 19 likely pathogenic or pathogenic mutations, 11 missense, 3 nonsense and 5 indel variants, in 16 patients. From these 16 patients, 7 had syndromic HL (7/19, 37%), including 2 cases with Usher syndrome, 1 case with Waardenburg syndrome, 1 case with Branchio-oto-renal syndrome, 1 case with Alport syndrome and 2 cases with unclear syndromes, and 9 had nonsyndromic HL (9/29, 31%). Five other variants were detected that were classified as variants of unknown clinical significance. Overmore, our findings support recent evidence that disqualifies mutations in the MYO1A gene that were previously associated with DFNA48 as a cause of hearing loss. In summary, our results demonstrate that targeted next generation sequencing offers an efficient, reliable and cost-effective tool for the molecular diagnosis of syndromic and nonsyndromic HL.

P-MonoG-162

Confirmation of DIAPH1 as the gene underlying autosomal dominant hearing loss DFNA1 and localization of its protein in the organ of Corti

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Non-syndromic sensorineural hearing loss (NSHL) is a genetically extremely heterogeneous condition. In about 20% of cases, NSHL is inherited in an autosomal dominant manner (ADNSHL). Since the identification of a splice site mutation in a family mapped to the DFNA1 locus on chromosome 5q31 in 1997, there has been no independent convincing report of DIAPH1 mutations causing deafness. DIAPH1 is assumed to play a role in the polymerization of actin, the major component of the cytoskeleton of hair cells. However, DIAPH1 localization in the organ of Corti has not yet been determined. By targeted next-generation sequencing of a panel comprising 66 genes associated mostly with non-syndromic deafness, we have identified a heterozygous 2-basepair deletion in DIAPH1, c.3624_3625delAG (p.Ala1210Serfs*31), that co-segregates with ADNSHL in a three-generation German family with five affected individuals. Like the mutation of the original DFNA1 family, this frameshift mutation localizes in exon 27. No mutation was detected in the other deafness genes. Hearing loss in this family was mostly noted between 3 and 4 years of age, but was probably congenital (the youngest patient had failed newborn hearing screening). According to continuous audiologic evaluation of the two youngest patients, hearing impairment initially particularly affected low to mid frequencies and was progressive (from mild to moderate at the age of three months to severe at 8 and 11 years of age), compatible with the phenotype described in the original DFNA1 family. By immunohistochemistry, we found that DIAPH1 is expressed in the organ of Corti, more specifically in the inner pillar cells and at the base of the outer hair cells (likely Deiters cells). Moreover, DIAPH1 was expressed in neuronal structures of the cochlea, such as spiral ganglion neurons and in the distal end (central part) of the cochlear nerve. We confirm DIAPH1 as DFNA1 gene and the distinct auditory phenotype associated with that locus. In view of a recently reported homozygous nonsense mutation in exon 16 of DIAPH1 underlying recessive microcephaly, ADNSHL-causing (truncating) mutations may be confined to the C-terminus of the gene.

P-MonoG-163

UNC80 mutation causes severe intellectual disability with dystrophy. Result of a clinico-exomic board round.

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Both clinical genetics and genome-wide sequencing substantially gain in diagnostic power if they inform each other. For demonstration we report the discovery of a novel syndromic type of intellectual disability in a diagnostic setting that involves a series of more 100 clinical exomes. A 7 year-old boy presented with feeding difficulties, severe dystrophy, hypotonic tetraparesis, but rigid spine and positive Babinski sign, absence of speech development, mildly characteristic facial features (prominent forehead, large ears), and cryptorchidism. NALCN-associated Infantile Hypotonia with Psychomotor Retardation and characteristic Facies (IHPRF, OMIM #615419) was considered as one of the differential diagnoses but was not confirmed by exome data. However, discussion between clinicians and exome analysts directed the attention to a homozygote frameshift mutation of UNC80 whose gene product interacts with the gene product of NALCN. The effect of UNC80 mutation was then confirmed in two non-related patients. While sometimes the information flow between clinicians and exome analysts is simple or unidirectional (e.g., the phenotype indicating a single candidate gene or the exome revealing an incidental diagnosis), genome-wide diagnostics may be as intricate as other elaborate diagnostic procedures thus requiring board rounds that join clinicians and geneticists.

P-MonoG-164

Repeat-primed PCR in myotonic dystrophy type 1 and 2 molecular testing: its usefulness and limitations

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Both genetic types of myotonic dystrophy, DM1 and DM2, are caused by microsatellite expansions. The DM1 associated CTG tract in the DMPK gene is basically a simple repeat, while the CCTG repeat in the CNBP gene, associated with DM2, is a part of a complex repetitive motif (TG)n(TCTG)n(CCTG)n, in which each of the elements are highly polymorphic. Repeat-primed PCR represents one of the commonly used techniques for determination of the presence or absence of expanded DM1/DM2 alleles. However, as the structure of the two repeat motifs is different, there are features of repeat-primed PCR which are unique for each type of DM. This contribution reviews our six year experience with PCR based (conventional and repeat-primed PCR) DM1/DM2 testing in Slovakia. Surrounding sequences and the purity of the repeat motif represent crucial features for the results of repeat-primed PCR applications. As we mentioned above, the DMPK CTG motif is generally pure, however, in rare cases sequence interruptions can be present and can modify both the conventional and repeat-primed PCR results. In opposite, the CNBP CCTG tract is generally interrupted, while uninterrupted alleles are rather rare. Because of the complexity of the CNBP repeat motif and the generally present interruptions in the CCTG part, repeat-primed PCR reaction should be designed in a proper way - it is better to avoid amplification through the (TG)n(TCTG)n part of the motif. Since our results suggest the potential benefit of the simultaneous use of conventional PCR and repeat-primed PCR performed in both directions, we have designed several multiplex reactions to parallelise the required testing reactions. Moreover, fluorescent labelling of both primers in conventional PCR may also improve the accuracy and reliability of the diagnostic assays based on these methods. Although the simultaneous dual-labelled conventional PCR and bidirectional repeat-primed PCR testing approach improves the DM diagnostic procedure it has also limitations which should be kept in mind during assay design and result interpretation. Of course, modifications described in our contribution are not restricted only to DM testing and may also be useful in PCR/repeat-primed PCR testing of several other repeat expansion disorders.

P-MonoG-165

Pathogeneity of ABCB6 in dyschromatosis universalis hereditaria: A novel missense mutation and clinical overlap with Dowling-Degos disease

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Background: Hereditary pigmentary disorders (HPD) are rare and associated with alterations in melanin synthesis, the number of melanocytes as well as in development, transport and transfer of melanosomes. Affected patients present with a heterogeneous spectrum of clinical signs. Hence, it is challenging to provide a precise diagnosis since there may be considerable overlap between currently recognized entities. To date, the diagnosis of pigmentary disorders is usually made by combining clinical symptoms and histological findings. Recently, however, several mutations in different genes have been identified as underlying cause of HPD, with additional genes still awaiting identification.

Methods: In sporadic and familial cases of patients diagnosed with Dowling-Degos disease (DDD) we performed whole exome sequencing to identify causative mutations and novel disease genes.

Results: In two unrelated sporadic cases, we identified a novel missense mutation in the ABCB6 gene, encoding ATP-binding cassette, sub-family B, member 6. Mutations in this gene are known to cause the genodermatosis dyschromatosis universalis hereditaria (DUH).

Conclusions: In both patients studied here, molecular genetic confirmation of presumed DDD had been asked. Interestingly, our findings do not only provide additional support for ABCB6 as a disease gene for DUH but also for the first time suggest a clinical overlap between this disorder and DDD. Currently, mammalian cell culture based experiments are ongoing for further characterization of the new mutation. Identification of new causative mutations for HPD will lead to a better pathogenetic understanding and advance diagnostic

procedures in these diseases by using molecular genetic tools - thereby paving the way for the future development of potential targeted therapies.

P-MonoG-166

Translational control of the major AZFa gene, DDX3Y, and its X homologue in human male germ cells: diagnostic for mature sperm production?

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The major AZFa gene on the human Y chromosome, DDX3Y and its X homologue, DDX3X, are functionally conserved from yeast to men and involved in the translational control of transcripts functional for the nuclear cell cycle control. AZFa deletions including DDX3Y cause a complete absence of male germ cells (i.e. Sertoli cell-only syndrome) but no somatic pathology because the protein is only translated in spermatogonia. The homologous DDX3X protein is however expressed in spermatids and in multiple somatic tissues. Accordingly, we revealed germ cell-specific translational control mechanisms for both gene transcripts functioning during human spermatogenesis (Jaroszynski et al. 2011; Rauschendorf et al. 2011, 2014) and now asked the question whether their impairment might interfere also with mature sperm production?

For this purpose we set up a large clinical database of 150 infertile men with idiopathic azoospermia entering our TESE (testicular sperm extraction) program for mature sperm sampling from their testicular tissue which displayed different germ cell pathologies. With aid of quantitative expression assays for a set of genes identified to be solely expressed during a specific germ cell phase we found out that the amount of DDX3Y protein in spermatogonia is variable and probably controlled by the transcripts 5´UTR extension. In male germ cells DDX3X transcripts also include a long 5´UTR and are processed for polyadenylation mainly in their proximal 3´UTR in contrast to their somatic DDX3X transcript pattern (Rauschendorf et al. 2014). Moreover, a minor fraction of these transcripts contained a long 3´UTR extending ~17kb which subsequently becomes spliced at distinct sites producing six different short 3´UTR splicing variants (Rauschendorf et al. 2014).

We observed a severe impairment of the expression pattern of these DDX3X 3´UTR splicing products in infertile men with a mixed testis atrophy; i.e., with no sperm in their ejaculate but still a low amount in at least some of their testis tubules diagnosed in the testicular biopsy. Our data suggest a functional contribution of DDX3Y to the premeiotic differentiation process of spermatogonia, whereas a constant level of DDX3X expression seems to be required for the postmeiotic spermatid maturation process. Although its impairment seems not to interrupt the postmeiotic human germ cell maturation process itself, it may result in the reduction of mature sperm cells eventually causing azoospermia.

