VORTRÄGE

KEYNOTE LECTURE

Human Olfactory Receptors: Their Role in Odor Perception and as New Targets for Diagnosis and Therapy

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Scents indicate things, make promises, attract attention and stimulate imagination, feed anxieties and hopes: they are the salt in the atmospheric soup. We regard seeing and hearing as more important sensory functions, because they contribute more to conscious, cognitive processes of perception - but at moments of the greatest enjoyment we close our eyes and taste the scent, smell the taste. Before the spirit and beauty of a person can fascinate us, our nose must become infatuated.

The olfactory system in the nose acts as a window, monitoring environmental chemical information and convert chemical stimuli in electrical nerve impulses which are conducted along the olfactory sensory neuron to their glomerular target in the brain. Olfactory receptors (ORs) activation shows the distinguished (cAMP-based) transduction pathway for odorant perception. In 1991 Buck and Axel discovered the olfactory gene family, the largest gene family in the human genome, and postulated an exclusive expression in the olfactory epithelium. However, recent whole genome sequencing data from our and other labs show that ORs have been found in every tissue of human body which was analyzed by next generation sequencing. The importance of such ectopic expression of ORs is raised since the physiological function of some of ORs was characterized. When identifying additional expression profiles and functions of OR in non-olfactory tissue, there are limitations posed by the deorphanization of ORs concerning the activated ligands and by the small number of antibodies available. In contrast to the olfactory sensory neurons which are believed to express all 350 functional OR genes (only one OR type per cell), cells in non-olfactory tissues tend to express more than one individual OR gene per cell. In addition, some of the signaling pathways in non-olfactory tissues seem to involve completely different components in comparison to the olfactory neurons.

What is the functional role of these ectopically expressed olfactory receptors? Evidences rapidly accumulate that ORs participate in important cellular processes outside its primary sensorial organ where they function in odor detection and discrimination. In our lab the functional expression of the first was demonstrated in spermatozoa (2004). In the meantime we could show the existence and function of ORs in the cardiovascular system (heart, blood cells), the gastrointestinal system (small intestine, liver, pancreas), the genito-urinary system (kidney, testis, spermatozoa, prostate), the respiratory system (lung, smooth muscle cells), the skin (keratinocytes, melanocytes) and sensory organs (retina). Interestingly we found a broad spectrum of important functions like cell-cell communication and recognition, tissue injury, repair and regeneration, cancer growth, progression and metastasis, nutrient sensing and muscle contraction. Nevertheless the functional importance of ectopic ORs is still not sufficiently understood. Studies seeking to determine the function of ectopic ORs are still in its infancy and require further intensive exploration. However, the potential of ORs to serve a target for a wide range of clinical approaches is indeed given. This hold promises that the knowledge gained by future investigations would lead to deepen our understanding of OR function in health and disease and may provide the basis for the development of applications in diagnosis and therapies in near future.

PLENARY SESSIONS

Plen 1

X chromosome structure and regulation

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Complex mechanisms of dosage compensation regulate the mammalian X chromosome due to the presence of one copy in males (XY) and two in females (XX). X inactivation silences one X chromosome in

females in early development, leading to specific epigenetic and structural changes. The inactive X chromosome becomes condensed and forms a bipartite structure within the nucleus, as we have shown by chromatin conformation analyses. Specific long non-coding RNAs are implicated in the formation of this unique structure. The inactive X chromosome is preferentially located near the lamina or the nucleolus. Genes that escape X inactivation tend to be located at the periphery of the condensed inactive X chromosome. Such genes are more highly expressed in females, and thus associated with sex-specific differences manifested even in early development. We have found that significant sex bias in gene expression are associated with escape from X inactivation in human tissues from normal males and females, and in tissues from individuals with sex chromosome aneuploidy, including Turner or Klinefelter individuals.

Plen 2

The identification and characterization of mutations causing neurodevelopmental diseases

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A central challenge in human disease genetics is the identification of pathogenic mutations. One key approach to distinguishing benign and pathogenic mutations is to use population genetic data to identify regions of the human genome under purifying selection. Here I describe how the residual variation intolerance scoring framework has been applied to identifying pathogenic mutations in and outside protein encoding regions of the genome. Next I report how these are related approaches are being used to identify pathogenic mutations in large-scale scale studies in epilepsy and other neurodevelopmental diseases. Finally, I discuss how the identification of genetic causes of disease can inform treatment choices.

SYMPOSIA

S1-01

Modeling disorders of sterol biosynthesis with reprogrammed stem cells: New insight into lipid-protein interactions, disrupted signaling mechanisms, and cellular phenotypes

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Autosomal recessive disorders of cholesterol synthesis constitute a group of malformation syndromes that severely affect nervous system development and function, including Smith-Lemli-Opitz syndrome (SLOS) and lathosterolosis (LATH). While reduced cholesterol content due to mutations within the cholesterol synthetic enzymes 3β-hydroxysterol **Δ**7-reductase (DHCR7; SLOS causative) and 3β-hydroxysteroid **Δ**5-desaturase (SC5D; LATH causative) is common to these disorders, broad cellular and phenotypic differences of unknown origin exist both between and within patients diagnosed with these diseases. Thus, while the associated biochemical defects are well defined, neither the specific mechanisms underlying neurological abnormalities nor the role of decreased cholesterol versus sterol precursor accumulation in disease pathogenesis have been clearly delineated. To identify cellular phenotypes and causative signaling pathways, we derived induced pluripotent stem cells (iPSCs) from SLOS and LATH subjects to model these diseases *in vitro*. SLOS subjects were known carriers of the most common *DHCR7* mutations, including the intronic splice acceptor mutation c.964-1G>C and the missense mutation p.T93M. While all iPSCs demonstrated the expected biochemical defects due to *DHCR7* or *SC5D* mutations, cellular assays uncovered a defect in neural stem cell maintenance resulting in accelerated neuronal formation in SLOS iPSCs. Further molecular and biochemical analyses demonstrated inhibition of cholesterol-Wnt interactions and loss of Wnt/β-catenin activity mediated cellular phenotypes. However, this cellular phenotype was exclusive to SLOS, as LATH iPSCs did not exhibit a neural progenitor defect or inhibition of Wnt/β-catenin activity. While this work demonstrates the utility of iPSCs for modeling rare diseases and identifies signaling deficits potentially underlying SLOS phenotypes, questions remain regarding cellular and functional consequences, the specificity of lipid-Wnt interactions, and the role of other disrupted signaling pathways in mediating developmental and functional deficits in these diseases. Unpublished work using a variety of approaches will be discussed comparing the specific effects of cholesterol

synthesis mutations on cell fate, functional activity, and lipid modulated signaling pathways to more precisely define the consequences of cholesterol synthesis defects and identify potential targets for patient therapy.

S1-02

Williams syndrome: findings from the dish

Thanathom Chailangkarn

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Induced pluripotent stem cell (iPSC) technology has become one of the major approaches for disease modeling since its first report in 2006. The ability to reprogram cells from somatic into embryonic stem cell-like state and to differentiate them into desired cell types in the culture dish has allowed scientists to carry out the study of several diseases in cells such as neurons which, in the past, could not be isolated from living subjects. Williams syndrome (WS), a genetic neurodevelopmental disorder where 25-28 genes are hemizygously deleted, is among those. Despite cardiovascular abnormalities, its unique neurological phenotypes i.e. hypersociability is of our interest. For several decades, research on different neurological aspects of WS has been conducted in a variety of models such as patient-derived cell lines (lymphoblastoid cells and fibroblasts), post mortem tissue, and mouse models. However, the lack of physiologically relevant cell types such as neural progenitor cells (NPCs) and neurons has left a critical gap in our knowledge the disease's cellular and molecular phenotypes.

To fill this gap, we took the advantage of the reprogramming technology to capture the genomes of WS subjects in iPSCs, which could be then differentiated into NPCs and neurons, enabling evaluation of whether the captured genome with hemizygous deletion of those genes leads to relevant neuronal cellular phenotypes. Dental pulp cells-derived iPSCs of classical WS, rare WS and typical developing (TD) subjects were neurally induced via dual-SMAD inhibition in order to generate NPCs and neurons. We discovered that classical WS NPCs exhibited increased apoptosis, and, therefore, doubling time, compared to TD neurons. This could possibly contribute to the reduction in cortical surface area in classical WS individuals as assessed by magnetic resonance imaging. Surprisingly, we found that rare WS NPCs behaved similarly to TD NPCs rather than to classical WS NPCs in terms of apoptosis. We confirmed that frizzled9, which is deleted in the classical WS but not in our rare WS genome, is responsible for such phenotype via gain- and loss-of-function assays. Moreover, classical WS neurons in general showed increased frequency of activity-dependent calcium transient compared to TD neurons. Finally, classical WS neurons expressing CTIP2, a cortical layer V marker, exhibited an increase in total dendritic length and number of dendritic spines compared to TD neurons, which was in agreement with the results obtained from WS cortical layer V neurons in post mortem brain. These findings in WS neurons offer new insights into the haploinsufficiency effect in cortical layer V neurons whose role is implicated in social behavior, suggesting an increase in neuronal activity that could possibly be linked to hypersociability observed in WS individuals. We demonstrated that iPSC technology holds great potential for disease modeling by revealing the missing pieces of cellular and molecular information needed for further drug screening and discovery.

S1-03

Tissue-specific mutation accumulation in human adult stem cells during life

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The gradual accumulation of genetic mutations in human adult stem cells (ASCs) during life is associated with various age-related diseases, including cancer. Extreme variation in cancer risk across tissues was recently proposed to depend on the lifetime number of ASC divisions, owing to unavoidable random mutations that arise during DNA replication. However, the rates and patterns of mutations in normal ASCs remain unknown. We determined genome-wide mutation patterns in >50 ASCs of the small intestine, colon and liver of human donors with ages ranging from 3 to 87 years by whole genome sequencing of clonal organoid cultures derived from primary multipotent cells. Our results show that mutations accumulate steadily over time in all of the assessed tissue types, at a rate of approximately 40 novel mutations per year, despite the large variation in cancer incidence among these tissues. Liver ASCs, however, have different mutation spectra compared to those of the colon and small intestine. Mutational signature analysis reveals that this difference can be attributed to spontaneous deamination of methylated cytosine residues in the colon and small intestine, probably reflecting their high ASC division rate. In liver, a signature with an as-yet-unknown underlying

mechanism is predominant. Interestingly, mutation spectra of driver genes in cancer show high similarity to the tissue-specific ASC mutation spectra, suggesting that intrinsic mutational processes in ASCs can initiate tumorigenesis. Notably, the inter-individual variation in mutation rate and spectra are low, suggesting tissue-specific activity of common mutational processes throughout life.

S2-01

Treatment of lysosomal storage disorders: clinical issues, successes and challenges

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Enzyme replacement therapies have been developed over the last 25 years for several of the lysosomal storage disorders (LSD's). The success of enzyme replacement therapy for Gaucher disease paved the way for the development of similar treatments for the mucopolysaccharidoses, Fabry and Pompe disease and lately also for neuronopathic lysosomal storage disorders by intrathecal or intracerebral injections. In addition, small molecule approaches have been developed including substrate reduction therapies and chaperones, which can be used orally. While in Gaucher disease enzyme as well as substrate reduction therapy results in reversibility of disease manifestations, with decreases in hepatosplenomegaly, normalization of blood counts and prevention of skeletal disease, this is unfortunately not the case for all patients affected with other lysosomal storage disorders. An important concept is the "window of opportunity for treatment" which is different for these disorders. For example, in Fabry disease, early fibrosis in the heart is related to unresponsive disease and unfortunately fibrosis may occur already in an early stage, sometimes even without prior hypertrophy. Whether earlier intervention will be beneficial is largely unknown. And then: what is early? Many unresolved questions exist at this stage, including the following:
- what is the natural history and "point of no return" for the different LSD's?
- what is the natural history and "point of no return" for subgroups of patients within one LSD's?
- what are the long term complications: treatments change the phenotype rather than cure the disease
- what is the influence of antibody generation on clinical effectiveness?
- how do we manage the extreme costs of these products, especially in light of the many unsolved issues with respect to effectiveness?
Surprisingly, so far healthcare professionals, governments and industry have failed to systematically address these issues, resulting in insufficient knowledge for potentially lifesaving treatments. Early conditional access, followed by a strict, transparent, independent, collaborative evaluation in addition to fair pricing should be explored.

S2-02

Mitochondrial transcript processing and its disorders

Michal Minczuk

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Mitochondrial respiratory chain deficiencies exhibit a wide spectrum of clinical presentations. These pathologies result from defective mitochondrial energy production and can be caused by either mutations in the mitochondrial DNA (mtDNA) or mutations in nuclear genes coding for mitochondrially-targeted proteins. The underlying pathomechanisms can affect numerous pathways involved in mitochondrial biology including expression of mtDNA-encoded genes. Recent studies have revealed that expression of the mitochondrial genes is extensively regulated at the post-transcriptional stages that entail nucleolytic cleavage of precursor RNAs, RNA nucleotide modifications, RNA polyadenylation, RNA quality and stability control. These processes ensure proper mitochondrial RNA (mtRNA) function, and are regulated by dedicated, nuclear-encoded enzymes. Recent growing evidence suggests that mutations in these nuclear genes, leading to incorrect maturation of RNAs, are a frequent cause of human mitochondrial disease. I will discuss the current knowledge and most recent discoveries related to a subset of nuclear-encoded genes coding for proteins involved in mitochondrial RNA maturation, for which genetic variants impacting upon mitochondrial pathophysiology have been reported.

Metabolic role of peroxisomes in humans: functional interplay with other subcellular organelles and genetic diseases of peroxisome metabolism

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Eukaryotic cells contain a large number of different subcellular organelles which all perform separate tasks and thereby contribute to the overall performance of individual cells. Metabolism also requires the active participation of a variety of different subcellular compartments including the cytosol, mitochondrial, peroxisomes, lysosomes and the endoplasmic reticulum. The peroxisome is a good example of a subcellular organelle which plays an indispensable role in metabolism, but is nevertheless completely dependent on the interaction with other organelles to fulfill its role in metabolism. This is true for all major functions of peroxisomes, including: (1.) ether phospholipid synthesis; (2.) fatty acid beta-oxidation; (3.) fatty acid alpha-oxidation, and (4.) glyoxylate detoxification. With respect to peroxisomal fatty acid oxidation peroxisomes catalyze the chain-shortening of certain fatty acids including very-long-chain fatty acids, but requires the active help of mitochondria to catalyze the degradation of acetyl-CoA and the reoxidation of NADH as produced in peroxisomes. Furthermore, with respect to ether phospholipid biosynthesis peroxisomes heavily rely on the endoplasmic reticulum to complete formation of ether phospholipids whereas fatty acid alpha-oxidation also requires the functional interplay between peroxisomes and mitochondria and the same is true for glyoxylate detoxification. Recent evidence holds that the interaction between peroxisomes and the different subcellular organelles, including mitochondria and endoplasmic reticulum, is mediated by specific tethering protein complexes which bring organelles physically together thereby allowing metabolism to proceed smoothly. The importance of peroxisomes in metabolism is stressed by the existence of a large group of single peroxisomal enzyme deficiencies of which X-linked adrenoleukodystrophy is best known. Our current state of knowledge with respect to the role of peroxisomes in metabolism and the peroxisomal enzyme deficiencies will be presented at the meeting.

Huntington's disease: RNA-sequencing, small RNA-sequencing, ChIP-sequencing and GWAS data.

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Huntington's disease (HD) is a dominantly transmitted neurodegenerative disease of midlife onset. Recently several different unbiased genome wide studies in HD have been performed. These analyses point to a variety of pathological pathways that are associated with important features of the disease, including age at onset, CAG repeat size, and the extent of neuropathological involvement. Genome wide association studies (GWAS) have identified several regions of the genome that contain genes that are associated with the age at onset for HD. The strongest of these is located at 15q13.3 for rs146353869 for which a very rare allele (MAF=1.1%) is associated with an approximate 6-year younger age at onset for carriers of the minor allele. The same locus contains an independent effect for rs2140734 where a more common minor allele (MAF=30.2%) is associated with a 1.4 year older age at onset for carriers of this allele. These single nucleotide polymorphisms are in the region of FAN1, MTMR10 and several other genes; some of which are not expressed in brain and are not likely candidates for HD modification. eQTL analysis has not resolved which gene may be implicated. Other GWAS implicated loci include rs1037699 at 8q22.3 and rs144287831 at 3p22.2. We have sought to combine the information derived from multiple platforms to gain additional insight into the pathways that may be implicated in HD pathogenesis. In this strategy, we have performed mRNA-sequencing, small RNA-sequencing and ChIP-sequencing using the H3K4me3 mark for active transcription and the repressive mark H3K9me3 in human HD brain samples with GWAS genotyping. While the striatum is most involved in HD, the extent of neurodegeneration in post-mortem tissue precludes meaningful comparison between disease and control samples, and consequently we studied prefrontal cortex (BA9). Several common pathways were seen across these three platforms. mRNA-sequencing and miRNA-sequencing data identified altered transcriptional profiles implicating developmental pathways involving the HOX genes and related homeo-box domain genes (e.g. PITX1, POU4F2, etc.). Notably, microRNAs located in HOX gene clusters were among those most increased and levels of these correlate with pathological involvement in the striatum. These genes, associated with early embryonic development, are commonly silent in normal adult brain, and were among the most differentially expressed genes in HD brain. These prominent statistical effects are driven

by the near total absence of expression in normal brain. Pathways implicated in mRNA-seq and ChIP-seq studies, included immune function and regulation of gene expression. These associations were very strong, indicating a large immune reactive response in the HD brain which may be a major influence contributing to neurodegeneration. In many instances enrichment of H3K4me3 at transcription start sites was not accompanied by a corresponding increase in expression. The apparent inconsistency suggests that common regulatory mechanisms in the HD brain are disrupted and this may contribute to a complex interplay of factors contributing to the neurodegenerative process. Often findings in human HD brain samples conflicted with those reported in HD transgenic mouse models, suggesting that one may wish to be cautious in interpreting the significance of either type of study in isolation.

S3-02

The causal pathogesis of Huntington disease, new therapeutic approaches

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Huntington disease (HD) is an autosomal dominant neurodegenerative disease characterized by progressive loss of voluntary motor control, psychiatric disturbance, cognitive decline and death 15-20 years after motor onset. HD is uniquely caused by a polyglutamine encoding CAG expansion in the huntingtin gene (HTT), which allows for identification of pre-manifest mutation carriers as much as decades before onset and should facilitate development of disease modifying therapies. Yet over 20 years after identification of the HD mutation, available therapies offer only symptomatic relief and are fraught with side effects. Development of safe small molecule therapies for HD has been hindered by difficulties identifying and validating tractable drug targets within the disorder's complex pathogenesis. Teva Pharmaceuticals is developing potential novel treatments based on a mechanistic understanding of disease pathways common to neurodegenerative diseases. The progress of these studies will be reviewed.

S4-01

Rare complete gene knockouts in adult humans

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Loss-of-function mutations cause many mendelian diseases. Here have create a catalog of autosomal genes that are completely knocked out in humans by rare loss-of-function mutations. We sequenced the whole genomes of over 29,000 Icelanders and imputed the sequence variants identified in this set into a total of 151 chip-genotyped and phased Icelanders. Of the genotyped Icelanders, around 10% are homozygotes or compound heterozygotes for loss-of-function mutations with a minor allele frequency (MAF) below 2% in close to 2000 genes (complete knockouts). Genes that are highly expressed in the brain are less often completely knocked out than other genes. Homozygous loss-of-function offspring of two heterozygous parents occurred less frequently than expected (deficit of 136 per 10,000 transmissions for variants with MAF <2%, 95% confidence interval (CI) = 10–261). We are currently systematically phenotyping such human complete knock out. This phenotyping lasts 4 hours and attempts to cover most of the observable diversity in a non-invasive and cost efficient manner. I will demonstrate how using systematic phenotyping can advance the knowledge on individual gene knockout. We use results from in-house transcriptomics, existing animal models and complementary approaches to assess the observation in human. We will also discuss the scrutiny in other population in order to detect such complete knock-out. We will exemplify the impact of founder population and consanguinity in such an odissey.

S4-02

Homozygosity and The Human Variome: More Than Meets the Eye

Fowzan S Alkuraya

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After the recent explosion in sequencing throughput, variant interpretation has quickly become the bottleneck in our effort to usher in the era of genomic medicine. While homozygosity for apparently pathogenic

variants in the context of disease states is a well-established phenomenon, homozygosity can uncover many medically relevant aspects of the human variome that are difficult to study otherwise. For example, seemingly benign variants may prove pathogenic in the homozygous state. This includes variants with benign prediction using in silico tools as well as variants in dominant genes with no phenotype in carriers because they represent bona fide recessive inheritance. Variants that are associated with one phenotype in compound heterozygous states may express themselves quite differently phenotypically when homozygous. Furthermore, previously reported pathogenic variants can be challenged when their presence in homozygosity is associated with no abnormal phenotype, thus improving the specificity of the annotation of the morbid genome. Homozygosity for LOF variants is a special scenario that allows us to study naturally occurring human "knockouts", a powerful tool to study the physiological context of genes in humans. Finally, homozygosity in the context of autozygosity provides a robust mapping tool that can greatly aid in the identification of relevant variants, especially those that exert their pathogenic effect in ways that defy detection by our usual algorithms. By expanding the spectrum of phenotypes that are studied, one can unlock the full potential of homozygosity to understand the medical relevance of the human variome in it its full range from embryonic lethal to essentially benign.

S5-01

Inter-generational inheritance of diet–induced obesity and diabetes and identification of epigenetic factors in mammalian gametes

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The inheritance of epigenetic information in mammals across generations has been controversial. Some reports provided initial evidence that a paternal high fat diet may propagate obesity and glucose intolerance in offspring, but potential confounders such as molecular factors present in seminal fluid, paternal-induced alterations in maternal care or transmission of microbiomes were not ruled out in these studies. We have shown in mice that a parental high fat diet (HFD) renders offspring derived via in vitro fertilization (F1) more susceptible to develop excessive overweight and type 2 diabetes (T2D) in a gender and parent-of-origin specific mode. Female, but not male, offspring from obese parents became significantly more obese during a HFD challenge than female offspring from lean parents. Body weight trajectories and distribution patterns of individual body weights in female offspring from one obese and one lean parent demonstrate that paternal and maternal germline propagate obesity in a roughly equitable and additive fashion, but likely different mode of action. In contrast, a more deteriorated state of HFD-induced insulin resistance was observed in both F1 genders, albeit predominantly inherited via the maternal germline.

Towards the identification of epigenetic information in sperm and oocyte from HFD and low fat diet fed parents, we are currently analyzing their transcriptome and methylome signatures. The status of this analysis will be presented.

We report for the first time epigenetic inheritance of an acquired metabolic disorder via mammalian oocytes and sperms excluding confounding factors. Such an epigenetic mode of inheritance may contribute to the observed pandemic increase in obesity and T2D prevalence rates, especially in an environment where nutrition is abundant.

S5-02

From rare to common: New findings in monogenic obesity with implications for polygenic, common obesity

Heiko Krude

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Early onset and severe obesity can be inherited via loss of function mutations within the melanocortin pathway of hypothalamic body weight regulation. The most prominent player in this signalling pathway is the fat cell hormone leptin. Leptin gene mutations were the first to be linked to monogenic early onset obesity. After binding of leptin to leptin receptors in the arcuate nucleus of the hypothalamus the neuropeptide MSH is processed from the precursor POMC and acts as a ligand at the MC4 receptor. Mutations in the leptin receptor gene, the POMC gene and the MC4 receptor gene were subsequently diagnosed in further patients with extreme early onset obesity. While leptin mutation patients can be treated with recombinant leptin – as shown already in the late 1990s – all other monogenic obesity forms are leptin resistants, and additional leptin failed to decrease body weight. Only recently POMC gene deficient patients were successfully treated with the MSH-analogue Setmelanotide (Kühnen et al. 2016).

Common severe obesity is defined by the lack of disease causing monogenic defects. A plethora of GWAS

identified a large number of SNPs in common obesity associated with the individual BMI but only to a low amount of not more then 25%. However, almost all these common obese patients are characterized by high leptin levels suggesting sufficient generation of leptin in the increased fat tissue and a state of leptin resistance. Several new data concerning the contribution of epigenetic and genetic variants in the POMC gene locus argue for a role of the melanocortin pathway also in common obesity and imply, therefore, a potentially new treatment option also in common obesity based on MSH-analogues.

S6-01

Genetics of gene expression in immunity and infection

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Genome-wide association studies have highlighted the role of genetic associations with susceptibility to common inflammatory diseases, highlighting potential new insights into disease pathogenesis and opportunities for therapy. However understanding the functional basis of these associations and delivering translational utility remains a significant challenge to the field. Non-coding regulatory genetic variants are most commonly implicated in such studies. Recent work highlights how such variants are also major drivers of diversity in the immune response transcriptome. This talk will discuss approaches we are taking to try and establish functional links between immune phenotype-associated regulatory genomic and epigenomic variation, and specific modulated genes and pathways. I will describe insights from the application of expression quantitative trait (eQTL) mapping to define genomic modulators of the global transcriptomic response in different primary immune cell populations and to specific innate immune stimuli in health and disease. This work highlights the extent of local and distant context-specific eQTL, enabling resolution of immunoregulatory variants and the identification of specific modulated genes involving disease associated loci. Examples will be described showing how mapping trans-regulatory loci can be a powerful approach for discovery and dissection of gene networks informative for disease. I will also show how we have applied analysis of the genetics of gene expression in patients with sepsis admitted to intensive care, revealing new insights into disease pathogenesis. Further progress in this area will require characterisation of associated variants in the context-specific disease relevant epigenomic landscape in which they may act, requiring careful consideration of relevant immune cell types and environmental modulators to study, together with evidence establishing mechanism, for example based on mapping chromatin interactions and application of genome editing techniques.

S6-02

Fine-mapping the MHC region in autoimmune diseases

Soumya Raychaudhuri

Associate Professor of Medicine and Biomedical Informatics, Harvard Medical School, MA, USA
Associate Member, Broad Institute, Professor in Genetics, University of Manchester

The major histocompatibility complex is the single locus that explains more disease risk than all other loci combined for a large number of autoimmune diseases, include psoriasis, type I diabetes, rheumatoid arthritis, and ankylosing spondylitis. Here we describe efforts to fine-map the MHC locus in a range of these different diseases. For these efforts we have been using HLA imputation based on a panel of over 5,000 HLA typed individuals to infer HLA genotypes into large pre-existing genome-wide association (GWAS) data sets. We describe how we identified the individual amino acid positions for rheumatoid arthritis, type I diabetes, and other diseases that drive autoimmune disease risk. Most of these individual positions are within the binding groove and interact directly with antigens that may be triggering disease risk. We focus specifically on DRB1 position 13, which drives risk of multiple diseases. Next we demonstrate how HLA alleles interact with each other in a non-additive fashion to confer unexpectedly high or low risk of disease. Finally, we will go onto describe efforts to fine-map disease alleles in other loci outside of the MHC, where power is more limited, using complementary approaches integrating functional genomic data with genetic data.

EDU 1

Genodermatosen – der (un)geklärte Fall

Moderation: Judith Fischer, (Freiburg)

Die Vorstellung von geklärten und ungeklärten Fällen soll in diesem Jahr zum ersten Mal zu einem übergeordneten Thema erfolgen. Wir haben hierzu das Thema GENODERMATOSEN ausgewählt. Zu Beginn der Sitzung wird es einen 30-minütigen Übersichtsvortrag von Herrn Prof. Dr. Henning Hamm, leitender Oberarzt der Klinik und Poliklinik für Dermatologie, Venerologie und Allergologie, Universitätsklinikum Würzburg, mit dem Thema „Update zu Genodermatosen" geben. Für die geplanten sechs Fallvorstellungen im Anschluss daran, bitten wir um Anmeldung von interessanten Fällen und Zusendung von 6-8 Folien bis spätestens zum 22.03.2017 an judith.fischer@uniklinik-freiburg.de

EDU 2

Hirnfehlbildungen – der (un)geklärte Fall

Moderation: Dagmar Wieczorek (Düsseldorf), Gabriele Gillessen-Kaesbach (Lübeck)

Die Vorstellung von geklärten und ungeklärten Fällen soll in diesem Jahr zum ersten Mal zu einem übergeordneten Thema erfolgen. Wir haben hierzu das Thema Hirnfehlbildungen ausgewählt. Zu Beginn der Sitzung wird es einen 30-minütigen Übersichtsvortrag von Frau Dr. Nataliya DiDonato, Institut für Klinische Genetik, Medizinische Fakultät Carl Gustav Carus, Dresden, mit dem Thema „Häufige Hirnfehlbildungen – ein Überblick" geben. Für die geplanten sechs Fallvorstellungen im Anschluss daran, bitten wir um Anmeldung von interessanten Fällen und Zusendung von 6-8 Folien bis spätestens zum 22.03.2017 an dagmar.wieczorek@uni-duesseldorf.de.

Pro & Contra I

Die Zukunft der nicht-invasiven genetischen Pränataldiagnostik ("NIPD") – ethische Aspekte

Organisation: Jörg T. Epplen (Bochum)

Es diskutieren Professorin Dr. Christiane Woopen (Medizinethik, Köln) und Professor Dr. Christian Netzer (Humangenetik, Köln), Moderation: Professor Dr. Christoph Rehmann-Sutter (Bioethik, Lübeck)

Kommerzielle Anbieter von NIPD-Tests stehen in einem nationalen und internationalen Konkurrenzkampf um Marktanteile. Daraus entsteht nicht nur ein Druck, die Tests günstiger und besser (sicherer und präziser) zu machen, sondern auch ein Druck, das Testmenu über die gegenwärtig angebotenen Tests hinaus zu erweitern, die Tests früher in der Schwangerschaft durchführen zu können und die Tests direkt Konsumentinnen anzubieten, vielleicht vom Ausland aus. Die bisher etablierte Regulierung der Pränataldiagnostik in Deutschland würde damit unterlaufen. Der Arztvorbehalt, der heute nach Gendiagnostikgesetz vorgeschrieben ist, mit der persönlichen genetischen Beratung, könnte unter Druck geraten. Es stellen sich neue ethische und regulatorische Fragen, die in diesem Panel diskutiert werden sollen.

Fragen:
Was soll im Rahmen der NIPD alles getestet werden dürfen? Warum soll sich das Testangebot von Amniocentese und Chorionzottenbiopsie unterscheiden?
Wie unterscheidet sich NIPD in ethischer Hinsicht von der Amniocentese und Chorionzottenbiopsie?
Wie kann und soll die NIPD reguliert werden?

Pro & Contra II

„Mein Genom gehört mir" – Aufklärung, Einwilligung und Datenmitteilung bei genomweiten Analysen

Sabine Rudnik, Johannes Zschocke (Innsbruck)

Mit dem Einzug von genomischen Analysen (whole genome sequencing = WGS, whole exome sequencing = WES) in der genetischen Diagnostik und Forschung stellt sich aus verschiedenen Perspektiven die Frage, welche Informationen an Patienten oder Probanden zurückfließen dürfen oder sollen und wie dies in Aufklärung und Einwilligung vermittelt wird. In dieser Pro- und Contra-Runde soll offen über die Erwartungen von Patienten/Probanden, realistische Ressourcenplanung und kommunikative Strategien diskutiert werden.

Wie kürzlich aus juristischer Perspektive veröffentlicht wurde[1], haben einwilligungsfähige Patienten nach dem Patientenrechtegesetz ein Recht auf die Herausgabe ihrer Rohdaten aus WGS/WES, so dass sich die Frage stellt, ob und wie detailliert man im Vorfeld über den Inhalt der Daten aufklärt. Nach rechtsethischer Auffassung kann auch im Forschungskontext die Herausgabe von Rohdaten nur dann verwehrt werden, wenn ein „unverhältnismäßiger oder unzumutbarer Aufwand" entsteht oder die „Forschungsfreiheit hierdurch unverhältnismäßig eingeschränkt" wird. Die Autoren schlagen folgende Handlungsempfehlung bei einem entsprechenden Probandenwunsch vor: 1. Telefonischer Kontakt mit der Projektleitung, 2. Schriftliche Aufklärung über Natur und Implikationen der Rohdaten, 3. Angebot einer individuellen Beratung, 4. Unterzeichnung einer Erklärung, 5. Herausgabe einer vollständigen Kopie der Rohdaten auf sicherem Weg. Es stellt sich mithin die Frage, wie dieses Angebot in der Praxis umgesetzt und wie z. B. mit den Daten von Minderjährigen umgegangen werden soll.

Rein logistisch erfordert schon der Weg von der reinen Laboranalyse, bei der zahlreiche genetische Varianten generiert werden, bis zur Bestätigung als diagnostisch valider Befund, der dem Patienten / Probanden rückgemeldet werden kann, erhebliche personelle und finanzielle Ressourcen. Diese stehen im diagnostischen Kontext nur sehr bedingt zur Verfügung und sind in Forschungsprojekten bisher gar nicht abgebildet. Nach einer aktuellen Stellungnahme der Deutschen Forschungsgemeinschaft[2] sollen humane Genomsequenzierungen die Möglichkeit der Rückmeldung von Analyseergebnissen enthalten. Als Orientierung für einen verantwortungsvollen Umgang mit dieser Frage wird auf die Projektgruppe EURAT[3] verwiesen. Dennoch bleibt das Problem der Einordnung mitteilungswürdiger Ergebnisse aus Forscher- und Probandensicht und der Bereitstellung der für Aufklärungs- und Rückmeldungsalgorithmen erforderlichen Ressourcen. Darüber hinaus ist bis heute nicht geklärt, welche kommunikativen Prozesse eine ausreichende Basis für ein informiertes Einverständnis darstellen.

Diese und weitere Fragen möchten wir mit Frau Prof. Dr. med. Dr. phil. Eva Winkler (Nationales Centrum für Tumorerkrankungen, Heidelberg), Herrn Dr. phil. Martin Langanke (Theologische Fakultät, Universität Greifswald) und Herrn PD Dr. phil. Peter Burgard (Universitätskinderklinik Heidelberg) kontrovers diskutieren. Die Organisatoren werden in die Fragestellung einführen, anschließend sind Impulsreferate (ca. 10 min) der Referenten vorgesehen, bevor eine Debatte mit dem Publikum angeregt wird.

Talk nach 12

Quo vadis Humangenetik – Der neue EBM

Moderatoren: Christian Netzer (Köln) und Bernd Wollnik (Göttingen)

Am 01. Juli 2016 ist der überarbeitete EBM für den Fachbereich Humangenetik in Kraft getreten. In diesem sind weitreichende Änderungen humangenetischer Leistungen verankert worden. Ziel der Überarbeitung war es laut Kassenärztlicher Bundesvereinigung, die genetischen Leistungen im EBM an den aktuellen Stand von Wissenschaft und Technik anzupassen. Im Rahmen von vier kurzen Impulsvorträgen zu unterschiedlichen Aspekten des neuen EBMs sollen sowohl die vorgenommenen Änderungen, als auch deren Auswirkungen auf die Humangenetik und humangenetische Leistungen einem breiteren Publikum vorgestellt werden. Dabei ist es nicht unsere Intention, eine reine EBM-Informationsveranstaltung zu sein und Interpretationshilfen für die neuen Leistungslegenden der Abrechnungsziffern zu liefern. Wir möchten vielmehr eine konstruktive Diskussion anregen, die die grundsätzlichen Stärken und Schwächen des neuen EBM näher beleuchtet. Um die anschließende Podiumsdiskussion möglichst lebhaft und interessant zu gestalten, bitten wir schon einmal vorab um die Zusendung von Fragen und Kommentaren zum Thema, welche dann von den Moderatoren in die Diskussion eingebracht werden (via Email an: bernd.wollnik@med.uni-goettingen.de oder christian.netzer@uk-koeln.de). Diskussionsbeiträge vor Ort sind natürlich auch sehr erwünscht.

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[1] Fleischer et al. Das Recht von Patienten und Probanden auf Herausgabe ihrer genetischen Rohdaten. MedR 2016; 34:481-491

[2] Deutsche Forschungsgemeinschaft: Humane Genomsequenzierung – Herausforderungen für eine verantwortungsvolle Anwendung in der Wissenschaft. Stellungnahme, Bonn 2016.

[3] Projektgruppe „Ethische und rechtliche Aspekte der Totalsequenzierung des menschlichen Genoms" (EURAT). Stellungnahme, Heidelberg 2015.

DFG-Workshop

DFG-Fördermöglichkeiten: Individuelle Förderprogramme der DFG für jede Karrierestufe

Moderation: Astrid Golla (Bonn), Bernhard Horsthemke (Essen)

In dem Workshop werden die individuellen Förderprogramme der Deutschen Forschungsgemeinschaft (DFG) für jede Karrierestufe vorgestellt, vom Forschungsstipendium über die eigene Stelle zu den Exzellenzprogrammen Emmy Noether- und Heisenberg-Programm. Der Workshop richtet sich besonders an Nachwuchswissenschaftlerinnen und Nachwuchswissenschaftler.

In this workshop you will be introduced to the individual funding programs of the DFG (German Research Foundation) which can apply for every career stage, from research grants through the own work position to the promotion of excellence with the Emmy Noether and Heisenberg programs. The workshop is especially addressed to young researchers and young scientists.

Dr. Astrid Golla
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Oral-History-Projekt

Geschichte der Humangenetik in der BRD – Zeitzeugenerinnerungen

Moderation: Heiner Fangerau, Felicitas Söhner (Düsseldorf)

In den 1960er Jahren erlebte die Humangenetik wie eine Reihe anderer medizinischer Disziplinen auch eine erhebliche institutionelle Ausweitung. In den 1960er und 1970er Jahren wurde beispielsweise an den Universitäten der BRD das Gros der humangenetischen Lehrstühle, in den ausgehenden 1970er und 1980er Jahren auch in der DDR, eingerichtet. 1969 ging vom Symposium „Genetik und Gesellschaft" im Rahmen des Marburger „Forum Philippinum" die Initiative aus, in der ganzen Bundesrepublik genetische Beratungsstellen zu gründen und damit die genetische Forschung (wieder) medizinisch nutzbar zu machen.

In dieser Phase der Etablierung der Humangenetik auf akademischer und praktischer Ebene setzt das geplante Zeitzeugenprojekt ein. Es will die Entwicklung der Humangenetik in ihrem Selbstverständnis als Quer- und als Längsschnittfach (Pfadenhauer 2003:66) im deutschsprachigen Raum ab den 1970er Jahren mit Hilfe von Expertengesprächen dokumentieren und analysieren.

Im Forschungsprojekt sollen zwei Komplexe von Fragestellungen bearbeitet werden: ein wissenschaftshistorischer, in dem die Entwicklung und Anwendung von diagnostischen und therapeutischen Techniken im Mittelpunkt steht, und ein sozialhistorischer, in dem es um die Etablierung und den Ausbau der Institutionen der Humangenetik sowie um den Verlauf der das Fach betreffenden gesellschaftlichen Debatten geht.

Neben der Gründung von Instituten und der Fachgesellschaft sowie der Normierung der Ausbildung für ärztliche und naturwissenschaftlich ausgebildete Humangenetiker sollen die Funktion der historischen Reflexion und Bearbeitung der facheigenen nationalsozialistischen Vergangenheit in den 1980er Jahren für die Etablierung des Fachs und der schwierige institutionelle Trennungsprozess von der Anthropologie mit ihren Wirkungen auf das Selbst- und Fremdbild der Humangenetik analysiert werden (Weingart, Kroll, Bayertz 1992). Zusätzlich zur Entwicklung in der BRD sollen dabei auch die Entwicklung des Fachs in der DDR und mögliche deutsch-deutsche Kooperationen zur Sprache kommen (in Jena befand sich z.B. in den 70er Jahren das zentrale Referenz-Institut für genetische Beratung in der DDR (Vogel 1999:416).

Das Projekt kann sich auf zahlreiche Arbeiten zur Geschichte der deutschen Humangenetik stützen (Vgl. Kröner 1997, 1998; Cottebrune 2006, 2008; Weingart 1988; Bennike 1992).Dabei werden auch die Kontinuitäten und Brüche der Nachkriegs-Humangenetik zur Eugenik beachtet (Weingart, Kroll, Bayertz 1992; Kühl 2014) und die Entwicklung der deutschen Humangenetik im internationalen Kontext gezeichnet (McKusnick 1975; Harper 2008; Motulsky 2010).

Schwerpunktmäßig werden Methoden der Oral History eingesetzt (Obertreis 2012; Ritchie 2014). Als Grundlage dienen 25 themenzentrierte, halbstrukturierte Experteninterviews. Hinzu kommen die (auto-)biographische Literatur zu den Protagonisten der deutschsprachigen Humangenetik und Primär- und Sekundärliteratur zu Teilaspekten des Untersuchungsfeldes.

Im Vortrag werden das Projekt, seine Genese und die Ziele des Vorhabens vorgestellt werden.

Qualitätsmanagement Workshop

QW Molekulargenetik

Moderation: Dieter Gläser (Neu-Ulm), Jochen Decker (Freiburg)

Für diesen Workshop haben wir einen Vertreter der EMQN (European Molecular Quality Network) als Sprecher eingeladen. Er/Sie wird über die Erfahrungen mit dem NGS-Ringversuch berichten und darstellen, wie sich die zukünftigen Aktivitäten der EMQN gestalten. Zum einen bietet sich hier die Chance, Fragen und Probleme, die im Zusammenhang mit den Ringversuchen der EMQN stehen, zu stellen. Zum anderen ist es wünschenswert, Anregungen einzubringen, die der EMQN-Vertreter für die Diskussionen im Management Board mitnehmen soll. Unsere Interessen im EMQN Management Board werden derzeit von Clemens Müller-Reible und Jochen Decker bestens vertreten. Wir freuen uns auf eine rege Teilnahme.

Dialog mit SHG

Gespräch mit Vertretern von Selbsthilfegruppen

Moderation: Klaus Zerres (Aachen)

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Rita Lawrenz, *Arbeitkreis Down-Syndrom Deutschland e.V. (rita.lawrenz@down-syndrom.org)*
Heinz-Jürgen Brosig, *Deutsche Huntington-Hilfe e.V. (hj.brosig@dhh-ev.de)*
Hans Joachim Jentzsch, *LEONA e.V. (hans-joachim.jentzsch@leona-ev.de)*

In einem Gespräch mit Vertretern einzelner Selbsthilfegruppen sollen Themenbereiche wie Probleme von Menschen mit Behinderung im Alltag. Wie behindertenfeindlich ist unsere Gesellschaft heute? Einstellung zu den zunehmenden Möglichkeiten genetischer Diagnostik (prädiktive Diagnostik, PND, NIPD, PID) und deren Folgen. Erwartungen an die Humangenetik thematisiert werden. Teilnehmer haben die Möglichkeit, Fragen zu stellen.

SEL

SEL-001

The tumor genome of germinal center B-cell lymphomas other than Burkitt Lymphoma

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Germinal center (GC)-derived B-cell lymphomas are a heterogeneous group of B cell malignancies. Within the ICGC MMML-Seq consortium we have performed whole genome sequence analysis of 182 non-Burkitt GC-derived B-cell lymphomas including diffuse large B-cell lymphomas (DLBCL), follicular lymphomas (FL), FL/DLBCLs, double hit DLBCL and B-cell lymphomas, not otherwise specified (NOS). Several recurrently mutated genes have been identified, which include known lymphoma genes as well as genes not previously associated with B-cell lymphomas. Large differences have been observed in the pattern of recurrently mutated genes between the different subtypes and, in particular for the DLBCL, also within the subtype, pointing to the presence of multiple alternative driver mechanisms in GC-derived B-cell lymphomas. The density of somatic single nucleotide variants (SNVs) varied widely across the genome. We extracted three types of regions with increased SNV density: Kataegis (rainfalls, high SNV density at single sample level), Keraunos (hotspots, very high SNV density at cohort level but not at single sample level), and Psichales (intermediate SNV density at single sample level). Psichales occurred in late replicating regions of the genome and most likely reflects the increased mutation rate in regions with closed chromatin that has been observed in many cancer types. Keraunos and Kataegis clusters could be subdivided into those typical for (aberrant) somatic hypermutation (SHM) (early replicating, close to transcription start sites and high fraction of SNVs hitting the RGYW motif) and those non-typical for SHM. Cohort-wide mutational signature analysis has revealed ten known signatures including clocklike signatures, DNA repair defect signatures, an APOBEC signature and the B-cell specific signature S9 (AID and polymerase η). In addition, we discovered two new signatures (L1, L2). L1 was highly enriched in the SHM-typical Keraunos and Kataegis clusters and explains the high fraction of RGYW-affecting variants in (a)SHM target regions. L2 is enriched in clusters of mutations which do not show the typical properties of (a)SHM and at the immunoglobulin loci and might be related to class switch recombination. Signature S9 is depleted in SNV clusters and enriched in late replicating regions, and might be the fingerprint of diffuse, genome-wide activity of AID and polymerase η. Finally, we showed that the occurrence of some SNV clusters is conditional on the occurrence of other clusters and introduced the concept of SHM by proxy.

In summary, our whole genome sequencing analysis provides novel insights into the different mutational and oncogenic mechanisms active in the various subtypes of GC-derived B-cell lymphomas.

SEL-002

Mutations in three genes encoding proteins involved in hair shaft formation cause uncombable hair syndrome

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Uncombable hair syndrome (UHS), also known as "pili trianguli et canaliculi" or "Struwwelpeter-Syndrom" is a rare anomaly of the hair shaft which occurs in children and improves with age. UHS is characterized by dry, frizzy, spangly and often fair hair that stands away from the scalp in multiple directions and is impossible to comb. Most of the cases reported are sporadic but autosomal dominant or recessive inheritance patterns were also observed without being linked to any molecular genetic cause.

The first family we examined originated from the U.K. and had two affected and two unaffected siblings. We performed whole exome sequencing (WES) and identified a homozygous missense variant within PADI3 (peptidylarginine deiminase 3). PADI3 is mainly expressed in skin and hair follicles. By Sanger sequencing of PADI3 in additional patients, we identified homozygous and compund heterozygous mutations in 7 patients. As a next step, we performed WES in further unelucidated UHS cases and identified homozygous nonsense mutations in TGM3 (transglutaminase 3) and in TCHH (trichohyalin), respectively.

Elucidation of the molecular outcomes of the disease causing mutations by cell culture experiments of PADI3 and TGM3 and tridimensional protein models demonstrated clear differences in the structural organization and activity of mutant and wild type proteins. By immunofluorescence analysis, we could demonstrate a diffuse homogenous cytoplasmic distribution of the WT PADI3, whereas in the mutants the proteins were observed to form large aggregates throughout the cytoplasm. By use of human anti-citrullinated protein autoantibodies, we could show a strong labelling in the WT whereas the staining of the mutants was barely above background. In order to demonstrate the importance of PADI3 in hair shaft formation, we generated Padi3 knockout mice. Electron microscopy observations revealed morphological alterations in hair coat of Padi3 knockout mice. For TGM3, we performed a transglutaminase activity assay. The analysis results revealed that the WT had a significantly higher transglutaminase activity in comparison to the truncated protein.

Here, we report for the first time the identification of UHS causative mutations located in the three genes PADI3, TGM3 and TCHH. The two enzymes responsible for posttranslational protein modifications, and their target structural protein, are all involved in hair shaft formation through their sequential interactions. These findings provide valuable information regarding the pathophysiology of UHS and contribute to a better understanding of this protein interaction cascade. This could be of further value for cosmetics and pharmaceutics industries paving the way for development of novel products.

SEL-003

Mutations in EBF3 disturb transcriptional profiles and cause intellectual disability, ataxia and facial dysmorphism

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From a GeneMatcher-enabled international collaboration, we identified ten individuals with intellectual disability, speech delay, ataxia and facial dysmorphism carrying a pathogenic variant in the *EBF3* gene detected by whole-exome sequencing. Five missense, two nonsense, one 9-bp duplication, and one splice-site variant in *EBF3* were found; the mutation occurred *de novo* in eight individuals, and the missense variant c.625C>T [p.(Arg209Trp)] was inherited by two affected siblings from their healthy mother who is a mosaic. EBF3 belongs to the early b-cell factor family (also known as Olf, COE, or O/E) and encodes a transcription factor involved in neuronal differentiation and maturation. Structural assessment predicts perturbing effects of the five amino acid substitutions on DNA-binding of EBF3. Transient expression of EBF3 mutant proteins in HEK 293T cells revealed mislocalization of all but one mutant in the cytoplasm in addition to nuclear localization. By transactivation assays, all EBF3 mutants showed significantly reduced or no ability to activate transcription of the reporter gene under control of the *CDKN1A* promotor that corresponds well with loose association of EBF3 mutants with chromatin as demonstrated by *in situ* subcellular fractionation experiments. Finally, RNA-seq and ChIP-seq experiments demonstrate that EBF3 acts as a transcriptional regulator at cis-regulatory sequences and EBF3 mutant had reduced function due to partial disruption of the DNA-binding domain. These findings demonstrate that EBF3-mediated dysregulation of gene expression has profound effects on neuronal development in humans and add EBF3 to the growing list of genes in which mutations cause syndromic forms of intellectual disability.

Biallelic Mutations in the 3' Exonuclease TOE1 Cause Pontocerebellar Hypoplasia and Uncover a Role in snRNA Processing

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Deadenylases are best known for degrading the poly(A) tail during mRNA decay. The deadenylase family has expanded throughout evolution and, in mammals, consists of 12 Mg^{2+}-dependent 3' end ribonucleases with mostly unknown substrate specificity. Pontocerebellar hypoplasia type 7 (PCH7) is a unique recessive syndrome characterized by neurodegeneration with ambiguous genitalia (MIM%614969). We studied 12 human families with PCH7, uncovering biallelic, loss of function mutations in *TOE1*, which encodes an unconventional deadenylase. Toe1-morphant zebrafish displayed mid- and hind-brain degeneration, modeling PCH-like structural defects *in vivo*. Surprisingly, we found TOE1 associated with incompletely processed small nuclear (sn)RNAs of the spliceosome, which is responsible for pre-mRNA splicing. These pre-snRNAs contained 3' genome-encoded tails often followed by post-transcriptionally added adenosines. Human cells with reduced levels of TOE1 accumulated 3' end-extended pre-snRNAs, and immuno-isolated TOE1 complex was sufficient for 3' end maturation of snRNAs. Our findings reveal the cause of a neurodegenerative syndrome linked to snRNA maturation and uncover a key factor involved in processing of snRNA 3' ends.

WS1 MONOGENIC DISEASES

WS1-001

A recurrent mutation affecting the REtention and Splicing complex cause a severe progeroid phenotype with lipodystrophy in mouse and man.

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Lipodystrophy is a feature in several progeroid syndromes. In the present study, we investigated the molecular genetic cause of a novel progressive phenotype with severe lipodystrophy, short stature, cataracts and hearing loss. All three affected individuals, originating from two independent consanguinous families, died within the first decade of life.

Whole exome sequencing revealed the same homozygous premature termination codon in all affected individuals affecting a component of the REtention and Splicing complex. By RT-PCR amplification we found corrective splicing of the affected transcript leading to a gene product with an in-frame deletion. Patient-derived fibroblasts displayed striking changes in nuclear morphology and accumulations of unknown material in cytoplasmic vesicles. To investigate the underlying pathomechanism, we generated ES-cells with a similar deletion in the murine gene using the CRISPR/Cas9 system, which were used to generate highly chimeric mutant mice. All chimeric animals showed a progressive reduction of body weight due to a lipodystrophy involving the typical fat depots. In histology, a reduction of the adipocyte diameter was evident. Surprisingly, we found no evidence for a generalized splicing defect by RNAseq of human and murine mutant cells. However, consistently differentially expressed genes were detected.

In summary, we describe a novel syndrome with lipodystrophy and progeroid features. We deciphered the causal mutation and showed that corrective splicing of the affected mRNA is very likely the cause for the survival of the affected individuals. The causality of the mutation was proven by a mouse model that partially recapitulates the human phenotype and will be used for further investigations.

WS1-002

Gerodermia osteodysplastica is a congenital disorder of glycosylation with impaired decorin glycanation leading to disorganization of extracellular matrix and TGF-β signalling.

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Gerodermia osteodysplastica (GO) is an autosomal recessive segmental progeroid disorder characterized by skin laxity and early-onset osteoporosis. GORAB, the responsible disease gene, encodes a small Golgi protein of poorly characterized function. Since a GorabNull full knockout was lethal Gorab was also conditionally inactivated in mesenchymal progenitor cells of the murine limb skeleton. These GorabPrx1 mice showed dramatically thinned, porous cortical bone and spontaneous fractures of long bones. Osteocyte numbers were increased, but an abnormal morphology and differentiation was evident. Concomitantly, collagen fibrils appeared disorganized in dermis and bone. Measurement of glycosaminoglycan contents of several tissues revealed a specific reduction of dermatan sulfate levels. Decorin, a major proteoglycan regulating the formation of collagen fibrils and various signalling pathways, showed strongly reduced dermatan sulfate content, while other proteoglycans were not changed. Moreover, we found elevated activation of TGF-β leading to enhanced downstream signalling, which was also reproduced in GO patient-derived skin fibroblasts. Our data unravel a role of GORAB in the posttranslational modification of decorin. Gerodermia osteodysplastica can be regarded as a congenital disorder of glycosylation affecting proteoglycan synthesis, thus explaining the phenotypic overlap with other disorders of this group.

FRMPD4 is associated with X-linked non-syndromic hearing loss in humans

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Although many genes have already been identified as causing non-syndromic hearing loss (NSHL), diagnostic rates of approximately 50% among hearing impaired patients suggest that many more genes are remaining to be identified. NSHL is the most common sensory deficit that has a prevalence between one and two per 1000 newborns. Furthermore, it demonstrates classic genetic heterogeneity with as many as 1% of coding genes in the genome anticipated to be involved in non-syndromic forms of deafness. Autosomal recessive (75-80%) and autosomal dominant (15-20%) forms dominate inheritance patterns of deafness; however, in a small fraction of cases, X-linked deafness (1-4%) can be observed.

Whole exome sequencing of a German family with diagnostically unresolved NSHL revealed a novel missense variant predicted as pathogenic in the gene FERM and PDZ domains containing protein 4 (FRMPD4) on chromosome Xp22.2. This gene, also known as Preso1, was first described as a regulator of dendritic spine morphogenesis. Previous screening of pathogenic CNVs in array based comparative genomic hybridization among families with heterogeneous X-linked intellectual disability (XLID) showed duplication of Xp22.2 including part of FRMPD4 which implicated the gene in XLID. Interestingly, a segregating truncating and a de novo missense mutation in FRMPD4 have associated this gene with XLID, a phenotype not observed in our family.

Mouse expression studies localize Frmpd4 to spiral ganglion neuron peripheral dendrites of the developing cochlea. In addition, we analyzed frmpd4 knockdown and loss-of-function zebrafish mutants for innervation and structural defects in the otic vesicle and lateral line neuromasts. Posterior lateral line neuromasts are observed with reduced axonal outgrowth that is also likely reduced in the lateral line nerve. Abnormal innervation is also apparent in the otic vesicle. Fluorescent neuromast labeling marked a significant reduction of overall otic vesicle and lateral line neuromasts in mutants versus wild type zebrafish. Scanning electron microscopy revealed a pronounced absence of kinocilia in posterior lateral line neuromasts of frmpd4$^{-/-}$ zebrafish. Furthermore, adult frmpd4 mutants show significantly delayed acoustically evoked behavioural responses compared to wild type fish indicating hearing impairment. Investigation of transgenic Drosophila insertion mutants detected a mild auditory phenotype i.e. a reduction in mechanical amplification gain and associated reduction in antennal fluctuation power. Our results associate FRMPD4 with X-linked hearing loss and suggest mutations in this gene are correlated with pleiotropic effects in a wide range of evolutionarily distinct species. Our investigations hint to an evolutionarily conserved mechanism/function of FRMPD4 for mechanosensitivity to sound and suggests that an overlapping association to two distinct functions of the FRMPD4 gene (neural cognitive and sensorineural) can be distinguished.

WS1-004

Intercalated Cell Depletion and Vacuolar H+-ATPase Mistargeting in an Ae1 R607H Knockin Model

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The kidney maintains acid-base homeostasis and electrolyte balance through highly specialized cells. In the distal nephron acid secretion is mediated by type A intercalated cells (A-ICs), which contain V-type ATPase-rich vesicles that fuse with the apical plasma membrane on demand. Intracellular bicarbonate generated by luminal H+ secretion is removed by the basolateral anion-exchanger AE1. Dysfunction of type A intercalated cells results in distal renal tubular acidosis (dRTA) and human mutations in V-ATPase subunits and AE1 are causative for this condition.

For the AE1 R607H mutation a dominant-negative trafficking mechanism was proposed to explain AE1-associated dominant dRTA based on studies in MDCK monolayers. To test this hypothesis in vivo and to test potential rescue strategies correcting this mistargeting defect, we have generated a R607H knock-in mouse strain, which corresponds to the most common dominant dRTA mutation in human AE1, R589H. Heterozygous and homozygous R607H knock-in mice displayed incomplete dRTA characterized by compensatory upregulation of the Na+/HCO3- cotransporter, NBCn1. As expected for the R607H mutation, red blood cell Ae1-mediated anion-exchange activity and surface polypeptide expression were unchanged. Surprisingly, basolateral targeting of the mutant Ae1 in A-ICs was preserved in contrast to previous studies in MDCK cells. Instead, we found Ae1 expression in A-ICs strongly reduced in a R607H dosage-dependent manner. Additional cell culture studies in two widely used immortalized renal cell lines verified that targeting and half-life time of mutant AE1 protein was indeed preserved. Surprisingly, ATPase expression was reduced and its plasma membrane targeting upon acid challenge compromised. Ultrastructural analysis revealed a loss of apical vesicles in A-ICs, while we observed lysosomal inclusions and multilamellar bodies. Accumulation of p62- and ubiquitin-positive material in A-ICs of knock-in mice suggest a defect in the degradative pathway, which may ultimately lead to loss of A-ICs. Highlighting the expression of Ae1 specifically in A-ICs, type B intercalated cells were unaffected. We propose that reduced basolateral anion-exchange activity in A-ICs inhibits trafficking and regulation of V-type ATPase, compromising luminal H+ secretion and possibly also lysosomal acidification. Our findings illustrate the considerable, context-dependent complexity of AE1-related kidney disease.

WS1-005

Mutations in the leukemia inhibitory factor receptor (LIFR) gene and Lifr deficiency cause urinary tract malformations

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Congenital anomalies of the kidneys and urinary tract (CAKUT) are the most common cause of chronic kidney disease in children. As CAKUT is a genetically heterogeneous disorder and most cases are genetically unexplained, we aimed to identify new CAKUT causing genes. Using whole-exome sequencing and trio-based *de novo* analysis, we identified a novel heterozygous *de novo* frameshift variant in the leukemia inhibitory factor receptor (*LIFR*) gene causing instability of the mRNA in a patient presenting with bilateral CAKUT and requiring kidney transplantation at one year of age. *LIFR* encodes a transmembrane receptor utilized by IL-6 family cytokines, mainly by the leukemia inhibitory factor (LIF). Mutational analysis of 121 further patients with

severe CAKUT yielded two rare heterozygous *LIFR* missense variants predicted to be pathogenic in three unrelated patients. LIFR mutants showed decreased half-life and cell membrane localization resulting in reduced LIF-stimulated STAT3 phosphorylation. *LIFR* showed high expression in human fetal kidney and the human ureter, and was also expressed in the developing murine urogenital system. *Lifr* knockout mice displayed urinary tract malformations including hydronephrosis, hydroureter, ureter ectopia, and, consistently, reduced ureteral lumen and muscular hypertrophy, similar to the phenotypes observed in patients carrying *LIFR* variants. Additionally, a form of cryptorchidism was detected in all *Lifr*^{-/-} mice and the patient carrying the *LIFR* frameshift mutation. Altogether, we demonstrate heterozygous novel or rare *LIFR* mutations in 3.3% of CAKUT patients, and provide evidence that *Lifr* deficiency and deactivating *LIFR* mutations cause highly similar anomalies of the urogenital tract in mice and humans.

WS1-006

Loss of cdkl5 associated with deficient mammalian target of rapamycin (mTOR) signaling in mice and human cells

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We and other groups have shown that mutations in the X-linked cyclin-dependent kinase-like 5 (CDKL5) gene cause a severe neurodevelopmental disorder with clinical features including intellectual disability, early-onset intractable seizures and autism, that are closely related to those present in Rett syndrome (RTT) patients. RTT is caused by mutations in the X-linked MECP2 gene. CDKL5 is a serine/threonine kinase and to date knowledge about its functional roles is scarce. We searched for CDKL5 interacting proteins by yeast-two hybrid screens. One of the candidates identified in these screens is a subunit of the phosphatidylinositol-4,5-biphosphate 3-kinase (PI3K). The results obtained in yeast could be confirmed in vitro in mammalian cells and in mouse brain by immunoprecipitation experiments and by co-localization studies. PI3K phosphorylates membrane lipids which act as docking sites to recruit targets upstream of mTOR and thereby regulate among major cellular processes synaptic plasticity, which is the cellular basis for learning and memory. Alteration of mTOR signaling has also been demonstrated to be an important pathobiochemical feature in RTT. To test whether common deficits in mTOR signaling could be responsible for the molecular pathogenesis underlying both syndromes, we generated and studied a novel Cdkl5 knockout (Cdkl5 -/Y) mouse model and performed in vitro experiments in human cells. In Cdkl5 -/Y knockout mice loss of Cdkl5 is accompanied by reduced phosphorylation levels of critical components of the mTOR signaling cascade. These findings point at a regulatory role of CDKL5/Cdkl5 on mTOR activity and function. To gain further insights into the possible mechanism through which CDKL5/PI3K interaction could regulate mTOR signaling, we used HEK-T cells as cellular model. Following knock-down of CDKL5, the amount of PI3K protein was significantly reduced compared to controls. To evaluate the contribution of our findings to pathogenesis, we performed rescue experiments in CDKL5 knock-down HEK-T cells using wild-type and patient-specific mutant CDKL5 constructs. Further experiments are ongoing to clarify the molecular mechanism by which CDKL5 regulates PI3K protein level in the cells.

WS2 CANCER GENETICS I

WS2-001

Inferring expressed genes by whole-genome sequencing of plasma DNA

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The analysis of cell-free DNA (cfDNA) in plasma represents a rapidly advancing field in medicine. cfDNA consists predominantly of nucleosome-protected DNA shed into the bloodstream by cells undergoing apoptosis. We performed whole-genome sequencing of plasma DNA and identified two discrete regions at transcription start sites (TSSs) where nucleosome occupancy results in different read depth coverage patterns for expressed and silent genes. By employing machine learning for gene classification, we found that the plasma DNA read depth patterns from healthy donors reflected the expression signature of hematopoietic cells. In patients with cancer having metastatic disease, we were able to classify expressed cancer driver genes in regions with somatic copy number gains with high accuracy. We were able to determine the

expressed isoform of genes with several TSSs, as confirmed by RNA-seq analysis of the matching primary tumor. Our analyses provide functional information about cells releasing their DNA into the circulation.

WS2-002

Characterization of splice-site variants by targeted cDNA-Sequencing

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RNA-splicing is an important mechanism for eukaryotic gene expression and regulation. Defective splicing significantly contributes to monogenic disease in humans. Indeed, the mutational space for variants affecting splicing is larger than for coding variants. Several computational methods have been developed to predict a variant's effect on splicing but lack predictive value outside the canonical splice sites and do not predict aberrant transcripts. Thus the plethora of DNA variants generated by recent advances in "Next-generation" based sequencing (NGS) can be scored for a possible splicing effect, but a laborious wet-lab based confirmation and characterization is still required. RNA-Seq is widely used for quantification of gene expression and can be used to detect splicing events, but is limited for this use by the variable and often low read coverage of individual genes. Thus, a standardized NGS based approach to characterize potential splice variants is lacking.

Hence we investigated the utility of hybridization based gene-panel enrichment and NGS of cDNA. Based on results of computational simulation we selected twenty RNA-samples of patients with a known pathogenic splice-site variant in an inherited cancer predisposition gene. These variants were previously characterized by RT-PCR in our lab or in the literature. After rRNA depletion and DNA digestion we performed first and second strand cDNA synthesis followed by "tagmentation"-based library preparation, targeted enrichment using the TruSight Cancer panel and sequencing on an Illumina Miseq platform. A computational pipeline was established to enable automated detection of aberrant splicing events by implementing different alignment and splice-junction detection algorithms together with filtering against control data sets. We also considered variant calling for the detection of allelic imbalance and gene-level expression analysis in this data.

Using this approach we were able to confirm or improve the previously characterized transcript-level effect for all variants analyzed in the 14 affected cancer genes (BRCA1, BRCA2, CHEK2, RAD51C, PALB2, NBN, MLH1, MSH2, APC, PTEN, STK11, TSC1, EXT1 and NF1). For example, for the STK11 variant (c.597+1G>A in intron 4) conflicting results were previously reported in the literature; based on RT-PCR experiments one publication postulated exon-skipping while another suggested intronic read-through. Our data revealed that both aberrant transcripts are present resulting in nonsense mediated decay and reduced expression. This exemplifies the higher accuracy and robustness of targeted cDNA-Seq compared to RT-PCR.

Overall, our results show that targeted cDNA-Seq using gene panels is a simple, versatile and readily available tool to analyze possible aberrant transcription. This approach can also be used to identify deep intronic variants affecting splicing.

WS2-003

RNA-based pathogenicity evaluation of variants of uncertain significance (VUS) in breast/ovarian cancer families

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Breast and ovarian cancer (BC/OC) predisposition has been associated with a number of high- and low-penetrance susceptibility genes. Advances in sequencing technology has made multigene testing a practical option when searching for genetic variants associated with risk for BC/OC. Variants of uncertain significance (VUS), though, represent a major problem. We now studied 581 patients fulfilling criteria for BRCA1 and 2 testing using the next generation sequencing based TruSight Sequencing Cancer Panel on a MiSeq platform (Illumina). Data was analyzed after remapping with BWA to hg19 (GRCh37) using SeqNext software (JSI) for variants in 14 known high and moderate penetrance susceptibility genes (BRCA1/2, ATM, CHEK2, PALB2, RAD51C, RAD51D, NBN, CDH1, TP53, MLH1, MSH2, MSH6, PMS2).

Besides 106 deleterious mutations we also identified 89 VUS. Of these, 11 variants (1 each in BRCA1, BRCA2, PALB2, RAD51C, RAD51D, CDH1, and MLH1, and 2 in CHEK2 and MLH1, respectively) affect possible splicing sites. In addition, 5 synonymous and nonsynonymous variants outside the splicing sites (1 in BRCA1, BRCA2 and CDH1, respectively, 2 in RAD51D) were not reported in Exome Variant Server or Exome

Aggregation Consortium (ExAC) databases, so far. No families were available to study familial segregation. For all these variants a potential effect on splicing efficiency was predicted by three different computational algorithms (BDGP: Splice Site Prediction by Neural Network, NetGene2 Server and the Human Splice Finder (HSF 3.0) algorithm).

We took advantage that these genes are ubiquitously expressed to investigate possible effects of these variants on mRNA splicing using easily accessible peripheral blood. As mRNA is notoriously unstable, we used PAXgene tubes for stabilizing RNA immediately after drawing the samples. The subsequent RT-PCR analysis showed that 9 of the 11 variants located at potential splicing sites indeed affect splicing. Thus 8 of these variants could be classified as deleterious (IARC class 5), while one CHEK2 variant could not be unequivocally classified as the RT-PCR analysis identified only 20% of the mutant transcript indicating continued usage of the constitutive splice acceptor site. This led to the classification as a probably hypomorphic allele. The variants in CDH1 and MLH1 did not affect splicing and were classified as benign (IARC class 1). None of the 5 rare synonymous and nonsynonymous exonic variants showed any effect on splicing. In conclusion, this analysis allowed the disambiguation of 10 out 11 VUS at potential splice sites into a definite category (either IARC class 5 or 1).

This work highlights the importance of computational splicing prediction and validation using RT-PCR of peripheral blood RNA to assess the pathogenicity of VUS. This in turn, allows more accurate genetic counseling and clinical management of affected families.

WS2-004

A novel IDH1 R132H edited glioma in vitro model reveals altered metabolism and enhanced dependence on NAD+-regeneration in IDH1 R132H cells

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Gliomas present the major group of neoplasia in the central nervous system. They typically show invasive growth and high recurrence rate and are currently not curable. IDH mutations are detected in nearly 70% of low grade gliomas and are considered to play a key role in low grade glioma development. While it is known that IDH1/2 mutation leads to high-levels of 2-Hydroxyglutarate (2-HG) that functions as an oncometabolite, little is known about the influence of IDH1/2 mutations on energy metabolism and metabolic reprogramming in the tumor cells. Since patient derived IDH mutant cells do not grow in cell culture, previous studies from our group and others used transduced cell lines that overexpress IDH1. In order to develop in vitro models with reduced side effects, we used CRISPR/Cas to introduce the IDH1R132H mutation in a patient derived glioblastoma cell line. The edited cells expressed IDH1R132H in Western Blot and expression levels of IDH1 were comparable to the expression in wild type cells. The mutation was stable in long time culture experiments, without signs of senescence. Moreover we found elevated 2-HG levels, proving that the IDH1R132H neoenzymatic function is present in our cell lines. Thus, we were able to edit and culture genomic IDH1R132H mutated glioma cells for the functional analysis of the IDH1R132H mutation for the first time without the effects of overexpression models. Edited IDH1R132H cell lines showed extended doubling times compared to wildtype cells. Measurement of Krebs cycle metabolites using mass spectrometry revealed elevated glutamate levels. We found enhanced ATP-levels that could be a consequence of decreased ATP consumption. Additionally, the cells showed reduced viability compared to wildtype cells when cultivated in glycolysis inhibiting media, pointing out the enhanced dependency on glycolysis in IDH1R132H cells. These results indicate changes in tumor cell metabolism and energy household induced by the IDH1R132H mutation. Since we and others could show that IDH1R132H can alter NAD+ and NADPH levels, we tested if the IDH1R132H mutated cells are more susceptible to selective inhibition of NAD/P regenerating enzymes. esiRNA-Silencing of NAMPT specifically decreased cell viability in IDH1R132H but not wildtype cells with a concomitant increase of dead cells. In conclusion, we developed a suitable in vitro model to study the effect IDH1R132H. We currently test different treatment strategies that selectively target the metabolism of IDH1R132H in our model system. Our results indicate a potential treatment against IDH1R132H mutated glioma utilizing the impaired NAD+/NADPH-regeneration of the tumor cells.

WS2-005

HOXB13 as a susceptibility gene for hereditary prostate cancer: Associations with disease risk, clinical presentation and family history in Germany

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HOXB13, a member of the embryonic homeobox transcription regulators, has been identified as the first susceptibility gene specific for prostate cancer (PrCa). The founder missense mutation G84E, which likely originated from Finland, can be found in most populations of European ancestry. We determined the frequency of *HOXB13* G84E for the German population, assessed in a cohort of 379 unrelated cases, each with positive family history of PrCa, 367 sporadic PrCa cases and in 1015 controls. Additional 646 affected relatives from PrCa families were included to explore association with aggressive disease in subgroups with high Gleason score (>7), advanced tumor stage, or PSA at diagnosis >20ng/ml. Carriers of G84E were rare in controls (0.4 %) and showed increased frequencies in both sporadic (1.6 %) and familial PrCa cases (3.2 %). Estimated risks were OR = 4.2 (p = 0.026) and OR = 8.3 (p = 0.0003), respectively. The risk effect size increased with the number of affected individuals per pedigree: OR = 12.6 (p < 0.0001) for 3 or more, and OR = 14.4 (p < 0.0001) for 4 or more affected men. The strongest association with clinical features was observed between G84E and advanced tumor stage (OR = 9.2; p < 0.0001). In conclusion, the observed frequency of *HOXB13* G84E mutation carriers in our study cohort was intermediate as compared to the common prevalence in Scandinavia and the rare occurrence in mixed European populations from the US. The risk estimates of *HOXB13* G84E and the stronger effect sizes in families with increasing number of affected relatives were in line with a high penetrant germline predisposition. The association between G84E status and tumor stage may be of greater interest for clinical practice, but needs further validation. The absolute penetrance of the *HOXB13* G84E mutation should be investigated in further studies in order to elucidate its suitability as a genetic predictor for PrCa.

WS2-006

Smoking-associated DNA methylation markers predict lung cancer incidence

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Newly established blood DNA methylation markers that are strongly associated with smoking might provide new options for lung cancer (LC) screening. We aimed to assess the performance of the top hits from previous epigenome-wide association studies in prediction of LC incidence.

In a prospective nested case-control study, DNA methylation at AHRR (cg05575921), 6p21.33 (cg06126421), and F2RL3 (cg03636183) were measured by pyrosequencing in baseline whole blood samples of 143 incident LC cases identified during 11 years of follow-up and 457 age- and sex-matched controls without diagnosis of LC until the end of follow-up. The individual and joint associations of the 3 markers with LC risk were estimated by logistic regression, adjusted for potential confounders including smoking status and cigarette pack-years. The predictive performance was evaluated for both the individual markers and their combinations derived from multiple algorithms.

Pronounced demethylation of all 3 markers was observed at baseline among cases compared to controls. Risk of developing LC increased with decreasing DNA methylation levels, with adjusted ORs (95% CI) of 15.86 (4.18–60.17), 8.12 (2.69–4.48), and 10.55 (3.44–32.31), respectively, for participants in the lowest quartile of AHRR, 6p21.33, and F2RL3 compared to participants in the highest 2 quartiles of each site among controls. The individual 3 markers exhibited similar accuracy in predicting LC incidence, with AUCs ranging from 0.79 to 0.81. Combination of the 3 markers did not improve the predictive performance (AUC = 0.80). The individual markers or their combination outperformed self-reported smoking exposure particularly in light smokers. No variation in risk prediction was identified with respect to age, follow-up time, and histological subtypes.

AHRR, 6p21.33, and F2RL3 methylation in blood DNA are predictive of LC development, which might be useful for identification of risk groups for further specific LC screening, such as CT examination.

WS3 ENHANCERS, MODIFIERS AND RARE VARIANTS

WS3-001

Dynamic chromatin interactions at the PITX1 locus control enhancer activity during development and disease

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Over the past decades the search for disease causing variants has been focusing exclusively on the coding genome. This highly selective approach has been extremely successful however, recent data have revealed the importance of the non-coding genome in fundamental processes such as gene regulation, 3D chromatin folding, and pinpointed its role in disease.

In this study, we systematically investigate the cis-regulatory landscape of PITX1, a homeodomain transcription factor that is exclusively expressed in the hindlimb. Mutations and non-coding structural variations at the PITX1 locus have been shown to associate with a variety of congenital limb defects including club feet, polydactyly, and arm-to-leg transformation (Liebenberg syndrome). We performed in vivo enhancer reporter essays in transgenic mice and identified several limb enhancer elements at the Pitx1 locus; surprisingly they all showed both forelimb and hindlimb activity, although Pitx1 is never expressed in the forelimb. Capture Hi-C experiments revealed a hindlimb-specific chromatin-organization at the Pitx1 locus, which enables its promoter to contact several enhancers bearing a pan-limb activity only in the hindlimb. This tissue-specific chromatin folding plays a determinant role to refine the unspecific limb regulatory landscape toward a highly controlled and hindlimb delimited transcriptional output. To gain a better understanding of the pathology of PITX1 associated limb defects, we used CRISPR/Cas9 to generate a set of deletions and inversions in the Pitx1 cis-regulatory landscape in mice. genetic perturbations of the regulated 3D chromatin conformation lead to an ectopic forelimb expression of Pitx1, resulting in an arm-to-leg transformation in mice and in human patients respectively.

Our data further highlight the role of non-coding mutations affecting chromatin folding in congenital disease and give new insights into the regulation of Pitx1 during development and the pathomechanism of associated limb defects.

WS3-002

Mutations affecting coding or regulatory regions of SMC2 cause dysregulation of condensins resulting in a phenotype reminiscent of cohesinopathies

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Cornelia de Lange Syndrome (CdLS) is a dominantly inherited malformation syndrome caused by mutations in genes encoding subunits (SMC1A, SMC3, RAD21) or regulators (NIPBL, HDAC8) of the cohesin complex. This DNA-bound complex regulates several chromatin-related processes such as chromosome segregation, DNA-damage repair, transcription and chromatin structure.

The project presented initially started with two children and their mother who showed clinical features reminiscent of CdLS. While various sequencing approaches failed to identify the disease-causing mutation, a 60 kb spanning deletion co-segregating with the phenotype was identified by array-CGH. Besides the last exons of CYLC2, encoding a sperm head protein, no other genes were affected. Subsequent in-silico analyses predicted the existence of a ~3 kb tissue-specific regulatory element within this region, located approximately 1 Mb distant from the next protein-coding gene SMC2, which encodes a subunit of the cohesin-related condensin complex.

Significant reduction of SMC2 expression was verified in patient´s fibroblasts by qPCR analysis. Accordingly, a strong dysregulation of SMC2 was observed in HEK293 and SH-SY5Y cells deficient for the putative 3 kb regulatory element, which was deleted by CRISPR/Cas9 genome editing. Reporter gene assays further highlighted the functional relevance of the identified regulatory element in regulating the SMC2 gene

promoter. Interestingly, we could prove on protein as well as on mRNA level that alterations in SMC2 expression are correlated with the dysregulation of other condensin subunits such as SMC4 in patient's samples as well as in CRISPR/Cas9-generated cells.

In a large exome sequencing project we have identified a SMC2 frameshift mutation in an additional family with two patients who show clinical features overlapping with those seen in our initial family. Quantitative PCR analyses in fibroblasts of both subjects also showed significant reduction of SMC2 and SMC4 expression, which is consistent with our findings in the first family.

To further investigate whether alterations in condensin gene expression are specific for the dysregulation of SMC2, we have decreased SMC2 levels in different cell types by siRNA. Quantitative protein as well as mRNA analyses revealed reduced SMC4/SMC4 expression.

Our data show for the first time the coordinated expression of different condensin subunits and its relevance for human disease.