P-MonoG-167

Autosomal dominant congenital spinal muscular atrophy: a recognizable phenotype of BICD2 mutations

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Heterozygous BICD2 gene mutations (OMIM 609797) were recently identified as the genetic cause for autosomal dominant congenital spinal muscular atrophy (DCSMA). BICD2 mutations result in impaired dynein-mediated microtubular transport of motor neurons. The BICD2 phenotype comprises patients with congenital contractures and slowly progressive weakness mainly of the lower limbs. In addition, two families were described with completely different phenotypes, one with late onset (40-65 years) SMA and one with lower limb spastic paraplegia and hyperreflexia starting in adulthood (20-40 years). Until now, 10 families with DCSMA were reported in the literature.

In our study we sought to determine the diagnostic relevance of BICD2 mutations in a selected group of 25 index patients with neurogenic arthrogryposis multiplex congenita (AMC) (n=4), childhood onset autosomal dominant proximal SMA (n=3), and congenital or infantile onset distal motor neuropathy (n=18). In two unrelated patients within the AMC group we identified two new missense mutations that were bioinformatically predicted to be possibly damaging and disease causing. Family 1 with the heterozygous BICD2 mutation c.2515G>A, p.G839R, had patients in two generations with a striking clinical discordance that still remains unexplained, as all three brothers of the first generation, who had a late onset proximal SMA starting at 50-65 years of age, were deceased and not available for BICD2 testing. There were three affected brothers in the 2nd generation (aged 65, 59, and 52 years) who had an identical phenotype of DCSMA with congenital clubfoot deformity and muscular hypoplasia of the lower limbs. Motor development was significantly delayed, walking

was possible and progression was slow but clearly present. In particular shoulder girdle muscles became affected later in life, resulting in a recognizable posture with scapular winging and well preserved lower arm muscles. The youngest brother died suddenly because of a heart attack and underwent necropsy. First neuropathological examination revealed moderate loss of alpha motor neurons in the cervical part of the spinal cord. Microglial cells (CD68) were activated and present in the ventral horn and in other parts of the grey matter of the spinal cord.

Patient 2 was the only affected person with DCSMA in his family. Compared with family 1 he was more severely disabled and underwent operative corrections of knee and hip contractures. When examined at age 16 years, he was only able to walk with splints. The patient carried a de novo heterozygous BICD2 mutation (c.2202G>T, p.K734N) which was not detected in his parents.

To conclude, in our series BICD2 mutations were associated with a distinct phenotype of congenital lower limb contractures and muscular hypoplasia followed by only slow progression of weakness. There is clear evidence of anterior horn cell loss without peripheral or central nervous dysfunction in our patients. Our results give further insights into this rare motor neuron disease.

P-MonoG-168

A family case of hereditary pancreatitis with a novel complex mutation in the PRSS1 gene

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Hereditary pancreatitis is characterized by recurrent episodes of pancreatic inflammation. It progresses from acute (on average by the age of ten years) to chronic pancreatitis (on average by the age of 20 yrs) with irreversible pancreatic changes and an increased risk to develop pancreatic cancer (after the age of 50 yrs). Patients with autosomal dominant pancreatitis often carry mutations in the PRSS1 gene encoding cationic trypsinogen (protease, serine, 1). Gain-of-function mutations lead to an intrapancreatic premature conversion of cationic trypsinogen into active trypsin or to a resistance of trypsin to inactivation by autolysis. The two common mutations p.N29I (c.86A>T) in exon 2 and p.R122H (c.365G>A) in exon 3 account for approx. 90% of the PRSS1 mutations in hereditary pancreatitis.

We report on a pancreatitis family with a novel complex mutation in the PRSS1 gene. The 17-year-old index patient, his 48-year-old affected father as well as his so far asymptomatic 14-year-old sister carry the heterozygous PRSS1 mutation c.[343T>A; 347G>C; 351A>C; 354A>C; 360C>T; 365G>A; 366C>T; 390C>T] which has not yet been described in databases and literature. Since it contains the known pathogenic dinucleotide substitution c.365_366GC>AT which is predicted to lead to the mutation p.R122H on the protein level, we regard the novel complex mutation as pathogenic. Thus, the index patient's sister is very likely to develop pancreatitis later on. A BLAST search with the mutated PRSS1 nucleotides c.343_390 revealed a perfect match in the protease, serine, 3 pseudogene 2 (PRSS3P2). We therefore suppose that the familial complex mutation may be the result of a gene conversion event with PRSS3P2 as sequence donor. Gene conversion in the T cell receptor beta locus harboring the tandemly arrayed trypsinogen genes has been repeatedly postulated as a likely cause of several PRSS1 mutations.

P-MonoG-169

Founder mutation in CCDC23 in Syrian and Pakistani families with autosomal recessive intellectual disability

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Autozygosity mapping in a Syrian consanguineous family with two children with autosomal recessive intellectual disability (ARID) followed by exome sequencing revealed one candidate mutation in CCDC23: c.82C>T: p.Gly28X. A search of the CARID database (consortium of autosomal recessive intellectual disability), curated by our Erlangen group, unveiled that the same variant had been identified by the group of Hans van Bokhoven from Nijmegen in a consanguineous Pakistani family with two children with intellectual disability. The variant is located on the identical haplotype, suggesting a founder mutation. The families in Syria and in Pakistan did not report foreign ancestral origin. The variant is very rare as it was excluded in 372 healthy Syrian adults and it was also absent from the ExAc Browser beta databse but based on historical events, there are several possibilities of a gene flow between Syria and Pakistan.

Detailed clinical examination of all four patients from both families showed mild to severe intellectual disability, microcephaly, and muscular weakness and/or hypotonia. Further, some patients showed chest abnormalities, coordination difficulties, and cerebral atrophy. We performed molecular modelling and found that this premature stop codon in CCDC23 would probably not lead to nonsense mediated decay, but rather delete the second coiled coil domain, and is thus predicted to be deleterious through loss of dimerization of the protein.

It has been reported that the protein encoded by CCDC23 is essential for the secretion of vasohibin-1 that is involved in angiogenesis, also in brain. The pathophysiology is still unclear, but based on the known functions of VASH1 that is produced and secreted by vascular embryonic cells, this may be related to embryonic cells proliferation and migration. CCDC23 probably has additional unknown functions. To further prove the pathogenicity of the variant in CCDC23, we created expression constructs with wild type and mutant CCDC23 in order to measure vasohibin-1 concentration in cell culture medium. We expect this to be reduced when transfecting the cells with the mutant CCDC23.

Taken together, our data indicate that bi-allelic mutations in CCDC23 lead to severe intellectual disability, growth retardation, and muscular weakness. Further families with different mutations are necessary to confirm the pathogenicity and will hopefully allow better ascertaining the phenotype.

P-MonoG-170

Genetic screening for disease-associated mutations in human retinal diseases using whole exome sequencing (WES)

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Monogenic diseases of the retina and vitreous affect approximately 1 in 2000 individuals. They are characterized by tremendous genetic heterogeneity and clinical variability of symptoms involving more than 20 different clinical phenotypes and mutations in more than 200 genes. Clinical manifestations of retinal degenerations (RD) range from mild retinal dysfunctions to severe congenital forms of blindness. A detailed clinical diagnosis and identification of the underlying mutations are crucial for genetic counseling of affected patients and their families, for understanding genotype-phenotype correlations, and developing therapeutic interventions.

We make use of WES and have established a reliable and efficient high-throughput analysis pipeline of next generation sequencing (NGS) data to identify disease-causing mutations in in patients with retinal diseases. In this study, we sequenced 43 cases with different clinical phenotypes, including Retinitis pigmentosa, Leber congenital amaurosis, Stargardt disease, Best macular dystrophy, Bardet-Biedl syndrome and Usher syndrome. Thirty-three of these were isolated/sporadic. In 10 cases, samples from additional family members were available. Our data indicate that this approach enables us to genetically diagnose 53% (n=23) of the patients with mutation(s) in known disease-associated genes. Thus, 46% (n=20) of the remaining cases, which do not carry mutation(s) in a known gene, might lead to the identification of novel disease-associated genes and biological pathways underlying the disease phenotype. A total of 33 mutations were identified in these cases, some of which were found in more than one case. Amongst the identified mutations, 60% (n=20) were previously described in literature while 40% (n=13) are novel. The disease-causing mutations included missense mutations (42%), nonsense (30%), frameshift insertions or deletions (9%) and mutations predicted to interfere with splicing (18%). In conclusion, WES can efficiently and rapidly identify mutations in families affected with different forms of retinal diseases with a high diagnostic accuracy. We identified novel and recurrent mutations in 14 genes. The most frequent mutant alleles were detected in ABCA4 (27%) followed by CEP290, BEST1 and CRB1 (each 12%). Functional studies of the respective mutations are needed to understand the underlying disease mechanisms, which in future might lead to the development of therapeutic approaches.

P-MonoG-171

A patient-specific induced pluripotent stem cell model for intellectual disability caused by ST3GAL3 deficiency

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ST3GAL3 encodes the Golgi enzyme β-galactoside-α2,3-sialyltransferase-III that in humans predominantly forms the sialyl Lewis a (sLea) epitope on proteins. Mutations in this gene were previously identified in patients with non-syndromic as well as a syndromic form of autosomal recessive intellectual disability (ARID). In cellular and biochemical test systems, these mutations were previously shown to cause ER-retention of the Golgi enzyme and drastically impair ST3GAL3 functionality.

To further elucidate the molecular and cellular mechanisms involved in ARID caused by lack of ST3GAL3 function we generated induced pluripotent stem cell (iPSC) lines based on fibroblasts derived from a patient with West Syndrome, who carries a mutation in exon 12 (c.958G>C, p.(Ala320Pro)) of ST3GAL3, as well as a healthy sibling, using lentiviral reprogramming. Genomic integrity of iPSCs was validated by array CGH. After differentiation into cortical neurons, we investigated transcriptional, glycosylational, morphological and developmental alterations of patient and healthy sibling cells at sequential developmental time points by RNASeq, lectin blotting, FACS, and immunocytochemistry. Stem cell and cortical identity of iPSCs and their differentiated progeny were validated by immunocytochemistry and qPCR.