WS3-003

Neurocalcin delta suppression protects against spinal muscular atrophy in humans and across species by restoring impaired endocytosis

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Homozygous *SMN1* loss causes spinal muscular atrophy (SMA), the most common lethal genetic childhood motor neuron disease. *SMN1* encodes SMN, a ubiquitously expressed housekeeping protein, which makes the primarily motor neuron-specific phenotype rather unexpected. SMA individuals harbor low SMN expression from one to six *SMN2* copy genes, which is insufficient to functionally compensate for *SMN1* loss. However, rarely individuals with homozygous absence of *SMN1* and only three to four *SMN2* copies are fully asymptomatic, suggesting protection through genetic modifier(s). Previously, we identified plastin 3 (PLS3) overexpression as an SMA protective modifier in humans and showed that SMN deficit impairs endocytosis, which is rescued by PLS3 overexpression. Here, we identify reduction of the neuronal calcium sensor Neurocalcin delta (NCALD) as a protective SMA modifier in five asymptomatic *SMN1*-deleted individuals carrying only four *SMN2* copies. We demonstrate that NCALD is a Ca^{2+}-dependent negative regulator of endocytosis, as NCALD knockdown improves endocytosis in SMA models and ameliorates pharmacologically induced endocytosis defects in zebrafish. Importantly, NCALD knockdown effectively ameliorates SMA-associated pathological defects across species, including worm, zebrafish and mouse. In conclusion, our study identifies a previously unknown protective SMA modifier in humans, demonstrates modifier impact in three different SMA animal models and suggests a potential combinatorial therapeutic strategy to efficiently treat SMA. Since both protective modifiers restore endocytosis, our results confirm that endocytosis is a major cellular mechanism perturbed in SMA and emphasize the power of protective modifiers for understanding disease mechanism and developing therapies.

WS3-004

Retinoic acid catabolizing enzyme CYP26C1 is a genetic modifier in SHOX deficiency

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Mutations in the homeobox gene SHOX cause SHOX deficiency, the most frequent monogenic cause of short stature. The clinical severity of SHOX deficiency varies widely, ranging from short stature without dysmorphic signs to mesomelic skeletal dysplasia (Léri-Weill dyschondrosteosis, LWD). In rare cases, individuals with SHOX deficiency are asymptomatic. To elucidate the factors that modify disease severity/penetrance, we studied a three-generation family with five affected individuals with LWD using whole genome linkage analysis and whole exome sequencing. The variant p.Phe508Cys of the retinoic acid catabolizing enzyme CYP26C1

co-segregated with the SHOX variant p.Val161Ala in the five affected individuals, while the SHOX mutant alone was present in three asymptomatic individuals. Two further independent LWD cases with SHOX deficiency and damaging CYP26C1 variants were identified. The identified damaging variants in CYP26C1 affected its catabolic activity, leading to an increased level of retinoic acid. We also provide evidence that high levels of retinoic acid significantly decrease SHOX expression in human primary chondrocytes and zebrafish embryos. Individual morpholino

knock-down of either gene shortens the pectoral fins, whereas depletion of both genes leads to a more severe phenotype. Together our findings demonstrate that SHOX and CYP26C1 act in a common molecular pathway controlling limb growth and describe CYP26C1 as the first genetic modifier for SHOX deficiency.

WS3-005

Heart valve dysfunction in men and mice is caused by loss of function mutations in Adamts19, a novel marker for valvular interstitial cells

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On a global perspective defects of the cardiac valves are one of the most common heart abnormalities in humans, with a substantial number of them requiring surgical intervention at least once in their life. Several mechanisms have been proposed ranging from acquired to developmental causes, but thus far the majority can not be explained on the molecular level. Here we report on the identification of a unique human family affected by multiple dysfunctional cardiac valves early in life. Genetic screening revealed a homozygous deletion of the first eight exons in ADAMTS19, a novel candidate gene for valvular heart defects. To investigate its role in heart valve development, we designed a transgenic mouse model that reconstitutes the loss of function (LOF) in Adamts19 found in the human pedigree. Cardiac valves initially form through a process called Endothelial-to-Mesenchymal transition (EMT) then subsequently elongate and mature during early juvenile life. Expression analysis throughout embryonic and postnatal stages of Adamts19-/- mice revealed an expression in all cardiac valves after valve formation. High resolution, digital echocardiography showed that mice without Adamts19 expression develop dysfunctional aortic valves early in life, reminiscent of the human phenotype. Notably, the expression of Adamts19 in the valve was restricted to valvular interstitial cells and not observed in endothelial cells. Functional analysis using proteomic approaches suggest that the presence of Adamts19 is necessary to maintain extracellular matrix remodelling during valve development and its maturation. Not only do the LOF mice fully recapitulate the human phenotype, they also highlight Adamts19 as a novel marker for valvular interstitial cells to specifically target initial post-EMT processes as well as serve as an important model to understand an ageing valve phenotype in humans.

Exome sequencing of 55 bipolar disorder patients with rapid cycling implicates novel candidate genes in disease development

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Bipolar disorder (BD) is a severe neuropsychiatric disorder characterized by recurrent episodes of mania and depression. BD has a lifetime prevalence of about 1% and a high heritability of about 70%. Although recent genome-wide association studies identified the first susceptibility genes contributing to disease development, the cumulative impact of common alleles with small effect may only explain around 38% of the phenotypic variance (Lee et al. 2011). In consequence, rare variants of high penetrance have been suggested to additionally contribute to BD susceptibility.

In the present study we focused on BD patients with rapid cycling (RC). RC is a course specifier of BD defined as having at least four recurrent episodes of acute illness within one year. Since RC showed strong evidence for familiarity, we hypothesized that BD patients with RC might represent a more defined etiological subgroup and that rare variants of high penetrance might contribute to the development of RC in BD patients.

We selected 55 unrelated BD patients with RC of German origin and performed exome sequencing using the Illumina HiSeq2500 platform.

For data analysis, the VARBANK pipeline of the Cologne Center for Genomics was used. We filtered for rare (minor allele frequency <0.1%), heterozygous and non-synonymous variants that were predicted to be possibly damaging or disease causing by at least 4 of 5 applied prediction tools.

After these filtering steps, we identified a total of 110 different genes which harbored rare functional variants in at least three independent patients. Gene set analysis for these genes using ConsensusPathDB revealed 165 enriched pathways (q<0.05) including actin cytoskeleton and calcium ion binding.

Subsequently we applied the Residual Variation Intolerance Score (RVIS) and identified 41 genes which were ranked among the 25% most intolerant genes in the genome. These genes included the previously reported genome-wide significant BD risk genes *SYNE1* and *MLL2*.

In addition, we identified novel, promising candidate genes which have not previously been implicated in BD development such as ryanodine receptor 3 (*RYR3*, affected in six patients) and huntingtin (*HTT*, 4 patients). Both genes are ranked among the 0.5% most intolerant genes of the genome. *RYR3* encodes a brain expressed intracellular cation channel that mediates the rapid release of Ca2+ from the endoplasmic reticulum, thus making it a highly plausible candidate gene for contributing to RC. Abnormal expansion of a trinucleotide repeat in the *HTT* gene causes Huntington disease which is a neurodegenerative disease characterized by motor, cognitive and psychiatric symptoms.

The seven most promising genes are currently being followed up by resequencing in larger cohorts of 2500 independent BD cases (including 250 patients with RC) and 2500 controls of European ancestry using the single molecule molecular inversion probes (smMIPs) technology.

WS4 INTELLECTUAL DISABILITY

WS4-001

De novo truncating mutations in the last and penultimate exon of PPM1D cause a novel intellectual disability syndrome

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Intellectual disability (ID) is a highly heterogeneous disorder with more than 600 known genes involved, yet, a genetic diagnosis remains elusive in ~35-40% of patients with moderate-severe ID. Recent meta-analyses statistically analyzing de novo mutations identified in >5,000 ID patients highlighted PPM1D as a candidate ID gene. PPM1D is a type 2C phosphatase that functions as a negative regulator of cell stress response pathways by mediating a feedback loop of p38-p53 signaling, thereby contributing to growth inhibition and suppression of stress induced apoptosis.

We identified 14 patients with mild-moderate ID and a de novo truncating PPM1D mutation. Deep-phenotyping of the patients revealed in addition to ID overlap for behavioural problems (ADHD and anxiety disorder), hypotonia, broad based gait, facial dysmorphisms and periods of fever and vomiting. PPM1D is shown to be expressed during fetal (brain) development and in the adult brain. All mutations were located in the last, or penultimate exon, suggestive of escaping nonsense-mediated mRNA decay. Both PPM1D expression analysis and cDNA sequencing in patient EBV-LCLs support the presence of a stable, but truncated transcript, consistent with this hypothesis. Exposure of patient's cells to ionizing radiation resulted in normal p53 activation suggesting that p53 signaling is not affected by the truncated protein. However, a cell growth disadvantage was observed. Thus, we show that de novo truncating PPM1D mutations in the last and penultimate exon cause syndromic ID which provides novel insights in the role of cell cycle checkpoint genes in neurodevelopmental disorders.

De novo truncating variants in ASXL2 are associated with a unique and recognizable clinical phenotype

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The *ASXL* genes (*ASXL1*, *ASXL2* and *ASXL3*) participate in body patterning during embryogenesis and encode for proteins that are involved in epigenetic regulation and assembly of transcription factors to specific genomic loci. Germline *de novo* truncating variants in *ASXL1* and *ASXL3* have been respectively implicated in causing Bohring-Opitz and Bainbridge-Ropers syndromes, resulting in overlapping features of severe intellectual disability and dysmorphic features. To our knowledge, *ASXL2* has not yet been associated with a human Mendelian disorder.

In this study, we performed whole-exome sequencing in six unrelated probands with developmental delay, macrocephaly, and dysmorphic features. All six had *de novo* truncating variants in *ASXL2*. A careful review enabled the recognition of a specific phenotype consisting of macrocephaly, prominent eyes, arched eyebrows, hypertelorism, a glabellar nevus flammeus, neonatal feeding difficulties, hypotonia and developmental disabilities. Although overlapping features with Bohring-Opitz syndrome and Bainbridge-Ropers syndromes exist, features that distinguish the *ASXL2*-associated condition from *ASXL1*- and *ASXL3*-related disorders are macrocephaly, absence of growth retardation and more variability in the degree of intellectual disabilities.

We were also able to demonstrate with mRNA studies that these variants are likely to exert a dominant negative effect, since both alleles are expressed in blood, with the mutated *ASXL2* transcripts escaping nonsense mediated decay. In conclusion, *de novo* truncating variants in *ASXL2* underlie a new neurodevelopmental syndrome, with a clinically recognizable phenotype. This work expands the germline disorders that are linked to the *ASXL* genes.

WS4-003

Functional characterization of novel GNB1 mutations as a rare cause of global developmental delay

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Global developmental delay (GDD), often accompanied by intellectual disability, seizures and other features is a severe, clinically and genetically highly heterogeneous childhood-onset disorder. In cases where genetic causes have been identified, de-novo mutations in neuronally expressed genes are a common scenario. These mutations can be best identified by exome sequencing of parent-offspring trios. De novo mutations in the guanine nucleotide-binding protein, beta 1 (GNB1) gene, encoding the Gβ1 subunit of heterotrimeric G proteins, have recently been identified as a novel genetic cause of GDD. Using exome sequencing, we identified 14 different novel variants (2 splice site, 2 frameshift, and 10 missense changes) in GNB1 in 16 pediatric patients. One mutation (R96L) was recurrently found in three ethnically diverse families with an autosomal dominant mode of inheritance. Ten variants occurred de novo in the patients. Missense changes were functionally tested for their pathogenicity by assaying the impact on complex formation with Gγ and resultant mutant Gβγ with Gα. Signaling properties of G protein complexes carrying mutant Gβ1 subunits were further analyzed by their ability to couple to dopamine D1R receptors by real-time Bioluminescence Resonance Energy Transfer (BRET) assays. These studies revealed altered functionality of the missense mutations R52G, G64V, A92T, P94S, P96L, A106T, and D118G but not for L30F, H91R, and K337Q. In conclusion, we demonstrate a pathogenic role of de novo and autosomal dominant mutations in GNB1 as a cause of GDD and provide functional evidence for a loss-of-function mechanism underlying the disease.

WS4-004

Comprehensive phenotyping and trio-exome analysis of 50 children with neurodevelopmental disease

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Whole Exome Sequencing (WES) has been proven as a powerful analytical tool to dissect the genetic basis of human hereditary disorders. Here, we report on a prospective deep phenotyping and trio-WES study of 50 children affected by previously undiagnosed and diverse complex neuropediatric disorders. All children underwent a standardised and comprehensive clinical work-up in a single centre that included detailed clinical evaluations by pediatricians and clinical geneticists, extensive laboratory and metabolic analyses, analyses of cerebrospinal fluid, MRI of the brain and EEG, followed by trio-WES analysis. This systematic approach allowed to identify a pathogenic mutation in a known disease gene in altogether 21 children (42%) and discovered a convincing candidate disease gene in additional 22 children (44%). Taken together, this translates into a successful genetic diagnosis of up to 86% in this cohort. In 3 children with mutations in a known disease gene (3/21 = 14.3%) the molecular diagnosis substantially influenced the clinical management and drug treatment. We further document an expansion of the phenotype in known disease entities in 4 individuals. The extraordinary high gene discovery rate in our cohort emphasizes the potential of trio-WES even in a clinically inhomogeneous group of individuals with likely genetic disease. However, this requires a multidisciplinary approach including deep and sometimes reverse phenotyping, research-based interpretation of trio-WES identified genetic alterations, extensive review of the literature, use of several mutation prediction and protein-modelling tools, as well as openness and exchange of data with national and international researchers and clinicians working on similar diseases.

Exome sequencing of pooled DNA samples for large-scale screening in individuals with sporadic intellectual disability

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High throughput sequencing has enabled identification of many novel disease genes and empowered diagnostic testing for heterogeneous disorders, especially for intellectual disability (ID) where more than 1000 genes have been implicated. Due to this extreme heterogeneity gene panels are ineffective, and expensive exome or genome sequencing is necessary. Furthermore, many affected individuals have to be sequenced to confirm candidate genes and to refine the phenotypic spectrum. We now explored if pooling strategies could satisfy the need for a genome-wide, simple, cheap and fast screening technology.

After initial evaluation of available computational methods by virtual pooling of exome data or simulated reads using different pooling fractions, we decided to exome sequence 96 individuals with sporadic ID in 8 pools of 12 samples each. This was suggested to be the optimal combination with a 90% detection rate. DNA was mixed in equimolar concentrations and submitted for exome sequencing. Read data was aligned to the human reference, and variants were called using a ploidy of 24. Resulting variant calls in 893 known ID genes (SysID database) were then filtered for loss-of-function (LOF) variants and for missense variants that were either previously reported as pathogenic or computationally predicted to be deleterious. Furthermore, we screened 523 ID candidate genes and 1694 haploinsufficiency intolerant genes for LOF variants. Subsequently, Sanger sequencing was used to determine the individual carrying each variant in the respective pool and to test segregation in the parents.

This approach resulted in the identification of 15 pathogenic variants (assumed or confirmed de novo) in known ID genes (AHDC1, ANKRD11, ATP6V1B2, CASK, CHD8, KCNQ2, KMT2A, KRAS, MED13L, RIT1, SETD5, TCF4, WAC, ZBTB18), two pathogenic variants inherited from a symptomatic or healthy parent, respectively, (ZMYND11, IFIH1), and a homozygous variant in the recessive TRAPPC11 gene. This included 13 loss-of-function and 5 missense variants. Additionally, we identified 4 de novo variants in candidate genes. In our ID cohort this resulted in a high mutation detection rate of 23%. Thus, detection of rare variants from exome sequenced DNA pools (Pool-seq) is feasible and has a high detection rate similar other screening approaches. Compared to affected-only exome sequencing this method can reduce costs by more than 90% with only marginal increase in Sanger-sequencing costs and significantly speed up wet lab work with an acceptable increase in computational complexity. In contrast to targeted sequencing methods like molecular inversion probes or hybridization-based panels, our method has the advantage of allowing flexible re-analysis of the same data for new genes.

In conclusion, we established exome Pool-seq as a method for large-scale, cost-efficient and flexible sequencing in highly heterogeneous but well characterized disorders like ID.

Three years of experience with targeted next-generation sequencing of developmental delay

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Next-generation sequencing (NGS) has opened up new possibilities especially in the search for disease-causing mutations in disorders with common clinical features but a heterogeneous genetic background. The identification of the underlying genetic defect provides a clear diagnosis for patients more and more influencing their management and occasionally even their therapy, and it is the prerequisite for prenatal or preimplantation decisions in the affected family. NGS panels are used widely in clinical settings to identify genetic causes of various monogenic disease groups, such as intellectual disability (Hu *et al.* 2015), neurodevelopmental and neuromuscular disorders, among others. However, many new challenges have been introduced both at the technical level and at the bioinformatic level, with consequences including new requirements for interpretation of results, and for genetic counseling. We report on our experience with a targeted NGS panel comprising over 1200 brain related genes (MPIMG-1-Test) in the routine clinical diagnostics of patients with syndromic and non-sydromic forms of developmental delay as well as patients with neuromuscular disorders.

202 patients (age 1-71; mean 16) with syndromic (S) or non-syndromic (NS) developmental delay or with neuromuscular symptoms (NM), seen at the genetic counselling unit of our institute, were analyzed with targeted exon enrichment and NGS. Chromosomal re-arrangements and copy number variations were excluded in all 202 patients previously by conventional karyotyping and high resolution array CGH analysis. A

modified version of the Medical Resequencing Analysis Pipeline (MERAP, Hu *et al.*, 2014) was used to check all detected variants against common Databases. For exclusion of technical artifacts and segregation testing of all likely disease-causing variants, Sanger sequencing was performed according to standard protocols.

Class 5 mutations have been identified in 15/202 (NS: 7; S: 8) patients (i.a.: *NDST1, PTPN11, ARID1B, ALS2, GAMT*). 38 (NS: 15; S: 18; NM: 5) genes where likely associated with the patient's phenotype (i.a.: *PACS1, TAF1, KDM5C, PEX1, GAN, ATP7A, ERLIN2, ALT1*) but previously unreported (class 4). Further 24 variants (NS: 14; S: 8; NM: 2) were classified as class 3 because of yet lacking evidence of pathogenicity; segregation analysis and functional studies to reclassify these variants are pending. Classification of pathogenicity was carried out according to the standards of Richard *et al.* 2015. All variants were confirmed by Sanger sequencing.

Our findings so far support the clinical utility (overall detection rate: ~26%, S: 35%, NS: 20%, NM: 25%) of targeted next generation sequencing in identifying monogenic causes in a genetically heterogeneous cohort of patients. Identifying the underlying cause, especially in childhood developmental delay, can often take the burden of guilt from the family and offer possibilities for risk prediction and prenatal and preimplantation choices in the family.

WS6 COMPLEX GENETICS

WS6-001

Contribution of Neandertal alleles to the heritability of psychiatric and cognitive phenotypes

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Palaeontological genomic analyses have shown that interbreeding between anatomically modern humans and Neandertals occurred in Europe and Asia 40000-50000 years ago. Approximately 1.5-2% of the modern European and Asian genome consists of introgressed DNA from Neandertals. Some of these introgressed regions have been suggested to contribute to several traits and phenotypes including major depression and other mood disorders.

In order to further assess the role of Neandertal ancestry in cognition and the contribution of genetic risk for psychiatric disorders, we performed genome-wide analyses of Neandertal alleles in publicly available Psychiatric Genomics Consortium (PGC) GWAS summary statistics with samples sizes ranging from about 6000 to 293723 individuals for the following phenotypes: Educational attainment, attention deficit hyperactivity disorder (ADHD), anorexia nervosa, anxiety disorders, autism spectrum disorder, bipolar disorder, major depressive disorder and schizophrenia. We estimated the proportion of heritability explained by SNPs in Neandertal introgressed regions using stratified LD Score regression (LDSC) and two sets of previously inferred Neandertal introgressed regions. In a secondary analysis, we investigated whether specific functional annotations such as 3'UTR, promoter regions or histone marks within Neandertal regions were significantly associated with selected phenotypes.

We identified a modest enrichment of heritability in Neandertal introgressed regions in anorexia nervosa, autism spectrum disorder, bipolar disorder and major depressive disorder, although none of the results were statistically significant. Several functional annotations, such as H3K4me1 histone marks within Neandertal introgressed regions, appeared significantly enriched for SNPs contributing to the heritability of anorexia nervosa and autism spectrum disorder. In bipolar disorder, DNaseI digital genomic footprinting regions, H3K9ac histone marks and super enhancer regions within Neandertal regions appeared particularly enriched for heritability. On the other hand, both sets of Neandertal regions were slightly depleted of SNPs contributing to the heritability of schizophrenia. For example, one set of Neandertal regions that contained 33% of all analysed SNPs only contributed to 29% of the variance of risk (standard error: 0.04; p-value: 3.86×10^{-3}). In comparison to the rest of the genome, Neandertal introgressed regions also contributed less to the heritability of educational attainment, ADHD and anxiety disorders, although these findings were not statistically significant.

To our knowledge this is the first study to systematically investigate the extent to which SNPs attributable to Neandertal introgressed regions contribute to the heritability of several psychiatric/cognitive phenotypes. We are currently increasing our power to detect SNP heritability in Neandertal regions by applying the LDSC method to larger PGC datasets.

WS6-002

Novel insights into male-pattern baldness pathobiology via integration of differential hair follicle miRNA and mRNA expression profiles with GWAS data

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Male pattern baldness (MBP) is a highly heritable condition and the most common form of hair loss in men. The phenotype is characterized by a distinct pattern of androgen-dependent progressive hair loss from the scalp that is restricted to hair follicles (HF) in the frontal and vertex scalp area. The molecular mechanisms that underlie this characteristic pattern and the differences in androgen-sensitivity between HF subpopulations in the frontal/vertex and the occipital scalp remain however elusive.

To gain novel insights into the underlying biology and contributing genes and pathways, we systematically investigated for a differential expression (DE) of miRNA- and mRNA-genes in HF samples from the frontal and occipital scalp area of 24 healthy male donors. Array-based genome-wide miRNA and mRNA profiling revealed expression of 823 miRNAs and 21,247 mRNAs in human HF, of which 144 miRNAs (17%) and 3,230 mRNAs (15%) showed a DE between HF subpopulations. The strongest DE miRNAs included miR-4674, miR-6075 and miR-3185. Among the strongest DE mRNAs were the WNT-signaling inhibitor *DKK1*, the protein kinase *PAK1* and the retinoid acid receptor *RORA*. A subsequent pathway-based analysis in miRPathDB revealed that DE miRNAs targeted numerous interesting pathways. Among them the WNT- and mTOR signaling pathway which have been implicated in the control of hair follicle cycling, a mechanism that is disturbed in MPB affected HF and other plausible candidate pathways such as estrogen, thyroid hormone signaling or epidermal growth factor binding which have not yet been implicated MPB pathobiology.

To yield further evidence for an involvement of DE miRNAs and mRNAs in the developement of MPB, we subsequently integrated our expression data with association data from a large GWAS meta-analysis on MPB (N=22,518). Of the DE miRNAs and mRNAs, only 1 miRNA (miR-193b) and 49 mRNAs were located within 1 Mb of one of 63 genome-wide significant MPB risk loci. Notably, the analysis revealed a co-localization of DE miRNA, DE mRNA, and nominally significant association signals ($P<10^{-5}$) at 9 other genomic loci, pointing towards a role of these genomic regions in MPB pathogenesis. Among them a locus on chromosome 3q22.2 that comprises the genes encoding the Ephrin-Type-B Receptor 1 (*EPHB1*) and the prostaglandin transporter *SLCO2A1*. Interestingly, ephrins have been shown to be regulated by androgens and to play a role in HF formation, proliferation and hair cycling. And expression of prostaglandin D2, which is transported by SLCO2A1, has been found to be upregulated in balding scalp where it inhibits hair growth. In summary, our systematic analysis of differential miRNA and mRNA expression and the subsequent integration with genetic association data identified 9 novel potential risk loci for MPB and numerous candidate genes and pathways that are likely to play a role in MPB pathogenesis and emphasizes the importance of data integration of large-scale omic-analyses.

WS6-003

Male-pattern baldness and its association with coronary heart disease

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Male-pattern baldness (MPB) is characterized by a progressive hair loss from the frontal and vertex scalp that affects ~80% of men at the age of 80 years. Epidemiological studies have shown positive associations between MPB and coronary heart disease (CHD) and related phenotypes such as blood pressure (BP), diabetes (DM) or elevated blood lipid levels. The results however vary with regard to the associated pattern of hair loss (frontal or vertex) and the assessed endpoint measures for CHD. And so far no study has investigated for a shared genetic determinant between the traits. Using data from the Heinz Nixdorf Recall study (N=1,675 males) and a large meta-analysis on MPB (N=22,518), we aimed at a systematic investigation of the

association between MPB and CHD on (i) an epidemiological and (ii) a genetic level. In the age-adjusted model, we found a moderate positive association of MPB and new-onset of CHD (Hazard Ratio [HR]=1.2, 95%confidence interval [CI]:0.8;2.0) as well as DM (Prevalence Rate Ratio [PR]=1.5, 95%CI:1.0;2.1). While the risk for CHD was increased for frontal balding (HR=1.5, 95%CI=[0.8-2.7]), men with vertex balding showed a higher BMI (ß=1.4kg/m2), elevated fasting triglyceride (ß=8.0mg/dL) and lower HDL-C levels (ß=-2.7mg/dL). To assess the genetic overlap between MPB and CHD, we created a risk score (RS) from 63 MPB lead SNPs (P<5x10^{-8}) and tested for association with CHD and related traits phenotypes. No significant associations were observed. However, an age-stratified analysis revealed a 4% per allele risk increase for CHD (HR=1.04, 95%CI:0.97;1.17) and a decrease in fasting triglyceride levels (ß=-0.5). We next used LD score regression analysis in to test for genome-wide genetic correlation between MPB and CHD. The analysis revealed no significant correlations with cardiometabolic (N=3), lipid (N=4) or metabolic traits (N=103). Finally, to investigate for a genetic overlap at single loci, we compared the MPB risk loci with reported GWAS signals for CHD. The analysis identified seven overlapping associations between MPB and BP (N=3); QT-interval length; atrial fibrillation; sudden cardiac arrest; and DM. For the majority of loci, the direction of effect differed between MPB and CHD, opposing previous epidemiological findings. Positive associations were identified between MPB and diastolic BP (*FGF5*, 4q21.21) and sudden cardiac arrest (*ATF1*, 12q13.12). Interestingly, FGF5 is known to stimulate cell growth and proliferation in multiple cell types, including cardiac myocytes and hair follicle (HF) cells, and ATF1 is a HF expressed regulator of cell growth and differentiation that has been shown to prevent foam cell formation, which suggests that FGF5- and ATF1-signaling contribute to both traits. Thus, our data support an association between MPB and CHD related phenotypes and suggest that MPB deserves further evaluation as an additional risk factor for CHD.

WS6-004

Pleiotropic effect of genetic variants associated with complex diseases and traits in age-related macular degeneration

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Purpose: Age-related macular degeneration (AMD) is the leading cause of vision loss in western societies and is caused by both environmental and genetic risk factors. With regard to the latter, several associated risk loci harbor genes involved in the complement system, high density lipoprotein metabolism or extracellular matrix homeostasis. These pathways are known for their pleiotropic role in other conditions, such as cardiovascular disease, auto-immune diseases and cancer. Here we aimed to investigate the extend of overlap between the genetic risk of various complex diseases and traits and the genetic risk for AMD.

Methods: First, we catalogued 2,331 previously published, genome-wide significant variations associated with 82 complex diseases or traits. Next, we computed a genetic score by calculating the (weighted) sum of risk increasing alleles for each disease or trait. Consequently, a higher genetic score indicates that an individual has more risk/trait increasing alleles of a given disease or trait. For each score, we computed the association with late stage AMD using a dataset provided by the International AMD Genomics consortium (IAMDGC) including 16,144late stage AMD cases and 17,832 controls. We also assessed the association of each variation individually with late stage AMD risk in order to identify novel disease loci with strong evidence for pleiotropy.

Results: Nineteen genetic scores of complex diseases and traits were significantly associated with AMD risk (FDR < 0.01). Most notably, all genetic scores related to autoimmunity were elevated in AMD patients (P < 5.85x10-09), while scores related to cardiovascular disease were reduced in AMD patients compared to controls (P < 3.10x10-05). We also found that the genetic scores of melanoma and related malignancies were higher in AMD patients (P < 8.43x10-05). In addition, 32 out of 2,331 variants, which were used to compute the genetic scores, were significantly associated with AMD (FDR < 0.01), implicating 25 novel, pleiotropic loci in AMD risk.

Conclusion: Our findings demonstrate a substantial overlap between the genetic risk of complex diseases/traits and the genetic risk for AMD and provide evidence for 25 novel, pleiotropic loci associated with AMD. While our findings highlight common disease pathways that may facilitate to develop multi-use drug targets, they also challenge the notion that gene/genome manipulation could be applied in general terms to eradicate risk for a defined complex disease.

Worldwide genetic association study of exfoliation syndrome and glaucoma identifies common genetic variants at five new susceptibility loci

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Exfoliation syndrome (PEX), a complex systemic disorder of the extracellular matrix, is the commonest cause of secondary glaucoma in aging population and thus a major cause of blindness globally, affecting 60-70 million subjects worldwide. Inside a large, international collaboration project a genome-wide association study (GWAS) was carried out on 9,035 PEX cases and 17,008 controls, recruited from 24 countries across six inhabited continents, with replication in a further independent 4,585 cases and 92,829 controls from 15 countries. Significant association was observed at seven loci, of which two confirmed the already known associated loci at the genetic markers mapping to LOXL1- and CANA1A- gene, five are new (P<5x10-8). The five new loci map to chromosomes 13q12 (rs7329408 near FLT1-POMP-SLC46A3, P=9.41 x 10-16), 11q23.3 (rs11827818 near TMEM136–ARHGEF12, P=1.21 x 10-10), 6p21 (rs3130283 at AGPAT1, P=2.12 x 10-9), 3p24 (rs12490863 at RBMS3, P=3.9 x 10-8) and 5q23 (rs10072088 near SEMA6A, P=3.64 x 10-8). To determine the pathophysiological role of the three most significantly associated loci (13q12, 11q23.3, 6p21), we investigated the expression and localization of the six related genes (FLT1, POMP, SLC46A3, TMEM136, ARHGEF12 and AGPAT1) by qRT-PCR, immunohistochemical- and Western-blot analysis in genotyped ocular tissues of PEX and control patients. All six genes displayed moderate mRNA expression in all ocular tissues analysed, with highest levels in iris, ciliary body, and retina. However, only POMP showed a trend towards reduced expression in the presence of the rs7329408 risk allele, in both PEX and control patients. In general, both mRNA and protein expression of POMP and TMEM136 were significantly reduced up to 45% (p<0.005) in anterior segment tissues in PEX eyes compared to controls. No differences in mRNA and protein expression were detected for the remaining genes analysed. Immunofluorescence analysis showed that POMP, a proteasome maturation protein, is ubiquitously expressed in most ocular cell types and that TMEM136, a transmembrane protein of unknown function, is primarily localized to endothelial cells of blood vessels and aqueous outflow structures. Additionally, protein staining intensities for POMP and TMEM136 were markedly reduced in anterior segment tissues of PEX eyes compared to controls and co-localized to abnormal accumulation of PEX material on ocular surfaces and in blood vessel walls. Thus, at least two of the newly identified loci provide new biological insights into the pathology of PEX syndrome/glaucoma and highlight a role for impaired proteasome function as well as vascular and trabecular endothelial dysfunction in the disease pathogenesis.

Nonsyndromic cleft palate only – evidence for a limited contribution of common variants in contrast to nonsyndromic cleft lip ± palate

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Cleft palate only (CPO) is a common congenital malformation which might occur as part of a syndrome or in an isolated form, i.e., nonsyndromic CPO (nsCPO). NsCPO has a prevalence of 1:2500 and is considered multifactorial with genetic as well as environmental factors contributing to the disorder. In a recent study we identified the first genome-wide significant locus for nsCPO which has been independently confirmed in another study. In order to discover more nsCPO risk loci we performed a genome-wide imputation study with GWAS data from 550 case-parent trios with European, Asian and African ancestry which was retrieved from dbGaP upon approved data access. Notably, this GWAS dataset had not yet been imputed, and we hypothesized that we can increase power to identify novel genetic associations by increasing the marker density and follow-up of suggestive findings by independent replication. Genome-wide genotypes were imputed using IMPUTE2 based on 1000 genomes haplotypes, and SNPs were selected based on info-score > 0.4 and minor allele frequency > 1%. The imputation did not reveal any genome-wide significant SNP,

however, 83 SNPs at 26 loci showed P-values $< 10^{-5}$. Loci with more than two variants below this threshold (n=19) were to be replicated using the MassARRAY system (Agena Bioscience). Three independent samples were used: Two case/control replication cohorts from Central Europe (92 cases, 335 controls) and Yemen (37 cases, 231 controls), and one European case-parent trio replication cohort (EuroCran study; 223 trios). In a first round we genotyped 18 SNPs at eleven loci. One variant, rs6809420 at chr. 3q13, showed P< 0.1 in the replication cohort and after combining replication and GWAS data, resulted in a decrease of P-value from 1.14×10^{-03} to 3.22×10^{-04}. This indicates that this locus, which includes candidate genes such as *IGS11*, a known cell-adhesion molecule with yet unknown function in craniofacial development, might harbour a common risk variant with low effect size. We are currently performing a second round of genotyping, results of which will be presented at the conference. Of note, for nonsyndromic cleft lip with/without cleft palate (nsCL/P), the most frequent form of orofacial clefting, 20 risk loci have been detected by GWAS so far, with some of them reaching (nearly) genome-wide or significant P-values in samples much smaller than 550 cases. In the imputed nsCPO dataset none of the presently known nsCL/P risk loci showed a P-value $< 10^{-5}$. Our data so far confirm previous molecular and epidemiological findings, that nsCPO is genetically distinct from nsCL/P. Furthermore, the results indicate that common variants alone might not contribute to the same extent to nsCPO as compared to nsCL/P.

WS5 TECHNOLOGY

WS5-001

Improving the identification of disease-causing mutations with automated image analysis – current results of the PEDIA study

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Over the past years, prioritization strategies that combined the molecular predictors of sequence variants from exomes and genomes of patients with rare Mendelian disorders with computer-readable phenotype information became a highly effective method for detecting disease-causing mutations.

The drawback of phenotype-based prioritization, however, is that they require a deep and comprehensive feature description to gain good performance. But in routine diagnostics, the naming of phenotypic features varies among clinicians, and sometimes a comprehensive phenotypic overview is not possible because of missing terminology.

These gaps can be reduced by including a new layer of phenotypic information using facial recognition technology to detect dysmorphic features from two-dimensional photographs. Automated image analysis is in principle able to identify any deviation from the norm and to quantify it objectively. We therefore developed an approach that combines Facial Dysmorphology Novel Analysis (FDNA) technology with standard phenotypic and genomic features to identify pathogenic mutations in exome data.

We have started collecting data from a diverse spectrum of patients with molecularly confirmed diagnoses in a multi-center study, and we present the current results. At the time of abstract submission more than 300 patients from over 10 contributing institutions were evaluated and used for simulation of a training set of exomes.

Automated facial recognition yields the correct diagnosis amongst the first ten suggested syndromes in more than two thirds of the cases and shows a high correlation with syndrome predictions that were based on expert annotated features. Hereby, we could also confirm the diagnosis in cases with only subtle facial features.

Consequently, we used classical machine learning approaches to integrate scores based on the image analysis, phenotypic description and exome sequence of the patients and could predict the pathogenic mutation among the top 10 positions in a prioritized exome in more than 90% of the monogenic cases in our cohort.

Hence, our results show that computer-assisted facial recognition is not only a promising technology that could be applied in the routine diagnostic workflow, but also a technology that allows diagnosis in cases with non-typical clinical presentation and boosts the diagnostic yield in exome studies.

WS5-002

The added value of rapid exome sequencing in critical clinical situations

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For critical clinical situations, turnaround times (TATs) of exome sequencing need to be fast in order to have an impact on clinical decision making. We therefore set out to develop a fast exome sequencing approach (max. 14 days). Urgent exomes are preferably sequenced as trios to enable de novo analysis and assist data interpretation. DNA library preparation is performed using the SureSelect QXT protocol (v5, Agilent), and sequencing is done on a NextSeq500 (Illumina) with a high coverage (200-300x). Automated file handling allows rapid BWA mapping, GATK variant calling and annotation.

A total of one-hundred samples have been sequenced until now using this rapid procedure: 28 trio's, 1x mother and child, 2x parents plus 2 children, and 6 single cases. Six trios with known aberrations were used for experimental setup. Of the remaining 31 families, in 14 families (possible) pathogenic SNVs were identified, of which some still need further follow up, whereas 17 families remained negative after inspection of SNVs and small indels. For CNV analysis, a trio based reference- free CNV approach is still under development. Preliminary data show that all control CNVs (53kb-6Mb) are detected correctly, and retrospective CNV analyses of the other samples identified three possibly de novo CNVs that need further follow up.

Shorter TATs days were already beneficial for some patients, i.e. an adult male suffering from myelofibrosis and autoinflammatory symptoms. A STING-like phenotype (=stimulator of IFN genes) was suspected, with a possible involvement of the JAK/STAT pathway. Urgent exome sequencing was performed and results were available within 9 days. Interestingly, both a somatic variant in MPL (=trombopoetine receptor > myelofibrose) and a heterozygous variant in ACP5 (TRAP, known immune dysregulation disorder) were identified, both fitting to the patients phenotype. Based on these results the medication of the patient was changed, resulting in a substantial improvement of the patients constitution.

In conclusion, we have implemented a rapid exome sequencing workflow for urgent cases. The rapid identification of pathogenic variants already had implications on patient treatment, underlying the added value of a fast genetic diagnosis.

WS5-003

Ultra-sensitive mosaic mutation detection in blood DNA of healthy individuals – new insights into age-related clonal hematopoiesis

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It was recently shown that that clonal hematopoiesis can be driven by somatic point mutations. These acquired mutations occur with normal aging in up to 10% of older (>65y) individuals (1-3) and few reports in younger individuals.

Here we present a targeted re-sequencing assay that combines high throughput with ultra-high sensitivity based on single-molecule molecular inversion probes (smMIP) (1). We have now analyzed DNA from 2000 healthy blood donors from 5 different age groups (20-29; 30-39; 40-49; 50-59 and 60-69y), with no previous diagnosis of cancer, for somatic mutation in 100 loci. Those loci included 50 known drivers of clonal hematopoiesis (2-4) and 50 novel or candidate loci.

The improved assay allows low-frequency variant detection with variant levels down to <0.1%. This improved sensitivity allowed the identification of somatic mutations in a limited set of loci in >15% of old individuals, but also report those mutations in individuals of the youngest age group. Most prevalent mutations include known hotspot mutations in DNMT3A and ASXL1.

Here we show that somatic drivers of clonal hematopoiesis are more prevalent and occur in younger individuals than previously reported. These somatic events are age-related. However, the high prevalence and their occurrence in relatively young individuals implicates their origin as a common biological process involved in normal aging.

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WS5-004

CNV detection from targeted next-generation panel sequencing data in routine diagnostics

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Gene dosage abnormalities account for a significant proportion of pathogenic mutations in rare genetic disease related genes. In times of next generation sequencing (NGS), a single analysis approach to detect SNVs and CNVs from the same data source would be of great benefit for routine diagnostics. However, CNV detection from exon-capture NGS data has no standard methods or quality measures so far. Current bioinformatics tools depend solely on read depth which is systematically biased. We developed a novel approach based on: 1. utilization of five independent detection tools to increase sensitivity, 2. different reference sets for different kits and normalization against samples from the same sequencing run to improve robustness against workflow conditions, 3. definition of special quality thresholds for single exon events to minimize false negatives, 4. identification of reliable regions by assessment of capture efficiency using a reference set of CNV negative patients to minimize false positives. A CNV is called in a reliable region if at least two out of five tools are concordant for the respective CNV. The pipeline shows a sensitivity of 80% and a precision of 95%. Within routine gene panel diagnostics we analyzed a total of 1088 patients indicated to have rare Mendelian diseases for SNV and CNVs. In 32 patients a CNV was detected in genes associated with the respective individual phenotype. Interestingly, in several cases the CNV completed the patients report as it was detected in genes with a recessive mode of inheritance where previously only a heterozygous pathogenic SNV was found. Overall, with the additional analysis of CNVs we increased the diagnostic yield from 15% (class 4, 5 single nucleotide events) to 18%. However, there are still issues in the detection of CNVs from NGS data for routine diagnostics. CNV pipelines are very prone to errors caused by enrichment inconsistencies compared to SNV detection tools. The assessment of sensitivity and specificity is difficult due to the lack of datasets to validate CNV detection pipelines. Originally, the analysis of CNVs was performed mainly in patients with mental retardation disorders, resulting in a paucity of CNV data linked to other Mendelian diseases. Moreover, the identification of the actual size and thus the assessment of pathogenicity of a CNV is difficult, because targeted NGS gene panels do not cover all genes. In conclusion, NGS data is a suitable data source for the simultaneous detection of SNVs and CNVs for clinical diagnosis; however, with the current tools it is only applicable in accurately validated regions.

WS5-005

Identification of transposon insertions in whole-exome sequencing data

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45% of the human genome consists of transposable element derived sequences, the most abundant of which are L1 and Alu elements, followed by endogenous retroviruses. Several hundred of these elements remain active, leading to insertion frequencies of up to one in 20 live births for Alu elements and posing a threat to genome integrity.

While most studies on transposons employ whole-genome sequencing (WGS) or target-enrichment based sequencing approaches, the most commonly used form of diagnostic high-throughput sequencing is currently whole-exome sequencing (WES). We were therefore interested in investigating transposon insertions in WES data as a possible source of disease causing mutations.

We developed a software to call non-reference transposon insertions from single-end WES datasets by split-read mapping and analyzed 385 exomes this way. On average, 188 non-reference insertions were identified in each exome, with an average of 1.7 sites per patient identified in <= 0.5% of other patients. Of these rare variants, 91% were deemed plausible by visual inspection. Automated confidence calls of the software were concordant with visual inspection in 87% of cases. In 5% of cases a plausible insertion was awarded a lower score by the algorithm and in another 5% not called at all. In 1% of cases the automated call

appeared to be falsely positive, in another 1% at the wrong position within the same 100bp window. Laboratory validation of 11 convincing insertions revealed a 73% true positive rate, leading to an estimated specificity of 67%. When performing calls for reference L1 insertions on 10 exomes, 65% (49% - 70%) of known elements whose flanking regions were covered by at least two reads were correctly identified, leading to a sensitivity of 65%. We thus estimate the average number of non-reference transposon insertions in our WES dataset to be 194 (153 – 235).

67% and 22.2% of sites identified were associated with Alu and L1 elements, respectively, with the majority of calls stemming from evolutionary young transposons still assumed to be active. 10.3% of sites were located within the CDS, 3.4% in the UTRs of genes, 0.5% spanned an intron/exon border, 48.5% were intronic and 37.3% of insertions were found in intergenic regions.

We then chose 8 insertions within intronic (7) or UTR (1) regions for further analysis. Seven were not detected in the mRNA. One intronic Alu element insertion, however, was spliced into the mRNA of NABP1, leading to a frameshift mutation and a premature stop codon, potentially altering or abolishing gene function.

In summary, we have shown that transposon insertions, both common variants as well as rare or de novo variants, can be detected in WES data. Such insertions in coding or regulatory regions of disease-relevant genes might therefore explain some of the cases in which no pathogenic coding mutation can be identified by WES.

WS5-006

The influence of human genetic variation on Epstein-Barr virus sequence diversity: a genome-to-genome approach

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Genome-wide association studies (GWAS) have identified common genetic polymorphisms that associate with clinical manifestation and immune response parameters of various infections. We here present an alternative approach, using variation in the virus sequence as phenotype, which is specific by nature and unique to genomic research in infectious diseases, for genome-to-genome (G2G) association studies. Building on the unprecedented possibility to combine large-scale human and viral genomic data, we explored interactions between human genetic variation and viral sequence diversity in individuals infected with Epstein-Barr virus (EBV). The major goal is the identification of key genetic players in the evolutionary 'arms race' between pathogen and host.

EBV is the pathogenic agent of infectious mononucleosis and is associated with a broad spectrum of lymphoid and epithelial malignancies, including lymphomas and nasopharyngeal carcinomas. There is also evidence for a role of EBV in the pathoetiology of multiple sclerosis. Its genome is approximately 190 Kbp long and encodes around 80 proteins, not all of which have been definitely identified or characterized.

It is known that high loads of EBV are present in patients with advanced human immunodeficiency virus (HIV)-induced immunodeficiency. We therefore selected 780 immunosuppressed patients included in the Swiss HIV Cohort study (SHCS) with low CD4+ T cell counts, and quantified EBV copy number in peripheral blood mononuclear cells (PBMCs). 290 cell samples contained more than 2,000 viral copies in total and were subjected to target isolation and subsequent enrichment using the SureSelect method by Agilent Biotechnologies, followed by Illumina whole-genome sequencing. After data processing and quality control, variable amino acids were called as binary variables, resulting in >200 variable positions per individual in average.

The same patients also underwent genome-wide genotyping to obtain host genetic variation, followed by imputation based on the Haplotype Reference Consortium reference panel.

The association analyses are currently ongoing, and we will present the results at the conference. We use logistic regression to test for association between host single nucleotide polymorphisms (SNPs) and binary EBV amino acid variants. Bonferroni correction is applied for multiple testing correction on the sides of both host and pathogen. Stratification is taken into consideration by including principal components (PCs) for the host, and phylogenetic PCs for the virus.

This project will offer a global description of the adaptive forces acting on EBV during natural infection. We have shown before for HIV that a virus genome associates much more strongly with human genetic variants than clinical endpoints. The analysis of all signals resulting from the interaction between human and viral genomes has the potential to identify novel host defense mechanisms, which could serve as future diagnostic and therapeutic targets.

WS9 BASIC MECHANISMS AND EPIGENETICS

WS9-001

Paternal body mass index (BMI) effects on sperm methylation and transmission into the next generation

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Background

The prevalence of metabolic disorders, in particular obesity has dramatically increased worldwide. Genetic variants explain only a minor part of this obesity epidemics induced by physical inactivity and over nutrition. Epidemiological studies in humans and animal models of diet-induced obesity indicate that epigenetic changes associated with adverse parental and/or intrauterine factors may contribute to the missing heritability of metabolic disorders. Possible adverse paternal effects are likely transmitted by the sperm to the next generation. To prove this hypothesis, we have systematically analyzed the effects of paternal obesity on the sperm epigenome and its implications for the next generation.

Results

To study the possible transmission of paternal BMI effects to the next generation, methylation levels of eight paternally expressed imprinted genes (PEG1, PEG3, PEG4, PEG5, PEG9, PEG10, NESPAS and IGF2), two maternally expressed imprinted genes (MEG3 and H19), and the obesity related gene HIF3A were quantified by bisulphite pyrosequencing in sperm of 109 donors (undergoing IVF/ICSI) and 121 fetal cord blood (FCB) of resulting offspring (conceived by IVF/ICSI with the same sperm samples). HIF3A showed a significant positive correlation between sperm methylation and paternal BMI. This effect on the sperm epigenome was replicated in an independent cohort of 188 sperm samples. For HIF3A, paternal BMI also showed a significant positive correlation with FCB methylation. On the other hand, PEG5/NNAT exhibited a significant negative correlation between paternal BMI and FCB methylation. In contrast to pyrosequencing, deep bisulphite sequencing (DBS) allows one to study DNA methylation at the single molecule level and enables us to distinguish between maternal and paternal alleles in FCB samples with an informative SNP. Epimutations which are defined as alleles showing >50% aberrantly (de)methylated CpG sites can also be identified with DBS. Upon performing DBS on sperm samples, we observed a higher epimutation rate in the high BMI (28-40) group when compared to the low BMI (19-24) group across the four studied genes (PEG1, HIF3A, H19 and NESPAS). We are presently analyzing DBS data in selected cord blood samples with an informative SNP to separately quantify methylation at the paternal and maternal alleles. It is important to decipher the methylation of the paternal allele when studying whether sperm methylation alterations are transmitted to the offspring.

Conclusions

Our results suggest that male obesity is associated with modification of the sperm DNA methylome, which may affect the epigenome (in fetal cord blood) of the next generation.

WS9-002

Extent and genetic basis of inter-individual DNA methylation differences in human monocytes

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Allele-specific DNA methylation occurs at functionally different regions: 1) at imprinting control elements, 2) on the silent X chromosome in females and 3) across the genome and probably dependent on the DNA sequence in *cis*. The latter is termed haplotype-dependent allele-specific methylation and may contribute to inter-individual phenotypic variation. In a previous study on monocyte to macrophage differentiation, we showed that DNA methylation differences between individuals were greater than between the two cell types. To study the genetic basis of these inter-individual differences in DNA methylation, we analysed the methylome obtained by whole genome bisulfite sequencing (WGBS) of monocytes from five unrelated donors. For identifying differentially methylated regions (DMRs), we created two synthetic methylomes: one with the

highest methylation values of each CpG in the five samples and one with the lowest methylation values. Defining a DMR as a region of at least 4 CpGs with a methylation level difference of at least 0.8, we identified 157 DMRs, which cover 1165 CpGs and fall into different chromatin states, where methylation is inversely correlated with active histone marks. Using the Hardy-Weinberg law, we estimate that there are 692 DMRs with a MAF>0.05. We hypothesized that *cis*-acting DNA polymorphisms could be responsible for the inter-individual variation of the DMRs methylation levels. We genotyped 2.5 million SNPs in the five donors and found that 82/157 (52%) DMRs have methylation levels highly correlated (>0.9) with the genotype of at least one nearby SNP (± 6 kb window). This correlation was verified in 6/7 DMRs by targeted bisulfite sequencing in monocytes from 4 individuals used for WGBS and from 2 additional individuals. To validate our results in a larger population and possibly find correlating SNPs outside the ± 6 kb window for the remaining DMRs, we performed genome-wide association studies (GWAS) using SNP genotypes and Illumina 450k CpG methylation data from blood samples of 1131 individuals from the Heinz Nixdorf Recall Study. These methylation arrays encompass only 51 CpGs contained in 30 of our DMRs, showing that they fail to identify a great number of potentially important regions. We certified that for these 51 CpGs, monocyte and whole blood DMRs methylation levels were correlated, and performed a GWAS with ~500,000 SNP for each of the 51 CpGs. For 48/51 CpGs, the correlation peak was near the CpG position. For each GWAS, the SNP with lowest p-value (in most cases $p<1E^{-200}$) was designated as lead-SNP. SNPs in high linkage disequilibrium ($r^2>0.8$) to the lead-SNPs were located within the corresponding DMR or 16 bp to ~116 kb from it. Many regions are bound by CTCF and other transcription factors. It is likely that SNPs affect the binding of these factors and thus the methylation state of the region. We conclude that these inter-individual differences in DNA methylation are mainly driven by genetic factors.

WS9-003

The dystonia 6 (DYT6) protein THAP1 recruits the histone deacetylase HDAC3 to mediate gene repression

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Dystonia describes a heterogeneous group of neurological movement disorders characterized by contractions in various muscles resulting in abnormal postures, involuntary twisting and repetitive movements. Dystonia 6 (DYT6), a primary torsions dystonia that first has an impact on cranio-cervical muscles causing problems with speaking and eating, is caused by mutations in the THAP1 gene (Thanatos-associated domain-containing apoptosis-associated protein 1). THAP1 belongs to the family of THAP proteins that are characterized by the presence of an evolutionarily conserved specific DNA-binding THAP zinc finger motif at their N-terminus. In humans 12 THAP family members are known, designated THAP0 to THAP11. Interestingly, most of the DYT6-causing mutations affect this THAP domain. While we have previously described THAP1-mediated repression of specific target genes, the molecular mechanisms how THAP1 regulates promoter activity are rather unknown. It is known, that other members of the THAP family such as THAP7 and THAP11 interact with the histone deacetylase HDAC3 to mediate transcriptional repression.

We have performed yeast-two-hybrid and GST pulldown assays to identify a specific interaction of THAP1 with HDAC3. By the use of truncated THAP1 fragments we were able to narrow down HDAC3 binding to the N-terminal THAP-domain. For further functional characterization we have decreased HDAC3 levels by siRNA treatment or chemical inhibition and used Taqman analyses to quantify the effect on THAP1-target genes expression. Thus, a significant increase of THAP1-target genes expression was detected in those cells treated with HDAC3 siRNA. To further investigate whether the observed increase in gene expression is due to alterations of histone acetylation within the promoter regions we performed chromatin immunoprecipitation (ChIP) assays followed by qPCR using antibodies specific for different acetylated N-terminal residues of histone 3 as markers for transcriptional active promoters. By this, we detected an increased acetylation within the promoter regions of THAP1 target genes that are dysregulated in cells treated with decreased HDAC3 levels.

Our data show for the first time a functional interaction of the 'dystonia 6 protein' THAP1 with the histone deacetylase HDAC3 and therefore give new insights into the molecular mechanisms of THAP1-mediated gene repression. Interestingly, previous functional studies as well as structure analyses revealed that only a subset of the DYT6-causing mutations affecting the N-terminal THAP domain alter THAP1-binding to DNA. In ongoing studies we want to investigate the consequences of DYT6-causing mutations on THAP1-HDAC3 complex formation and its relevance in the molecular pathology of dystonia.

The human RHOX gene cluster: target genes and functional analysis of gene variants in infertile men

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Reproductive homeobox (RHOX) genes are clustered on the X chromosome and share a unique 60 amino acid helix-turn-helix DNA binding homeodomain. They were identified in several species as having important roles in reproductive tissues, notably in the testis. The human RHOX cluster is composed of three genes: RHOXF1 and two copies of RHOXF2 (RHOXF2A, RHOXF2B) which are referred to as RHOXF2/2B. RHOX proteins are expressed exclusively by germ cells in human testis and aberrant RHOX methylation is associated with several sperm parameters. Because little is known about the molecular mechanism of RHOX function in humans, the aim of the study was to identify target genes of human RHOX proteins and to investigate the impact of RHOX mutations on protein function.

Using gene expression profiling, we identified genes regulated by members of the human RHOX gene cluster. Some genes were uniquely regulated by RHOXF1 or RHOXF2/2B, while others were regulated by both of these transcription factors. Several of these regulated genes encode proteins involved in processes relevant to spermatogenesis, e.g. stress protection and cell survival. One of the target genes of RHOXF2/2B is RHOXF1, suggesting cross-regulation to enhance transcriptional responses. The potential role of RHOX in human infertility was addressed by sequencing RHOX in a group of 250 patients with severe oligozoospermia. This revealed two mutations in RHOXF1 (c.515G>A and c.522C>T) and four in RHOXF2/2B (-73C>G, c.202G>A, c.411C>T and c.679G>A), of which only one (c.202G>A) was found in a control group of men with normal sperm concentration. Functional analysis demonstrated that c.202G>A and c.679G>A significantly impaired the ability of RHOXF2/2B to regulate downstream genes. Molecular modelling suggested that these mutations alter RHOXF2/F2B protein conformation.

By combining clinical data with in vitro functional analysis, we demonstrate how the X-linked RHOX gene cluster may function in normal human spermatogenesis and we provide evidence that it is impaired in human male fertility.

RIT1 controls actin dynamics via complex formation with RAC1/CDC42 and PAK1

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RIT1 belongs to the RAS family of small GTPases. Germline and somatic *RIT1* mutations have been identified in Noonan syndrome (NS) and cancer, respectively. We show that NS-associated RIT1 mutants intensified signal flux through the MEK-ERK pathway upon growth factor stimulation. By using heterologous expression systems, we identified the p21-activated kinase 1 (PAK1) as novel effector of RIT1. We found that RIT1 interacts with the RHO GTPases CDC42 and RAC1, both of which are crucial upstream regulators of PAK1. Disease-causing *RIT1* mutations enhance protein-protein interactions and uncouple complex formation from growth factors. Expression of both wild-type RIT1 and its mutant forms resulted in dissolution of stress fibers and paxillin-containing focal adhesions from the cell center and increased cell movement. We conclude that RIT1 is a potent regulator of actin dynamics, and dysregulated RAC1/CDC42-PAK1 signaling controlling cell adhesion and migration may be one aspect of the molecular basis of NS.

WS9-006

ZBTB48 is a vertebrate telomere-binding protein limiting telomere length

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Telomeres are short repetitive TTAGGG sequences that cap the ends of chromosomes. These stretches of DNA are covered by proteins and RNAs which together protect the putative double strand break from DNA repair mechanisms and facilitate replication. However, telomeres shorten with every cell division due to the end replication problem. The ribonucleoprotein telomerase counteracts this process by de novo elongation of telomeric repeats but its expression is mostly confined to the germ line and stem cells. Even in the latter its activity is usually not sufficient to completely prevent telomere shortening. All cancer cells are also faced with this challenge and while the majority of cancer cells rely on telomerase, approximately 15% of cancers ensure sufficient telomere length via the recombination-based alternative lengthening of telomeres (ALT) mechanism. To better understand telomere biology we aimed to identify novel telomeric factors by systematically screening for telomere-binding proteins in cell lines from 16 different vertebrates. Here, we identified and characterized ZBTB48, a zinc finger protein, as a novel direct telomere-binding protein across the vertebrate lineage. ZBTB48 is directly binding to telomeric DNA *in vitro* and it is localizing to telomeres *in vivo* via one specific zinc finger domain in both telomerase- and ALT-positive cancer cells. Interestingly, ZBTB48 knock-out cells have longer telomeres, suggesting that ZBTB48 limits telomere elongation. In addition, the combination of ChIPseq, RNAseq and proteome analysis revealed a transcription factor activity for a small, but specific set of target genes of ZBTB48, linking its telomeric functions to mitochondrial metabolism. In conclusion, ZBTB48 is a novel direct telomere binding protein with transcription factor activity that acts as negative regulator of telomere length.

WS7 CLINICAL GENETICS

WS7-001

Discrepancies between clinical indications and molecular results in imprinting disorder diagnostics

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The correlation between defects at specific imprinted loci and distinct imprinting disorder (ID) was accepted for a long time. However, it is now put into question because of a growing number of patients with Multilocus Imprinting Disturbances (MLID), i.e. the aberrant methylation at more than one imprinted locus. In particular, MLID is present in individuals with Silver-Russell syndrome (SRS) and Beckwith-Wiedemann syndrome (BWS), and it has meanwhile turned out that patients with opposite phenotypes can share common epimutation patterns. On the other hand, MLID always occurs as mosaicism and varies in different tissues of the same individual. Interestingly, the majority of MLID carriers show only one specific ID phenotype, though loci of other IDs are affected in addition to the one specific for the phenotype. We become aware of a growing number of patients with unexpected and even contradictory molecular findings in respect to the clinical diagnosis for referral. Amongst others, we detected the SRS specific ICR1 hypomethylation in 11p15 in two of our patients referred as BWS. In the first case, the ICR1 hypomethylation was detected only in lymphocytes but was not present in buccal swab DNA. The patient only had a slight asymmetry, but showed normal growth and did not exhibit any other feature compatible with BWS, nor with SRS. The reason for the lack of clinical features is unclear, but is comparable to the observation in monozygotic, but clinically discordant SRS and BWS twins. Here the unaffected twin often carries the epimutation only in lymphocytes whereas the affected one shows the alteration in additional tissues. A reason might be sharing of hematopoietic stem. It can be postulated that the patient presented here is born after an (undetected) twin pregnancy with early loss of the affected twin. In the second case, the initial diagnosis of BWS was made due to asymmetry, though overgrowth or other features were not present. Further clinical ascertainment did not confirm this diagnosis, but growth of the patient was in the lower percentiles, in concordance with the ICR1 hypomethylation. These cases as well as further cases in our cohort confirm that there is an urgent need to provide detailed clinical data upon requesting molecular diagnostics for imprinting disorders. In fact, the growing number of patients with unexpected results

complicates the interpretation and illustrates the broad phenotypic range, but also provides further insights in the etiology of IDs and setting of imprinting marks

WS7-002

Nephrotic syndrome associated with brain anomalies - new lessons on the heterogeneity of Galloway-Mowat syndrome

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The eponymic name Galloway-Mowat syndrome (GAMOS; OMIM 251300) has been coined for the association of early-onset nephrotic glomerulopathy, microcephaly with variable brain anomalies, and facultative diaphragmatic hernia. It is supposed to be inherited as an autosomal recessive trait and clinical as well as genetic heterogeneity has been suggested. In 2014, WDR73 mutations were identified as a cause of GAMOS, but only a few cases have been reported to date.

Over the last 15 years, we have collected DNA samples and clinical data from 56 unrelated families with one or more children affected by GAMOS or a GAMOS-like syndrome (glomerulopathy plus variable anomalies of brain morphology or function as inclusion criteria), including 15 consanguineous families. In this cohort, we performed whole exome sequencing followed by targeted analysis by Sanger and NGS multigene panel resequencing.

In a total of 25 families of this cohort (46%) the probable underlying genetic defect could be identified. In affected individuals from two consanguineous families, homozygous mutations of WDR73 could be found (Vodopiutz et al., 2015). Thus, this gene accounted for only 4% of cases of our cohort. The affected child of another family had a novel homozygous mutation in ARHGDIA. This gene has previously been described in three families to cause early-onset steroid-resistant nephrotic syndrome (Gupta et al., 2013; Gee et al., 2013), but there is some evidence that non-specific brain anomalies may also be part of the ARHGDIA-associated phenotype. Fourteen and three index patients from unrelated families had mutations in one autosomal (OSGEP) and one X-linked gene (LAGE3), respectively, both encoding for components of the KEOPS protein complex that has been implicated in transcription, telomere maintenance and chromosome segregation. No human phenotype has previously been assigned to mutations in this complex. Notably, eight unrelated families with an identical mutation originated from the East Asian population where the carrier frequency for this allele is 0.0008. In one consanguineous family with multiple affected children the disease segregated with a homozygous mutation in the SGPL1 gene encoding for sphingosine-1-phosphate lyase. In four families, the kidney phenotype could be attributed to mutations in genes for non-syndromic nephrosis (NPHS1, PLCE1, one novel gene), while the brain phenotype was apparently independent.

In conclusion, the molecular genetic findings in this cohort confirmed that GAMOS is exceedingly heterogeneous, and still in almost half of the patients with a GAMOS-like phenotype the genetic cause remained unclear. On the basis of our findings we are now able to define new biologic mechanisms that are critically involved in both, brain development and integrity of the glomerular filtration barrier. Genotype phenotype correlations are emerging. Finally, we demonstrate that GAMOS can also be inherited as an X-linked trait.

WS7-003

CAD Mutations and Uridine-Responsive Epileptic Encephalopathy

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We report biallelic mutations in CAD, encoding an enzyme of de novo pyrimidine biosynthesis, in four patients with developmental disability, epileptic encephalopathy, anaemia, and anisopoikilocytosis. Two

children died after a neurodegenerative disease course. Treatment of two surviving children with oral uridine led to immediate cessation of seizures in both. A four-year-old girl, who was previously in minimal conscious state, started to communicate and walk with assistance after nine weeks of treatment. A three-year-old girl likewise showed developmental progress. Blood smears normalised and anaemia resolved. Our findings support the efficacy of uridine supplementation rendering CAD deficiency a treatable neurometabolic disorder.

WS7-004

Delineation of the GRIN2A phenotypic spectrum

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Alterations of the N-methyl-D-aspartate (NMDA) receptor subunit GluN2A, encoded by the gene GRIN2A, have been associated with a spectrum of neurodevelopmental, speech and epilepsy disorders. We identified 48 previously unreported patients with heterozygous pathogenic variants in GRIN2A, including 30 novel variants. After re-evaluation of all published GRIN2A cases, 104 previously reported patients met the ACMG criteria for being pathogenic or likely pathogenic. Thus, we are able to collectively review genotypes and phenotypes of 152 individuals with GRIN2A-related disorders. We show that the known phenotypic spectrum is expanded and ranges from near-normal development to severe and unspecific encephalopathy, comprising any disorder of speech development. Furthermore, some patients do not display seizures. In contrast to previous reports, GRIN2A missense variants cluster within the functionally most relevant domains. We are the first to describe genotype-phenotype correlations in GRIN2A-related disorders, where carriers of pathogenic missense variants tend to have more severe neurodevelopmental phenotypes compared to carriers of truncating variants. The most severe end of the phenotypic spectrum was found to include novel features, such as infantile spasms and arthrogryposis and was associated with pathogenic variants in the pore-forming domain of GRIN2A.

WS7-005

PSENEN mutations underlie Dowling-Degos disease associated with acne inversa

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Pigmentation disorders (PDs) comprise a large group of rare and heterogeneous disorders that are mainly characterized by various coloration abnormalities affecting single parts of the body or the complete integument. The large group of PDs includes the autosomal dominant inherited hyperpigmentation disorder Dowling-Degos disease (DDD). DDD is genetically heterogeneous, and to date causal mutations in three genes, namely *KRT5*, *POFUT1* and *POGLUT1* have been identified. After exclusion of mutations in these genes, we performed exome- and Sanger-sequencing in six unrelated DDD-patients/families and identified six heterozygous truncating mutations in *PSENEN* encoding the presenilin enhancer protein 2. On closer examination of the histological sections, we came upon a novel feature that distinguished these individuals from previous DDD-cases by the presence of follicular hyperkeratosis. To assess the functional significance of *PSENEN* mutations in DDD pathogenesis, we performed mammalian cell culture based studies and knockdown experiments of *PSENEN* homolog *psenen* in zebrafish larvae (zfl). Knockdown of psenen in zfl resulted in a phenotype with scattered pigmentation, which mimicked human DDD. *In vivo*-monitoring of pigment cells in the developing zfl suggested that disturbances in melanocyte migration and differentiation underlie DDD pathogenesis.

Interestingly, six of the *PSENEN* mutation carriers presented with co-morbid acne inversa (AI), an inflammatory hair follicle disorder. All individuals had a history of nicotine abuse and/or obesity, which are known trigger-factors for AI. Although *PSENEN* mutations have been identified in a small subset (<1%) of familial AI previously and the co-manifestation of DDD and AI has been reported for decades, our study is the first to demonstrate experimentally that mutations in *PSENEN* indeed can cause co-manifestation of DDD and

AI, most likely triggered by predisposing factors for AI. Thus, the present report describes a clinically and histopathologically novel DDD subphenotype in *PSENEN* mutation carriers, which is associated with an increased susceptibility to AI.

WS7-006

Protein substitution therapy for autosomal recessive congenital ichthyosis (ARCI)

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Autosomal recessive congenital ichthyosis (ARCI) is a rare cornification disorder with a heterogeneous genetic background. So far mutations in 10 genes have been described to cause ARCI. Among those the transglutaminase 1 gene (TGM1) is mutated in the majority of patients (around 30%), and its gene product, TGase1, is therefore primarily targeted in our approach for protein substitution. Patients with ARCI have an impaired skin barrier function, most of them are born with a collodion membrane and suffer subsequently from varying degrees of hyperkeratosis, erythema, transepidermal water loss and infections. The disease can be life threatening neonatally but lacks a causative therapy and is still only treated symptomatically. Therefore, our aim is to develop a personalised, causative therapy where the defective protein is substituted topically via a nanocarrier.

Therapeutic, human TGase1 was synthesized in HEK 293 cells and assessed by Western blot and flow cytometry analysis. Enzyme activity was measured by in vitro assay. TGase1 was then coupled to a polyglycerol-based nanogel (dPG-NG) containing the thermoresponsive linker poly(N-isopropyl)acrylamide (PNIPAM), stabilising the enzyme as well as adding a thermal protein release trigger at 35°C, which is favorable for cutaneous applications. Immunocytochemical stainings for TGase1 on monolayered basal keratinocytes that lack TGM1 expression confirmed the successful uptake of extrinsic TGase1 into the cells. Further analysis over time showed that the enzyme was no longer detectable after 48h and consequently led us to define a treatment schedule for the following experiments. 3D full thickness skin models were used as in vitro system to determine barrier function and enzyme activity after treatment with varying concentrations of the dPG-NG/TGase1 complex. Three different sets of skin models were used for these experiments: normal models mimicking the healthy skin with an intact barrier function, models where TGM1 was knocked down, and models made with ARCI patient cells with TGM1 mutations. Franz cell tests on treated skin models lacking intrinsic TGase1 confirmed the impaired barrier activity in disease models, demonstrated an improved barrier function after repeated treatments with dPG-NG/TGase1 and showed restored TGase1 activity using an in situ assay. Furthermore, first toxicity tests using MTT revealed high biocompatibility of dPG-NG/TGase1 after treatment of 2D and 3D cell cultures.

These findings are successful steps for an advanced topical drug delivery system and are a promising approach for causative therapeutic intervention in ARCI. After further optimization concerning protein dosage and thorough toxicity tests, we will adapt this system also for the use with other proteins involved in ARCI.

WS8 CANCER GENETICS II

WS8-001

Whole-genome and transcriptome sequencing of pediatric Burkitt lymphoma patients treated in population-based prospective clinical trials

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Burkitt lymphoma (BL), including its leukemic variant Burkitt leukemia (B-AL), is the most common type of pediatric B-cell lymphoma accounting for 30-40% of new cases. Its biological hallmark is the IG-MYC translocation involving MYC and mostly the immunoglobulin heavy (IGH) locus or more rarely one of the immunoglobulin (IG) light chain loci. At the cytogenetic level the IG-MYC translocation is the sole abnormality in around 40% of cases. Overall, BL is characterized by a low genomic complexity.

The aim of the present study was to analyze the genomic and transcriptomic landscape of pediatric/adolescent Burkitt lymphoma by sequencing according to the guidelines of the International Cancer Genome Consortium.

A total of 39 samples of BL/B-AL from pediatric/adolescent patients entered this sequencing study. All patients were treated in population-based prospective clinical trials. Inclusion criteria were besides availability of suitable materials, consent to participate in the study and appropriate diagnosis: age at diagnosis (≤ 19 years), the presence of IG-MYC rearrangement detected by FISH and/or whole genome sequence (WGS), absence of rearrangements of BCL2 or BCL6 genes. We performed WGS of tumor and matched control as well as transcriptome sequencing of the tumor cells according to the standards of the ICGC (www.icgc.org).

The pathognomonic IG-MYC translocation was detected in 37of 39 of the cases using WGS, but was observed in all cases by FISH. An IGH-MYC juxtaposition was detected in 34 patients and its variants IGK-MYC and IGL-MYC in 1 and 4 cases, respectively.

We identified two different expression patterns of MYC transcripts which were associated with the translocation breakpoint location. On the one hand the canonical MYC transcript and on the other hand an alternative transcript with a transcription start site before the second exon. The latter produces an mRNA which contains 486 nucleotides not included in the canonical transcript but nevertheless it encodes the identical protein.

The integration of single nucleotide variants (SNV) and copy number aberrations (CNA) identified a total of 128 recurrently (≥2 samples) mutated genes. MYC, ID3, TP53, CCND3, SMARCA4, ARID1A, FBXO11, DDX3X were mutated in ≥20% of samples. In 33/39 (85%) cases, the ID3/TCF3 complex was altered. Mutations in one or more of the ATP-binding cassette (ABC) family members ABCC5, ABCC6 and ABCC12 were present in 7/39 (18%). On the other hand, recurrent (≥2 cases) breakpoints of structural variants (SV) affected genes like MYC, GPC5, PCDH15, LSAMP, MDS2 and FBXO11. Interestingly, SNVs and SVs of the FBXO11 gene were mutually exclusive.

Overall Burkitt lymphoma showed a low genomic complexity with a low number of SNVs and SVs. However, the integration of CNAs, SNVs and SVs allowed us to identify recurrently affected genes, which are involved predominately in the PI(3) kinase pathway, tonic BCR signaling, and cell cycle regulation, chromatin composition and germinal center development.

WS8-002

Epigenetic variation between clinical and genetic subgroups of Burkitt lymphoma

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Burkitt lymphoma (BL) is the most common mature aggressive B-cell lymphoma in childhood. The genetic hallmark of BL is a chromosomal translocation involving the *MYC* oncogene and one of the immunoglobulin loci leading to MYC deregulation. Three epidemiologic variants of BL are differentiated: endemic (eBL), which occurs predominantly in Equatorial Africa and is associated with EBV-infection, sporadic (sBL), which occurs in westernized countries and immunodeficiency-associated. In addition, Burkitt leukemia (B-AL) is differentiated from BL in cases with more than 25% of the bone marrow cells being lymphoma cells. Another rare BL-variant is *MYC*-positive precursor B-cell acute lymphoblastic leukemia coexpressing TdT and MYC (TdT+BL). Finally, we recently described a *MYC*-negative variant which shows a typical alteration on chromosome 11 (mnBLL). The aim of the present study was to examine the epigenetic landscape of these BL variants.

To this end, we analyzed the DNA methylation of 116 BL (60 sBL, 29 eBL, 10 B-AL, 15 mnBLL, 2 TdT+BL) using the HumanMethylation450 BeadChip and contrasted the findings to 24 diffuse-large B-cell lymphoma (DLBCL) and 30 follicular lymphoma (FL). The majority of lymphoma were recruited in the framework of the ICGC MMML-Seq and MMML projects. The eBL were obtained from the NCI Ghana Burkitt Project. As controls, we used public available DNA methylation data from 93 B-cell populations of various differential stages. Furthermore, we investigated whole-genome bisulfite sequencing (WGBS) data of 12 sBL and 6 B-AL in comparison to 4 germinal center B-cell populations from healthy donors to decipher differentially methylated regions (DMR). These are defined as 10 or more CpGs differentially methylated between two groups.

Unsupervised DNA methylation analysis of BL, FL and DLBCL revealed that all BL variants cluster apart from the non-BL cases. Thus, supporting on epigenetic level that all analyzed BL samples are BL variants. Multigroup comparison (σ/σ_{max}=0.4, q<1e-13) separated the BL variants roughly in 3 groups: eBL, EBV-positive sBL and all other BL variants. Furthermore, this analysis revealed eBL to harbor a massive hypermethylation in comparison to all other BL variants. Comparison of the DNA methylation using the HumanMethylation450 BeadChip data of sBL and B-AL revealed 199 CpGs to be differentially methylated (σ/σ_{max}=0.4, q<0.005). In contrast, using the WGBS data of the same samples a total of 4712 DMRs could be identified which were mostly located in enhancer and polycomb target regions.

In conclusion, we show that all analyzed BL variants share a similar DNA methylation profile. Interestingly, DMRs between sBL and B-AL were mainly located in enhancer and polycomb regions. In contrast, eBL showed a massive hypermethylation in comparison to the other BL variants. Thus, the differences identified by DNA methylation analysis can improve the understanding of the biological and clinical differences of the BL variants.

WS8-003

Epigenetic analyses of T-cell large granular lymphocytic leukemia identify differentially methylated genes pointing to potentially deregulated signaling pathways

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Introduction

T-cell large granular lymphocytic leukemia (T-LGL) is a mature T-cell leukemia which often arises in the context of autoimmune disease. Genetic changes like recurrent chromosomal aberrations are rare. Recent studies identified somatic *STAT3* and *TNFAIP3* mutations in T-LGL cells. However, the molecular events driving leukemogenesis remain largely unknown.

Objectives

The goal of our study was to characterize the epigenetic basis of T-LGL to better understand leukemogenesis and potentially identify druggable pathways or diagnostic biomarkers for T-LGL.

Materials & Methods

We analyzed the DNA methylome of FACS sorted tumor cells of 11 T-LGL cases in comparison to benign αβ T-cell subsets. The Infinium Human Methylation 450 Bead Chip was used for analysis. We annotated our data with the publicly available chromatin segmentation data of CD8^{+}T-cells from the IHEC/BLUEPRINT Project. The expression levels of selected genes were tested by reverse transcription real-time PCR.

Results

Supervised analysis of T-LGL compared to benign CD8^{+} memory cells resulted in 1,083 CpG loci significantly (q < 0.001) differentially methylated. The annotation with the chromatin segmentation data of CD8^{+}T-cells from the BLUEPRINT project revealed enrichment of changes in methylation in distinct genomic regulatory elements in T-LGL. These differentially methylated functional regions were enriched for a set of transcription factor binding sites, known to be relevant in other lymphoid neoplasms. By bioinformatic analysis of methylation data and integration with gene expression data we identified hypermethylated and hypomethylated genes (e.g. *BCL11B*, *THEMIS*, *ZEB2*, *HIVEP3*) which point to candidate pathways potentially deregulated in the pathogenesis of T-LGL.

Conclusion

Our study identified DNA methylation changes in a set of candidate genes involved in various signaling pathways, which could potentially be used for diagnosis, prognosis and may become targets for novel treatment options.

Imbalance mapping and exome sequencing of paired primary diagnostic and relapse samples from MYC-positive Burkitt lymphomas

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Burkitt lymphoma (BL) is a mature aggressive B-cell lymphoma genetically characterized by a chromosomal translocation leading to IG-MYC juxtaposition. Treatment of BL is usually very successful particularly in children, with a cure rate of over 90% even among patients with advanced stage disease. However, the prognosis of the remaining patients experiencing disease progression and/or relapse is still very poor. BL has an overall low genomic complexity, thus secondary chromosomal changes in addition to the IG-MYC translocation are rare. However, genomic complexity has been associated with aggressive disease and poor prognosis in various lymphomas including BL. Because little is currently known about the underlying genetics of disease progression in BL we aimed at characterizing the molecular changes and characteristics that might lead to the relapse of BL.

Sequential tumor biopsies from initial diagnosis (ID) and follow-up were available from a total of 8 patients (4-15 years at ID), which were divided into two groups: Five patients experienced a relapse from their initial BL diagnosed 58-210 days after ID (group 1). In contrast, three patients developed twice a BL, i.e. presented with BL as secondary neoplasms diagnosed 3-5 years after ID (group 2). DNA extracted from archival formalin-fixed, paraffin-embedded (FFPE) tissue was used to analyze genome-wide copy number alterations (CNA) using the OncoScan® platform (Affymetrix) and mutational landscape by whole exome sequencing (WES).