Neuronally differentiated patient-specific iPSCs displayed increased adherence properties compared to the respective healthy sibling derived cells. FACS analysis of neuronally differentiated cells furthermore showed that only about 3% of all patient cells expressed the neurodevelopmental marker Tbr1 compared to 44% in control cells.

Tbr1 is a transcription factor regulating regional and laminar identity in the developing neocortex and is expressed in postmitotic projection neurons and Tbr1 mutant mice show atypical layering and lack of a subplate in rostral cortical areas. Tbr1 expressing neurons are generated by a subsequent transition from radial glia cells over intermediate progenitors. Temporary down scaling of focal adherence in radial glia cells is one main key part in this neurogenesis.

In view of our results we hypothesize, that the increased adherence properties, that might be caused by the altered glycosylation in patient cells, lead to a temporarily decreased neurogenesis of Tbr1 expressing neurons during embryogenesis. This might impede proper cortical organization in patients and could thus explain the occurrence of cognitive impairment.

P-MonoG-172

Biallelic mutations in dopachrome tautomerase (DCT) cause isolated congenital nystagmus and oculocutaneous albinism

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Oculocutaneous albinism (OCA) is a genetically heterogeneous group of diseases characterized by variable hypopigmentation of the skin, hair, and by ophthalmologic abnormalities. Visual deficits include various degrees of foveal hypoplasia, reduced pigmentation of the retina and iris, photophobia and nystagmus. Variable expressivity exists with congenital nystagmus being the sole symptom in some patients carrying mutations in OCA-associated genes. OCA is inherited in an autosomal-recessive manner and caused by mutations in genes encoding for proteins that are involved in melanin biosynthesis or melanosome homeostasis. Mutations in the four most frequent OCA-associated genes (TYR, OCA2, TYRP1, SLC45A2) account together for around 50% of all OCA patients. Within the melanosomes the three key enzymes of melanin synthesis are TYR (tyrosinase), TYRP1 (tyrosin-related protein-1) and DCT (dopachrome

tautomerase) (also known as TYRP2). DCT had been a promising candidate gene for OCA since years as two recessive and a semi-dominant Dct mutant allele were described in the slaty mice with their greyish coat colour but evidence for a pigmentation phenotype caused by mutations in DCT in humans is lacking.

Here, we report on a consanguineous Turkish family with three individuals with isolated congenital nystagmus. By exome sequencing we identified biallelic mutations in DCT in this family segregating with the phenotype. Furthermore, we screened a cohort of patients with congenital nystagmus and/or OCA and identified an unrelated individual affected by OCA harbouring mutations in DCT on both alleles. Based on the key role of DCT in melanin synthesis and the phenotype observed in mice we hypothesize that biallelic mutations in DCT are associated with a pigmentation disorder in human with a phenotypic spectrum ranging from congenital nystagmus to OCA.

P-MonoG-173

Failure in PP2A/mTOR signaling, local protein synthesis and its consequences on functional connectivity in neurons from patients with Opitz BBB/G syndrome

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Opitz BBB/G syndrome (OS) is a X-chromosomal recessive disorder that is characterized by a defective formation of the ventral midline. Typical symptoms are hypertelorism, cleft lip and palate, congenital heart defects and hypospadias. In addition many patients present with aplasia/hypoplasia of the corpus callosum and vermis hypoplasia, exhibit learning disabilities and/or are mentally retarded. Mutations in the MID1 gene on the X-chromosome are responsible for Opitz BBB/G syndrome. The MID1 protein functions as an ubiquitin ligase and plays an important role in the regulation of microtubule-associated PP2A and mTOR-dependent protein translation. The MID1 protein complex is transported along the microtubules to destinations where mTOR/PP2A-controlled local protein synthesis is necessary. The phenotype of OS patients underlines the role of the MID1 protein in cell types where local protein synthesis and intracellular protein segregation are essential for the cell function. Such cell types include migrating neural crest cells on one hand and neurons on the other. Therefore we suggest that the MID1 ubiquitin ligase located at the switch between mTOR and PP2A activities plays a fundamental role in neuronal morphology and function.

We have generated iPS cell lines of OS patients carrying 5 different mutations (OS1-OS5). OS1 and OS3 carry missense mutations in the B-Box1 (c.388G>A, p.A130T) and the coiled-coil domain (c.884T>C, p.L295P), respectively. OS2 carries an insertion of four nucleotides in the FNIII domain (c.1445insCAAA, p.N482Tfs*7) leading to a frame shift. OS4 carries a splice site mutation affecting exon 8 (c.1655+1G>A) which encodes the B30.2 domain. OS5 carries a deletion of four nucleotides in coding exon 9 (c.1801_1804delCTCC, p.L601Gfs*20). Isogenic control cells will be generated by CRISPR genomic editing in order to correct the MID1 gene mutations. For one OS patient we have derived iPS clones from his mother, some of which carry the inactive copy of the mutated MID1-Gen and can be used as isogenic controls. We have already successfully differentiated these iPS into embryoid bodies containing neuronal rosettes. Differentiation into neuronal stem cells (NSCs) will be carried out by manual collection, dissociation and replating of neuronal rosettes as proliferating neural precursor cells (NPCs). These NPCs will be further differentiated into 2D and 3D cortical neuron cultures that will be used to (i) analyse mTOR signaling, (ii) study morphology of OS patients' derived neurons and (iii) look at the synaptic function and connectivity of such neurons.

P-MonoG-174

Molecular Basis of Aggressive Periodontitis

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Periodontitis is an inflammation disorder leading to teeth attachment loss and degradation of the alveolar bone. Chronic periodontitis (CP) is a very common form of the disease affecting 30-50% of the adult population. Aggressive periodontitis (AP) is a rare disorder with a prevalence of approximately 0,1%. AP is characterized

by an early age of onset, rapid rate of progression and high inflammation activity leading to loss of teeth before the age of 35, if untreated.

Here we report on a Tyrolean four-generation family indicating an autosomal dominant inheritance pattern for AP. The result of our genome-wide linkage analysis revealed one large co-segregating chromosomal region in affected individuals. The interval is 12 Mb in length and contains approximately 300 genes. In addition, we performed whole exome sequencing in four affected family members to identify possible common variants in the linkage region. Assuming a monogenic cause for the disease we have been looking for an unknown, heterozygous mutation in the coding sequence and exon/intron boundaries. Identified variants were filtered with common parameters including alignment against various mutation databases and prioritized based on their translational and predicted functional effects.

In our functional studies we have already shown a significant reduction of chemotactic and phagocytic activity of neutrophils in affected individuals. In regard to

these preliminary results we propose a mutation contributing to an immunological dysfunction. Signs of Ehlers-Danlos-syndrome (EDS) present in some family members may also suggest a mutation resulting in disturbed connective tissue deposition.

P-MonoG-175

A missense mutation in the PISA domain of HsSAS-6 causes autosomal

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Asymmetric cell divisionis essential for normalhuman brain development. Mutations in several genes encoding

centrosomal proteins that participate in accurate cell division have been reported to cause autosomal recessive

primary microcephaly (MCPH). By homozygosity mapping including three affected individuals from a consanguineous

MCPH family from Pakistan, we delineated a critical region of 18.53 Mb on Chromosome 1p21.3-

1p13.1. This region contains the gene encoding HsSAS-6, a centrosomal protein primordial for seeding the formation

of new centrioles during the cell cycle. Both next-generation and Sanger sequencing revealed a homozygous

c.185T>Cmissense mutation in the HsSAS-6 gene, resulting in a p.Ile62Thr substitution within a highly

conserved region of the PISA domain of HsSAS-6. This variant is neither present in any single-nucleotide polymorphism

or exome sequencing databases nor in a Pakistani control cohort. Experiments in tissue culture cells

revealed that the Ile62Thr mutant of HsSAS-6 is substantially less efficient than the wild-type protein in sustaining

centriole formation. Together, our findings demonstrate a dramatic impact of the mutation p.Ile62Thr on

HsSAS-6 function and add this component to the list of genes mutated in primary microcephaly.

P-NORMAL VARIATION / POPULATION GENETICS / GENETIC EPIDEMIOLOGY / EVOLUTIONARY GENETICS

P-NormV-176

Variability and Standardization of the Human Genome Sequence

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When the sequence of the human genome was announced to be completed in its first version in 2001, the knowledge about the variability from individual to individual was still very rudimentary. In the years to follow, the variability of the genome sequence with respect to allele frequencies of common, but also of rare and very rare variants, became more and more evident, especially due to the advent of high throughput genotyping and sequencing technologies. The haploid genomic fragments - the current “official” reference sequence is assembled from - are bearing the alleles from the given individual, be it common alleles, but also be it rare alleles. Being aware of this, the question immediately arises, which reference sequence could serve as the optimal “standard” human genome sequence. The most widely accepted consensus sequence for the human genome momentarily is the one deposited under the name Genome Reference Consortium Human Build 37 (GRCh37), also known as hg19 assembly. This sequence is a mosaic of sequences from about 13 individuals of diverse backgrounds (2001 Nature 409 p.860). The accuracy of this human genome sequence has been estimated, after the first redrafting, to be better than 1 error in 100.000 bases however, without taking into consideration the gaps caused by structural rearrangements. Since the first version, a number of revisions have been published. Despite all re-assembly efforts and the enormous advancements in sequencing technologies, this reference is still not meeting the requirements of a good standard. Minimal requirements for a standard are (1) a minimum number of errors, considering all current sources of sequence data and (2) the representation of the major allele at polymorphic positions. The second point will be depending on the population, the sequence should serve as a reference for, since a minor allele in one population may be the major one in another. These considerations are leading to the proposal, to define, besides a universal standard human genome sequence, also standards for individual populations. When resequencing a 100 kb region of the human genome, using long range PCR amplicons from pools of DNA of 50 individuals each, we identified one site, obviously representing an erroneous entry in the genome sequence. And for a large number of sites, obviously the minor alleles are listed as the reference. Our experiment shows an efficient way for eliminating errors from the genome sequence with rather small effort, but also for determining the major alleles in a population. It also demonstrates that the current reference genome is flawed and points to the necessity of derivation of reliable human genome sequence standards.