Analysis of the CNA in the 5 paired BL samples (group 1) revealed an increase in genomic complexity in 4/5 pairs as in ID a mean of 8 CNA was detected in contrast to 13.4 CNA in relapse samples (p=0.113). Of note is that in all pairs, the relapse shared almost all CNA which were present in ID. WES analysis of group 1 showed similar results in all analyzed pairs. In total, 46.6% of mutations (median number of mutations = 106) were shared in ID and relapse. Nevertheless, a considerable amount of mutations were unique in ID and relapse with a median of 18 (11.8%) and 51 (41.7%) mutations, respectively. On the other hand, mutations detected in samples from patients with secondary neoplasm (group 2) were mostly unique to ID (= 109, 43.9%) whereas only 26.1% of all mutations were shared in ID and secondary neoplasm samples (= 49). Furthermore there were no shared CNA in the corresponding samples identified by OncoScan® analysis.

To sum up, the OncoScan® and WES analysis, of the paired BL group (1) provide strong evidence for a linear clonal evolution, meaning relapses may directly evolve from the previous lymphoma clone rather than a common precursor. In contrast, results obtained for patients with secondary neoplasm (group 2) showed no indication for linear but rather for divergent evolution. Thus, analysis of recurrent mutations shared in ID and second neoplasm samples can provide important information about disease progression and are therefore subject of ongoing analysis.

In Runx1 mutated AML the number of Runx1 mutations, loss of the wild-type allele and the number and kind of additional mutations impact on prognosis

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AML with mutated RUNX1 show a distinct pattern of cytogenetic and molecular genetic abnormalities and an adverse prognosis. We analyzed the impact of multiple RUNX1 mutations and RUNX1 wild-type (WT) loss on associated genetic alterations and survival. For this, 467 AML cases with RUNX1 mutations (mut) were split in (1) RUNX1 WT loss (n=53), (2) >1 RUNX1mut (n=94) and 1 RUNX1mut (n=323). 163 cases were selected for mutation analyses of 28 genes.

In cases with 1 RUNX1mut, +8 was frequently found, whereas in WT loss +13 was the most abundant trisomy (+8: 66% in 1 RUNX1mut vs. 31% in WT loss, p=0.022; +13: 15% vs. 62%, p<0.001). Cases with >1

RUNX1mut showed an intermediate distribution (+8: 44%, +13: 50%). Missense mutations were the most abundant mutation type in WT loss cases (53% vs. 31%, p=0.006), whereas in 1 RUNX1mut, frameshift mutations were found more frequently (45% vs. 28%, p=0.016). In cases with >1 RUNX1mut, both were observed at similar frequencies (missense: 36%, frameshift: 38%). Mutation analyses of 163 selected cases revealed 411 additional molecular mutations. 95% of cases showed at least one RUNX1-accompying mutation (range: 0-6). The median of accompanying mutations was n=2 in the total cohort and in cases with 1 RUNX1mut and >1 RUNX1mut, whereas it was n=3 in RUNX1 WT loss. SRSF2 (39%), ASXL1 (36%), DNMT3A (19%), IDH2 (17%), SF3B1 (17%), TET2 (17%) and BCOR (16%) were revealed as most frequently mutated genes. Cases with RUNX1 WT loss showed a higher frequency of ASXL1mut compared to the other cases (50% vs. 29%, p=0.009), while U2AF1mut were absent from this group (0% vs. 10%, p=0.019). Median overall survival (OS) in the total cohort was 14 months. WT loss (OS: 5 months) and >1 RUNX1mut (14 months) showed an adverse impact on prognosis compared to 1 RUNX1mut (22 months; p=0.002 and p=0.048, respectively). Mutations in ASXL1 and KRAS and the presence of ≥2 additional mutations also negatively impacted OS (10 vs. 18 months, p=0.028; 1 vs. 15 months, p<0.001; 12 vs. 20 months, p=0.017). In univariate Cox regression analysis RUNX1 WT loss (HR=1.6; p=0.024), ≥2 additional mutations (HR=1.9; p=0.019), ASXL1mut (HR=1.6; p=0.030) and KRASmut (HR=4.4; p=0.001) had an adverse impact on OS. Multivariate Cox regression analysis revealed an independent adverse effect on OS for RUNX1 WT loss (HR=1.6; p=0.039) and KRASmut (HR=4.2; p=0.001). For 216/467 cases we received samples during course of the disease. In none of these cases, an evidence for a RUNX1 germline mutation was found by analyzing the mutation loads, thus all RUNX1 mutations are somatically acquired. Taken together, we found strong differences between the subgroups in regard of cytogenetic and molecular genetic aberrations as well as regarding prognosis. Thus, not only the presence and number of RUNX1 mutations but also the conservation of an intact RUNX1 allele as well as the number and kind of additional mutations is biologically and clinically relevant.

WS8-006

Atypical paroxysmal nocturnal hemoglobinuria presenting with autoinflammatory symptoms is caused by germline and somatic mutations involving PIGT

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Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired disorder of the blood-forming system.

Typically, affected hematopoietic stem cells (HSCs) in PNH harbor a single somatic loss-of-function mutation in the X-linked *PIGA* gene. Previously, a PNH patient with a different molecular etiology has been described and herein we report three more cases of this new subgroup: A predisposing germline mutation in *PIGT*, which is an autosomal gene of the glycosylphosphatidylinositol (GPI)-anchor synthesis pathway, is followed by a second somatic hit. By means of deep sequencing and array-CGH, we observed acquired deletions of 8 Mb to 18 Mb on chromosome 20q in PNH cells that include PIGT as well as a region that is commonly deleted in myeloproliferative neoplasms and myelodysplastic syndromes and that is known to be differentially methylated. This results in a complete loss of expression of certain genes at this locus which is also thought to contribute to the clonal expansion.

The deficiency of GPI-anchored proteins on PNH cells results in a lack of the complement regulatory proteins CD59 and DAF/CD55 on the cell surface and leaves them more vulnerable to the C5b-9 membrane attack complex.

In contrast to classical PNH without any fully synthesized GPI-anchors, *PIGT* mutations impair the transamidase that links the substrate to the anchor and thus result in an accumulation of unbound GPI molecules.

This difference in the pathophysiology can also be visualized in flow cytometric analysis of peripheral blood: While CD55 and CD59 surface levels are reduced in all PNH cells, the atypical PNH cells due to a transamidase deficiency can be discriminated by a specific antibody, T5 mAb, that binds free GPI anchors.

Besides the classical PNH symptoms of anemia, thrombosis, and hemolysis, patients with *PIGT* mutations also manifest with additional autoinflammatory symptoms, such as urticaria, fever, arthralgia and meningitis, and it is hypothesized that the free GPI-anchor that accumulates in affected cells is causally related to autoinflammation. Based on these findings, we propose the new entity of atypical PNH.

POSTER

P-BASIC MECHANISMS AND EPIGENETICS

P-BasEpi-001

Preventive Genetic Counseling in Neurogenetic Disorders Needs a Better Collaborative Approach Between Genetic and Neurology Clinics - A Report of Four Siblings with Unverricht–Lundborg disease:

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Genetic counseling is the process of helping people to understand and adapt the medical, psychological and familial implications of genetic contributions to disease. For parents with a previous child or other family member with a known genetic syndrome expands options for preimplantation or prenatal diagnosis for the current or the future pregnancies. However, timely referral by health providers to genetic counselor and for discussing with couples regarding possible options is important. Additionally, other factors such as personal decision making especially due to high price of some genetic services and uncertain results cause considerably delays to genetic testing.

There are more than 200 various types of inherited neurological disorders in which alterations in genes lead to an inherited condition such as Huntington disease, inherited forms of Alzheimer disease, ataxia, muscular dystrophies and epilepsies. The knowledge of the causative gene mutations in the affected individual is critical in the possible prenatal diagnosis in other members of the pedigree. Therefore a multidisciplinary care team, including neurologist and genetic counselor for the conditions diagnosed as inherited neurological disorders is critical in prenatal setting and consideration of an effective management.

Here, our report of four siblings affected by a rare form of inherited epilepsy (Unverricht–Lundborg disease) with an autosomal recessive pattern highlights the importance of the needs for a better collaborative approach in the neurogenetic setting. In fact, the birth of four successive siblings affected by similar neurogenetics disorders in a specific family is showing the need for more attention to this important issue, especially in terms of intersectoral collaboration.

P-BasEpi-002

Molecular Mechanisms Underlying the Initiation, Progression and Invasion of Colorectal Cancer: A Network Biology Approach

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Abstract

Objectives: Many attempts have been conducting to decipher molecular mechanisms underlying the initiation, progression and invasion of colorectal cancer (CRC). Here, we proposed the possible molecular mechanisms responsible for CRC initiation, progression and invasion using a network biology approach.

Materials and Methods: In order to investigate the underlying CRC pathogenesis, the dataset GSE21510 consisting of normal tissues, stage I, stage II, stage III and stage IV of CRC were obtained from Gene Expression Omnibus (GEO) and further examined. The differentially expressed genes (DEGs) were subjected to protein-protein interaction databases and a PPI network was constructed for each CRC stage. Topological analysis of resulted PPI networks revealed functional hub genes and involved in CRC development. Furthermore, the overlap genes between four studied CRC stages were determined and deeply evaluated to identify deregulated biological networks during CRC development. A standard Real-time PCR was performed to validate the in silico findings utilizing SW620 and NCM460 cell lines.

Results: The most important hub genes (CDK1 for stage I, UBC for stage II, ESR1 for stage III and ATXN1 for stage IV) and sub-networks were identified in CRC stages. Moreover, several novel biomarkers were also introduced for each CRC stage. Gene ontology (GO) and signaling pathway enrichment uncovered the important roles of Wnt, MAPK and JAK-STAT signaling pathways in regulation of CRC pathogenesis. Functional annotation of overlap genes revealed that cell cycle regulating genes are the most highly regulated genes during CRC initiation, progression and invasion. In vitro analyses confirmed deregulation of ATXN1 and CDK1, two hub genes of stage IV, in metastatic colon SW620 cells compared to normal colon NCM460 cell line.

Conclusions: Our study provides a new insight into the distinct molecular mechanisms underlying the pathogenesis of CRC. The functional hub genes, sub-networks, prioritizes key pathways and novel CRC biomarkers were also provided that can be useful in therapeutic programs.

P-BasEpi-003

Frequency of SCA8, SCA10, SCA12, SCA36, FXTAS and C9orf72 repeat expansions in symptomatic SCA negative for the most common SCA subtypes

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Targeted next-generation sequencing approaches as well as next-generation whole exome sequencing are becoming more widespread in routine molecular diagnostics for patients with ataxia. However, since NGS at present is not suitable to detect (trinucleotide) repeat expansions, a pre-NGS testing for common polyglutamine expansion SCAs seems mandatory. But also SCA subtypes caused by expansions in non-coding regions of genes like SCA8, SCA10, SCA12, and SCA36 as well as other ataxias known to be associated with repeat expansions like the fragile X-associated tremor ataxia syndrome (FXTAS) should be taken into account before applying NGS-based diagnostics. In order to find an optimal diagnostic strategy in future more information about the frequency and phenotypic characteristics of rare repeat expansion disorders associated with ataxia would be helpful. We therefore analyzed a cohort of 441 patients with symptoms of cerebellar ataxia, dysarthria and other unspecific symptoms who were referred to our center for SCA diagnostics and showed alleles in the normal range for the most common SCA subtypes SCA1-3, SCA6, SCA7, and SCA17. These patients were screened for expansions in SCA8, SCA10, SCA12, SCA36 and FXTAS as well as for the pathogenic hexanucleotide repeat in the C9ORF72 gene. No expanded repeats for SCA10, SCA12 or SCA36 were found in the analyzed patients. Five patients with ataxia of unknown etiology showed SCA8 CTA/CTG combined alleles (83-129) that are discussed to be potentially pathogenic. One 51-year-old male patient with unclear dementia syndromes was diagnosed with a large GGGGCC repeat expansion in C9ORF72. And the analysis of the FMR1 gene identified one patient with a permutation (>50 CGG repeats) and seven patients with alleles in the grey zone (41 to 54 CGG repeats), thus suggesting that individuals with FMR1 repeat expansions in the gray zone may also present with neurological signs.

P-BasEpi-004

Characterizing the role of imprinted genes in normal B-cells and germinal center derived B-cell lymphomas

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Some genes are subject to the mechanism of imprinting, i. e. their expression depends on parental origin. They primarily function in the control of proliferation, fetal development and cellular differentiation. Constitutional imprinting disorders are in part also associated with an increased tumor risk. Loss of imprinting has been also described as somatic event in tumorigenesis. While this phenomenon has been broadly analyzed in solid tumors, data on alterations of imprinting in lymphatic neoplasms are largely missing.

We analyzed the RNA expression of 321 transcription units/regions known or supposed to be subject to imprinting in two cohorts of normal B-cells and germinal center derived B-cell lymphomas. The first cohort (MMML) contains 686 samples: 56 Burkitt lymphomas (BL), 600 non-Burkitt lymphomas (non-BL, including various subtypes like follicular and diffuse large B-cell lymphoma) and 30 normal germinal center B-cell samples (GCBC, as controls). The second cohort (ICGC MMML-Seq) comprised 201 samples with 20 BL, 176 non-BL and 5 GCBC samples. Gene expression was analyzed with Affymetrix U133A GeneChips in the MMML cohort and by RNA sequencing in the ICGC MMML-Seq cohort. Results of the transcriptional analyses in the ICGC MMML-Seq cohort were compared to the DNA methylation available from a subset of the analyzed samples (Kretzmer et al., Nat Genet, 2015).

Of the 321 transcription units 114 sites, corresponding to 64 transcription units, were present on the applied array used for the analysis of the MMML cohort. A two group comparison revealed 53 significantly differentially expressed sites corresponding to 31 transcription units between BL and non-BL including the PLAGL1 and PEG10 genes. In total, 19 and 16 sites corresponding to 16 and 10 transcription units are differentially expressed between BL versus GCBC and non-BL versus GCBC, respectively. Comparison of gene expression in the ICGC cohort revealed 70 differentially expressed sites corresponding to 68 transcription units between BL and non-BL (overlap with MMML cohort: 24/31 differentially expressed transcription units), including again PEG10 and PLAGL1, 37 differentially expressed sites corresponding to 37 transcription units between BL and GCBC (overlap with MMML cohort: 7/16 differentially expressed transcription units) and 44 differentially expressed sites corresponding to 39 transcription units between non-BL and GCBC (overlap with MMML cohort: 6/10 differentially expressed transcription units). These differences in gene expression we detected did not correlate with DNA methylation changes at the corresponding transcription regulatory sites.

From our results we conclude that altered expression of imprinted genes indeed plays a role in tumorigenesis of germinal center derived B-cell lymphomas. However, the altered transcriptional regulation of these genes seems not to rely on the usual epigenetic mechanisms known from constitutional imprinting disorders.

P-BasEpi-005

Investigating the expression of genes associated with autism spectrum disorders to identify sex related differences

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Neurodevelopmental disorders such as autism, attention deficit and hyperactivity syndrome as well as language problems and learning difficulties have a higher prevalence in male individuals compared to females. Autism is characterized by impairments in social interaction, communication deficits and restricted and

repetitive behaviors. Boys are more frequently affected than girls; the ratio of affected boys compared to girls is 4:1 for autism and 11:1 for Asperger syndrome.

In this study we aim to elucidate the reason for this gender difference by following up two hypotheses: (1) risk genes for autism spectrum disorders (ASD) might be expressed at different levels in males and females and (2) ASD risk genes might interact with sexually dimorphic pathways.

First, we investigated the expression of genes associated with autism spectrum disorders, including the Shank gene family, in the brain of male and female mice to identify sex-dependent differences. The RNA expression levels were analyzed in five different brain regions (cortex, hippocampus, striatum, cerebellum, thalamus) at different developmental stages (E15, E17, P1, P7, P12 and adult) in male and female mice. We identified a sex dimorphic expression of Shank1 and Shank3, but not of Shank2. Due to the fact that early brain development is strongly influenced by sex hormones (estrogen, testosterone), we further investigated the influence of these hormones on Shank expression in human neuroblastoma cells (SH-SY5Y) and primary mouse hippocampal neurons.

A better understanding of the sex differences in the brain might help to explain the vulnerability for neuropsychiatric disorders like autism and paves the way to discover putative risk or protective factors for these disorders.

P-BasEpi-006

Imprinting defects in Temple syndrome are caused by a failure in imprint establishment and/or maintenance

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Temple syndrome (TS14) is a rare imprinting disorder characterised by low birth weight and height, muscular hypotonia and feeding difficulties in the infant period, early puberty and short stature with small hands and feet and often truncal obesity. In a subset of patients with TS14, the disease is caused by an imprinting defect (ID) affecting the paternal allele of the imprinted region 14q32. The ID results in aberrant methylation of the three known differentially methylated regions (DMRs), the germline-derived primary *DLK1/MEG3* intergenic (IG-)DMR (*MEG3/DLK1*:IG-DMR), the postfertilization-derived, secondary DMR at the *MEG3* promoter (*MEG3*:TSS-DMR), and the postfertilization-derived, secondary intragenic *MEG8*-DMR (*MEG8*:Int2-DMR). The *MEG3/DLK1*:IG-DMR and the *MEG3*:TSS-DMR are methylated on the paternal chromosome and hypomethylated in patients with TS14 and an imprinting defect. The *MEG8*:Int2-DMR is unmethylated on the paternal chromosome and hypermethylated in these patients. Both the *MEG3/DLK1*:IG-DMR and the *MEG3*:TSS-DMR act as imprinting control centres, although the *MEG3/DLK1*:IG-DMR functions as an upstream regulator of the *MEG3*-DMR. So far, the function and regulation of the *MEG8*-DMR is unknown. The hypomethylation of the paternal allele in TS14-ID patients at the *MEG3/DLK1*:IG-DMR and the *MEG3*:TSS-DMR point to a failure in the establishment of the methylation imprint or to maintain the methylation imprint after fertilization. In this case, the incorrectly imprinted chromosome 14 would be inherited from either the paternal grandfather or grandmother. To prove this assumption we are investigating the grandparental origin of the affected chromosome 14 in our cohort of ten TS14-ID families by studying the parent-of-origin specific methylation of the three DMRs in combination with informative single nucleotide variants (SNPs). At the moment we have identified three families informative for the *MEG3/DLK1*:IG-DMR, two families for the *MEG3*:TSS-DMR and two families for the *MEG8*:Int2-DMR. So far we have obtained results in two families for the *MEG3*:TSS-DMR. We found that in one case the allele harbouring the ID was inherited from the paternal grandmother, but in the second case from the paternal grandfather, indicating that the ID occurred after erasure of the parental methylation imprints. A complete lack of methylation observed in the majority of TS14-ID patients is therefore likely due to a problem in establishing methylation on the paternal chromosome, whereas in rare cases with methylation mosaicism, the ID is probably due to a problem to maintain the paternal imprint after fertilization.

P-BasEpi-007

SPOC1 is involved in the suppression of L1 transposable elements in the germline

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SPOC1/PHF13 is a gene located on human chromosome region 1p36.31 and mouse chromosome 4qE2. The protein was first described in patients with epithelial ovarian cancer, where its expression correlated with tumour progression and reduced survival time. SPOC1 is a reader of the epigenetic mark H3K4me2/3,

dynamically associates with chromatin during mitosis and plays a role in chromosome condensation. SPOC1 deficient mice show a pronounced hypoplasia of the testis with a progressive loss of germ cells. Although loss of SPOC1 leads to a significantly reduced chromatin condensation of the sex chromosomes in meiosis, the protein is not expressed in spermatocytes but in the undifferentiated precursor cells, the spermatogonial stem cells (SSCs).

Here, we present ChIP-Seq data of mouse testis tissue demonstrating that SPOC1 strongly binds to evolutionary young L1 elements in undifferentiated spermatogonia. We show that in HEK cells overexpression of SPOC1 leads to repression of transposition activity of LINE-elements strongly indicating a role of SPOC1 in L1 element suppression.

The cell has developed several lines of defence against retrotransposition to maintain genomic integrity, including DNA methylation. These defence mechanisms are most elaborate in spermatogonial stem cells since transposition events in these cells would have a dramatic impact on the next generation. Therefore, the repression of retrotransposition is of fundamental importance for germ cell development and ultimately the quality of the gametes.

Moreover, we present meDIP results showing that the methylation levels of L1 sequences decrease upon *Spoc1*-knockout and demonstrate that the histone methyltransferase G9a is strongly upregulated in preleptotene meiocytes of *Spoc1$^{-/-}$* mice. G9a is expressed from spermatogonia until early meiosis where it regulates H3K9 di-methylation and has been shown to be involved in the repression of L1 element in mouse spermatogonia. We are able to demonstrate that H3K9me2 levels are unaltered in *Spoc1$^{-/-}$* mice, suggesting a potential functional link between G9a and SPOC1 that does not affect the catalytic activity of G9a. Since G9a can regulate *de novo* and maintenance DNA methylation of L1 promoters, SPOC1 could function in targeting G9a to L1 sequences.

In conclusion our data implicate the epigenetic reader SPOC1 in the suppression of LINE elements during germ cell development.

P-BasEpi-008

Characterization of the expression of the imprinted Kcnk9-gene in specific brain regions and the phenotypic analysis of Kcnk9-knockout mice

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Kcnk9/KCNK9 is a maternally expressed imprinted gene whose mutations are responsible for the maternally inherited Birk-Barel mental retardation dysmorphism syndrome. It encodes a member of the superfamily of K+channels with two pore-forming domains and is involved in the modulation of the resting membrane potential and excitability of neuronal cells. So far, only homozygous *Kcnk9* knockout mice with inactivation of both parental alleles were phenotypically characterized. These mice displayed cognitive deficits as well as a reduction of K+ leak current by 50%. In the light of maternal-specific imprinted expression of *Kcnk9/KCNK9* and the maternal inheritance of the Birk-Barel mental retardation dysmorphism, a thorough phenotypic analysis of heterozygous *Kcnk9* knockout mice with inactivation of only the maternally inherited allele is also warranted.

As first aim of our study, we characterized the parental allele-specific expression of Kcnk9 in various regions of the mouse brain.

Quantification of Allele-Specific Expression by Pyrosequencing (QUASEP) method was performed for different brain areas from several developmental stages of (C57BL/6xCast/Ei) F1 hybrid mice. Exclusive expression from the maternal Kcnk9 allele was detected in the dentate gyrus, hippocampus, mesencephalon, medulla oblongata, thalamus and pons. Biallelic expression with, however, a strong bias towards the maternal *Kcnk9* allele (94-99% of the transcripts) was observed in the olfactory bulbs, cortex, cerebellum, striatum and olfactory tubercles.

As the second aim of our study, the phenotypes of wildtype, heterozygous *Kcnk9* knockout mice with maternal inherited knockout allele *(Kcnk9KOmat)* and homozygous *Kcnk9* knockout mice (*Kcnk9KOhom*) were comparatively examined in a behavioral test battery. Due to the already known cognitive defects of *Kcnk9KOhom* animals and especially the phenotype of the patients with Birk-Barel syndrome, it was assumed that Kcnk9KOmat and Kcnk9KOhom animals show deficits in some of the tests. The spontaneous alternation in the Y-Maze test was significantly reduced by approximately 10-20% in *Kcnk9KOmat* and *Kcnk9KOhom* mice compared to wildtype mice indicating a clearly impaired working memory. In addition, *Kcnk9KOmat* and *Kcnk9KOhom* mice displayed a reduced prepulse inhibition of startle response compared to wildtype mice indicating an impairment of sensomotoric gating, a process to filter out irrelevant information. Acoustic startle response as a measure of anxiety levels was also significantly decreased, but only in *Kcnk9KOhom* mice. Our findings shall further elucidate the role of *Kcnk9/KCNK9* in brain physiology and pathophysiology and open new avenues for treatment of cognitive dysfunctions in Birk-Barel syndrome.

P-BasEpi-009

Different disease expression in monozygotic twins with compound heterozygous mutations in the SPG11 gene

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A pair of monozygotic 22-year-old twins suffering from hereditary spastic paraplegia 11 (SPG11) is described.

Patients underwent thorough clinical examination and Magnetic resonance imaging (MRI) and MR-Spectroscopy (MRS) at 3 Tesla. Genetic testing was performed by Sanger sequencing and alternative splicing by RNA analysis.

Clinically the patients presented a similar spectrum of symptoms with a higher level of disability in one of the patients. MRI studies including morphometry and regional microstructural analysis by diffusion tensor imaging (DTI) of the corpus callosum (CC) revealed marked thinning and corresponding increases of axial diffusivity (AD), radial diffusivity (RD) and apparent diffusion coefficient (ADC) and reduction of the fractional anisotropy (FA) as compared to healthy controls in all CC sections, particularly in the anterior callosal body. There was marked supratentorial white matter reduction and to a lesser extent grey matter reduction in both patients. Involvement of the cortico-spinal tracts was reflected by FA and RD alterations and cervical cord atrophy. The more strongly affected patient showed a higher degree of callosal microstructural damage and cervical cord atrophy. Genetic testing of the SPG11 gene revealed two mutations in compound heterozygous state, a known frameshift mutation as well as a novel synonymous exonic splice site mutation.

This study shows similar but distinct clinical and imaging findings in monozygotic twins suffering from SPG11, suggesting individual downstream genetic effects.

P-BasEpi-010

Targeted RNASeq to uncover impact of genomic mutations

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Next generation sequencing techniques tremendously improved our ability to identify sequence variants. However fixing disease causing mutation still lack behind because of several reasons: inappropriate gene specific data bank, insufficient prediction tools, incomplete analysis and others. In addition identified sequence variants are a mixture of severe disease causing mutations and a myriad of variants of unknown pathogenicity. In addition an unknown number of silent mutations, neutral polymorphism and sequence variants deeply buried in introns might severely influence splicing of the premature RNA molecule. By solely analysis of the DNA sequence this impact onto the integrity of the mRNA is completely ignored. In order to catalog the mRNA isoforms derived from genes of our interest we started to set up RNASeq technologies in our routine lab. To reduce the amount of data, to improve the power of analyses and to identify rare isoforms of transcripts we use targeted RNASeq to characterize the mRNA molecules originating from those genes we are interested in (e.g.: hereditary breast cancer core genes (10 genes), hereditary colon cancer (23 genes), primary ciliary dyskinesia (PCD)(40 genes)). Genes involved in PCD offer the invaluable advantage that the respiratory epithelium where these genes are normally expressed can be sampled from the inferior turbinate of the nose by brush biopsy either from healthy probands or from patients suffering from PCD. In addition to direct preparation of RNA from these cilia, cilia carrying cells or tissue can be cultured and manipulated to investigate ciliogenesis. Data resulting from RNASeq experiments are analyzed by established informatics tools (TopHat, Cufflinks and derivatives thereof). We will show results from our work in progress and we hope to convince people to intensify RNA analyses even in routine labs to uncover hidden mechanism and/or mutations impacting mRNA splicing and thereby causing human disease.

P-BasEpi-011

The role of telomere length in Multiple sclerosis and Huntington disease

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Telomeres are located at the ends of chromosomes and have an essential role in the maintenance of genome stability. After each cell division, a small part of this specialized sequence is lost. When telomeres reach a critically reduced length, the cell either dies through apoptosis or enters a state of permanent cell cycle arrest. It has been demonstrated that telomere biology is directly linked to basic biological phenomena such as aging, tumorigenesis and maintenance of DNA integrity. It is known that oxidative stress accelerates telomere shortening in cells, resulting in premature cell senescence. Shorter leukocyte telomeres have been observed in type II diabetes or degenerative disease like dementia and Alzheimer disease as well in chromosomal instability syndrome, such as Fanconi anemia (FA) and Nijmegen breakage syndrome (NBS).

Any link between telomere length and inflammation has not yet been extensively studied in autoimmune diseases. Accelerated length shortening might be related to autoimmune disease predisposition. Yet the reasons for this shortening are likely manifold, including the individual genetic background, oxidative stress and chronic inflammation. In order to shed light on these relationships, we investigate genomic DNA extracted from blood of patients diagnosed with multiple sclerosis and from patients with Huntington disease. The samples were divided into age groups. StepOne Q-PCR was applied to detect the relative telomere length as a function of age. Initially identified differences in telomere lengths still have to be confirmed in larger cohorts.

P-BasEpi-012

*** Epigenetic signatures of gestational diabetes mellitus on ATP5A1, PRKCH, SLC17A4, and HIF3A cord blood methylation

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Background: Intrauterine exposure to gestational diabetes mellitus (GDM) confers a lifelong increased risk for metabolic and other complex disorders to the offspring. GDM-induced epigenetic modifications modulating gene regulation and persisting into later life are generally assumed to mediate these increased disease risks. To identify candidate genes for fetal programming, we compared genome-wide methylation patterns of fetal cord bloods (FCBs) from GDM and control pregnancies.

Methods and Results: Using Illumina's 450K methylation arrays and following correction for multiple testing, 65 CpG sites (52 of which are associated with genes) displayed significant methylation differences between GDM and control samples. Three of four candidate genes, ATP5A1, PRKCH, and SLC17A4, from our methylation screen and one, HIF3A, from the literature were validated by bisulfite pyrosequencing. The GDM effect on FCB methylation was more pronounced in women with insulin-dependent GDM who had a more severe metabolic phenotype than women with dietetically treated GDM. However, the effect remained significant after adjustment for the maternal BMI and gestational week in a multivariate regression model.

Conclusions: Our study supports an association between maternal GDM and the epigenetic status of the exposed offspring. Consistent with a multifactorial disease model, the observed FCB methylation changes are of small effect size but affect multiple genes/loci. The identified genes are primary candidates for transmitting GDM effects to the next generation. They also may provide useful biomarkers for the diagnosis and prognosis of adverse prenatal exposures and assessing the success of interventions during pregnancy.

P-BasEpi-013

Differential hSNM1B/Apollo expression and mutagen sensitivity in lymphoblastoid cell lines

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The nuclease hSNM1B/Apollo has a dual function in both DNA-repair and maintenance of telomeres. As to the repair of DNA interstrand crosslinks (ICL), hSNM1B/Apollo is linked to the Fanconi Anemia (FA) pathway and cells depleted for hSNM1B/Apollo (siRNA) resemble those from patients with FA. We have identified a single nucleotide polymorphism, rs6674384, which is associated with quantitative differences in

hSNM1B/Apollo expression (mRNA). We analyze whether the differential expression relates to the degree of cellular sensitivity to the DNA interstrand crosslinks inducing mutagen mitomycin C (MMC) and ionising radiation (IR), which induces, among other lesions, DNA double strand breaks. All experiments are realized using lymphoblastoid cells derived from generally healthy donors. Results of RT-PCR analysis of hSNM1B/Apollo expression and of the cell viability assays will be presented and discussed in the context of the potential usefulness of considering rs6674384 in predicting individual sensitivity to mutagens relevant in anti-cancer treatment.

P-BasEpi-014

NAHR events causing type-1 NF1 microdeletions are not associated with an increased mutation rate in breakpoint-flanking regions

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Large deletions of the *NF1* gene and its flanking regions are the most frequent recurrent mutations in patients with neurofibromatosis type 1 (NF1). Different types of large *NF1* deletions have been identified which are distinguishable in terms of their size and breakpoint position. Most frequent are type-1 *NF1* deletions spanning 1.4-Mb and characterized by breakpoints located within the low-copy-repeats NF1-REPa and NF1-REPc which exhibit 97.5% sequence homology within 51-kb. Type-1 *NF1* deletions are caused by non-allelic homologous recombination (NAHR). Two NAHR hotspots have been identified termed PRS1 and PRS2 which encompass 5-kb and 4-kb, respectively. Approximately 80% of all type-1 NF1 deletion breakpoints cluster within the PRS1 and PRS2 NAHR hotspots. In this study, we analysed whether the NAHR events causing type-1 *NF1* deletions would be associated with an increased de novo mutation rate of sequences located in breakpoint-flanking regions. To do so, we sequenced the deletion breakpoint-flanking regions in the patients and compared these sequences with the homologous regions amplified from DNA of the patients' parents who are not affected by NF1. However, in the germline of these parents, the deletions were mediated by NAHR and then transmitted to their offspring. The parental haplotypes within the PRS1 or PRS2 regions of NF1-REPa and NF1-REPc were analysed by long-range PCR and direct sequence analysis. The comparative analysis of parental haplotypes with the sequences flanking the deletion breakpoints in the patients revealed the absence of any de novo mutations in breakpoint-flanking regions of 19 PRS2-mediated and 6 PRS1-mediated type-1 *NF1* deletions. We conclude that although NAHR is a mutational mechanism causing large *NF1* deletions, there is no evidence for a local mutagenic effect of these recombination events. Hence it is unlikely that NAHR underlying type-1 *NF1* deletions involves error-prone translesion polymerases that would increase the de novo mutation rate in breakpoint flanking regions.

Furthermore, the detailed haplotype analysis of PRS2, a highly active NAHR hotspot mediating the majority of large *NF1* deletions, revealed that non-allelic homologous gene conversion (NAHGC) between NF1-REPa and NF1-REPc, which results from non-crossover resolution of recombination intermediates, is the major driving force responsible for the haplotype diversity in this region. Remarkably, the haplotype diversity patterns observed for NF1-REPa and NF1-REPc were markedly different indicating that during NAHGC, NF1-REPa is disporportionately more often the donor sequence used to repair mismachtes in heteroduplex regions than NF1-REPc. We also noticed a correlation between haplotype diversity and the number of PRDM9 A-allele binding sites suggesting that haplotype diversity and hence the NAHGC rate within PRS2 in NF-REPa is regulated by PRDM9.

P-BasEpi-015

*** Comparative whole genome bisulfite sequencing analysis in patients with single locus and multi locus DNA imprinting disturbances and their parents

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DNA methylation aberrations at differentially methylated region of imprinted genes interfere with the naturally parental-specific mono-allelic expression. That leads to a bi-allelic or absent expression of the imprinted gene, a cause of imprinting disorders (IDs).

We aimed at analyzing the genome wide DNA methylation pattern of two patients with IDs, namely transient neonatal diabetes mellitus (TNDM) and multi locus imprinting disturbance (MLID), and their respective parents.

The DNA methylation profiles of these individuals were obtained by whole genome bisulfite sequencing (WGBS) on B cells sorted by magnetic cell isolation. The sequencing libraries were prepared as described in Kretzmer et al. [1] and sequenced on an Illumina HiSeq 2000 machine. WGBS data were processed with the methylCtools toolkit. Briefly, bisulfite-treated sequences were aligned with BWA-mem using a three-letter approach, and the methylation ratios were quantified for ~26.9 million out of 28.2 million CpG sites (coverage>5) genome-wide. Quality control was performed to assess the quality of the DNA methylation profiles and genetic fingerprinting was performed on the WGBS data confirming sample origin and family relationship. The WGBS data was further compared to already existing Genome-Wide Human SNP Array 6.0 (SNP array) and HumanMethylation 450k Bead Array (450k) data, resulting in a good accordance with Pearson's correlation coefficients >0.97.

We detected an overall DNA methylation level around 75% in all samples. Already known DNA methylation alterations, e.g. hypomethylation in PLAGL1 were validated by WGBS. By searching for differentially methylated regions (DMRs), defined as regions composed of at least five consecutive CpG loci showing a methylation difference between patient and corresponding parents above 30%, we identified 442 DMRs in the MLID trio and 1776 DMRs in the TNDM trio. In the MLID trio 238/442 DMRs showed increased and 204 DMRs decreased DNA methylation in the patient's sample. Of 442 DMRs, 325 are located in regions potentially associated with transcriptional regulation. Further analysis revealed that 34/442 DMRs are associated with imprinted genes. In the TNDM trio, we detected 1705/1776 DMRs to show hypermethylation in the patient compared to her parents and 71/1776 DMRs with lower DNA methylation. In these trio 1166/1776 DMRs are associated with regions potentially correlated to transcriptional regulation and 13 DMRs with imprinted genes

Summarized, our results show that WGBS is a well suited and valid method for analyzing DNA methylation. While the overall DNA methylation levels does not differ between the analyzed patients and parents, a detailed analysis of smaller regions revealed the existence of 442 respectively 1776 differentially methylated regions between the analyzed MLID and TNDM patient and their parents.

[1] Kretzmer et al. Nat Genet. 2015 Nov;47(11):1316-25.

P-BasEpi-016

Characteristic mutational profile in children of individuals exposed to ionizing radiation

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The DNA damaging effects of ionizing radiation are deliberately used in cancer therapy as well as feared in accidents related to nuclear technology.

Despite its influence on the exposed organism, irradiation was believed to have no major effect on succeeding generations, as irreparable DNA damages were thought to result in cell death.

Recently, however, genome-wide mutation screenings in offsprings of male mice that were irradiated with high dosages showed an accumulation for certain de novo events.

We therefore focused on these mutational classes in a small cohort of human individuals that were conceived while or after their fathers were exposed to high frequency radiation.

In the whole genome sequences of 22 such offsprings we could confirm de novo rates for single nucleotide variants in the order of 10^{-8} per base as previously reported.

Interestingly, however, we found de novo translocations of paternal origin as well as increased numbers of clustered de novo mutations that resemble the results from animal studies.

This characteristic mutation profile might thus be used as an indicator of irradiation exposure in one of the individual's parents.

P-BasEpi-017

Effects of Mutation Localization on Autosomal Dominant Hypophosphatasia

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Hypophosphatasia (HPP) is a hereditary metabolic disorder of the bone. It is caused by impaired activity of the tissue-nonspecific isoenzyme of alkaline phosphatase. Its function is essential for bone and tooth mineralization. The ALPL enzyme forms homodimers and consists of three domains: the calcium binding domain, the catalytic center and the homodimeric interface/crown domain. Five clinical forms are distinguished depending on the patient's age when clinical symptoms occur. Early onset of the disease normally accompanies a severe clinical course, in this case HPP is normally transmitted as an autosomal recessive trait. HPP due to a single mutation in the ALPL gene and inherited as an autosomal dominant trait can cause milder forms. So far detailed knowledge of the milder forms is lacking.

39 patients with a mutation in the ALPL gene were interviewed in a standardized questionaire concerning the different disease manifestations: teeth, bone fractures, pain of bones and muscles and quality of life. Subgroups were formed with regard to the localization of the mutations in the three protein domains.

Patients with mutations clustering in the catalytic site of the molecule showed the most severe odontohypophosphatasia: one individual had premature primary tooth loss, 31% of patients showed adult tooth loss, 77% suffered from dental caries. The majority had the first manifestation before the age of 18. Persons suffering from mutations in the two other domains reported a relatively high quality of live with low pain of muscles and bones. Unexpectedly in all groups there was no significant difference in the portion of patients with bone fracture.

Conclusion: The clinical signs of dominant HPP are mostly unspecific. Especially dental problems like severe adult teeth loss, an early manifestation of dental caries or enlarged pulp chambers can be a sign of odontohypophosphatasia and a dominant inherited mild HPP. Mutations in the catalytic site of the ALPL molecule are associated with a more severe odontohypophosphatasia.

P-BasEpi-018

Screening of non-neoplastic lymphatic tissues from children for the IGH-MYC fusion using a highly sensitive 4-color FISH-assay

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Burkitt lymphoma is a mature B-cell lymphoma which on the genetic level is characterized by the Burkitt translocation t(8;14)(q24;q32) juxtaposing the IGH locus in 14q32 next to the *MYC* locus in 8q24. In a minor part of Burkitt lymphomas, immunoglobulin light chain variants of the translocation result in overexpression of *MYC*. Despite being pathognomonic for Burkitt lymphoma, the IG-*MYC* juxtaposition alone is not sufficient on its own for a malignant transformation of the cell. Other IGH rearrangements like the IGH-*BCL2* fusion, typical for follicular lymphoma, were detected in a significant number of healthy individuals. For the IGH-*MYC* translocation, only scarce data in healthy individuals exist. This is most likely due to scattering of the breakpoints which are far more difficult to target by PCR than the IGH-*BCL2* translocation. Therefore, we aimed at investigating if *MYC*-translocation positive cells can also be detected in normal B-cell maturation. Considering the epidemiology of Burkitt lymphoma being the most common B-cell lymphoma in children, we focused on samples from young individuals. On the one hand, we analyzed non-neoplastic tissue specimen of bone marrow (n=14) (age range 3-18, median age 8.5 years) and lymph nodes (n=19)(age range 2-18 years, median age 12 years). On the other hand, considering the typical clinical presentation of Burkitt lymphoma, we included non-neoplastic tissue specimen containing Peyer Patches (n=25)(age range 48hours-20years, median age 17years). The specimen were analyzed using a four-color fluorescence in situ hybridisation (FISH) assay with probes flanking the breakpoints on chromosomes 8 and 14. In this setting, a positive result comprised the break on both chromosomes (seen as signal split for each locus) and fusion of the involved genes (leading to two different fusion signals).

The assay was first validated on controls of cells with a normal male karyotype from healthy individuals as well as on five Burkitt cell lines and each five FFPE embedded t(8;14) negative and positive tissues as negative and positive controls. The assay was then applied for the screening of a IGH-*MYC* fusion in the above mentioned paraffin-embedded tissues. Successful hybridizations of overall 9, 15 and 17 FFPE sections from

bone marrow, lymph nodes and Peyer Patches respectively could be obtained. A translocation t(8;14)(q24;q32) was not detectable in any of the investigated tissues.

With the established assay we were able to provide a highly sensitive tool for the detection of the translocation t(8;14)(q24;q32). However, we did not detect normal B-cells carrying this translocation. This does not exclude that such cells exist. Alternatively, the growth advantage conferred by *MYC* may promote the acquisition of secondary genetic changes. This may result in a rapid tumorigenesis, that if occurring these cells only present as full blown Burkitt lymphoma.

P-BasEpi-019

Myotonic Dystrophy: links to the nuclear envelope

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Myotonic dystrophies (DM) are slowly progressing multisystemic diseases with a predominant muscular dystrophy - making DM the most frequent muscular dystrophy in adulthood. DM is caused by heterozygous DNA-repeat expansions in the DMPK gene (DM1) or the CNBP gene (DM2). The repeat-containing RNA accumulates in ribonuclear foci and splicing factors are sequestered to these foci, resulting in abnormal regulation of alternative splicing. DM patients show overlapping phenotype presentations with progeroid laminopathies, which are caused by mutations in nuclear envelope proteins.

In search for molecular signatures of this overlap, we found an enrichment of nucleoplasmic reticuli in DM1 and DM2 patient myoblasts. Additional, we found an alternative splicing of the LMNA gene - both effects that are associated with progeroid laminopathies. This implies possible shared pathomechanism between DM and progeroid laminopathies.

P-BasEpi-020

Investigations on the stability of the CpG85 imprint in human RB1

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Retinoblastoma is a tumor of the retina occurring in young children up to the age of five. It is caused by biallelic inactivation of the tumor suppressor gene RB1. We have shown that human RB1 is an imprinted gene and as such characterized by differential DNA methylation of a CpG island (CpG85) in RB1 intron 2. CpG85 is not methylated on the paternal allele and acts as a promoter for the alternative RB1 transcript, RB1-E2B. It is argued that expression of RB1-E2B is causative of the observed skewing of regular RB1 expression in favor of the maternal allele.

A true gametic differentially methylated region (gDMRs) is established in only one of the parental germ lines. It is supposed to be stable during early embryonic development and to be passed on to all daughter cells. We could show that CpG85 is free of methylation in human sperm. Publicly available methylome data on oocytes revealed that CpG85 is fully methylated in human oocytes. These data are in agreement with CpG85 being a maternal methylated gDMR. We showed that the level of CpG85 methylation is 60 percent in blood, as expected. However, in eight tissues of three individuals we observed a gain of methylation at CpG85 ranging from 60 to 65 percent in liver and skin, and increasing to 70 to 85 percent in the other tissues (heart, kidney, muscle, brain, lung and spleen). Interestingly, the degree of methylation was lower in fetal tissue than in adult tissue, as determined for brain and muscle. We also observed gain of methylation at CpG85 in two human embryonic stem cell lines and induced pluripotent stem cells. This is consistent with the finding of complete methylation at CpG85 in eight different retinoblastoma cell lines. We therefore conclude that CpG85 is an unstable DMR.

In oocytes, DNA methylation of gDMRs is established by transcriptional read-through from an upstream promoter. Therefore, we hypothesize that gain of DNA methylation at CpG85 is caused by run-through transcription from the upstream regular RB1 promoter. To test this hypothesis, we generated a genetic model carrying modifications in the RB1 promoter and in CpG85 using CRISPR/Cas9 technology. Data on the establishment of the model and first results will be presented.

P-BasEpi-021

The CTLH ubiquitin ligase complex is involved in ciliogenesis

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The Gid/CTLH protein complex with its seven core protein members is conserved in all eukaryotic cells. In Saccharomyces cerevisiae it functions as an ubiquitin ligase complex and regulates the metabolic switch from gluconeogenesis to glycolysis (1). Recently, we could show that the vertebrate Gid/CTLH complex also functions as an ubiquitin ligase, however substrates and exact function remain unknown (2). A growing number of components of the ubiquitin protein system (UPS) are described to be regulators of ciliogenesis (3). Defects in such genes are considered to cause ciliopathies, genetic disorders with typical phenotypic variations in patients and model organisms (4). First data supports our hypothesis that the CTLH complex plays a major role in ciliogenesis, e.g. the CTLH subunit RMND5A localizes to the basal body which is a modified centriole of primary cilia in NIH-3T3 cells and Rmnd5 knock down in *Xenopus laevis* leads to defects in cilia formation of epidermal multiciliated cells.

P-BasEpi-022

Regulation of HDGF gene expression by hsa-miR-129-5p in hepatocellular carcinoma

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Background and aims: Hepatocellular Carcinoma (HCC) is characterized by genetic and epigenetic changes that lead to a deregulation of important tumor suppressors and oncogenes in a multistep process. One of these epigenetic changes is the elevated expression of histone-deacetylases (HDACs) which contribute to a transcriptional repression of certain genomic regions by remodeling the chromatin structure. Thereby, not only the expression of tumor-relevant genes is affected, but also the expression of microRNAs (miRNAs). Selected miRNAs have been shown to play important roles in carcinogenesis. We aimed to identify miRNAs deregulated by histone deacetylation and to understand their functional consequences in HCC tumorigenesis.

Methods: Histone acetylation was induced by the global HDAC inhibitor trichostatin A (TSA) in four HCC cell lines (HLE, HLF, Huh7, HepG2) and two immortalized liver cell lines (THLE-2 and THLE-3) in order to identify differentially expressed miRNAs and messenger RNAs (mRNAs) by global expression profiling. Findings were validated by transfection of microRNA mimics and siRNA-mediated knockdown in HCC cell lines, quantitative PCR, Western blotting and luciferase reporter assays.

Results: After HDAC-inhibition, hsa-miR-129-5p was significantly upregulated. The miR-129-5p holds tumor suppressive potential and its expression is reduced in different types of tumors. One predicted target gene of miR-129-5p is the *Hepatoma-derived Growth Factor* (*HDGF*). This mitogenic growth factor is highly expressed in a variety of cancers, for example in HCC, and its expression correlates with a poor prognosis, irrespective of the tumor type. HDGF is a multifunctional protein that is involved in several signaling pathways, contributing to proliferation and metastasis of cancer cells, induction of angiogenesis and inhibition of apoptosis. Incubation of HCC cells with TSA or transfection with miR-129-5p reduced expression of HDGF. Luciferase assays indicate a direct regulation of *HDGF* by miR-129-5p. Moreover, expression of the death receptor *Fas*, which is a potential downstream target of HDGF, is also regulated by the miR-129-5p.

Conclusions: The altered expression of the tumor suppressor miR-129-5p due to chromatin remodeling may play a fundamental role in hepatocarcinogenesis. We expect that histone deacetylation and putative target genes of epigenetically deregulated miR-129-5p can be targeted by new therapeutic agents.

The microRNA-449 family inhibits TGF-β-mediated liver cancer cell migration by targeting SOX4

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Introduction: Modulation of microRNA expression is considered for treatment of hepatocellular carcinoma (HCC). Therefore, we characterized the epigenetically regulated microRNA-449 family (miR-449a, miR-449b, miR-449c) with regards to its functional effects and target genes in HCC.

Methods: After transfection of miR-449a, miR-449b, and/or miR-449c, tumor-relevant functional effects were analyzed using in vitro assays and a xenograft mouse model. Binding specificities, target genes, and regulated pathways of each microRNA were identified by microarray analyses. Target genes were validated by luciferase reporter assays and expression analyses in vitro. Furthermore, target gene expression was analyzed in 61 primary human HCCs compared to normal liver tissue.

Results: Tumor suppressive effects, binding specificities, target genes, and regulated pathways of miR-449a and miR-449b differed from those of miR-449c. Transfection of miR-449a, miR-449b, and/or miR-449c inhibited cell proliferation and migration, induced apoptosis, and reduced tumor growth to different extents. Importantly, miR-449a, miR-449b, and, to a lesser degree, miR-449c directly targeted SOX4, which codes for a transcription factor involved in epithelial-mesenchymal transition and HCC metastasis, and thereby inhibited TGF-β-mediated cell migration.

Conclusions: This study provides detailed insights into the regulatory network of the epigenetically regulated microRNA-449 family and, for the first time, describes distinct tumor suppressive effects and target specificities of miR-449a, miR-449b, and miR-449c. Our results indicate that particularly miR-449a and miR-449b may be considered for miRNA replacement therapy to prevent HCC progression and metastasis.

Novel mutation in the ATM gene and activaton of two kryptic splice sites in an 52 year old female patient with gastric cancer

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Gastric cancer is a global public health concern, ranking as the third leading cause of cancer mortality. Familial aggregation of gastric cancer is common in about 10% of the cases, and about half of these can be attributed to hereditary germline mutations. However, for most gastric cancer cases, whether genetic events contribute to cancer susceptibility remains unknown.

Here we present a case report of a patient with gastric cancer, a family history of breast cancer and a novel mutation leading to complex cryptic splicing in the ATM gene.

NGS panel sequencing and CNV/MLPA analysis of 18 genes associated with gastric and breast cancer were performed. Sequencing revealed a novel mutation in intron 27 of the ATM gene, ATM,c.4109+1G>A in an heterozygous state (nomenclature according to HGVS; reference sequence NM_000051.3). In silico-analysis by ALAMUT (Version 2.8.1) predict the loss of the donor splice site of intron 27 of the ATM gene. cDNA-analysis was performed and revealed the loss of exon 27 of the ATM by a complex activation of two kryptic splice sites. A premature stop codon was generated giving rise to a truncated protein that leads to a pathogenic variant.

The results of the genetic analysis are discussed in the context of the clinical findings. Identification of the underlying genetic causes of gastric cancer will give a better view of the mechanisms that contribute to the pathophysiology of the disease.

P-BasEpi-025

Identification of a Novel PAX4 Gene Mutation in a Young German Woman with Gestational Diabetes Mellitus

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About 2-5% of all pregnant women develop gestational diabetes mellitus (GDM) during their pregnancies and diabetes complicating pregnancy is associated with adverse maternal and perinatal outcomes, notably, risk of fetal macrosomia and neonatal hypoglycemia and development of diabetes after pregnancy. GDM is considered to result from interaction between genetic and environmental risk factors.

The case of a 35-year old female German patient with a novel mutation in the PAX4 gene (rare MODY gene Type 9) as a cause of gestational diabetes mellitus is presented. We describe clinical, biochemical and genetic features of the patient, who developed GDM and gave birth to her child by cesarean section.

MODY genes type 1-11 were analyzed. Sequencing the PAX4 gene revealed a novel mutation in exon 8, PAX4,c.778delC, p.(Leu260Cysfs*24); reference sequence NM_006193.2), a deletion of a cytosine leading to a truncated, non-functional protein. To date, no small deletion has been detected in the PAX4 gene.

Identification of the underlying genetic causes of GDM will give a better view of the mechanisms that contribute to the pathophysiology of the disease. Furthermore, early identification may improve options to prevent GDM and complications for the mother and her child. The results of the genetic analysis are discussed in the context of the clinical findings.

P-BasEpi-026

Epigenetic age acceleration in germinal center B cell lymphomas

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The modulation of DNA methylation is highly flexible and plays an important role during cell differentiation. Furthermore, the DNA methylome alters considerably during aging. Age related changes in the DNA methylation of regulatory genes are assumed to have a major impact on carcinogenesis (Teschendorff, 2010). Moreover, it was demonstrated that the chronological age of a human donor can be predicted with high accuracy by analyzing the DNA methylation of a specific minor set of CpG loci which are aberrantly methylated during aging (Horvath, 2013).

Hence, we intended to investigate the effect of epimutations identified in different lymphoma entities in comparison with the influence of epigenetic changes in sequential B cell differentiation stages on the epigenetic age. Additionally, our aim was to analyze whether entity-specific differences in the resetting of the epigenetic clock are generated during lymphomagenesis or derive from modified DNA methylation in the germinal center B cells of origin.

To address these issues, we performed DNA methylation profiling (HumanMethylation450 BeadChip) of 72 Burkitt lymphoma samples (age 2-76 yrs), 119 diffuse large B cell lymphoma samples (age 3-93 yrs) and 103 follicular lymphoma samples (age 22-80 yrs) from the ICGC MMML-Seq and MMML-consortium (Kretzmer et al., 2015) and the Hematopathology Section Kiel as well as 145 peripheral blood samples of healthy individuals (0-63 yrs) available from the SAME project and current publications of our group (Kolarova et al., 2015; Friemel et al., 2014). In addition, we received 61 B cell subpopulation samples (0-66 yrs) covering different stages of B cell differentiation that were measured in the same way (Kulis et al., 2015; Lee et al., 2012). The epigenetic age of the samples was predicted using the "Online Age Calculator" accessible at https://dnamage.genetics.ucla.edu and compared with the corresponding chronological age of the donors.

In fact, the epigenetic age of peripheral blood samples of healthy donors was in high accordance with their chronological age (Pearson´s r 0.968, p-value <0.001) while the correlation between epigenetic and chronological age of sequential B cell differentiation stages was slightly lower (Pearson´s r 0.893, p-value<0.001).

In contrast, the predicted epigenetic age of the Burkitt lymphoma samples was significantly higher than the corresponding chronological age. This deviation may be interpreted as "epigenetic pre-aging".

Nevertheless, the epigenetic age of diffuse large B cell lymphomas and follicular lymphomas tended to be less affected.

In conclusion, we found significant epigenetic pre-aging in Burkitt lymphoma samples that seems to be induced during lymphomagenesis and does not derive from altered DNA methylation patterns in the germinal center B cells of origin. Moreover, no significant shift of the epigenetic age was observed for the other lymphoma entities, healthy blood samples and B cells of sequential differentiation stages.

P-BasEpi-027

Identification of type-1 NF1 deletion breakpoints mediated by rare PRS2 haplotypes

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Neurofibromatosis type 1 (NF1) is a hereditary cancer syndrome with an incidence of 1 in 3000. In 5% of all NF1 patients, large deletions encompassing the *NF1* gene and its flanking regions are causing the disease. The majority of all large *NF1* deletions are of type-1; they encompass 1.4-Mb and are mediated by nonallelic homologous recombination (NAHR) with crossover. The breakpoints of type-1 deletions are located within the low-copy repeats NF1-REPa and NF1-REPc which exhibit high sequence homology to one another. Previous studies suggested that type-1 deletion breakpoints cluster within the paralogous recombination sites PRS1 and PRS2 spanning 5-kb and 4-kb, respectively.

In our present study, we investigated 218 patients with type-1 *NF1* deletions using long-range PCRs to detect breakpoints located within PRS1 or PRS2. According to these analyses, 157 (72%) of the breakpoints are located within PRS2 and 34 (15.6%) in PRS1. However, 27 (12.4%) of the type-1 deletions were not positive for these deletion-junction PCRs. We surmised that some of these deletions may have breakpoints within the 14-kb region located between PRS1 and PRS2. This 14-kb region also exhibits high sequence homologoy between the NF1-REPs which is a prerequisite for NAHR. Indeed, 12 of the 27 type-1 *NF1* deletions exhibited breakpoints within this 14-kb region as determined by the analysis of seven overlapping deletion-junction PCRs. However, the breakpoints of 15 deletions remained unidentified since positive deletion-junction PCR products could not be amplified. To identify the breakpoints of the 15 deletions, we performed custom-designed microarray CGH analysis with a high resolution of probes located within and flanking the NAHR hotspots PRS1 and PRS2. The array analysis suggested that 11 of the 15 deletions exhibit breakpoints within PRS2, even although previously performed breakpoint-spanning PCRs with primers designed according to the reference sequence of the human genome have been negative in these 11 cases. Since PRS2 exhibits high sequence diversity resulting from frequent nonallelic homologous recombination events without crossover, we surmised that haplotype diversity is responsible for the failure of the breakpoint-spanning PCRs performed with primers designed according to the reference sequence. Therefore we characterized the haplotype diversity of PRS2 in 30 human individuals and designed deletion-junction PCR primers that facilitate the amplification of rare PRS2 haplotypes. So far, we have identified the breakpoints of four of the 11 type-1 *NF1* deletions predicted to have been mediated by PRS2 according to the array results. We are confident to identify further breakpoints by extending these analyses using primers suitable to amplify rare PRS2 haplotypes. Our findings indicate that the characterisation of NAHR hotspots in terms of haplotype diversity is a premise to identify the breakpoints of NAHR-mediated microdeletions by means of deletion-junction PCRs.

P-BasEpi-028

*** Array-based DNA methylome analyses of primary lymphomas of the central nervous system

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Primary lymphomas of the central nervous system (PCNSL) are defined as diffuse large B-cell lymphomas (DLBCL) that are confined to the central nervous system (CNS). Although PCNSL cannot be distinguished from DLBCL by their morphology as well as their histology, they differ in prognostic outcome. The aim of the present study was to compare the epigenomic landscape of PCNSL and DLBCL.

To this end, we analyzed the DNA methylation of a total of 26 PCNSL (cryopreserved or formalin fixed and paraffin embedded (FFPE)) using the Infinium HumanMethylation450 BeadChip Array (Illumina) and contrasted these findings to 79 DLBCL (Kretzmer et al., 2015). As controls, we used publicly available DNA methylation data from a total of 50 normal brain samples derived from different regions of the CNS (Gilbert et al., 2105; Jaffe et al., 2016; Kurscheid et al., 2015; Mur et al., 2013; Wockner et al., 2014).

After normalization of the data we performed thorough filtering and removed the random SNPs, all loci located on gonosomes, as well as those loci with a detection p-value >0.01 in at least one of the samples, leading to 457,951 loci entering subsequent analyses. When comparing the DNA methylation profiles of PCNSL versus DLBCL we identified 8279 differentially methylated loci (σ/σ_{max}=0.4; q<1e-4). In order to remove those loci which represent a "brain signature", we compared DLBCL versus brain (σ/σ_{max}=0.4; q<0.05) based on the list of the previously identified 8279 loci. After removing this "brain signature", we ended up with 2231 loci that are differentially methylated between PCNSL and DLBCL.

In a next step we wanted to make sure that the differences in methylation at these 2231 loci are not due to differences in starting material (cryopreserved versus FFPE) which is known to influence the outcome of the BeadChip analysis. Therefore, we compared the DNA methylation profiles of five cryopreserved versus FFPE samples (derived from the same tissue samples) and identified 318 differentially methylated loci (σ/σ_{max}=0.4, q<0.05). Only five loci of both lists overlapped, which were subsequently removed from further analysis so that we ended up with a final list of 2226 loci which are differentially methylated between PCNSL and DLBCL.

In order to analyze the biological implications of the differentially methylated loci we evaluated an enrichment of known functional groups (Kulis et al., 2015). Remarkably, CpG loci that are differentially methylated during normal B-cell maturation were significantly depleted. In turn we saw an enrichment of loci located in heterochromatin. In summary, we detected more than 2000 loci that are differentially methylated between PCNSL and DLBCL, which do not play a functional role in normal B-cell differentiation.

P-BasEpi-029

Replication study of GWAS-identified genetic modifiers of age at Huntington's Disease onset

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Although there is a strong correlation between CAG repeat length and age at onset (AO) of motor symptoms, individual Huntington disease (HD) patients may differ dramatically in onset age and disease manifestations despite similar CAG repeat lengths. Since the modifier variations described so far only account for a small fraction of the heritable contribution to the AO, the identification of loci and genes using genome-wide methods appears highly promising. Against the background of incomplete understanding of the HD disease pathophysiology, the hypothesis-free approach of GWAS offers an ideal starting point for the search of modifier genes. Recently, a combined analysis of all GWA data to HD modifiers identified different loci with genome-wide significant signals for association to residual age at motor onset [GeM-H. Consortium]. Interestingly, none of the most significant association signals and none of the trending SNPs in the European GWA analysis corresponded to any previously suggested candidate modifier genes. In order to be able to better assess these data, we tried to replicate the top ten associated GWAS variants in a comprehensive cohort of 505 German HD patients. We only found modest association with one of the top ranked SNPs (rs72810940), all remaining variations showed no correlation with the AO. This inconsistency highlights once again the difficulties of modifier searching in HD or any other monogenic disorder, which faces the same challenges as the genetic characterization of complex disorders.

P-CANCER GENETICS

P-CancG-030

Follow-up of a CFTR association with Barrett's esophagus and esophageal adenocarcinoma

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Esophageal adenocarcinoma (EA) represents one of the most rapidly increasing cancer types in high-income countries. Barrett's esophagus (BE) is a premalignant precursor of EA and has an estimated prevalence of 5-6% in the population. However, only 0.1 to 0.3% of BE patients develop EA. Within an international consortium, we carried out a GWAS meta-analysis in 6.167 BE patients, 4.112 AE patients and 17.159 controls (Gharahkhani et al., Lancet Oncology, 2016). In a comprised BE/AE analysis, we identified 14 genome-wide significant risk loci, of which seven were previously unreported. The strongest associated new risk variant was identified for rs17451754 ($P=4.8\times10^{-10}$), which maps within intron 21 of the *CFTR* gene. *CFTR* encodes a protein that functions as a chloride channel and that is mutated in patients with cystic fibrosis (CF). Mutations in CF lead to abnormal viscous secretions with altered chemical composition, resulting in dysfunction of the respiratory system and the gastrointestinal tract. The most common CF mutation is ΔF508, a deletion of three nucleotides in CFTR that results in the loss of a single codon for phenylalanine on protein position 508. Interestingly, CF patients show a highly increased incidence of gastroesophageal reflux, which represents the major risk factor for BE and AE. In view of the phenotypic overlap for gastroesophageal reflux and cystic fibrosis, and for gastroesophageal reflux and both BE and AE, combined with the association of *CFTR* risk variants in patients with BE and AE, it seems plausible that a common pathophysiological mechanism is triggered by *CFTR*. In order to test this hypothesis, we analyzed the association of ΔF508 in a European case-control cohort with BE and AE patients. For this, we performed a genotyping assay of ΔF508 in 1.037 BE patients, 1.609 AE patients and 941 controls. We could not observe a significant association (P=0.57). This might be (i) due to insufficient sample power or (ii) due to the fact, that not ΔF508 but other genetic variants at the locus might explain the underlying functional mechanism of the association. Fine mapping of all genetic variation at the *CFTR* locus and extensive functional analysis are needed to find the causal variant that explains how the *CFTR* locus interferes with the pathomechanism of BE and AE. A recent functional study indicated *CFTR* as a tumor suppressor gene in murine and human intestinal cancer, providing further evidence for *CFTR* as a true disease gene for BE and AE.

P-CancG-031

BRCA1 mutation identified as the underlying cause in a family with hereditary pancreatic cancer

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Background

It has long been established that mutations in *BRCA2* predispose for pancreatic adenocarcinoma with *BRCA2* germline mutations identified in 6-10% of familial pancreatic cancer cases. Consequently, screening for pancreatic cancer has been recommended for mutation carriers with an affected first-degree relative since early detection has been shown to significantly improve 5-year survival from 4-7% to 24%.
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For *BRCA1* mutations, however, relevance in pancreatic tumorigenesis is still being discussed with several studies questioning an elevated risk of pancreatic cancer in families with BRCA1 mutations while others are suggesting that *BRCA1* may also play an important role in predisposing to pancreatic cancer. Clinical screening for pancreatic cancer commonly remains unavailable to *BRCA1* mutation carriers and it has even been questioned whether *BRCA1* should be analyzed in familial pancreatic cancer at all.
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Clinical report

Here we report on a 59 year old woman with metastatic pancreatic cancer whose sister had died of pancreatic cancer at 50 years of age. In this family we identified a pathogenic *BRCA1*-germline mutation (BRCA1: NM_007294.3:c.1292dupT,p.(Leu431Phefs*5)) by next-generation sequencing using a 94-gene panel. The index patients' tumor was available for genetic analysis and showed loss-of heterozygosity for *BRCA1*. This strongly suggests the *BRCA1* mutation to be causative of the pancreatic cancer development in this patient.
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When the family was first introduced to genetic counselling there was no evidence of breast- or ovarian cancer in any relatives. Only after identification of the mutation did the index person reach out to distant family members and it was thereby revealed that a distant branch of the family had independently been counselled for hereditary breast and ovarian cancer. In this part of the family, however, there had not been any cases of pancreatic cancer.
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Subsequent predictive testing was offered to healthy family members and 3 further mutation carriers could be identified. Two women were referred to breast cancer screening. Additionally, the mutation was identified in a relative with recurrent metastatic breast cancer at the age of 38 years. For her and the index patient PARP-inhibition therapy thus became a possible further treatment option.
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Conclusions

In conclusion we propose next-generation sequencing approaches including the analysis of *BRCA1* to be used in familial pancreatic cancer. We also argue that *BRCA1* mutation carriers with pancreatic cancer cases in their family should be offered the same screening program as *BRCA2* mutation carriers. Within the framework of a study this could allow for more precise risk stratification in the future.

P-CancG-032

An unexpected etiology of a malignant tumor of the thymus

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With an incidence of 0.2-0.3/100 000 malignant tumors of the thymus are a rather rare type of cancer. Here we report the case of a man of German descent, who presented with a thymoma at age 55. In the pathological report the thymus tumor was described as an extremely unusual thymoma with partial loss of keratin and massive proliferation of myoid cells. It was subsumed to a primary thymic, partially epithelial neoplasia, resembling an uncommon B2/B3-thymoma.

After the patient's death his widow looked for genetic advice concerning the risk of disease for her children. Detailed personal and familial history brought up surprising information: thymoma was one of four cancers in our patient. He developed adenocarcinoma of the colon at age 35, squamous cell cancer of the nose/upper lip at 54 years and in addition current cancer staging revealed a papillary renal cell carcinoma. According to family history his father and his uncle developed colon cancer with 58 and 66 years, the son of this uncle was diagnosed with colon cancer at age 47. This cousin of the propositus was referred to genetic counseling, because of MSI-high-status and loss of MLH1 and PSM2 in immunohistochemistry. He was found to have a deleterious mutation in exon 14 of MLH1-gene (c.2644C>A, p.Tyr548Stop) resulting in a premature termination of MLH1-protein.

Our patient has never been tested for HNPCC. However a post mortem performed immunhistochemical examination of thymic cancer cells revealed an almost complete loss of MLH1 nuclear expression suggesting the presence of a MLH1 germline mutation and indicated HNPCC.

Considering the loss of MLH1 in tumor cells it is more than likely that the development of thymoma was the consequence of deficient DNA mismatch-repair. There have been reports of rare tumors in HNPCC families in the last years (i.e. clear cell renal carcinoma and uterine sarcoma). Pande et al. reported one case of thymoma in their registration of cancer occurrences in 368 mutations carries from 176 HNPCC families.[1]

Our case emphasizes the importance of detailed family history and contributes to the discussion of widening the inclusion criteria for genetic counseling and testing for HNPCC. To this day the revised criteria of Bethesda are used to identify families at risk. We propose that the established criteria have to be revised and rare tumors should be included.

[1]Pande M, Wei C, Chen J, et al. Cancer spectrum in DNA mismatch repair gene mutation carriers: results from a hospital based Lynch syndrome registry. Familial cancer 2012; 11(3): 441-7.

P-CancG-033

Unknown partner genes in leukemias with rare translocations can be identified using targeted RNA sequencing

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In hematological malignancies fusion genes play an important role and function as therapeutic targets, impressively shown for e.g. BCR-ABL1 and ETV6-PDGFRB. Thus, the identification of fusion genes is the basis for precision medicine, selecting treatment based on genotype and providing markers for disease monitoring.

The aim of this study was to test the value of targeted RNA sequencing in a routine diagnostic work up.

51 cases were selected harboring rearrangements of KMT2A (n=10), RUNX1 (n=19), ETV6 (n=11), PDGFRB (n=6), NPM1 (n=2), RARA (n=2) and JAK2 (n=1) identified by chromosome banding (CBA) and FISH analyses. In none of the cases the partner gene could be identified using standard methods. Targeted RNA sequencing was performed using the TruSight RNA Fusion panel (Illumina, San Diego, CA) consisting of 7690 probes covering 507 genes known to be involved in gene fusions. This assay allows the capture of all targeted transcripts. Sequencing was performed on NextSeq (Illumina, San Diego, CA). Analysis was performed with the RNA-Seq Alignment App (BaseSpace Sequence Hub) using Star for Alignment and Manta for gene fusion calling with default parameters (Illumina).

In 32/51 cases with rearrangements involving KMT2A (n=10), RUNX1 (n=8), ETV6 (n=6), PDFGRB (n=4), RARA (n=2), NPM1 (n=1) or JAK2 (n=1) the partner genes were identified. These were in KMT2A rearranged cases: MLLT10 (n=2), MLLT1 (n=2), ITPR2, FLNC, ASXL2, DCP1B, MAML1 and ARHGEF12.

In RUNX1 translocated cases partner genes were PLAG1 (n=2), PRDM16, MECOM, ZFPM2, MAN1A2, N6AMT2, and KIAA1549L. PRDM1, MECOM and ZFPM2 have previously been described in the literature as RUNX1 partner genes but were not suspected in our cases as partner genes due to complex cytogenetic rearrangements. The other identified partner genes have not been described so far. Interestingly, PRDM1, MECOM, ZFPM2 and the newly identified PLAG1 are all members of the C2H2-type zinc finger gene family.

Partner genes identified in ETV6 rearranged cases were: ABL1, CCDC126, CLPTM1L, ERG, FOXO1 and CFLAR-AS1.

WDR48, ZBTB11, NFIA and MPRIP were identified as partner genes of PDGFRB and RPP30 in an NPM1-translocated AML.

In an ALL patient a JAK2-PPFIBP1 fusion was identified leading to classification as a BCR-ABL1-like ALL. In an APL patient showing an ins(17;11)(q12;q14q23) a ZBTB16-RARA fusion was identified and thus resistance to all-trans retinoic acid, arsenic trioxide, and anthracyclines can be predicted. Further in a case with t(17;19)(q21;q13) an IRF2BP1-RARA fusion was detected.

Conclusions: Targeted RNA sequencing was able to characterize rare gene fusions and provided the basis for the design of RT-PCR based assays for monitoring MRD. Targetable genetic aberrations were identified, which were not detected by CBA enabling more individualized treatment. Targeted RNA sequencing may be a valuable tool in routine diagnostics for patients with rearrangements unresolved by standard techniques.

P-CancG-034

Familial breast cancer and CHEK2 gene mutations

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Female carriers of a pathogenic mutation in the CHEK2 gene are reported to have a life time risk of about 20-45% to develop breast cancer. There is evidence for increased risks for contralateral breast cancer, male breast carcinoma and other types of tumors. In addition to well-known mutation CHEK2:c.1100del, other pathologic mutations are being identified in the gene due to the inclusion of the gene in most breast cancer gene panels for DNA testing. Between 2012-2016 the Center for Hereditary Breast and Ovarian Cancer Regensburg cares for 10 families with pathogenic or probably pathogenic mutations in the CHEK2 gene affecting nine female patients and one male patient (6 x c.1100del, 1 x deletion of exon 10, and 3 x variants considered as likely pathogenic: c.1408G> C, c.1561C> T, c.1169A> C).

The mean age of diagnosis of breast cancer (both sexes) was 45.1 years (range 25-61 years). The patient with the deletion of exon 10 was first diagnosed at 25 years of age and developed a contralateral breast carcinoma (DCIS) at 35 years of age. The male patient was diagnosed with breast cancer at 58 years of age and at 59 years with a renal carcinoma. One patient was diagnosed with a papillary thyroid carcinoma at age 26 years and developed breast cancer with 35 years.

In 8 out of the 10 families, breast carcinoma diagnosed with 51.3 years on average, was reported in the family history. In addition, there were additional malignancies such as prostatic carcinoma, thyroid carcinoma, colorectal cancer, gastric carcinoma, leukemia, cervical carcinoma and malignant melanoma. None of the affected family members was tested for the respective CHEK2 mutation.

The tumors with an initial diagnosis at 25 years and 35 years were estrogen-receptor-negative and progesterone-receptor-negative. The other 8 of the 11 breast cancers were positive for the estrogen receptor, 7 of the 11 tumors were positive for the progesterone receptor. The receptor status of the contralateral DCIS is unknown. Only the male breast carcinoma was Herceptin receptor positive, all other breast carcinomas of the CHEK2 mutation carriers were HER2 negative. Among our CHEK2 positive families we noticed the association with CHEK2 mutation and female breast cancer. We observed a contralateral breast cancer, male breast cancer and other tumors in our families as well. The majority of the observed breast cancers was estrogen and progesterone receptor positive and Herceptin negative.

P-CancG-035

Uniparental disomies detected as germline alterations in uterine smooth muscle tumors

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While benign uterine smooth muscle tumors are among the most frequent human symptomatic tumors, their malignant or borderline lesions are only rare findings. Both lesions can show somatic copy number alterations, but their patterns differ, thus constituting helpful diagnostic tools. Aimed at an advanced classification of the lesions we have performed molecular inversion probe array analyses of these tumors. Besides complex patterns of genomic alterations seen in nearly all cases, two of the lesions presented with copy number neutral uniparental disomies i.e. normal copy numbers with an apparent monoallelic origin. In one case, an UPD of part of the long arm of chromosome 22 was detected in a uterine leiomyosarcoma. The tumor showed genetic heterogeneity with gains and losses. In addition, the 11.45 Mb segment located at 22q12.1-q13.1 was clearly of monoallelic origin throughout all cells investigated. All other genetic alterations were restricted only to part of the cells of the sample thus reflecting the presence of tumor cells as well as normal bystander cells which in general characterizes mutations that had arisen during tumor development. In contrast, the UPD that was detected in all examined cells clearly suggests its germline occurrence. The second tumor was a leiomyoma-variant of the type with bizarre nuclei. Again, besides gains and losses an apparent germline UPD was found that covered a 3.71 Mb segment on chromosomal segment 8q11.21. UPD for even the whole arm of chromosome 22 repeatedly has been reported not to coincide with phenotypic manifestations. Nevertheless, the question arises whether or not the observed UPDs might be related to a familiar predisposition for uterine muscle tumors. Of note, as a result of genome-wide association studies SNPs on 22q recurrently have been found to be significantly associated with fibroid development.

Triple negativity is an independent predictor of germline mutations in breast cancer predisposing genes

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Breast cancer is the most common cancer in women. 12-15% of all tumors are triple-negative breast cancers (TNBC) lacking expression of estrogen receptor, progesterone receptor and human epidermal growth factor receptor 2. So far, TNBC have been mainly associated with mutations in BRCA1, although recent studies also found mutations in other breast cancer susceptibility genes. A BRCA1/2-centered perspective thus may ignore the significance of other predisposing genes, whose relevance appears obvious as DNA damage repair by homologous recombination is a complex process involving many proteins.

To determine the prevalence of mutations we performed panel-based germline mutation testing of 10 high and low-moderate penetrance breast cancer susceptibility genes (BRCA1, BRCA2, ATM, CDH1, CHEK2, NBN, PALB2, RAD51C, RAD51D and TP53) in 229 consecutive individuals affected with TNBC unselected for age at diagnosis or breast and ovarian cancer family history. Age at diagnosis ranged from 23 to 80 years with an average of 50.2 and a median of 48 years. In 60 women (26.2%) we detected a pathogenic mutation, with a higher frequency (31.3%) in the group manifesting cancer before 60 years. Deleterious BRCA1 mutations occurred in 14.8% of TNBC patients, predominantly frameshifting (24/34, 70.6%). The most frequent, both among BRCA1 mutations and in total, were the founder mutations c.5266dupC and c.2411_2412delAG. Deleterious BRCA2 mutations occurred in 5.7% of patients, all but one (c.1813dupA) being unique. While no mutations were found in CDH1 and TP53, 15 mutations (25%) were detected in one of the six other predisposition genes (PALB2, CHEK2, ATM, NBN, RAD51C, RAD51D). No individual presented more than one mutation.

Almost half of all deleterious mutations (42.5%) were detected in very young women aged 35 years or less. The median age at diagnosis was significantly younger for BRCA1 (40 years) and BRCA2 (41.5 years) carriers compared to patients without a mutation (p=2.746e-05; Mann-Whitney) or compared to non-BRCA1/2 mutation carriers (p=0.022). In contrast, patients with non-BRCA1/2 mutations were not significantly younger than mutation negative women (p=0.5288). Interestingly, family history had an independent influence on age at diagnosis. Taken as a whole, women with family history had a median age at diagnosis 6 years earlier than those without (p=0.00057). This difference was lost in mutation carriers while it remained in cases without mutation.

In summary, our data confirm and expand previous studies of a high frequency of germline mutations in genes associated with ineffective repair of DNA damage by homologous recombination in women with TNBCs. Many of these women would go untested with current restrictive criteria. In order that each patient receives therapies tailored to her genetic status, gene panel based mutation testing should be offered to all women diagnosed with TNBC, irrespective of age at diagnosis or family history.