P-NormV-177

Single nucleotide polymorphism creating a variable upstream open reading frame regulates glucocorticoid receptor expression

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It is known that glucocorticoid receptors are critically involved in major physiological and biological processes such as metabolism, development and stress response. A few genetic variants which influence corticosteroid sensitivity during stress response have been identified in the coding sequence of the glucocorticoid receptor gene NR3C1 so far.

However, the molecular regulatory mechanisms within the glucocorticoid receptor gene NR3C1 leading to such altered cellular stress responses are still incompletely understood.

Thus, in the present study we searched for functional upstream open reading frames (uORFs) and associated single nucleotide polymorphisms (SNPs) in the human NR3C1 gene and performed various in vitro stress experiments to investigate their influence on gene expression. The in silico analysis identified a SNP (rs10482612) being present heterozygously in about 1.2% of the world population and 1.8% of the European population, whose minor allele ‘A’ creates a uORF. Our functional analysis performed by reporter gene assays and qPCR confirmed that the minor allele ‘A’ of the SNP rs10482612 can indeed alter luciferase activity both during baseline conditions and cellular stress. The reduced expression of the glucocorticoid receptor might have an impact on stress response and other glucocorticoid receptor-related cellular pathways in SNP rs10482612 allele ‘A’ carriers.

P-NormV-178

First detailed reconstruction of the male Trachypithecus cristatus karyotype 44,XY1Y2

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Background: The chromosomal homologies of human (Homo sapiens = HSA) and silvered leaf monkey (Trachypithecus cristatus = TCR) have been previously studied by classical chromosome staining and by fluorescence in situ hybridization (FISH) applying chromosome-specific DNA probes of all human chromosomes in the 1980s and 1990s, respectively.

Results: However, as the resolution of these techniques is limited we used multicolor banding (MCB) at an ~250-band level, and other selected human DNA probes to establish a detailed chromosomal map of TCR. Therefore it was possible to precisely determine evolutionary conserved breakpoints, orientation of segments and distribution of specific regions in TCR compared to HSA. Overall, 69 evolutionary conserved breakpoints including chromosomal segments, which failed to be resolved in previous reports, were exactly identified and characterized.

Conclusions: This work also represents the first molecular cytogenetic characterizing a multiple sex chromosome system with a male karyotype 44,XY1Y2. The obtained results are compared to other available data for old world monkeys and drawbacks in hominoid evolution are discussed.

This work was supported by the China Scholarship Council.

P-PRENATAL DIAGNOSIS / REPRODUCTIVE MEDICINE

P-Prenat-179

Prenatal diagnosis of a concurrent deletion and duplication rearrangement in the 22q11 region – a case report

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The 22q11 region is known for the presence of several low-copy repeats (LCR22A-H). These LCRs are directly involved in the chromosomal rearrangements associated with e.g. the chromosome 22q11.2 deletion syndrome (velocardiofacial syndrome, DiGeorge syndrome), the Emanuel syndrome (supernumerary der(22)t(11;22) syndrome), and the cat-eye syndrome.

With an approximate incidence of 1/4000 live births the most common aberration in the 22q11 region is the deletion of a 3.0 Mb region flanked by LCR22A and LCR22D. In addition, microduplications of different sizes, occurring between LCR22A to LCR22H, have also been described. Recently, due to new high throughput techniques, several patients with concurrent rearrangements have been reported. These double chromosomal rearrangements are characterized by a high phenotypic variability. Therefore, clinical development, in particular after prenatal diagnosis of a double rearrangement in 22q11, is not completely predictable.

We report a prenatal case with concurrent 22q11 deletion (LCR22A-LCR22D) and 22q11 duplication (LCR22D-LCR22G) rearrangement detected after amniocentesis, which was prompted due to detected ultrasound abnormalities (incipient intrauterine growth retardation, Tetralogy of Fallot and microcephaly) in the 25th week of pregnancy. We will give you an insight into the difficulty of genetic counselling in this prenatal case. Additionally we will report the postnatal clinical status of our little patient. The current data help to improve the characterization of the phenotype caused by a double rearrangement in 22q11.

P-Prenat-180

Prenatal diagnosis of Beckwith-Wiedemann syndrome: distinct pattern of fetal anomalies

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So far, only few studies, mainly case reports, have described Beckwith-Wiedemann syndrome (BWS) before birth. Abdominal wall defects were only present in a third of all cases, overgrowth in about 80% and macroglossia in half of the cases, respectively. Other features involved a large spectrum of different findings such as polyhydramnios, placental enlargement, abdominal distension, renal anomalies, visceromegaly, cardiac defects, non-immune hydrops, single umbilical artery and elevated maternal serum AFP.

To examine the prenatal anomalies in fetuses with BWS proven by molecular genetic analysis twelve pregnancies seen at three tertiary referral centres (Universities of Tuebingen, Bonn and Cologne) were retrospectively assessed. The genetic mutation, the results of the second trimester ultrasound examination and the outcome of the pregnancies are shown.

In five, including three related cases, there was a maternal inherited CDKN1C mutation. A paternal UPD 11p15.5 was found in two cases, a genome-wide UPD in one case and a hypomethylation of IC2 was found four times.

Two pregnancies were terminated due to severe early preeclampsia at 22.0 and 20.4 weeks' gestation. In summary, six pregnancies ended in severe preeclampsia and seven of the ten live born fetuses were delivered preterm before 37 weeks' gestation, mean gestational age at delivery was 33.8 (SD 3.8) weeks' gestation and mean fetal weight was 3022 (SD 1208) g corresponding to the 86th centile.

Median gestational age at the time of ultrasound examination was 22.6 (range 19.0 – 29.7) weeks of gestation. In all cases, the head circumference (HC) and the femur length (FL) were within the normal range, but the HC-FL ratio was above the 95th centile in 75% of the cases. An exomphalos, macroglossia and visceromegaly were observed in 67%, 50% and 83% of the cases and in 58% and 83%, there was polyhydramnios and placentamegaly, respectively. The fetal pancreas was identified in three quarters of the cases. A third of the women had large, overstimulation-like ovaries, although each pregnancy was conceived naturally. In four cases, beta-hCG levels were measured and mean hCG-levels were 498,106 IU/l.

In this case study, we have shown that prenatal diagnosis of BWS cannot just rely on the triad of visceromegaly, macroglossia and exomphalos. Other important hints are discordant growth between head and femur, placentamegaly up to placental mesenchymal dysplasia, increased beta-hCG levels, overstimulation-like ovaries and visualization of the pancreas. Besides exomphalos, BWS should be considered if there is macroglossia, a distinct growth pattern, pancreatic hyperplasia, placentamegaly and substantially increased levels of beta-hCG. In our study, half of the pregnancies resulted in severe preeclampsia and the majority in preterm delivery, respectively. This should be part of the counselling, and should trigger careful monitoring of the subsequent course of pregnancy.

P-Prenat-181

The Influence of Low Molecular Weight Heparin Medication on Plasma DNA in Pregnant Women

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Non-invasive prenatal testing (NIPT) for common fetal trisomies, which analyses cell-free fetal DNA circulating in maternal blood, is a new option in prenatal care. Several hundreds of thousands of tests are being performed worldwide every year. Until today the fetal fraction in cell-free maternal plasma DNA is the most critical determinant for a successful NIPT analysis which is affected by certain parameters such as maternal weight or the presence of an aneuploidy. No other pregnancy-related factors, crucial for a reliable NIPT analysis, have been identified yet. We provide the first report describing an effect of drugs on circulating plasma nucleic acids and subsequent NIPT results. Low molecular weight heparin (LMWH) is widely used as

anticoagulants in placenta-mediated pregnancy complication. We show that in pregnant women on LMWH medication the cell-free plasma DNA contains a higher proportion of small DNA fragments featuring an unusually high guanosin-cytosin (GC)-content than in unaffected women. This apparently biases NIPT results so that they cannot be interpreted correctly and even may provide false results. For such cases our report provides guidance that blood sampling for NIPT should ideally occur right before the next application of LMWH when the LMWH level is lowest in blood.

P-Prenat-182

Prenatal diagnosis of a complex deletion and duplication of chromosome 2q due to a maternal paracentric double inversion

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We report a fetus with increased nuchal translucency, tetralogy of Fallot, pectus excavatum, anal atresia and facial dysmorphisms. Prenatal chromosome analysis after amnion cell culture and array CGH revealed a complex rearrangement of chromosome 2 with a 10.1 Mb deletion 2q22.1q23.2 and a 33.6 Mb duplication 2q32.2q36.1. The pregnancy was subsequently terminated. Parental karyotyping and further FISH analysis revealed a maternal derivative chromosome 2: der(2)(pter->q22.1::q36.1->q32.2::q23.2->q32.2::q23.3->q22.1::q36.1->qter). This rearrangement can be explained by a double inversion with the breakpoints 2q22.1/2q36.1 and 2q32.2/2q23.2. The parents already have two healthy children, in a current pregnancy the fetus inherited the derivative chromosome 2 in a balanced state. Double inversions are very rare complex chromosome rearrangements. We will review the literature.

P-Prenat-183

Homozygosity/ autozygosity mapping following exome sequencing revealed a mutation in the C5orf42 gene as a cause of Meckel-Gruber Syndrome in two fetuses of a multiplex consanguineous Arab family.

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Meckel-Gruber Syndrome (MKS) is a rare autosomal recessive lethal ciliopathy characterized by the triad of occipital encephalocele, bilateral polycystic kidneys and post axial polydactyly. Additional anomalies are frequently associated. The major diagnostic criteria of MKS include at least two of the typical manifestations. MKS is a genetically heterogeneous disease involving at least eleven different genes. Prenatal diagnosis relies primarily on fetal ultrasound findings and molecular analysis is used to confirm the presumptive diagnosis.

In the present case, we used a homozygosity/ autozygosity mapping following exome sequencing in a multiplex consanguineous family originating from Tunisia with two affected fetuses and a healthy daughter. In both affected fetuses the diagnosis of MKS was established through detection of a novel homozygous mutation p.Gly1248Asp in the C5orf42 gene (OMIM#614571).

We present clinical findings and moleuclar genetic data highlighting that ciliopathies represent a single clinical entity with a spectrum of overlapping symptoms and causative genes. C5orf42 is known as a causative gene of Oral-facial-digital syndrome type VI and of Joubert syndrome type 17. In 2013, Shaheen et al. reported a C5orf42-associated case of MKS in an Arab family with atypical presentation (lacking both polydactyly and renal involvement). A second hit in other ciliopathy related genes might explain the variable phenotype.

Our case provides evidence of the usefulness of NGS technology as a quick and accurate diagnostic tool for an otherwise genetically heterogeneous disease.

P-Prenat-184

Development of a non-invasive prenatal test (NIPT) assay for trisomy 21 (T21) based on comparative quantification of chromosome 21 and a reference chromosome via quantitative real-time PCR

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Current NIPT assays for T21 in clinical application use massively parallel sequencing (MPS) based strategies which are quite costly. Here we present proof of principle data of an easily affordable T21 specific NIPT assay based on quantitative real-time PCR (qPCR). The test consists in a further development of the previously applied PraenaTest® quality control assay QuantYfeX® which estimates the fetal DNA fraction in total cell-free DNA of maternal blood by utilising differentially methylated regions on the chromosomes of the unborn child and the mother. The measurement of the fetal fractions of chromosome 21 target loci and reference loci on other chromosomes revealed significantly increased ratios of fetal fraction between chromosome 21 and the reference chromosomes in T21 positive cases compared to euploid cases allowing a clear distinction of T21 positive and T21 negative samples. The test performance of this T21 qPCR assay was investigated by comparison with the respective results from PraenaTest® laboratory routine.

P-Prenat-185

Recent bioinformatic advances of non-invasive prenatal detection to enhance diagnostic accuracy and aneuploidy discovery

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Objective:

In recent years multiple independent studies have shown the ability of random massively parallel sequencing (MPS) of maternal plasma DNA to accurately detect common fetal aneuploidies. Here, we present the results of a new algorithmic approach improving the analysis of sequencing data applied to a previously un-blinded clinical study to demonstrate improved median absolute deviations (MAD)and coefficients of variance (CV) resulting in an enhanced discrimination of affected versus unaffected pregnancies compared to the currently approved PraenaTest® DAP.plus algorithm.

Methods:

In 2011 maternal blood samples were collected from 517 pregnant women with high aneuploidy risk and extracted cell-free plasma DNA was sequenced using Illumina sequencing platform HiSeq2000. The un-blinded study data consisting of 472 valid data sets was reanalyzed by a new bioinformatics algorithm based on a reference mappability score following GC normalization to correct for genomic library preparation and sequencing bias. A MAD-based z-score equation was used to identify fetal aneuploidies. Results of MPS based technique were compared with those from invasive procedures and the previously certified algorithm.

Results:

Overall, 39/40 samples were correctly classified as trisomy 21-positive (sensitivity: 97.5%; one-sided confidence interval: 88.7%) and 427/427 samples were correctly classified as trisomy 21-negative (specificity: 100%; one-sided confidence interval: 99.3%). Furthermore, 5 of 5 T13 cases and 8 of 8 T18 cases were correctly identified. The overall detection rate of trisomies 13, 18 and 21 is 98.11% (52/53). MAD and CV of chromosomes 13, 18 and 21 could be reduced by 32.4% or 30.6% in average, respectively. MAD based z-scores increased by 38.4%, 21.8% and 59.9% in the cases of chromosome 21, 18 and 13, respectively.

Conclusion:

Due to the improved bias correction followed by a significant reduction of statistical variance, the PraenaTest® allows an even more reliable detection of common autosomal trisomies; furthermore it enables the potential of decreasing read coverage without reducing detection accuracy and sequencing at lower costs and higher sample performance.

P-Prenat-186

Cystic fibrosis preimplantation diagnostics – Belarusian Center of Reproductive Medicine experience

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Cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations play a crucial role in male infertility. In published data 60-90% men with congenital absence of the vas deferens (CAVD) are CFTR gene mutations carriers. F508del is identified in 60-70% cases. Couples in which both partners carry CFTR gene mutations have a high risk (25%) of conceiving an offspring affected by cystic fibrosis (CF, #219700, autosomal recessive disorder) and should be advised to undergo prenatal or preimplantation genetic diagnostics (PGD) to prevent giving birth to an ill child and passing CF to future generations. We present our experience in CFTR gene mutations carriers testing and PGD for CF performed in 2013-2014.

601 men with azoospermia or severe oligozoospermia were examined using a molecular method. 16 (2.6%) heterozygote carriers of CFTR gene mutations were identified: dF508del (10 cases), CFTR2,3del (3 cases), 2184insA (2 cases), 1677delTA (1 case). 62.5% patients presented F508del variant of CFTR gene mutation. 2 couples (C1;2) showed heterozygous CFTR mutations genotypes in both partners: dF508del (C1); dF508del and CFTR2,3del (C2). Genetic counseling for outcome prognosis and CF testing discussion were done. These couples underwent testicular biopsy for acquiring spermatozoa followed by sperm cryopreservation, in vitro fertilization (IVF), ICSI and PGD. Embryos with 8 cells underwent biopsy, two blastomers were aspirated and multiplex PCR for known parental mutations was performed.

Results: two blastomers of each embryo were concordantly non-affected. Heterozygous carrier embryo (C1) and embryo with no CFTR mutations (C2) were transferred on day 5 after follicles aspiration. Both embryo transfers resulted in pregnancies. The pregnancy obtained in C1 developed successfully, prenatal ultrasound and serum markers screening results were unremarkable and a healthy boy was born – W.3900 g, L.54 cm, delivery per vies naturales, 39 weeks of gestation. The boy was tested for CFTR gene mutations – heterozygous, clinically healthy.In couple 2 a healthy pregnancy is progressing.Our results proved that genetic screening of infertile couples undergoing IVF for CFTR gene mutations is of great importance. PGD is a considerably less dangerous alternative for prenatal diagnosis giving infertile families with a high risk of cystic fibrosis the opportunity to have unaffected offspring without pregnancy termination risks.

P-TECHNOLOGY AND BIOINFORMATICS

P-Techn-187

SNiPA regulatory: A catalog of experimentally identified promoter and enhancer regions

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The majority of disease-linked genetic variants identified by genome-wide association studies are located in non-coding DNA, suggesting regulatory effects as possible molecular causes of complex diseases. In line with that, variants linked to multifactorial disorders have been found to substantially overlap with expression quantitative trait loci (eQTLs). However, eQTL data, which can give direct hints on genes affected by a variant through regulatory mechanisms, are only available for a handful of tissues and cell types. More recently, protocols to define and measure regulatory DNA segments on a genome-wide scale across hundreds of cell types have provided a great amount of data on regulatory regions. Nonetheless, mapping genetic variants to such regulatory elements as well as identifying the genes affected by changes to these elements remains difficult. To tackle this issue, we first collected data on regulatory DNA segments from various sources in our web resource SNiPA (www.snipa.org) and provide an interactive variant-based access to this information. In a second step, we used these accumulated data to build a new catalog of functional regulatory elements augmented with protein-DNA interaction data and target gene information. To this end, we combined ChIP-seq data clusters from ENCODE with DNase I hypersensitive sites and upstream antisense and enhancer RNA mappings across several hundred tissues to retrieve a set of enhancer and promoter regions. The validity of our approach was inspected using eQTLs located within those elements supporting the correlation of the expression of target genes with the activity patterns of the regulatory elements. We added this newly derived

regulatory catalog to the variant annotation compendium contained in SNiPA, further improving the convenient investigation of effect predictions for non-coding genetic variation.

P-Techn-188

Deep intronic variants in the factor VIII gene

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Haemophilia A (HA) is the most common X-linked bleeding disorder with an incidence of 1 in 5000 males. Different mutation types in the factor VIII gene (F8) lead to a decrease in coagulation factor VIII activity and variable severities of the patients' phenotypes. The causative alteration can be detected in the vast majority of HA patients by standard diagnostic screening methods targeting the coding regions of the F8 gene. Still, this approach fails in about 4 % of cases. This study intended to analyse the whole F8 gene including all intronic sequences in 16 HA patients by next generation sequencing (NGS) in order to screen for deep intronic variants.

Fifteen of the male index patients showed mild and one case a moderate HA phenotype with no abnormality in the coding sequences and splice sites of the F8 gene identified by standard diagnostic techniques. Patient 16 was pre-diagnosed with a duplication of exons 1-22 and a triplication of exons 23-25 of the F8 gene by multiplex ligation-dependent probe amplification (MLPA). Target enrichment for the entire genomic region of F8 was performed with the SureSelectXT system (Agilent) and sequencing on a MiSeq (Illumina). NGS data were analysed with GenSearchNGS (PhenoSystems) and revealed 23 deep intronic candidate variants in different F8 introns. Three of the single nucleotide variants (SNVs) have been described before as HA causing mutations. Six other variants were recurrent among the 15 cases studied. Several bioinformatic tools were used to score all candidate variants regarding their potential deleteriousness and the creation of de novo splice sites, also in comparison to already published deep intronic F8 mutations. In each of the patients, at least one SNV or CNV was predicted to be potentially pathogenic. The patient with moderate HA additionally showed a deletion of 9.2 kb in intron 1 with both breakpoints located in Alu-type repeats. The copy number variations (CNVs) of patient 16 could be well seen in the NGS data by comparing the coverage of the affected exons, but it wasn't possible to define the exact breakpoints.

In summary, this NGS approach proved an effective method to analyse the whole F8 gene for potentially pathogenic deep intronic variants and CNVs in a selection of 16 male HA patients. In general, this approach bears the potential to be applied as efficient one-step sequencing method of the complete F8 gene in molecular diagnostics of haemophilia A. Yet, in a diagnostic setting besides in silico prediction further functional studies like mRNA analysis would be required to confirm the causality of deep intronic variants. As this study was pseudonymized, mRNA analyses on patients' blood samples were not possible. Therefore, we are now trying to confirm causality of some of the candidate variants by mini-gene assays and subsequent RNA analyses.