*** Neural retina differentiation of hESCs as an in vitro model for retinoblastoma

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Retinoblastoma is the most common eye tumor of early childhood. Inactivation of both alleles of the retinoblastoma gene (*RB1*) results in the development of retinoblastoma. Our aim is to establish a human cell-based model for retinoblastoma. Using the CRISPR/Cas9 system we have generated human embryonic stem cells (hESCs) carrying a mutation either on one or both *RB1* alleles. All the detected mutations are located in exon 3 of the *RB1* gene and close to the splice donor site of this exon. Analyses on DNA, RNA and protein level were performed for three mutant and one double-mutant clone. The following genotypes were identified by deep sequencing (NM_000321.2(RB1_v001)): clone C2, c.364_380del, heterozygous; clones C7 and G3, c.372_378del, heterozygous; clone G4, c.[372_378del; c.367_368dup] (complex mutation on one allele), homozygous (loss of heterozygosity). The mutations of all four clones result in a premature stop codon in exon 4. On RNA level we detected expression of mutant *RB1* transcripts reflecting the genotype in all clones and an additional mutant *RB1* transcript with skipping of exon 3 in three clones. As the heterozygous clones also showed expression of the wildtype *RB1* transcript, RB1 protein (pRb) could be detected for these clones (C2, C7, G3) by western blot analysis. However, the double-mutant clone G4 showed no expression of pRb. So far,

we have characterized 3 heterozygous and one homozygous clone. Another three double-mutant clones are under investigation.

It has been shown that in 3D culture hESCs can be differentiated into neural retina containing organoids. We established this differentiation schedule and started comparative differentiation of wildtype H1 hESCs and the *RB1* null derivative (G4, *RB1*^{mt/mt}) into neural retina. During the first weeks of differentiation into neural retina organoids generated from the *RB1*^{mt/mt} hESCs have a smaller diameter and thinner retina layer compared to wildtype organoids. However, during the time-course the mutant organoids began to catch up. Thus, at later stages no difference in size and thickness could be observed anymore. Comparative immunostainings of cryosections at d19 show no difference in expression of the markers PAX6 and SOX2 between the wildtype and mutant hESCs. Further comparative immunostainings for markers specific for neural retina like e.g. RX and VSX2 at d19 and d33 are ongoing and will be presented.

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Exome sequencing identified potential causative candidate genes for unexplained Cowden syndrome

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Purpose: Cowden syndrome (CS) is a cancer predisposition syndrome characterized by the occurrence of breast cancer, epithelial thyroid cancer, endometrial carcinoma and various other findings such as mucocutaneous lesions and macrocephaly. CS belongs to the PTEN hamartoma tumor syndrome (PHTS) primarily associated with germline mutations in *PTEN*. In recent years, germline mutations in additional genes (*SDHB, SDHC, SDHD, PIK3CA, AKT1, SEC23B*) have been described in few patients; however, to date, in 20-75% of patients meeting clinical criteria for CS the underlying cause remains unclear.

Methods: To uncover predisposing causative genes, the exomes of 11 clinically well characterized, mutation negative patients with suspected CS were sequenced (Illumina HiSeq) using leukocyte DNA. Assuming a monogenic disease model, the called variants were filtered for rare (minor allele frequency ≤1% for homozygous/compound heterozygous variants and ≤0.01% for heterozygous variants according to dbSNP, EVS, and ExAC), truncating (nonsense, frameshift, highly conserved splice sites), and missense germline variants (predicted to be pathogenic by at least 2/3 in-silico tools). For data analysis and variant filtering the GATK software and the Cartagenia Bench Lab NGS Software were applied. All candidate genes were included in a Pathway Analysis (Ingenuity). In a first preliminary analysis, we focused on known cancer genes and genes interacting with *PTEN*.

Results: After stringent filtering steps, comparison with large datasets from population-based controls, and detailed manual inspection to exclude artifacts, 75 genes were affected by presumed biallelic variants (16 homozygous and 59 putative compound-heterozygous), one of these is a known cancer gene (*CBFA2T3*); in 17 genes biallelic variants were found in 2-6 patients. Heterozygous variants were found in 23 genes in 2-6 patients, but none of these are known cancer genes. In 132 genes, heterozygous truncating mutations occurred in only one patient, 4 of these are cancer genes (*MSH6, WRN, KDM5A, PML*). The phenotype of the patient with a *MSH6* frameshift deletion fulfilled key features of CS (early-onset metachronous papillary thyroid cancer, breast cancer, endometrial and colorectal cancer), however, the tumor spectrum is partly compatible with Lynch syndrome / HNPCC. Examination of the colorectal cancer demonstrated microsatellite instability and a loss of MSH6 protein expression. The pathway analysis of the remaining candidate genes identified several interacting partners of *PTEN* (*GRHL3, EHHADH, CSTF3*).

Conclusions: Preliminary data indicate that exome sequencing might identify potentially relevant causative genes for CS, some of which are recurrently mutated. The present work-up consists of the inclusion of further non-cancer genes, validation of variants by Sanger sequencing, testing of relatives to determine the phase of assumed biallelic variants and segregation with the phenotype where applicable.

Identification of a molecular signature for prognostic classification and individualized cancer therapy in adrenocortical carcinoma

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With 1 to 2 cases in 1 million inhabitants per year adrenocortical cancer (ACC) is a rare disease. Due to often late diagnosis and limited treatment options prognosis for patients are poor with a 5 year overall survival rate of 7 to 35%. Though knowledge about molecular genetic events in ACC increased over the last few years no reliable molecular prognostic factors, no effective targeted cancer therapy and no personalized treatment approach has emerged to date. That's why we intend to establish a reliable method to define a molecular signature of ACCs that could be used for a prognostic classification of adrenocortical cancers, for planning an individualized therapeutic approach and for the identification of known or potential targetable molecular events in the single patient.

In a retrospective study DNA from ACC and matched blood samples is sequenced to detect somatic single nucleotide variants (SNV), small insertions and deletions (InDel) and copy number alterations (CNV). Sequencing data are then compared to clinical data e.g. tumor stage, resection status, Ki67-index and time of progression free and overall survival to define molecular prognostic factors.

Target enrichment of 160 genes that are known to be associated with different entities of cancer is performed with the Human Comprehensive Cancer Panel (Qiagen) and sequenced on a NextSeq500 (Illumina). Data are analysed with GensearchNGS (PhenoSystems). *ZNRF3*, a gene that was also described to be involved in the development and the progression of ACC a few years ago, is sequenced separately with Sanger and analysed with Gensearch (PhenoSystems).

To date tumor samples and matched blood samples from 43 patients were analysed. One or more tumors comprise one or more SNVs or small InDels in 48 of 160 genes of the panel and in *ZNRF3*. SNVs and small InDels are most often found in TP53, CTNNB1 and *ZNRF3* with frequencies of 28%, 26% and 19% respectively. In 37 of 160 genes CNVs – duplications and deletions – occur. CDK4 is duplicated in over 50% of the cohort. *MDM2* gains are found in over 40%. One can also find three types of CN patterns: A quiet type with low number of copy alterations, a noisy one with high number of chromosomal breakages and a chromosomal one with high frequency of alterations of chromosomal arms.

While no correlation between SNVs and small InDels and clinical outcome could be found so far, CN patterns of the ACCs seem to correlate with progression free survival and overall survival. Patients with a noisy CN pattern have a shorter progression free and overall survival than patients with chromosomal and quiet type.

Though tendencies in the correlation of molecular markers and prognosis for patients suffering ACC can be recognized, further samples need to be analysed to confirm the results. It is planned to sequence another 60 tumor samples and matched blood samples for this retrospective study and to validate the results in a prospective study with another 100 patients.

The prostate cancer risk mutation G84E in HOXB13 is associated with TMPRSS2:ERG fusion negative adenocarcinoma

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The recurrent germline missense mutation G84E in the *HOXB13* gene has been demonstrated to predispose to hereditary prostate cancer (PrCa), despite the underlying pathogenic mechanism is not yet understood. Molecular examination of a first set of G84E positive tumors sought for somatic characteristics, and suggested that oncogenic ETS gene fusions may appear at unusually low frequencies as compared to the general prevalence of ETS fusions in PrCa (22 % vs approx. 50 %). Hypothesizing that *HOXB13* could predispose to ETS fusion negative PrCa, we have analyzed 942 cases from three European ancestry populations (Finland, Germany and US) for the coincidence of *HOXB13* G84E and the most common ETS fusion, *TMPRSS2:ERG*, in corresponding tumor samples. While the prevalence of *TMPRSS2:ERG* fusions was similar among the three study groups (range: 56.5% - 60.7%), the frequency of G84E genotypes differed markedly between US (1.5%), German (3.6%) and Finnish samples (8.3%). Despite the expected frequency gradient among study populations, all subsamples showed a strong enrichment of G84E mutation carriers among *TMPRSS2:ERG* fusion negative cases as compared to fusion positive cases (center adjusted OR = 4.96; 95%CI = 2.30 - 11.9; p = 0.0001). Consistent with the previous study, the crude frequency of the *TMPRSS2:ERG* fusion in *HOXB13* G84E carriers was 23.5 % (range 16.7 % - 28.6 %). Examination of disease characteristics highlighted age at diagnosis to be associated with *TMPRSS2:ERG* negative status (per year OR = 1.04, p = 0,00007) and by trend, also with the presence of the G84E germline variant (per year OR = 0.97, p = 0.14). Within the subtype of *TMPRSS2:ERG* fusion negative carcinoma carriers of G84E were diagnosed 3.5 years earlier as compared to non-carriers (61.6 ±1.4 years versus 65.1 ±0.4 years, p = 0.017). In conclusion, this study demonstrated a significant tumor subtype specific association for *HOXB13* G84E mutation carriers having a higher frequency of *TMPRSS2:ERG* fusion negative PrCa. Meta-analyses from case control comparisons suggested that subtype specific risk of *HOXB13* G84E for *TMPRSS2:ERG* negative PrCa could be as high as OR = 19.0, as compared to OR = 9.9, when PrCa is regarded as one entity regardless of fusion status. Finally, although *TMPRSS2:ERG* negative PrCa is usually known to be associated with later ages of diagnoses, *HOXB13* mutations may indicate a subgroup of earlier onset cases within the fusion negative entity.

Neuroendocrine tumor of the adrenal gland: an unusual manifestation of TSC

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We report on a young woman asking for the recurrence risk of the neuroendocrine tumor of her mother deceased at the age of 38.

Her mother clinically presented because of therapy-resistant hypertonia, dyspnoe, progressive edema in the legs and face and a caput medusae. MRI scan revealed a tumor (11x12 cm) in the right adrenal gland with lymph node metastases compressing the v. cava inferior and synchronous metastases in lung and liver. Laboratory examinations showed highly elevated levels of cortisol and adrenocorticotropin (ACTH). Cerebral

MRI was normal suggesting an ectopic ACTH secretion by a non-pituitary tumor. Histologically, an undifferentiated, largely necrotic tumor was described so that the neuroendocrine nature of the tumor could not be proven. She died within three weeks after diagnosis.

On suspicion of multiple endocrine neoplasia type 1 we initially performed a sequence analysis of MEN1 on tumor DNA by next generation sequencing without detection of a pathogenic mutation. Thereupon the molecular genetic panel analysis (NF1, RET, SDHB-D,TMEM127, TSC1, TSC2, VHL) uncovered the heterozygous mutation c.3379C>T (p.Arg1127Trp) in the gene TSC2. This mutation is already described as pathogenic (Hu et al. 2014). In the tumor DNA the allele frequency of the normal allele mounted up to 10%, whereas the allele frequency of the mutant allele came to 90% pointing to a loss of heterozygosity (LOH). The mutation was confirmed by Sanger sequencing. Taken all together, we assumed, that the mutation in the gene TSC2 represents a germline mutation.

Mutations in the suppressor genes TSC1 and TSC2 cause tuberous sclerosis, an autosomal-dominant disorder, resulting in hamartomatous tumors in the heart, brain, kidneys, skin and other organs. Once in a while it is discussed whether neuroendocrine tumors (NETs) represent a characteristic of TSC. There are some case reports describing NETs in the context of TSC, but mainly in connection with NETs of the pancreas (e.g. insulinoma) or the pituitary. To the best of our knowledge there exists only one case report of a bronchial carcinoid as a result of a germline mutation in TSC1 (Dworakowska et al. 2009) and no description of NET of the adrenal gland due to a mutation in TSC1 or TSC2. NGS provides the opportunity of wide-spread testing, even post-mortem, in order to get clarification for the descendants. Although in our case we could not distinguish if the mutation detected represents a germline mutation or a somatic mutation, we were able to offer a predictive testing to the daughter and other family members.

We report on a rare case of NET of the adrenal gland because of mutation in the gene TSC2.

This case illustrates that in the differential diagnosis of NETs, TSC genes should also be considered.

P-CancG-042

Does miR-371a-3p represent a valuable biomarker for the minimal-invasive detection of Germ Cell Neoplasia in situ?

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Germ cell neoplasia in situ (GCNis) is the precursor lesion of testicular germ cell tumours (TGCT). If detected clinically, this lesion may herald a pending TGCT. Unfortunately, the only way of diagnosing GCNis is by testicular biopsy and subsequent immunohistochemical examination. Therefore, non-invasive methods of diagnosis are required. miRNAs of the miR-371-3 and miR-302/367 cluster had been suggested as serum biomarkers of full-blown TGCTs. We aimed to explore the utility of these miRNAs for the detection of the pre-invasive stage of TGCTs and we looked to the expression of two miRNAs in serum samples of 27 GCNis patients.

Expression of miR-371a-3p and miR-367-3p was analysed in serum samples by quantitative PCR. The cohort of 27 GCNis patients consisted of 11 patients with a solitary testicle, who had undergone orchiectomy for contralateral TGCT, and 16 patients with two testicles, one of which with GCNis, but no concurrent TGCT. Twenty men with non-malignant testicular disease served as controls. Additionally, in situ hybridisation (ISH) with a probe against miR-371a-3p was performed on four testicular biopsy specimens known to harbour GCNis. Sequential step sections of the corresponding tissue blocks were analysed immunohistochemically, using Oct4 antibody to visualise GCNis.

The median expression value of miR-371a-3p in GCNis-patients was 5.2 (Interquartile range [IQR] = 35.8) which is significantly higher than the median expression of 0.0 (IQR = 0.0) in controls. Both of the two GCNis subgroups had significantly higher miR-371a-3p levels than controls, with a median expression of 18.2 (IQR = 37.3) and 2.7 (IQR = 32.7), respectively. Regarding miR-367-3p expression, there were no significant differences between GCNis and controls. Using a relative quantity of 5 as a cut-off value, the miR-371a-3p was able to detect 51.2% (95% confidence interval [95% CI] = 31.9 – 71.3%) of GCNis, while only 5% (95% CI = 0.1 – 24.9%) of the controls were positive. In the subgroup with previous TGCT 63.6% (95% CI = 30.8 – 89.1%) of GCNis could be detected and in the subgroup without previous tumour the rate was 43.8% (95% CI = 19.8 – 70.1%). The detection rates for all GCNis and for both subgroups were significantly higher than for the controls. ISH staining demonstrated the expression of miR-371a-3p in GCNis cells in two of the four cases.

In conclusion, this study indicates a new and minimal-invasive way of diagnosing GCNis by measuring serum levels of miR371a-3p. This approach is endorsed by the demonstration of miR371a-3p in GCNis cells by ISH staining. However, the sensitivity is still low and thus, the method certainly needs refinement possibly by applying a panel of additional microRNAs. Nonetheless, measuring serum levels of miR371a-3p may constitute a valuable aid in clinical assessment of men afflicted with high-risk factors of TGCT.

P-CancG-043

*** The miR-371a-3p is a highly specific and sensitive serum-based marker for the diagnosis and follow-up of testicular germ cell tumours

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Testicular germ cell tumours (TGCT) are a paradigm of curable malignancies. Clinical management largely relies on measuring the serum biomarkers. Inopportunely, the markers beta-HCG, AFP and LDH are only elevated in about 60% of patients. Therefore, microRNAs of the clusters miR-371-3 and miR-302/367 were proposed as novel serum-based markers. We evaluated four of the candidate miRNAs (miR-371a-3p, miR-372-3p, miR-373-3p and miR367-3p) with regard to their usefulness as TGCT markers.

Overall, serum samples from 166 TGCT-patients and from 106 controls were analysed using quantitative PCR. The first 50 consecutive patients and 20 controls were analysed for all four miRNAs. After ROC-analysis only the marker with the greatest discriminative power was studied further. The decline of miRNA expression after orchiectomy was quantified In 134 cases and in 27 metastasized cases the marker was analysed repeatedly during the course of chemotherapy. Additionally 10 cases with relapsing disease were studied.

The miR-371a-3p featured the highest discriminative power (area under the curve: 0.94; 95% confidence interval [95% CI]: 0.874 - 0.982). In the entire cohort, patients could be distinguished from controls with a sensitivity of 88.7% (95% CI: 82.5 - 93.3%) and a specificity of 93.4% (95% CI: 86.9 - 97.3%) with this marker. In patients without metastases the miR-371a-3p expression declined significantly after surgery. In metastasized cases the levels dropped sharply after chemotherapy. All of the 10 relapses had elevated miR-levels, and expression decreased upon chemotherapy. miR-371a-3p has significantly higher sensitivity than each one of the classical TGCT markers and than a combined panel of beta-HCG, AFP and LDH (87.8% vs 50.4%). In non-metastasised seminoma the miR-371a-3p expression depended significantly on tumour size.

miR-371a-3p is highly sensitive and specific for TGCT. It correlates with the stage of disease and with treatment effects and it therefore fulfils the prerequisites of a valuable serum-based biomarker. The significant association with tumour bulk in localised disease provides evidence for the TGCT being the primary source of miR expression. The sensitivity of miR-371a-3p surpasses that of classical TGCT markers by far, and thus it may become the new gold standard for serum diagnostics of TGCT in the coming years.

P-CancG-044

A novel MECOM missense mutation causes radioulnar synostosis with amegakaryocytic thrombocytopenia 2 (RUSAT2) and predisposes to myeloid leukemia

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Co-occurrence of radioulnar synostosis and amegakaryocytic thrombocytopenia (RUSAT) was initially described as an inherited thrombocytopenia syndrome that is caused by a mutation in *HOXA11*. In three simplex patients, de novo missense mutations in *MECOM* were reported as an alternative origin of the disease (RUSAT2). MECOM, identified as a common ecotropic viral integration site 1 (EVI1) in murine myeloid leukemia, is known as a key transcriptional regulator in hematopoiesis and sporadic myeloid leukemia. We report here on a novel MECOM mutation Cys766Gly (Uniprot Q03112-1) identified by whole exome sequencing in a family with RUSAT, hearing impairment, hand dysmorphisms, and patellar hypoplasia in four patients spanning three generations. Notably, two of four affected individuals in our family developed a myeloid malignancy. The novel MECOM missense mutation Cys766Gly affects a heavily conserved cysteine residue in C_2H_2-zinc finger motif 9 in the C-terminal zinc finger domain of MECOM. This residue is crucial for the tetrahedral coordination of a zinc ion stabilizing the zinc finger conformation and thus, is essential for DNA binding of the C-terminal zinc finger domain. Our findings reconfirm the causality of *MECOM* mutations and indicate that *MECOM* mutations also need to be considered in familial RUSAT patients. In addition, we report for the first time that *MECOM* germline mutations targeting the C-terminal zinc finger domain are associated

with an increased risk for myeloid malignancies. This extends the RUSAT2-associated phenotype and proposes that *MECOM* germline mutations can cause a genetic predisposition to myeloid malignancy.

(Z., B. and S., D. contributed equally to this work)

P-CancG-045

PSMC3IP germline variants in BRCA1/2 mutation-negative families with hereditary breast and ovarian cancer and early onset of cancer

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PSMC3IP located on chromosome 17q21 is a putative tumor suppressor gene that encodes for the nuclear PSMC3 interacting protein. The protein functions as coactivator of steroid hormone mediated gene expression and is important for RAD51 and DMC1-mediated homologous recombination during DNA repair of double-strand breaks. Recently germline variants in *PSMC3IP*, also known as *GT198*, *TBIP*, and *HOP2*, have been identified with low frequency in early onset familial breast and ovarian cancer (HBOC) patients and in a patient with apparently sporadic early onset breast cancer. Somatic variants in *PSMC3IP* are frequently observed in breast, ovarian, and fallopian tube cancers.

In this study, we analyzed a cohort of 166 *BRCA1/2* mutation-negative HBOC (n=158) or early onset sporadic breast cancer patients (n=8) for variants in *PSMC3IP*. We identified seven different heterozygous variants in 8 out of 166 index patients: c.-115G>A (rs191843707); c.-70T>A (rs752276800); c.-37A>T (rs199620968); c.-24C>G (rs200359709); c.519G>A p.(Trp173*); c.537+51G>C (rs375509656); c.*24G>A. These variants were not listed or at very low frequency (<1%) in the ExAc database. Carriers of *PSMC3IP* germline variants were mostly (6/8) affected by early onset breast cancer (median age of onset 36 years). For three out of seven different variants (c.-115G>A, c.519G>A, and c*24A>G), a possible impact on *PSMC3IP* expression or function was observed. The stop mutation c.519G>A p.(Trp173*) was found in two sisters, which were both diagnosed with unilateral breast cancer at age 33. The premature stop codon is located within the DNA-binding domain of PSMC3IP and is predicted to induce nonsense-mediated mRNA decay (NMD). Remarkably, c.-115G>A was already described in familial breast and ovarian cancer, and was found once in this study in a female that developed unilateral breast cancer at the age of 33 years. The variant c.-115G>A (rs191843707) was shown to induce a slightly, albeit significant decrease of reporter gene expression. The c.24*G>A variant was identified in a woman diagnosed with unilateral breast cancer at the age of 36 years. Luciferase reporter assays indicated an impaired effect of c.24*G>A on microRNA binding.

Germline variants in *PSMC3IP* are present in breast and ovarian cancer families. Whether mutated *PSMC3IP* is a new risk factor for early onset breast/ovarian cancer in families with HBOC and/or apparently sporadic early onset breast cancer remains to be shown.

P-CancG-046

Inherited DNA Repair Mutations: Are they Modifiers of BRCA1 and BRCA2 Penetrance and Age at Onset of Hereditary Breast and Ovarian Cancer?

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BACKGROUND: Inherited mutations in BRCA1 and BRCA2 are the most common causes of hereditary breast and ovarian cancer (HBOC). The risk of developing breast cancer by age 70 in women carrying a BRCA1 mutation is 57-65% and 45-55% in BRCA2 carriers. However, mutations in BRCA1 and BRCA2 only explain about 25% of all HBOC cases. The lifetime risk varies between families and even within affected individuals of the same family. The cause of this variability is unknown but it is hypothesized that additional

mutations or rare variants in genes that are possibly interacting with BRCA1/2 in different DNA-repair pathways contribute to this phenomenon.

METHODS: We obtained samples of 181 patients positive for BRCA1 or BRCA2 mutations and an age-of-onset (AOO) of breast cancer below 35 or above 60 years of age from the German Consortium for Hereditary Breast and Ovarian Cancer. Panel sequencing was done to screen germline DNA for mutations in 311 genes involved in different DNA-repair pathways. Variants were classified into five classes according to a modified version of Plon et al (2008). Only truncating mutations and known pathogenic missense mutations were considered pathogenic or likely pathogenic.

RESULTS: The patient group with an early AOO (93 women) had developed breast cancer at a mean age of 26.4 years (± 2.06) and the control group (88 women) had developed breast cancer at a mean age of 69.5 years (± 7.05). A total of 4,293 variants were detected in all patients and 54 of these (1.3%; 95% CI, 0.96%-1.64%) were presumed to be deleterious. Mutations were found in 45 genes other than BRCA1 and BRCA2. Mutations were mainly found in single-strand break repair (SSBR 29%), double- strand break repair (DSBR 26%) and checkpoint factors (13%). The rest were found in genes with other functions such as BRCA1/2 interactors, centrosome formation, and signal transduction. The putative mutations were found in 26 women of the control group (29.5%; 95%CI, 20.3%-40.2%) compared to 36 women of the patient group (38.7%; 95% CI, 28.8%-49.4%). The incidence of germline mutations in DNA-repair genes did not differ according to the age of onset (P=0.2). Prevalence of additional germline mutations in DSBR in patients (13%) was not significantly different from prevalence of DSBR mutations in controls 10.2% (P=0.6)

CONCLUSIONS: The preliminary results failed to show a difference in mutation load between the two cohorts of BRCA1/2 carriers sorted by age of onset. Larger studies are needed and may provide further insight into the role of mutation load in HBOC age of onset of BRCA1/2 carriers.

P-CancG-047

A low frequency haplotype spanning SLX4/FANCP is associated with early-onset breast cancer and reduced DNA repair capacity

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Many genes that harbor rare mutations which entail a medium or high risk for breast cancer (BC) belong to DNA double strand repair and have been identified by linkage analysis or by sequencing of candidate genes in BC families. In addition, a considerable number of common but low risk germline variants have been found in genome wide association studies. However, these predisposing factors yet only explain a fraction of BC cases.

With the intention to identify low frequency variants conferring an intermediate BC risk, we performed an association study using a candidate gene approach and testing DNA repair capacity with the micronucleus test (MNT) as an additional second phenotype.

rs3810813 in the SLX4/FANCP gene showed an association with BC that was pronounced in younger cases and was confirmed in a verification cohort (combined analysis of 1,448 cases, 2593 controls: OR=2.19 (1.45–3.30), p=0.00012 for cases ≤40 years). Genotyping additional SNPs and imputation revealed a specific European haplotype of ca. 150kb length that spans SLX4 and adjacent genes. It is tagged by 6 SNPs, of which 5 are in high linkage disequilibrium (D' >0.98, R^2 >0.8) and have identical minor allele frequencies (0.05), thus obscuring the exact localization of a causal variant.

The pattern of association is very similar for both phenotypes, BC and MNT, and covariate analysis revealed a significant interaction between BC and MNT (p=0.024). The strength of both associations was increased for younger age groups [<60 years: BC: OR=2.6(1.6-3.9), p=1.6×10-5; MNT: beta=37.8(17.9-57.8) p=5.3×10-4); <40 years: BC: OR=4.7(2.1-10.4) p=2.4×10-5; MNT: beta=53.2(17.1-89.3) p=0.023].

The observed mutual dependence of the two phenotypes allowed a considerably improved interpretation of the results: (i) the unknown causal variant on the haplotype can be assumed to be present mostly in cases, indicating a rare variant with a rather strong effect; (ii) using this information on the two phenotypes in the association between the MNT results and BC improved considerably the identification of the specific risk among cases (<60 years) who carried the haplotype. ROC curves for BC depending on MNT results revealed that the stratification on carriers of the haplotype increased the AUC from 0.65 (p=0.0007) to 0.94 (p=0.0001). Both associations can be best explained by a risk variant carried by a fraction of the haplotypes that is enriched

in early onset BC cases. SLX4 is the only gene in the tagged region which can be functionally related to both associated phenotypes, while for the other genes no connection to BC or DNA repair is reported.

P-CancG-048

Somatic mutations in Fanconi anemia associated myeloid neoplasms

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Background: Fanconi anemia (FA) is a rare inherited chromosomal instability syndrome associated with bone marrow failure as well as myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). One-third of FA individuals exhibit bone marrow cytogenetic clones, notably gains of 1q and 3q and/or loss of 7/7q, and 15% to 30% of FA patients developed MDS/AML. In recent years, the application of high throughput technologies has revealed recurrent somatic mutations in genes implicated in myeloid malignancies. As additional genetic maladies facilitating MDS/AML development in FA is lacking, we aimed to elucidate whether these mutations would be present in FA patients with MDS/AML.

Methods: Using Illumina TruSightTM Myeloid Sequencing Panel (San Diego, CA), we performed next-generation sequencing (NGS) on DNA extracted from bone marrow specimens from 17 FA MDS/AML patients registered in the European Working Group of Childhood MDS. The sequencing panel targeted 54 genes frequently mutated in hematologic neoplasms.

Results:. Ten of the 16 (62.5%) evaluable patients had 18 lesions (1 to 3 mutations per patient; 14 missense, 1 nonsense, 2 insertion and 1 duplication) in 13 genes. The presence of a somatic mutation did not appear to correlate with complex karyotype or -7/7q. All affected genes occurred in isolation with exception of RUNX1 and KRAS. While 13 of the mutations were pathogenic, 5 were variance of unknown significance. Mutations in genes involved in epigenetics (DNA methylation, chromatin maintenance and cohesin complex; IDH2, TET2, DNMT3a, IDH1, EZH2, RAD21 and ASXL1) and mutations in transcription factor genes (RUNX1, IKZF1 and ETV6) represented the most frequently affected genes. This was followed by mutations of genes encoding signaling molecules including the RAS pathway (KRAS and PTPN11). Altered RUNX1 was the most common lesion and occurred in individuals with AML, RAEB or RAEB-T. One patient with refractory anemia with ring sideroblasts (RARS) had a mutation in the spliceosomal gene, SF3B1.

Conclusions: While the most common mutations encountered in sporadic cases of MDS were in genes involved in RNA splicing and epigenetics, these two broad categories of genes appeared to have less influence in our FA patients. Most mutations were nonrecurring suggesting that there is no specific mutation pattern of these genes in FA-related MDS/AML. However, RUNX1 mutations and also mutations involved in genes of the RAS pathway appear to play a pathogenic role in FA MDS/AML development. Taken together, the data suggests that mutations in genes that cause clonal hematopoiesis in the population at large do not contribute significantly to FA hematopoietic clonal disease; however, particularly acquisition of RUNX1 and RAS pathway alterations promote malignant myeloid disease progression. More extensive studies analyzing more patients are necessary to further define the secondary hits leading to FA myeloid disease.

P-CancG-049

Isolation of GSCs and comparison between GSCs and glioblastoma tissue using SNP-array and gene expression analyses

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Objective

Glioblastoma stem-like cells (GSCs) carry stem cell features and therefore seem to be responsible for tumor initiating, maintaining, recurrence and chemo- and/or radiotherapy resisting. The current knowledge on

genetic and transcriptomic characteristics of these cells especially in comparison to glioblastoma tissue is still limited. The aim of this study is to compare the genetic and genomic profile of glioblastoma tissue and GSCs. Thereby, differences in involved genes and affected pathways on DNA level as well as on gene expression level are identified.

Material and methods

Peripheral Blood and tumor tissue were obtained from patients with glioblastoma. Tumor tissue derived explant cell culture and serum-free culture were established. Based on multi-parameter magnetic-activated cell sorting (MACS) technique, CD15 and CD133 labeled cell subpopulations of GSCs could be isolated. The tumor tissue, serum-free culture, and the isolated cell subpopulations as well as blood were analyzed by SNP array and gene expression (excluding blood) in a paired design. For preliminary characterization of GSCs in the serum-free culture we confirmed the stem cell features of GSCs by the expression of Nestin, SOX2, and CD133 (applying immunofluorescence staining).

Results

Our results of SNP array analyses showed genetic aberrations in all analyzed cellular entities (tumor tissue and cell subpopulations, e.g. gain of chromosome 7, loss of 10q23.31, loss of 10q11.1->q26.3, and complete loss of chromosome 10). Furthermore, distinct genetic differences between the cell subpopulations and tumor tissue were observed (e.g. loss of chromosome 4 and segmental uniparental disomy of 9p24.3->p21.3, only in cancer stem- like cell subpopulations). In addition, we detected many possibly candidate cancer genes and pathways which may have an influence on tumorigenesis. Gene expression analyses revealed strongest differences between fresh tumor tissue and serum-free culture based cells, where more than a third of investigated genes were affected. When contrasting fresh tumor tissue with stem cell marker positive serum-free cultured cells, 1,106 genes were upregulated in the stem cell marker positive cells, whereas 1,533 genes where upregulated in fresh tumor tissue. Within these genes, strongest enriched pathways in stem cell marker positive cells included positive regulation of cell cycle and cancer-related pathways, whereas in fresh tumor tissue predominantly immune-related pathways were found, e.g. myeloid leukocyte activation, inflammatory response and phagocytosis.

Conclusion

Differences between GSCs and tumor tissue using SNP array analyses and gene expression were detected. Our results may help to get more information about the molecular pathomechanisms of glioblastoma. It still needs more investigations on the field of genetic and genomic analyses between GSCs and glioblastoma tissue to identify novel potential targets for therapy development.

P-CLINICAL GENETICS, GENETIC COUNSELLING AND PRENATAL DIAGNOSIS

P-ClinG-050

*** Major impact of targeted NGS results on the clinical management of growth retarded patients referred as Silver-Russell syndrome

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Due to its heterogeneous etiology, primordial growth retardation is often a challenge for geneticists and clinicians in respect of diagnosis, therapy and prognosis. Thus, pinpointing its genetic origin is required for a personalized treatment and prognosis. One syndrome mainly characterized by intrauterine and postnatal growth is Silver-Russell syndrome (SRS), a clinically and molecularly heterogeneous disorder with a considerable overlap with other syndromes. In only 60% of patients with the characteristic SRS phenotype the diagnosis can be confirmed molecularly, but 40% of cases remain without molecular diagnosis. In fact, in clinically less well characterized patients referred for diagnostic testing, the detection rate is less than 20%. However, systematic investigations on the contribution of mutations in genes which may be considered in the differential diagnoses of SRS are still missing. We examined 60 patients referred for molecular testing of SRS but without molecular alterations associated with SRS by NGS. A targeted NGS approach comprising 26 genes implicated in the differential diagnoses of SRS or suggested as SRS candidate genes was performed. In 5 patients fulfilling the criteria of SRS accordingly to our recently developed clinical scoring system, disease-causing variants were found. These patients carried mutations in genes associated with Bloom syndrome, Mulibrey nanism, KBG syndrome, SHORT syndromes or IGF1R-associated short stature, respectively. Indeed, some of the differential diagnoses detected in our cohort have a major impact on clinical management, including cancer screening because of a high risk for tumor development. Furthermore, we did not identify any

pathogenic mutation in one of proposed SRS candidate genes (e.g. MEST, GRB10, COPG2), thus raising the question whether these genes are indeed involved in the etiology of SRS. We show that a (targeted) NGS approach is an important tool to identify the genetic cause in patients with unexplained growth retardation. Furthermore, our data show (positive) clinical scoring in SRS should not impede the consideration of differential diagnoses and other molecular causes.

P-ClinG-051

Heterozygosity for loss-of-function mutations in ARID2 cause Coffin-Siris syndrome

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Chromatin remodeling is a complex process shaping the nucleosome landscape, thereby regulating the accessibility of transcription factors to regulatory regions of target genes and ultimately managing gene expression. The SWI/SNF (switch/sucrose nonfermentable) complex remodels the nucleosome landscape in an ATP-dependent manner and is divided into the two major subclasses Brahma-associated factor (BAF) and Polybromo Brahma-associated factor (PBAF) complex. Somatic mutations in subunits of the SWI/SNF complex have been associated with different cancers, while germline mutations have been associated with autism spectrum disorder and the neurodevelopmental disorders Coffin-Siris (CSS) and Nicolaides-Baraitser syndromes (NCBRS). CSS is characterized by intellectual disability (ID), coarsening of the face and hypoplasia or absence of the fifth finger- and/or toenails. So far, variants in five of the SWI/SNF subunit-encoding genes ARID1B, SMARCA4, SMARCB1, ARID1A and SMARCE1 as well as variants in the transcription factor-encoding gene SOX11 have been identified in CSS-affected individuals. ARID2 is a member of the PBAF subcomplex, which until recently had not been linked to any neurodevelopmental phenotypes. In 2015, mutations in the ARID2 gene were associated with intellectual disability. In this study, we report on two individuals with private de novo ARID2 frameshift mutations. Both individuals present with CSS including ID, coarsening of facial features, other recognizable facial dysmorphisms and hypoplasia of the fifth toenails. Hence, this study identifies mutations in the ARID2 gene as a novel and rare cause for CSS and enlarges the list of CSS-associated genes.

P-ClinG-052

*** Identification of new TRIP12 variants and detailed clinical evaluation of individuals with non-syndromic intellectual disability with or without autism

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The ubiquitin pathway is an enzymatic cascade including activating E1, conjugating E2, and ligating E3 enzymes, which governs protein degradation and sorting. It is crucial for many physiological processes. Compromised function of members of the ubiquitin pathway leads to a wide range of human diseases, such as cancer, neurodegenerative diseases, and neurodevelopmental disorders. Mutations in the thyroid hormone receptor interactor 12 (TRIP12) gene (OMIM 604506), which encodes an E3 ligase in the ubiquitin pathway, have been associated with autism spectrum disorder (ASD). In addition to autistic features, TRIP12 mutation carriers showed intellectual disability (ID). More recently, TRIP12 was postulated as a novel candidate gene for intellectual disability in a meta-analysis of published ID cohorts. However, detailed clinical information characterizing the phenotype of these individuals was not provided. In this study, we present seven novel individuals with private TRIP12 mutations including two splice site mutations, one nonsense mutation, three

missense mutations, and one translocation case with a breakpoint in intron 1 of the TRIP12 gene and clinically review four previously published cases. The TRIP12 mutation-positive individuals presented with mild to moderate ID (10/11) or learning disability [intelligence quotient (IQ) 76 in one individual], ASD (8/11) and some of them with unspecific craniofacial dysmorphism and other anomalies. In this study, we provide detailed clinical information of eleven TRIP12 mutation-positive individuals and thereby expand the clinical spectrum of the TRIP12 gene in non-syndromic intellectual disability with or without ASD.

P-ClinG-053

Identification of multiple single gene disorders in one family by WES – a challenge from clinical and diagnostic perspective

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Background
Whole exome sequencing (WES) using next generation sequencing has proven to be a powerful tool in determining the underlying genetic cause of rare disorders. Here, we show, that clinical follow-up and diagnostic re-evaluation can be crucial for uncovering further disease-causing mutations.

Clinical report and genetic findings
We report follow-up data of a previously published consanguineous family with two children, a boy and a girl, suffering from severe encephalopathy, hypotonia, microcephaly and retinal dystrophy. WES had shown a homozygous intronic splice variant in *PGAP1* (c.1090-2A>G;p.?) causative for the symptoms. Both parents were heterozygous carrier for the *PGAP1* variant (Granzow, Paramasivam et al, Mol Cell Probes 2015). In the next pregnancy, the unborn child presented hydrops fetalis, omphalocele, short tubular bones and cystic kidneys. Chorionic villus sampling showed the fetus to be homozygous for the *PGAP1* variant. However, neither of these symptoms fit with a *PGAP1*-associated disorder. Additional WES of fetal DNA and re-evaluation in the family showed a homozygous nonsense variant in *IFT140* (c.G3577T;p.E1193*) consistent with a diagnosis of Mainzer-Saldino syndrome (MSS) which is characterised by the association of renal disease, retinal pigmentary dystrophy, cerebellar ataxia and skeletal dysplasia, as a second diagnosis in the fetus. Again, both parents were shown to be a heterozygous carrier for the *IFT140* variant. Yet, as omphalocele was not accounted for by any of the identified conditions, a third genetic cause cannot entirely be excluded. Alternatively, omphalocele may be a rare manifestation of MSS, or be the result of a combination of both disorders. The couple opted for induced abortion.

Discussion
It is estimated that an individual carries multiple heterozygous variants for autosomal recessive disorders in his or her genome. Especially in consanguineous families, this results in an elevated risk for children with more than one disorder. In recent publications of clinical exomes, double diagnoses have been reported in 0 to 12% of investigated subjects. Thus, the possibility of more than one causative gene should be carefully explored when working with WES and re-evaluation in case of additional clinical symptoms within a family should be considered. Also, follow-up of families with rare genetic disorders may lead the clinical geneticist beyond the assumed single cause to multiple single gene disorders in the same family.