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VarWatch - A Database of in limbo Genetic Variants from NGS

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Next Generation Sequencing (NGS) allows the timely interrogation of whole human exomes and even genomes at reasonable cost, thereby allowing a major breakthrough in the diagnosis of (mostly monogenic) genetic disorders. However, NGS often reveals genomic variants in a patient that cannot be interpreted conclusively regarding their clinical significance. Such 'in limbo' variants (meaning of 'transitory state') mostly have to await confirmation, for example, by a second independent report of the same or a closely related mutation in a patient with a comparable phenotype. For rare diseases, however, such a requirement seems difficult to meet by means of regular scientific communication such as publication in peer-reviewed journals. One solution to this problem would be the establishment of a public database of in limbo variants. However, collating such data in register-like fashion would face two major challenges: (i) to motivate NGS laboratories to contribute and (ii) to constantly ensure adequate data quality. Therefore, we propose the pilot implementation of a transient variant watch list (called 'VarWatch') that simultaneously serves as a query tool of previously logged mutations awaiting validation and as an entry point for new variants. Owing to its dual

function, VarWatch should acquire only high quality entries by way of queries made by qualified and authorized end users. Moreover, the prospect of efficiently clarifying unsolved diagnostic cases should be a sufficiently strong incentive for them to consult VarWatch and to participate in its give-and-take modus operandi. To avoid conflicts in a field also governed by economic interests, the primary goal of VarWatch will be to establish a repository that is free of any financial and political interests but at the same time integrates as many stakeholders from the NGS diagnostics community as possible. Initially, VarWatch will mostly help patients and their doctors in diagnosing individual cases. With time, however, this health care-orientated benefit will be complemented by an increasing scientific value. In fact, through its planned tight tuning with existing mutation repositories, such as the Human Gene Mutation Database (HGMD), VarWatch will become a fast and efficient facilitator of the scientific use of very valuable (but otherwise invisible) information from clinical care.

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Targeted next-generation sequencing of 1222 genes in routine diagnostics of patients with intellectual disability

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Next-generation sequencing has opened up new possibilities in the search for disease-causing mutations in Mendelian pedigrees. It has been clear for a long time that genetic factors are involved in intellectual disability (ID). This has been supported by recent findings of germ line de novo (DN) mutations and large-scale copy number variations in ID patients. Identification of underlying genetic defects not only provide a clear diagnosis for patients but also pave the way for future therapy development.

100 patients with intellectual disability/cognitive deficits, seen at the genetic counselling unit of our institute, were analyzed with targeted exon enrichment and NGS. The MPIMG-1-Test, which provides panel diagnostics for over 1200 brain related genes was established at our institute in 2013. For enrichment a TruSeq Custom Enrichment Kit (Illumina Inc.) was used. 2x300 basepair paired-end sequencing (Illumina MiSeq Reagent Kit v3) was carried out on an Illumina MiSeq system. Read alignment was performed with SOAP version 2.2. On average, more than 90% of the enriched exons were covered by at least ten reads. A modified version of the Medical Resequencing Analysis Pipeline (MERAP, Hu et al, 2014) was used to check all detected variants against dbSNP137, the 1000 Genomes Project, the Exome Variant Server, the OMIM catalog and the Human Gene Mutation Database. For exclusion of technical artifacts and segregation testing of all likely disease-causing variants, PCR and Sanger sequencing were performed according to standard protocols.

Confirmed disease-causing mutations have been identified in 12 out of the 100 patients. We found 5 cases with mutations in genes (NDST1, STIL, PTPN11, GAMT, SGSH) previously reported and recognized as causing of a disorder matching the patient's phenotype (sequence variation category 5). 7 cases were identified in genes likely associated with the phenotype (ZEB2, MLL2, HRAS, ITPR1, AFF2), but with a previously unreported sequence variation (category 4). In further 4 patients, likely gene disrupting not yet reported sequence variants (category 3) were identified in known disease-causing genes (BRCA2, CLCN2, DYRK1A), and 10 patients were found to have potentially disease-causing missense variants (category 3). Pathogenicity of the variants was supported by prediction tools (SIFT, PolyPhen2, MutationTaster) and conservation scores. All variants were confirmed by Sanger sequencing, while segregation analysis is still ongoing in 10 of these families. Among others identified sequence variants target chromatin modifiers (HDAC4, ZEB2), genes involved in the mTOR pathway (CDKL5), nuclear transcription factors (AFF2) and recurrent targets for de novo mutations in ID genes (HUWE1, DYRK1A, GRIN2B).

Our findings so far support the clinical utility of the MPIMG-1-Test and MERAP pipeline and underline the important role for NGS techniques in the clinical setting when addressing a group of disorders with extreme genetic heterogeneity.

P-Techn-191

An efficient and comprehensive strategy for genetic diagnostics of polycystic kidney disease

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Renal cysts are clinically and genetically heterogeneous conditions. Autosomal dominant polycystic kidney disease (ADPKD) is the most frequent life-threatening genetic disease and mainly caused by mutations in PKD1. The presence of six PKD1 pseudogenes and tremendous allelic heterogeneity make molecular genetic testing challenging requiring laborious locus-specific amplification. Increasing evidence suggests a major role for PKD1 in early and severe cases of ADPKD and some patients with a recessive form. Furthermore it is becoming obvious that clinical manifestations can be mimicked by mutations in a number of other genes with the necessity for broader genetic testing. We established and validated a sequence capture based NGS testing approach for all genes known for cystic and polycystic kidney disease including PKD1. Thereby, we demonstrate that the applied standard mapping algorithm specifically aligns reads to the PKD1 locus and overcomes the complication of unspecific capture of pseudogenes. Employing careful and experienced assessment of NGS data, the method is shown to be very specific and equally sensitive as established methods. An additional advantage over conventional Sanger sequencing is the detection of copy number variations (CNVs). Sophisticated bioinformatic read simulation increased the high analytical depth of the validation study and further demonstrated the strength of the approach. We further raise some awareness of limitations and pitfalls of common NGS workflows when applied in complex regions like PKD1 demonstrating that quality of NGS needs more than high coverage of the target region. By this, we propose a time- and cost-efficient diagnostic strategy for comprehensive molecular genetic testing of polycystic kidney disease which is highly automatable and will be of particular value when therapeutic options for PKD emerge and genetic testing is needed for larger numbers of patients.

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An alternative method for the analysis of deletions/duplications with MLPA© using the QIAxcel© Advanced System

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Multiplex Ligation-dependent Probe Amplification (MLPA) from MRC-Holland© is the gold standard for the detection of exonic deletions/duplications in genetic diagnostic testing. MLPA is based on a multiplex PCR, but different from a classical multiplex PCR it is a two-step process involving a hybridisation/ligation reaction for generating the amplification template as the first step. Every exon of a specific target gene is covered by one or more probe-pairs which, when both probes bind to the exon, are ligated. In the second step, the ligated probe-pairs will then be amplified and subsequently analysed using a capillary electrophoresis system. The resulting electropherogram shows a specific peak pattern for each sample. The ratio of the peak height between target and reference probes or even reference samples is used for the detection of exonic deletions/duplications. The fragment analysis is usually performed using capillary (Sanger) sequencing, which is a costly and time consuming method. In this pilot-study we present a faster and more cost effective alternative approach for the MLPA analysis by using the QIAxcel© Advanced System from Qiagen©. The QIAxcel© Advanced System is an automated DNA and RNA analyser, replacing the traditional gel analysis of DNA and RNA.

For our pilot study we used two different clinical samples: a) 12 FAP (familial adenomatous polyposis coli) patients with known mutation status as well as 5 healthy controls; b) 5 HNPCC (hereditary non-polyposis colorectal cancer, Lynch syndrome) patients with known mutation status as well as 3 healthy controls. For the MLPA analysis we used the SALSA MLPA P043 APC Probemix© for the FAP samples and the SALSA MLPA P003 MLH1/MSH2 Probemix© for the HNPCC samples. The visualisation of each MLPA-generated amplification product was performed using a Sanger sequencer as well as the QIAxcel capillary electrophoresis system. Utilizing a newly developed protocol we were able to detect exonic deletion/deletions with the same

resolution and accuracy as with Sanger sequencing. Based on our preliminary results using the QIAxcel Advanced system allows us to have reliable, fast and cost effective MLPA analysis.

P-Techn-193

Likelihood-based inference of relationship for family exome data

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Whole exome sequencing (WES) analysis has become a powerful strategy to search for causal variants underlying rare Mendelian disorders. However, the identification of the disease-causing mutation among thousands of potentially functional sites can be challenging. One way to reduce the list of candidate variants is to use inheritance information of additionally sequenced individuals from the same family. This strategy depends completely on correct pedigree information and may lead to false discoveries in case of sample swaps or label mix-ups.

We developed an algorithm based on likelihood ratios to discriminate between different relationship types (e.g. siblings, parent-child, unrelated) in family exome data. While various methods exist to derive likelihood ratios using highly polymorphic variant marker for paternity inference or sib-pair analysis, a method that combines these approaches on whole exome sequencing data is still missing.

Our approach compares likelihood ratios based on different hypotheses assuming a specific relationship between any pair of WES samples. In addition to the estimated pairwise kinship, visualization of the likelihood ratios also reveals the presence of technical replicates or samples with cryptic relatedness that are included in the study cohort, e.g. second-order relationship such as uncle-nephew.

The implemented algorithm was tested on family WES data of the 1000 genomes project, as well as on in-house data where we could confirm underlying degrees of relationships.

P-Techn-194

A pipeline for eQTL-analysis using Illumina microarray data

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GWAS enable the identification of common variants associated with different phenotypes. A majority of these variants are located in non-coding parts of the genome, which necessitates a subsequent functional annotation. Here, expression quantitative trait loci (eQTL) analyses have gained major attention. eQTLs are SNPs which affect the expression of associated genes either locally (cis) or distantly (trans). The observed eQTL effects are often tissue and context-dependent. Since genetic disorders affect a variety of different organ systems, a deeper understanding of specific regulatory processes in disease-relevant tissues is warranted to gain novel insights into the underlying biological mechanisms. Moreover, advances in high-throughput data generation with different existing microarrays and sequencing technologies as well as the increasing number of large collaborative efforts, highlight the importance of comparability between data sets and the need for a robust and fast pipeline for eQTL mapping over different tissues. Therefore, we aimed at establishing an automated workflow for identification of cis and trans-eQTLs from high-throughput genomic and transcriptomic data. To this end, genome-wide genotype and expression data was generated using Illumina's Omni-Family and HumanHT-12 bead arrays. To ensure comparability of the eQTL findings across studies and to enable integration with GWAS, genotypes were subsequently imputed (1000Genomes). Robustness of the workflow is guaranteed by application of different elaborate filter steps, which were individually tested and implemented. For expression data, this involves removal of non-expressed, ambiguous and low-confidence transcripts. Genotypes are filtered according to standard QC parameters (e.g. MAF, call rate, confidence score). eQTLs are then identified through integration of genotype and expression data with linear regression using MatrixEQTL. Inclusion of covariates in the regression analysis ensures controlling for confounding effects in the data. Suitable covariates are determined via principal component analysis and can be correlated to phenotype categories or batches by linear model fitting. This leads to an improved and highly reliable identification of eQTLs. The pipeline was successfully applied to identify tissue-specific eQTLs from stomach, hair and hippocampus. Here, we found more than 10,000 cis- and trans-eQTLs in the investigated tissues, with ~35% being tissue-specific. The data has been used to functionally annotate GWAS data for Barrett's esophagus, male pattern baldness and psychiatric disorders. Thus, our pipeline presents a standardized and easily applicable method for identification of functional genetic variance derived from microarray and RNAseq data, while still being computationally efficient and fast. As demonstrated, integration of eQTL data with GWAS

can help to elucidate underlying biological mechanisms at known disease risk loci and to identify novel candidate genes.

P-Techn-195

Comprehensive variant analysis in a single Clinical Grade NGS Experiment for diagnostic routine

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Next-Generation Sequencing of disease gene panels is a widely used technique in routine genetic diagnosis. Sufficient coverage of target regions is a well-known prerequisite of accurate variant detection. However, there are other issues that are less obvious but important to ensure correct variant detection. These issues include variants exposed to the end of reads, proper trimming of the primer sequences and special care of repetitive regions. Furthermore, the establishment of accurate analysis pipelines is often hampered by low numbers of confirmed variants. Two key points ensure accurate and comprehensive detection of variants: (1) understanding the limitations of the sequencing platforms, the chemistry used for enrichment/amplification, the sequence context of genes in the panel and the source or type of sample and (2) validating the analysis pipeline on a wide range of distinct variants.

Here, we introduce the Sophia Data-Driven Medicine (DDM) Platform, which offers clinical grade NGS data analysis of SNPs, Indels and large deletions or duplications (CNVs). Each analysis pipeline is designed specifically for a combination of sequencing platform, enrichment/amplification chemistry and particular genes in the given panel. Every single command as well as the whole analysis pipeline are under strict version control and subject to a daily routine testing as well as frequent revalidations. Each analysis pipeline is validated by real clinical samples with known variants. By facilitating the network of our users across Europe, we are able to validate our pipelines on large sample numbers >300 samples. To validate our CNV detection algorithm, we analysed >900 samples across 4 different centers and correctly identified all positive samples. Our deep understanding of a wide spectrum of different pathogenic variants allows us to obtain the CE-IVD quality mark for our specific analysis pipelines.

Our analysis pipelines have been applied to a broad range of different commercial and custom gene panels and reach very high sensitivity and specificity, sufficient to replace the traditional Sanger sequencing.

P-THERAPY FOR GENETIC DISEASES

P-Therap-196

Chediak-Higashi syndrome is an important differential diagnosis in oculocutaneous albinism with substantial clinical consequences

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Chediak-Higashi syndrome (CHS) is a very rare autosomal recessive disorder. It is characterized by partial oculocutaneous albinism, severe immunological defects, thrombocytopathy with bleeding tendency, and varying neurologic problems, all of which are signs of a defect in vesicle transport. CHS leads untreated to an early death. The only available treatment is allogenic hematopoietic stem cell transplantation (HSCT), which is effective in treating the hematologic and immune defects, however usually neurologic problems persist. Approximately 85% of affected individuals develop a haemophagocytic lymphohistiocytic disease like picture to enter an accelerated phase marked by lymphocytic infiltration of liver, spleen, lymph nodes, and bone marrow. The morphologic hallmark of this syndrome is the occurrence of giant and abnormal large granules in leukocytes and other granule-containing cells suggesting deficiency of intracellular vesicle formation and lysosomal dysfunction. Classical CHS is caused by biallelic mutations in the gene encoding lysosomal trafficking regulator (LYST).

Here we report on a four-years-old girl who presented at the age of five month with features indicative of oculocutaneous albinism (OCA). Firstly, the genes TYR, OCA2, and SLC45A2 responsibly for OCA type 1, 2, and 4, respectively, were completely analysed by Sanger sequencing. No mutation was identified. Subsequently, a differential diagnosis of CHS was made. Sanger sequencing of the whole coding region of LYST revealed two hitherto undescribed heterozygous mutations in the LYST gene: c.3556_3557delAG, p.His1187Serfs*12 in exon 8 and c.11080delG, p.Asp3694Ilefs*28 in exon 51. Analysis of both parents confirmed the compound heterozygote state of the mutations in the child. Thus, the diagnosis of Chediak-

Higashi syndrome could be established. After this the patient was treated with HSCT from a matched unrelated donor with a reduced intensity protocol (Fludarabin – targeted Busulfan – ATG) at the age of one year. Until now the development of the child is appropriate to her age and no neurological symptoms are occurred.

In the meantime, the mother is pregnant again. A prenatal diagnosis regarding CHS was offered.

P-Therap-197

On the way to genotype-specific treatment recommendations for LIS1- associated classic lissencephaly

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Classic lissencephaly is a rare brain malformation caused by defective neuronal migration during embryonic development. It is characterized by a smooth cerebral cortex with reduced or even absence of the typical gyration of the primate brain. Children with LIS1-associated classic lissencephaly typically remain at a neurodevelopmental age of about 3 months and suffer from an intractable seizure disorder. The aim of our study was to analyze the largest genotype specific subgroup with regard to their response to antiepileptic therapy in order to develop LIS1 specific treatment recommendations.

We retrospectively evaluated 21 patients (age: 8m - 24y) with genetically and radiologically confirmed LIS1-associated classic lissencephaly. Efficacy of current and earlier used anticonvulsant drugs and supportive therapies was evaluated by the families (n = 21) and responsible neuropediatricians (n = 16) using standardized questionnaires. Statistical analysis was performed for all drugs used by more than 5 patients.

All LIS1 patients showed pronounced developmental delay and developed drug resistant seizures, 86% within the first 6 months. Infantile spasms were the first seizure type in 82%. During the disease course the majority suffered from multiple seizure types, generalized tonic-clonic seizures being the prominent type. Antiepileptic medication decreased the median number of daily seizures from 3.5/d to currently 1.0/d. Lamotrigine, valproate and phenobarbital were rated the most successful anticonvulsant drugs with good or partial response rates for 84-100% of the patients. Lamotrigine was evaluated significantly better than oxcarbacepine, levetiracetam and sultiame for which no treatment response was noted in 46-100% of the LIS1 cohort ($p<0.05$).

In addition to pharmacological therapy, respiratory therapy, physiotherapy and hippotherapy were rated as very effective concomitant therapies in >80% of the LIS1 patients. In contrast, other commonly prescribed therapies such as speech therapy, early intervention programs and aid to the blind were rated as ineffective or only partially effective in ≥ 50% of the patients.

LIS1 mutations uniformly result in early drug resistant epilepsy. Further prospective studies are needed to elucidate the genotypic response to anticonvulsant therapy in a larger LIS1 cohort. According to our first evaluation LIS1 patients might benefit from genotype-specific antiepileptic treatment recommendations due to their unique histopathological brain malformation. In addition, further studies are needed to compare the results to other genotype-specific cohorts of classic lissencephaly, f.e. patients with DCX and TUBA1A mutations.

P-Therap-198

Shox2 dependent genetic program in embryonic stem cell derived pacemaker cells

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Stem cell differentiation is important in many biological processes including heart development, tissue repair and regeneration. The elucidation of molecular mechanisms that restrict the potential of pluripotent stem cells (ESCs) and promote cardiac lineage differentiation is of crucial relevance, since embryonic stem cells

represent a potentially unlimited source of cardiomyocytes and hold great potential for cell based heart therapies.

Previously we have demonstrated that a gene regulatory network involving the homeodomain transcription factor Shox2 controls the development and function of the native cardiac pacemaker. This prompted us to develop a murine ESC based cardiac differentiation model using the pacemaker specific marker gene Shox2 as a molecular tool. We isolated pluripotent Shox2+/+ and Shox2-/- murine ES cells from blastocysts of Shox2 deficient mice in order to investigate Shox2 dependent genetic pathways in ESC derived pacemaker cells. Subsequently, we compared several approaches utilizing different pharmacological treatments as well as fluorescent activated cell sorting sorting (FACS) to enrich our target population of cells during cardiogenic differentiation. Expression analysis on cell subtype specific marker genes revealed highest and purest enrichment of pacemaker cells after cell sorting. Based on this differentiation protocol, we compared expression profiles of differentiated Shox2+/+ and Shox2-/- ES cells using RNA-Seq technology and identified novel Shox2 dependent pathways during pacemaker differentiation.

Taken together, we established an ESC based cardiac differentiation model and successfully purified pacemaker cells, which may now provide a basis for studying biological pacemakers. In addition we identified novel genes involved in the development of specific cardiac subtypes.

P-Therap-199

In vivo investigations of the effect of short- and long-term recombinant growth hormone treatment on DNA-methylation in humans

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Treatment with recombinant human growth hormone (rhGH) has been consistently reported to induce transcriptional changes in various human tissues including peripheral blood. For other hormones it has been shown that the induction of such transcriptional effects is conferred or at least accompanied by DNA-methylation changes.

To analyse effects of short term rhGH treatment on the DNA-methylome we investigated a total of 24 patients at baseline and after 4-day rhGH stimulation. We performed array-based DNA-methylation profiling of paired peripheral blood mononuclear cell samples followed by targeted validation using bisulfite pyrosequencing. Unsupervised analysis of DNA-methylation in this short-term treated cohort revealed clustering according to individuals rather than treatment. Supervised analysis identified 239 CpGs as significantly differentially methylated between baseline and rhGH-stimulated samples ($p<0.0001$, unadjusted paired t-test), which nevertheless did not retain significance after adjustment for multiple testing. An individualised evaluation strategy led to the identification of 2350 CpG and 3 CpH sites showing methylation differences of at least 10% in more than 2 of the 24 analysed sample pairs. To investigate the long term effects of rhGH treatment on the DNA-methylome, we analysed peripheral blood cells from an independent cohort of 36 rhGH treated children born small for gestational age (SGA) as compared to 18 untreated controls. Median treatment interval was 33 months. In line with the groupwise comparison in the short-term treated cohort no differentially methylated targets reached the level of significance in the long-term treated cohort.

We identified marked intra-individual responses of DNA-methylation to short-term rhGH treatment. These responses seem to be predominately associated with immunologic functions and show considerable inter-individual heterogeneity. The latter is likely the cause for the lack of a rhGH induced homogeneous DNA-methylation signature after short- and long-term treatment, which nevertheless is well in line with generally assumed safety of rhGH treatment.

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P-Therap-200

Induced Pluripotent Stem Cell-Derived Keratinocytes for Patient Specific Models of Monogenic Keratinization Disorders

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Human primary keratinocytes have a very short lifespan 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 inducing pluripotency in adult skin fibroblasts and the posterior differentiation of iPSC into keratinocytes can offer an unlimited source of these cells. Because iPSC carry the same genetic information as the adult cells used for reprogramming, these cells provide a valuable tool for patient-based disease models. Hence, the goal of this project is to establish an iPSC-based in vitro skin model system for the development of new therapies for congenital skin diseases as well as the characterization of underlying pathomechanisms.

The focus of this project has been on autosomal recessive congenital ichthyosis (ARCI), a heterogeneous skin disorder characterized mainly by impaired function of the skin barrier, scaling of the skin and generalized erythema. Because only symptomatic treatments are available, efficient and possibly causal treatments for this disease are urgently needed.

We have now generated and characterized several iPSC lines originating from ARCI patient skin fibroblasts using the STEMCCA lentiviral system. These iPSC present typical embryonic stem cells features, such as alkaline phosphatase activity, increased expression of specific pluripotency markers measured by both immunocytochemistry and qRT-PCR and the ability to differentiate into cells from all three germ layers after embryoid bodies (EBs) formation. Genome integrity was assessed and confirmed by karyotyping and DNA microsatellite analysis. Whole genome expression data pointed to typical pluripotency gene expression profiles and is currently being further analysed.

Differentiation of these iPSC into basal epidermal keratinocytes is now in progress. Our strategy comprises two ways of differentiation, either directly from iPSC colonies, or after EBs formation. Our first results show an expression of early ectodermal marker TP63 as well as of KRT18, a marker for simple epithelia at early stages of ectodermal differentiation process. Several markers for basal keratinocytes are expressed later in differentiation, mainly KRT5 around day 25 and KRT14 around day 35, showing a commitment to the epidermal fate. The differentiated cells are also morphologically very similar to human keratinocytes. After further characterization, we have been using these iPS-derived keratinocytes to generate 3D full skin models in order to mimic the skin phenotype of ARCI patients. Our approach thus promises individualized cell models for the functional study of ARCI and for pharmacological interventions.

P-Therap-201

Thiamine-responsive megaloblastic anemia syndrome mimicking mitochondrial disease

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Background: Thiamine-responsive megaloblastic anemia (TRMA) is an autosomal-recessive disorder characterized by the triad of sensorineural hearing loss (SNHL), insulin-dependent diabetes mellitus (IDDM) and megaloblastic anemia with ringed sideroblasts. In addition, visual problems (optic atrophy, retinal dystrophy), cardiovascular abnormalities (heart defects, sudden death following high-output heart failure) and neurological deficits (stroke, epilepsy) may occur. SLC19A2 is the only gene in which mutations are known to cause TRMA.

We report a consanguineous family (Yesidi Kurds of Syrian descent) with 4 children, 3 of them affected by TRMA.

- A male child died at the age of 3 years following multi-organ failure, he was affected by IDDM and SNHL.
- A female child died at the age of 3 years following cardiomyopathy, she was affected by IDDM and SNHL. Complex 1-activity of the mitochondrial respiratory chain was reduced in muscle.
- A now four-year-old male is affected by SNHL (since the age of 8 months) and IDDM (since the age of 18 months). Up to now, no signs of cardiomyopathy, anemia or visual problems are apparent.

Based on multisystemic disease including IDDM, hearing loss and cardiomyopathy in three siblings, a mitochondrial disease was supposed, supported by the reduced activity of respiratory chain enzyme complex

1 in the affected female. However, no mutation could be identified in the mitochondrial genome (MGZ Munich). Finally, the homozygous mutation c.237C>A (p.Y79Ter) in the SLC19A2 gene (thiamine transporter) was identified (post mortem) (Prof C. Klein, LMU Munich) in the deceased affected boy and consecutively in his affected brother, proving the diagnosis of TRMA. The parents are heterozygous for this mutation. Following thiamine supplementation, exogenous insulin substitution was no longer necessary in the surviving child (glycated hemoglobin A1c 5.2%). Further surveillance is strongly recommended regarding diabetes, cardiac complications, vision, hematological disease, hearing loss and psychomotor development.

Conclusions: Thiamine deficiency due to TRMA (compromising thiamine-dependent enzymatic activities like mitochondrial function and oxidative metabolism) can mimic mitochondrial disease showing symptoms like IDDM, deafness and cardiac failure.

P-Therap-202

Cutaneous enzyme delivery for a novel personalized protein replacement therapy for patients with autosomal recessive congenital ichthyosis (ARCI)

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Autosomal recessive congenital ichthyosis (ARCI) is a rare congenital disorder of cornification. Patients show variable extent of erythema and scaling of the skin. Newborns may present with a collodion membrane, which makes the disease live threatening at birth. Pathophysiologically, the function of the skin barrier is impaired. ARCI can be caused by mutations in different genes including TGM1, ALOX12B and ALOXE3, which code for TGase1, 12R-LOX and e-LOX3, respectively, and which are mutated in around 50% of patients with ARCI. Here we describe an approach for a novel, personalized therapy with the goal of replacing the defective protein in the patient skin.

In a first step the three enzymes TGase1, 12R-LOX and e-LOX3 were produced in a mammalian expression system since correct folding of the proteins is necessary for activity of the enzymes. Production of TGase1 was checked by FACS and Western Blot analysis and enzyme activity was analyzed using a colorimetric assay with K5 peptide as a substrate. For efficient protein delivery into the epidermis TGase1 was coupled to thermoresponsive PNIPAM-dPG nanogel, which releases the protein at temperatures above 35°C. This characteristic is well suitable for delivery into the skin since the temperature at the skin surface is around 32°C and reaches 35°C only in deeper epidermal layers. In a next step the TGase1/nanogel particles were tested on cultured keratinocytes. Thereby we observed successful protein uptake into the cells and, most importantly, that the enzyme is still active after delivery. We then generated normal 3D full thickness skin models and disease models with TGM1 knockdown in keratinocytes and treated them with TGase1/nanogel. Characterization of TGM1 knockdown models before treatment showed hardly any TGM1 expression and activity, as well as a significantly reduced epidermal barrier function. After four times application of TGase1/nanogel within 8 days TGase1 deficient models showed efficient recovery of the barrier function as measured in a Franz-cell permeation test.

Our findings have revealed cutaneous protein replacement as a promising approach to personalized therapy for ARCI. After further experiments with patient cells, optimization of epidermal delivery and toxicity tests we will adapt our system also to other genes involved in the development of congenital ichthyosis.

P-Therap-203

Metformin as a potential candidate in HD therapy

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Huntington‘s disease (HD) is an autosomal dominant neurodegenerative disorder that leads to cognitive decline and affects motor abilities. In the prodromal phase of the disease patients develop mood swings, personality changes and subtle cognitive impairment. Close understanding of clinical signs and molecular mechanisms behind this early stage of HD is an important requisite for the development of a causal therapy.

An unstable glutamine (CAG) trinucleotide repeat expansion within the exon 1 of the Huntingtin gene leads to the disorder (Cummings et al., 2006). We have analysed a knock-in mouse model that carries 150 CAG repeats and the human exon 1 in the 5’ end of the murine huntingtin gene. By using a novel object recognition test with a 24 hour interval between sample and test phase we have found a profound deficiency of

hippocampus dependent long-term memory in heterozygous transgenics. This phenotype was detected as early as 12 weeks of age and is complementary to deficits that we have identified in the HdhCAG111 mouse model previously. Motor deficits as well as intranuclear aggregates are described at much later stages in both of these models. This early phenotype makes the two models ideal for drug screening.

Metformin is a biguanide, an AMPK antagonist and mTOR agonist. It is commonly used as an anti-diabetic drug. Side effects of metformin in glucose normal people have not been described. We have shown previously that by interfering with the mTOR kinase and its opposing phosphatase, PP2A, metformin regulates local protein synthesis in the brain and is able to suppress the production of disease making protein in HD. In addition, beneficial effects of metformin in HD e.g. prolongation of survival in males (Thong et al., 2006) and delay of HD-associated symptoms by improving mitochondrial functions (Adhihetty et al., 2010) have been found. By using the described test battery we are now testing hippocampus based long-term memory in heterozygous and WT HdhCAG150 males at 12 weeks with and without application of metformin. We hope to see an improvement in long-term memory in treated heterozygous mice compared to non-treated, which would make metformin an ideal candidate for a therapy of early stage HD.