Conclusion
Using WES, we have identified two independent single gene disorders in a consanguineous family demonstrating that clinical follow-up and diagnostic re-evaluation can be crucial for uncovering multiple disease-causing mutations in one family.

P-ClinG-054

De novo mutations of MYT1L in individuals with intellectual disability

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Submicroscopic deletions of chromosome band 2p25.3 have been reported in more than 20 patients. Common clinical features include intellectual disability /developmental delay, central obesity and behavioural difficulties. *MYT1L* became the main candidate gene for ID and obesity since it is deleted or disrupted in all published patients. However, reports of deletions affecting only this gene and even more so of deleterious *MYT1L* sequence variants are very rare. To our knowledge, until now only two patients with *de novo MYT1L* point mutations have been reported.

In the present study, we analysed a cohort of individuals with intellectual disability of unknown aetiology and their unaffected parents by whole exome sequencing. We identified *de novo MYT1L* sequence variants in two out of 311 patients. Patient 1 carried a nonsense mutation (c.1531G>T, NM_015025.2; Gly511*) whereas patient 2 carried a direct splice site mutation (c.2769-2A>G). According to prediction algorithms, both detected *MYT1L* variants are deleterious (patient 1: SIFT score 0, CADD score 42; patient 2: CADD score 24.6). In addition, patient 2 carried a de novo splice site variant in *SETD1B*. However, this variant is predicted to be benign (CADD score 2.5) as well as a known SNV (rs749218728, MAF 0.0000323).

A comprehensive clinical characterisation of the two patients yielded only mild or moderate intellectual disability, behavioural problems and muscular hypotonia as common clinical signs. Surprisingly, obesity was only present in patient 2. Postnatal tall stature and transient microcephaly were present in one patient each. This clinical picture is compared to the published phenotypes of patients with *MYT1L* point mutations, patients with microdeletions of only *MYT1L* and patients with larger 2p25.3 deletions. With the reduced penetrance regarding obesity, the clinical picture of patients with *MYT1L* mutations is becoming more and more unspecific.

P-ClinG-055

Familial microphthalmia associated with a novel homozygous RAX mutation

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The retina and anterior neural fold homeobox gene (RAX) controls the embryonic eye development and is involved in human autosomal-recessive microphthalmia. So far only a few compound heterozygous mutations in RAX have been described in microphthalmia patients. We report a first case of microphthalmia caused by a novel homozygous mutation in RAX. The 8-month-old patient was born to consanguineous parents and presented with extreme microphthalmia, panhypopituitarism and developmental delay. MRI of the brain showed bilateral agenesis of the anterior visual pathway and tractus opticus. Ocular ultrasound confirmed bilateral anophthalmia. Additionally, dysgenesis of the corpus callosum and an abnormal pituitary gland have been detected. The first child of these parents, who died shortly after birth, had also been diagnosed with bilateral anophthalmia. Using panel diagnosis of the disease associated genome, we identified the homozygous pathogenic variant c.112del, (p.138fs) in RAX. We performed a segregation analysis and confirmed that both parents are heterozygous for this variant. So far developmental delay and panhypopituitarism have not been described in association with RAX mutations. Therefore we conducted array comparative genomic hybridization and karyotyping in the index patient. Both tests gave normal results. Prenatal diagnosis by chorionic villus sampling in the next pregnancy excluded a homozygous carrier status for this RAX mutation.

P-ClinG-056

Borjeson-Forssman-Lehmann Syndrome in Females – a case report

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Mutations in the PHF6 gene are associated with Borjeson-Forssman-Lehmann syndrome (BFLS), an X-linked intellectual disability disorder affecting mainly males. Female carriers usually show no or mild clinical

signs. However, recent studies described females with de novo PHF6 gene defects (mutations, deletions) and severe phenotypes resembling Coffin-Siris syndrome (Zweier et al. 2013, Di Donato et al. 2014). Here, we report on a girl with a maternally inherited PHF6 mutation and a phenotype resembling those described previously in affected females. The mother had learning difficulties and mild dysmorphological features (hypertelorism, prominent forehead).

When seen at age 12 months, the proposita showed muscular hypotonia, was unable to sit and had limited head control (developmental delay 6 months). Dysmorphic features included scaphocephaly, hypertelorism, a small flat nose with anteverted nares, low set, prominent ears, a high, narrow palate, absent labia minora and linear skin pigmentation on the thighs. Ophthalmologic investigation identified strabism convergens of the left eye, hyperopia and an excavated papilla with a pale optical nerve. Ultrasound showed patent foramen ovale, tricuspid insufficiency and a unilateral incomplete duplication of the renal pelvis.

Karyotyping performed elsewhere was normal (46,XX). Results of a genome-wide SNP array analysis (Affymetrix CytoScan HD) were also normal. Using a targeted NGS approach for syndromic and non-syndromic developmental delay encompassing over 1200 brain related genes (MPIMG-1-Test), we identified a heterozygous LOF-mutation c.88C>T (p.Gln30*) in the PHF6 gene (encoding PHD finger protein 6). In addition, a human androgen receptor (HUMARA) assay using blood DNA showed a highly skewed X-inactivation (91:9). Segregation analysis indicated a maternal origin of the variant. The mother also had skewed X-inactivation in blood. Her husband and other daughter tested normal for the c.88C>T variant.

Here, we describe the first female patient with a maternally inherited PHF6 mutation and a severe phenotype. The mild phenotype in the mother might be due to different patterns of X-inactivation or to (undetected) mosaicism. This report represents the 12th case of a severely affected female patient with a PHF6 gene defect. Findings support the assumption (Zweier et al. 2013) that the female phenotypes of BFLS might be more common than previously estimated.

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P-ClinG-057

Pitfalls in diagnosis of Kleefstra Syndrome in a consanguineous child

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Kleefstra syndrome (KS) is an autosomal dominant disorder characterized by hypotonia, developmental delay, and distinctive facial appearance (microcephaly, brachycephaly, microglossia). Other common features include congenital heart and urogenital defects, epilepsy, microcephaly, and behavioral abnormalities. Deletions ranging from 0.04Mb to cytogenetically visible abnormalities in 9q34.3 usually account for 80% of cases [1].

We present a case study using molecular cytogenetic approaches on a 3 year old boy presenting with microcephaly-brachycephaly, macroglossia and absent speech development. The boy is the first child of healthy, consanguineous parents of Pakistani origin. Following an uncomplicated pregnancy, the hypotrophic newborn was delivered at 37 weeks weighing 2610g. In the third month of life, the baby had viral meningitis. Regular pediatric follow-up revealed psychomotor delay with hypotonia. Creatine kinase, lactate and fibroblast growth factor 21 measured in serum were high. Subsequent investigations at the age of 16 months included brain MRI, electroencephalogram and muscle biopsy that gave hints of a mitochondriopathy or potential neuropathy of axonal type.

Due to the suspicion of a complex mitochondriopathy, whole exome sequencing was performed using a SureSelect Human All Exon Kit (Agilent, 50Mb V5) on a HiSeq 2500 (Illumina). The analysis revealed a heterozygous microdeletion of 121 kb on chromosome 9q34.3 which was classified as an unclear variant (UV), EHMT1-gen was not affected. Re-examination of proband's DNA using array CGH detected a larger 180 kb heterozygous deletion in the 9q34.3 region (arr[hg19] 9q34.3(140,395,510-140,575,736)x1), encompassing exon 1 of EHMT1 (euchromatin histone methyltransferase 1; transcript NM_ 024757.4). Haploinsufficiency of this gene results in Kleefstra syndrome (OMIM 610253), a multisystem disorder due to either microdeletions in 9q34.3 encompassing EHMT1 or intragenic point mutations. MLPA analysis (EHMT1 MPLA-Kit P340) of parental DNA further indicated a de novo origin of the deletion in our proband. A similar deletion has been described previously in a case presenting with clinical features of Kleefstra syndrome [2] strengthening the importance to include the 5' part of EHMT1 in sequencing as well as CNV screening.

In summary, our study clearly shows that array CGH is a valuable complementary approach to NGS especially for poorly covered regions in NGS i.e. exon 1 of many transcripts.

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P-ClinG-058

A novel IFIH1 mutation in a family with Singleton-Merten syndrome and neurological manifestations

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We report on a three-generation family with variable manifestations including delayed and incomplete tooth eruption, early tooth loss due to short dental roots, acroosteolysis, osteoporosis, tendon ruptures, joint hypermobility, muscle weekness, glaucoma, neurological features, and psoriasis. After detection of elevated CD169/Siglec1-expression on monocytes and an upregulation of interferon-stimulated gene transcripts, Singleton-Merten syndrome was diagnosed. The novel heterozygous mutation c.992C>G (p.Thr331Arg) in IFIH1 was found in three affected family members. Singleton-Merten syndrome is a very rare autosomal dominant interferonopathy, so far described in not more than four families. Until now, only two different gain-of-function mutations in IFIH1 have been detected. Mutations in IFIH1 are also associated with Aicardi-Goutière syndrome and recently features of both conditions were found in the same family. Our findings expand the mutational spectrum of Singleton-Merten syndrome and demonstrate the high intrafamiliar variability associated with mutations in IFIH1.

P-ClinG-059

Identification of genetic defects in pulmonary arterial hypertension by a new next generation sequencing based panel diagnostic

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Background: Pulmonary arterial hypertension (PAH) is a rare autosomal dominant lung disease with reduced penetrance. In this study we developed a new PAH specific gene panel including major disease genes and further candidates. This next generation based diagnostic approach provides more comprehensive data than the current diagnostic procedure of Sanger sequencing only the three major genes (*BMPR2, ACVRL1, ENG*).

Methods: We included 37 patients with invasively confirmed PAH by right heart catheterisation and 5 relatives of further affected patients for genetic testing. A new PAH-specific gene panel was designed to enrich genes of interest. We used next generation sequencing to assess the coding sequence and intron/exon boundaries of 12 known disease genes and 17 candidate genes. Any potential pathogenic variants were reassessed by direct Sanger sequencing.

Results: Twenty-two of the 37 patients (59%) had a mutation in the gene *BMPR2, ACVRL1, ENG* or *EIF2AK4* identified by panel and Sanger sequencing. In addition, 12 unclassified variants were identified in 7 genes (known and candidate genes).

Conclusions: This new PAH-specific gene panel allowed for the first time the assessment of all known PAH genes and further candidates at once and markedly reduced overall sequencing time. The technique enables the identification of mutations in different genes within the same patient, which might act as modifiers increasing disease penetrance and accelerating PAH manifestation. Thus, this approach is about to change the routine diagnostic genetic testing in PAH patients.

Stroke as initial manifestation of ADA2-deficiency

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Homozygous or compound heterozygous mutations in CECR1 (cat eye syndrome chromosome region, candidate 1) have recently been identified to causing deficiency of adenosine deaminase 2 (ADA2; DADA2) with childhood polyarteritis nodosa (PAN) (omim # 615688). This inflammatory vasculitis affects the skin, and inner organs (predominantly kidneys and gastrointestinal tract) and also shows a high risk of ischemic stroke, brain hemorrhage as well as peripheral neuropathy. Using whole-exome sequencing it was also found that the six adult patients (aged 20-48) described by Sneddon in 1965 (Sneddon syndrome, omim # 182410) likewise carried compound heterozygous CECR1 mutations. Sneddon syndrome is characterized by a combination of dermatologic features (livedo racemosa) and ischemic brain infarctions. Recently, clinical and genetic data of more than sixty ADA2 patients have been reviewed and underlined the wide clinical variability in age at onset, clinical findings, outcome of neurological involvement, and additional hematological symptoms. Typically, stroke has been reported to follow systemic inflammatory disease and predominantly affects posterior and central brain areas. Here we describe one of the rare patients in whom acute mesencephalic stroke preceded systemic inflammation and presented as initial clinical symptom. Symptoms typical for ADA2 deficiency such as fever, livedo racemosa, abdominal colics, arthralgias, and Raynaud´s phenomenon were observed months later. CECR1 sequencing (NM_001282225.1) revealed two previously described pathogenic missense mutations: c.140G>C, p.(Gly47Ala) and c.506G>A, p.(Arg169Gln). Compound heterozygosity was confirmed by parental analysis. To the best of our knowledge, this combination of mutations has not been described until now. The p.(Arg169Gln) is considered as founder mutation in the Dutch population, but first phenotype-genotype analyses did not allow further prediction of clinical outcomes.

ADA2 deficiency should be considered in patients with childhood stroke despite the absence of systemic inflammation and cerebral vasculitis.

HADH mutation analysis in children with congenital hyperinsulinemic-hypoglycemia

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Congenital hyperinsulinism (CHI) has been described as heterogeneous entity caused by at least 9 different genes. In 1991, Tein et al. first described a defective activity of L-3-hydroxyacyl-CoA dehydrogenase in a 16-year old patient with hypoketotic hypoglycemic encephalopathy. Biochemical markers of L-3-hydroxyacyl-CoA dehydrogenase deficiency (SCHAD) are high 3-OH-glutarate excretion in urine and C4-OH-carnitine in plasma. The clinical presentation is very heterogeneous with regard to age of onset, severity of symptoms as well as response to medical treatment and leucine-sensitivity. In some patients even a near total pancreatectomy was performed. SCHAD dependent hyperinsulinism (HHF4) is a rare autosomal recessive disorder that is caused by mutations in the gene HADH. Here we describe 4 patients from 3 unrelated families out of a cohort of 136 CHI patients mainly from Central Europe.

Patients 1 and 2 are siblings from unrelated parents. The older brother manifested with hypoglycemic convulsions at the age of 5 weeks. A subtotal pancreatectomy was performed in an outside academic hospital. In further course he developed epilepsy and has been treated with diazoxide and anticonvulsants. The 2nd child was born with hypoketotic hypoglycemia and CHI was diagnosed in first days of life. Diazoxide treatment stabilized blood glucose and both children were referred to our pediatric endocrinology at the age of 8 years and 10 months, respectively. Mutational analysis revealed the homozygous variant c.547-3C>G within the region of the splice acceptor site in intron 4 of the HADH gene in both affected children. This change is neither registered in ExAC nor described in the mutation databases HGMD or in the literature and was predicted to disrupt proper splicing. We then completed mutational analysis in unidentified patients of our CHI cohort with diazoxide responsiveness and known or suspected consanguinity. Patient 3 was born to consanguineous parents and CHI manifested in the girl at neonatal age with hypoketotic hypoglycemia. She was successfully treated with diazoxide. Later, she developed convulsions and statomotoric developmental delay. The homozygous splice mutation c.636+471G>T in intron 5 of HADH was detected in the child. The parents were identified as heterozygous carriers. In patient 4, a girl born to consanguineous Turkish parents, CHI manifested

at the age of 6 months with hypoglycemic seizures. She responded well to diazoxide treatment. A homozygous missense mutation (c.406A>G; p.Lys136Glu) in exon 3 of HADH was detected in the patient and her parents were heterozygous carriers. HADH mutations in case 3 and 4 have been previously described in probands of Turkish descent and appear to be founder mutations in the Turkish population.

In conclusion, we recommend HADH mutation analysis to be considered in CHI children with unknown cause and known consanguineous pedigrees or originating from populations with higher prevalence of consanguinity.

P-ClinG-062

Practical experience of non-invasive prenatal diagnosis – More false positive results of sex chromosome aneuplodies than expected

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Since the introduction of non-invasive prenatal diagnosis (NIPD, e.g. Harmony® test) in 2013, this test is frequently demanded and routinely applied in prenatal centers and medical practices.

Mostly, NIPD is intended to detect autosomal trisomies (13, 18, 21), but also offers the possibility to analyze sex chromosomes. Therefore, also sex chromosome aneuploidies (SCA) (e. g. monosomy X (Turner syndrome), triple X, XXY (Klinefelter syndrome), XYY) are incidentally found.

So far, in our prenatal center SCA were detected in 7 pregnancies by Harmony® test, consisting of three pregnancies with monosomy X (Turner syndrome) and two pregnancies with Klinefelter syndrome (XXY). Triple X and XYY were detected one time each.

Of the three cases with suspected monosomy X, the diagnosis of Turner syndrome could only be confirmed in one case. This fetus also had a hydrops at week 10+0. For the other two fetuses, the chromosomal analysis of amniotic fluid revealed normal female karyotypes (46,XX).

In both cases with suspected Klinefelter syndrome, this diagnosis could be disproved by amniocentesis (karyotype 46,XY). In the pregnancies with assumed Triple X and XYY, the true fetal karyotype was not further determined yet.

From our experience, the rate of false positive results concerning the sex chromosome aneuploidies is noticeably higher than reported in two studies of Nicolaides 2014 and Hooks 2015. This has to be strongly considered in the counselling of patients who wish to know the fetal sex by NIPD.

P-ClinG-063

Congenital myopathies and congenital muscular dystrophies: Will genetic testing replace muscle biopsy in the near future?

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Congenital myopathies and muscular dystrophies are a group of inherited neuromuscular diseases with early onset and broad genetic and histopathological overlap. The diagnostic approach has considerably changed with next generation sequencing methods available. Here, we describe the diagnostic value of genetic and histological methods in a cohort of 117 index patients and hence the efficacy of diagnostic procedures.

78 of 117 patients had a muscle biopsy as a first-tier approach. In 54 of 78 patients muscle biopsy was informative, leading to a classification in subgroups of CM or CMD. However, in only a few of these cases biopsy led to a specific diagnosis (e.g. merosin deficiency). In 55 of 78 patients genetic testing (candidate gene sequencing or NGS) was performed additionally to muscle biopsy as a second-tier diagnostic step, while 39 patients of the whole cohort received genetic testing only. In almost two-thirds of these 94 patients genetic testing identified known pathogenic or most likely pathogenic variations. These findings illustrate that genetic testing is superior to muscle biopsy in accurately diagnosing CM or CMD.

In conclusion, we suggest that invasive muscle biopsy should be replaced by genetic testing as first-tier diagnostic procedure in patients with clinical signs of CM or CMD.

P-ClinG-064

Discrepant findings in uncultured and cultured amniotic cells suggest aberrant triploidy rescue in a growth retarded fetus with malformations

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Triploidy is a recurrent finding in prenatal diagnostics. In a small number of individuals, correction of triploidy has been suggested based on the finding of (mosaic) genome-wide uniparental disomy (UPD).

We here investigated uncultured und cultured amniotic cells (AC) and placental tissue from a fetus, in which ultrasound examination in the 17+0th week of gestation revealed growth retardation, left diaphragmatic hernia with parts of stomach and bowel localized in the chest, dextrocardia, short nasal bone and single umbilical artery. These findings were confirmed at the 18+1th week when the pregnancy was terminated. The pregnancy was conceived spontaneously by a 28-year-old mother and a 31-year-old father, both healthy and with uneventful family history. The parents were non-consanguineous and carry a normal karyotype. Microsatellite analyses of uncultured AC obtained at initial presentation showed for chromosomes 13, 18 and 21 a pattern suggesting triploidy with only biallelic presentation. While Y-chromosomal sequences were lacking the X-chromosome showed, unexpectedly a rather disomic pattern. Metaphase yield on cultured AC was low but showed a mosaic karyotype 48,XXX,+10[20]/47,XXX[17], which was confirmed for several chromosomes by interphase FISH. Remarkably, a triploid clone was cytogenetically not detected. Thus, we performed further analyses using microsatellite markers, OncoScan technology and FISH. These studies unraveled in uncultured AC a pattern suggestive of triploidy with the supernumerary chromosomal complement derived from a maternal isodisomy with the notable exception of the X chromosome. In cultured AC and placental tissue for all chromosomes, except X and 10, a diploid pattern was observed with alleles from both parents identical to those in the uncultured AC. Trisomy X was confirmed in both tissues with the supernumerary chromosome X being of paternal isodisomic origin. The trisomy 10 was seen only in cultured AC, and likely represents a pseudomosaic which nevertheless could not be proven due to insufficient yield of mitoses from the parallel cultures. Finally, retrospective interphase FISH on remnant uncultured AC showed two diploid clones, one disomic (approximately 40% of nuclei) and one trisomic (60%) for the X chromosome.

The most likely explanation for the findings is a mosaicism for one diploid clone with genome-wide maternal isodisomy and a second diploid but bi-parental cell line with paternal trisomy X. Given the identity of the (maternal) alleles in both clones our findings suggest that originally a triploid clone due to a maternal division error/ inclusion of a polar body II existed which underwent (erroneous) triploidy rescue resulting in one diploid biparental clone and one haploid clone of maternal origin that underwent haploid rescue resulting in genome-wide maternal isodisomy. The biparental clone with trisomy X either resulted from a sperm with two X-chromosomes or an erroneous X-duplication during trisomy rescue.

P-ClinG-065

Identification of a spastic ataxia of Charlevoix-Saguenay (SACS) patient by next generation sequencing (NGS): Pitfall of Sanger sequencing

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Spastic ataxia of Charlevoix-Saguenay (SACS) is an autosomal recessive neurodegenerative disorder and is caused by homozygous or compound heterozygous mutations in the SACS gene. First symptoms of SACS are walking difficulties due to unsteady gait. Further typical clinical features include spasticity, ataxia, pyramidal tract signs, nystagmus and dysarthria.

Here, we report on a 16-year-old female patient who initially presented with disturbances in motor abilities including frequent falls and high arched foot. Cranial MRT was normal while nerve conduction velocity was significantly reduced. The patient´s parents did not show any clinical features.

Since no PMP22 duplication was detected we performed a gene panel including 64 genes that are associated with hereditary motor and sensory neuropathies (HMSN) and related disorders by using targeted next generation sequencing. We identified the two heterozygous stop mutations c.9305T>A (p.Leu3102Ter) and c.9305dupT (p.Leu3102Phefs*8), located at the same position in the SACS gene. Sanger sequencing did not enable us to properly display that there is a transversion and a duplication of the same nucleotide at two different alleles. This exemplifies that, in contrast to Sanger sequencing, NGS can illustrate both alleles separately.

To conclude, this case was only resolvable by NGS which makes this method appropriate for the detection of compound heterozygous mutations, especially in the rare event when two mutations occur at the same position.

Characterization of aberrant mRNA-Splicing in Pompe disease by NMD inhibition

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Background The precise identification and characterization of genetic variants in monogenic diseases has a wide influence on diagnosis and therapy. About 10 % of pathogenic variants are splicing variants. Due to the complex mechanism of splicing regulation it is difficult to predict the effects of variants on mRNA splicing. Possible consequences are exon skipping, intron retention, generation of novel splice sites or the utilization of a cryptic splice site. Common consequences are a frame-shift and the generation of premature termination codon. This leads to RNA degradation via the nonsense mediated decay (NMD) pathway.

In a patient with the clinical symptoms of non-classical infantile Pompe disease and a confirmed acid alpha-glucosidase (GAA) deficiency, we detected two novel, exonic variants in the GAA gene. Both base pair exchanges suggested either an amino acid exchange or a splice defect as consequences. However, conventional investigation of the leucocyte mRNA of the patient and his parents was inconclusive. Degradation of the respective mutated RNA by NMD was suspected.

We developed an approach in order to characterize novel splicing mutations in a simple and non-invasive manner.

Material and method Isolated blood lymphocytes from patient and his parents were cultured in standard leucocyte medium supplemented with different concentrations of the NMD inhibitors ocadaic acid, anisomycin, and wortmannin for 24 h. Cells were harvested and RNA was isolated. The reverse transcribed cDNA was amplified in allele specific PCRs and qPCR assays.

Results Compared to the non-stimulated lymphocyte controls nonsense mediated RNA decay was inhibited by anisomycin. The consequences of aberrant RNA splicing were detectable: The maternal mutation results in exon skipping, the paternal mutation in intron retention. Furthermore NMD inhibition increases the amount of GAA-RNA in patient's lymphocytes as well as in the cells of his parents. The residual function of the resulting protein has to be investigated.

Discussion and conclusion RNA analysis in lymphocytes with and without NMD inhibition is a simple method for analysing splice defects in all monogenic disorders with expression of the disease causing gene in lymphocytes. A further advantage for the patient is the use of blood cells instead of fibroblasts, because a skin biopsy can be avoided and analysis times are reduced.

The exact characterization of pathogenic variants is an important aspect of diagnosis, prediction of disease severity and genetic counselling. In vitro NMD inhibition in lymphocytes of affected patients allows the characterization of splice defects. In the future successful inhibition of NMD in vitro might help to identify patients, who may profit from a therapeutic intervention with NMD inhibitors. Even expression of a partial protein with low or no activity reduces the risk for the patient to develop antibodies hampering enzyme/protein replacement therapy.

12q14 Microdeletion Syndrome: a family with short stature and Silver-Russel (SRS)-like phenotype.

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Introduction: The Silver-Russel syndrome (MIM 180860), first described independently by Silver and Russell in 1953, is a condition with intrauterine growth retardation, postnatal growth failure and other characteristic features, including relative macrocephaly (defined as a head circumference at birth ≥1.5 SD score (SDS) above birth weight and/or length SDS), prominent forehead, body asymmetry and feeding difficulties as recently defined in an international consensus statement.

Patients and Methods: We report here on 3 first degree relatives with a Silver-Russel syndrome phenotype who presented with prenatal- and postnatal growth retardation, feeding difficulties, a prominent forehead and a failure to thrive. Additional features such as dysmorphic facial features, periodically increased sweating, and scoliosis were present in one of the family members only, whereas learning problems and cardiac arrhythmia were present in one other. None of the patients had relative macrocephaly.

High resolution array-CGH was performed to screen for CNCs and MLPA to confirm the array-CGH result.

Results: No hypo-methylation of the imprinting center on 11p15 nor uniparental disomy of chromosome 7 and 14 were found in the index-patients. High-resolution array-CGH identified a 12q14.3 microdeletion of 1.67 Mb (arr[GRCh37 12q14.3(65,863,186–67,528,640)×1). The heterozygous loss was confirmed by MLPA in the index patient and the other two affected family members (i.e. her brother and mother). The deletion includes the genes HMGA2, LLPH, TMBIM4, IRAK3, HELB, GRIP1, and the pseudogene RPSAP52.

Conclusion: To the best of our knowledge this is the first report on familial presentation of a Silver-Russel syndrome due to a microdeletion in 12q14.3. None of the patients had relative macrocephaly. Supporting the hypothesis by Takenouchi et al. that the causative gene for relative macrocephaly resides centromeric to HMGA2, the region centromeric of HMGA2 is not included in the deletion in our family.

P-ClinG-068

Genotype-Phenotype correlation -The many facets of heterogeneous hearing loss in the context of molecular epidemiology

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Non-syndromic hearing loss (NSHL), with presently around 100 associated genes, is one of the most genetically heterogeneous disorders constituting nearly 70% of genetic deafness with a predominantly recessive inheritance pattern. Thirty percent of hearing loss (HL) can be connected as a part of over 600 distinct syndromes. Next Generation Sequencing (NGS) technologies have revolutionized pathogenic variant identification. Different strategies enhance pathogenic variant detection supporting detailed HL investigation to overcome the many ambiguities associated with clinical heterogeneity. Detection of the disease causing variant in correlation with the phenotype can be challenging in small families, in situations with ambiguous clinical histories and allelic heterogeneity.

Using a clinical and whole exome sequencing approach, we tested over 130 probands as part of a multicentre Iranian and German genetics of HL study that included 29 probands primarily with sporadic or dominant HL in a parent-child or parent-sibling trio context. The majority of these probands were pre-screened for defects in GJB2 and STRC. Libraries were prepared using TruSight One and Nextera Rapid Capture Exome enrichment and sequenced using the MiSeq and NextSeq 500 desktop sequencers (Illumina). Analysis was performed using GensearchNGS and an in-house exome analysis pipeline. Around 40% of cases were resolved from phenotype matching and segregation analysis. Interestingly, the fraction of resolved cases was much higher in our Iranian cohort (>50%) compared to our German cohort (>30%) which may be attributed in part to increased consanguinity in the Iranian families. We observed likely disease causing variants in syndrome-associated genes including EYA1 causing branchio-oto-renal syndrome, a phenotype that was retrospectively confirmed by acquisition of additional clinical information. With few exceptions, we observed a diverse collection of affected genes in probands from our German collected cohort. Contrastingly, the Iranian cohort revealed frequent mutations in MYO15A and OTOF. Furthermore, co-segregation of variants in MYO6 and TECTA, with expected dominant HL phenotype, was a hindrance overcome by extensive segregation testing. Familial locus heterogeneity was also observed by mutations in CIB2 and SLC26A4 segregating in different branches of the same extended pedigree. Success in the identification of disease causing variants in known HL genes is contingent upon analysis strategy, clinical information and opportunity for segregation testing. The ability to retrospectively connect an already apparent syndromic phenotype to a syndrome-associated gene without prior knowledge is a powerful application of comprehensive analysis that is not restricted to NSHL genes. This work provides an improved understanding of population-specific genetic epidemiology of hereditary HL and highlights the challenges in defining genetic causes in a highly heterogeneous disorder such as HL.

P-ClinG-069

Biallelic COL3A1 mutations results in autosomal recessive Ehlers-Danlos syndrome with additional neurological manifestations

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Vascular Ehlers-Danlos syndrome (type IV) is considered to be an autosomal dominant disorder caused by heterozygous mutations in COL3A1, which are missense or splice site variants in about 95 % of cases. We here report on a three-year-old female of non-consanguineous parents born with bilateral clubfoot as well as dysmorphic facial features, joint laxity, and mild contractures of finger joints. Developmental delay became evident. After trauma at 2 years of age she developed brain haemorrhage. MRI diagnosis at this age revealed an additional frontal aneurism as well as frontoparietal polymicrogyria. We identified novel compound heterozygous COL3A1 mutations: The nonsense mutation c.1282C>T (p.Arg428*) and the c.2057delC (p.Pro686Leufs*105) frameshift mutation leading to a premature stop codon. Further studies showed that the mutations were inherited from each parent who had no features for Ehlers-Danlos syndrome type IV. Only two other families have been reported so far with recessive mutations of this gene and a severe vascular phenotype and polymicrogyria. Biallelic mutations of COL3A1 seem to be accompanied with a significantly worse outcome compared with heterozygous mutations and polymicrogyria is an additional phenotypic feature.

P-ClinG-070

Recurrent Postzygotic Mosaic Mutation in the KRT10 Gene in Patients with Epidermolytic Epidermal Nevus

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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).

Here we describe five patients with epidermolytic epidermal nevi in different degrees of severity with the mosaic mutation c.466C>T (p.Arg156Cys) in *KRT10* gene. The same mutation has previously been described in patients with EI (Bygum et al. 2013). We analyzed DNA from peripheral blood and/or skin biopsies from affected and unaffected skin with Next Generation Sequencing (NGS) and Sanger sequencing methods. Using NGS we found this mutation in blood in mosaic states ranging from 6% to 23%. The mosaic could only be confirmed by Sanger Sequencing in the patient with the highest mosaic frequency of 23%. In four of our patients we investigated skin biopsies from affected and unaffected skin. It is noteworthy, that only one of four patients showed the mutation in heterozygous state of 50% in the affected skin, whereas the other patients presented a mosaic state also in the affected skin. To exclude a recurrent sequencing artefact at this position, we examined 100 control patients for this mosaic mutation using NGS. In none of these patients we found the same DNA change.

Patients with epidermolytic epidermal nevi have a higher risk to have children with a full-blown EI phenotype. Our results show the importance of NGS as the method of choice to explore the molecular genetic basis of epidermolytic epidermal nevi. Strikingly, all our patients carry the same mosaic mutation c.466C>T in *KRT10*. We suggest that this position is a hotspot for postzygotic mutations in *KRT10*.

P-ClinG-071

Prospective Evaluation of Predictive Huntington Testing: Why do at-risk individuals decide in favor of or against predictive DNA testing and how do they cope with the consequences?

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Huntington's disease (HD) is a rare autosomal dominant neurodegenerative disorder caused by expanded CAG repeats as diagnosed via direct DNA analysis. For asymptomatic individuals, predictive testing (PT) can facilitate life planning and diminish uncertainty, but it is also associated with substantial social and psychological challenges.

We present a prospective case series of counselees seeking predictive HD testing at the Huntington Centre North-Rhine Westphalia (Bochum, Germany) between 2010 and 2012. The international protocol including several pre-test sessions was followed throughout. The aim of this study was to prospectively follow the decision-making process of individuals at risk in our centre and explore their experiences following the decision as well as the impacts of mutation test results by means of standardized questionnaires and a semi-standardized telephone interview one year after the initial counselling session.

72 individuals participated in at least one of the three phases of the survey, including 31 individuals for the telephone interview. In our cohort, almost all interviewees reported a balanced emotional state one year after initial counselling, regardless of the decision for or against the test. The most important motivations for a decision in favor of PT were the ability to plan private life and to eliminate uncertainty. The most important motivations against PT were the fear of an increasing risk for others (e.g. offspring) and the fear to obtain an unfavorable HTT mutation result, followed by the considered, willful decision for "wanting to not know". Furthermore, we identified evidence for gender-specific aspects in decision-making in line with and expanding our previous observations.

This study represents one of the few comprehensive prospective evaluations regarding decision-making and coping strategies related to predictive testing for Huntington's disease. We submit that gender-related aspects should be heeded in genetic counselling during the predictive testing and counselling processes. Our findings could serve as a basis for more extended prospective evaluations with higher numbers of participants and longer follow-up intervals.

P-ClinG-072

Severe microcephaly and primordial dwarfism in a girl with Seckel-like phenotype - first case of a PLK4 deletion

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Background:

PLK4 (Polo-like kinase 4) has been designated as "master regulator" of centriole assembly. Complete loss of PLK4 is lethal in mice, whereas biallelic PLK4 mutations with some retained function have been described in a few patients with microcephaly, growth failure and retinopathy (MCCRP2, OMIM #616171). This is a heterogeneous entity overlapping with MCPH (primary microcephaly) and Seckel syndrome. During the last years several new genes have been discovered associated with this spectrum.

Clinical report and genetic findings:

We report on a 4 year old female patient with intellectual disability, primordial dwarfism (-6,9 SD), most severe disproportionate microcephaly (-15,6 SD), corneal clouding, myopia, teeth abnormalities and dysmorphism. Panel diagnostic by next generation sequencing for primary microcephaly, including all known genes for Seckel syndrome, was unremarkable. Microarray analysis (Affymetrix® CytoScan HD) revealed a heterozygous 65 kb deletion, spanning the PLK4 gene. This deletion was confirmed by MLPA (multiplex ligation-dependent probe amplification) analysis. Subsequent sequence analysis of the PLK4 gene showed a variant of unknown significance on the second allele. In silico analysis of this variant indicated a significant decrease of the relative splice efficiency at the splice donor site. RT-PCR analysis confirmed altered splicing, resulting in a predominant loss of exon 11 of the transcript and predicting truncation of the PLK4 protein. Interestingly, a residual wild-type transcript was also detectable in patient RNA, implying that this variant effects

splicing only partially. By analysis of the parents, the splice variant and the large deletion were proven to be compound heterozygous.

Discussion:

Up to now, only a few patients with PLK4 mutations have been described in the literature. The phenotype comprises primary microcephaly, primordial dwarfism and chorioretinopathy (MCCRP2). To our knowledge, we describe the first case of a PLK4 heterozygous whole gene deletion and at least partial biallelic inactivation of the gene, therefore expanding the genetic background of this disorder. Furthermore, we give a detailed phenotypic description of a further individual with PLK4 alterations. The girl does not show retinopathy so far. While generalised retinopathy was discussed to be one of the most prominent distinctive features between MCCRP2 and primary microcephaly/Seckel syndrome, we consider PLK4 rather to be a further candidate gene pointing towards Seckel syndrome.

Additional investigations on centriole function in patient-derived cells are in progress.

P-ClinG-073

Family analysis of patients with pathogenic variants in mitochondrial genes MT-ATP6 and MT-ATP8

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Pathogenic variants of mitochondrial DNA cause a wide range of severe congenital disorders with maternal inheritance and a high transmission risk for female carriers. We report on eight families with an index case presenting with the common pathogenic variant m.8993T>G (p.Leu156Arg) in the MT-ATP6 gene in virtually homoplasmic form. In five families the mutation was detectable in peripheral blood from the mother in heteroplasmic form. In three families with a sporadic case of Leigh syndrome the mutation was not detectable in peripheral blood (or urinary or buccal cells) from the mother, possibly indicating a de novo event. Furthermore, one family presented with a de novo nonsense mutation in the gene MT-ATP8, which was present in peripheral blood of the index case in about 70 % and was not detectable in the mother or the unaffected sister.

Two female carriers with a heteroplasmy level of 50 % asked for prenatal testing. Both pregnancies showed an apparently homoplasmic load of the mutation.

P-ClinG-074

Mutations in LZTFL1 (BBS17) may be associated with a severe renal phenotype

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Bardet Biedl Syndrome (BBS) is a ciliopathy that is characterized by obesity, retinal dystrophy, postaxial polydactyly, renal anomalies, male hypogonadism and learning disabilities. Because of the delayed onset of symptoms, the diagnosis is often established during late childhood. However, in some cases renal morphological changes detected by ultrasound may resemble those usually seen in autosomal recessive polycystic kidney disease (ARPKD). However, histologically BBS-kidneys differ distinctly from other polycystic disorders by cystic orientation, localisation, extension, structure and size. 21 BBS genes have been identified to date. Mutations in BBS2, BBS4, BBS6 and BBS10 have been found to cause an antenatal presentation of BBS that may in some aspects mimic Meckel Gruber Syndrome (MKS). There is increasing evidence that BBS, at least in some families, shows an oligogenic mode of inheritance with three mutations at two BBS loci.

Yet, only three patients in two families with BBS caused by mutations in LZTFL1 (BBS17) have been reported. Their diagnosis was established in childhood and all patients had mesoaxial polydactyly as a distinct manifestation. In contrast to previous LZTFL1 cases, in our family the diagnosis of ARPKD was suspected sonographically at 27 weeks gestation (wg). Pregnancy was terminated at 30 wg. Autopsy revealed postaxial polydactyly of both hands, enlarged spongy kidneys, hemivertebra T6 and some features of Potter's sequence. Histological examination of the kidneys showed multiple, not radially oriented thin walled cysts, internally lined by thickened PAS-positive basement membranes and microcystic dilatation of collecting ducts. Cystic changes were accentuated in the renal medulla. Corticomedullar differentiation was mainly preserved. The tentative diagnosis was BBS. Fetal DNA was investigated using a next generation sequencing panel which included 17 known BBS causing genes. Hereby a heterozygous nonsense variant (NP_065080.1: p.Glu260*) inherited from the mother and a heterozygous missense variant (p.Glu92Lys) inherited from the father of the LZTFL1

gene were identified. Furthermore a maternally inherited heterozygous missense variant of unknown clinical significance in BBS4 was detected (NP_149017: p.Pro443Ala).

Our case shows for the first time that mutations in LZTFL1 can lead to a severe prenatal presentation of BBS due to profound renal manifestations with a kidney histology that is not considerably milder but distinct from that observed in MKS. It is not clear to which extent the BBS4 variant may act as a disease modifier. This may challenge genetic counselling and prenatal diagnosis in a further pregnancy. Furthermore our case shows that mesoaxial polydactyly is not always present in BBS patients with LZTFL1 mutations and further studies are necessary to establish the frequency of mesoaxial polydactyly and other genotype phenotype correlations for BBS patients with LZTFL1 mutations.

P-ClinG-075

*** Targeted next-generation sequencing analysis in couples at increased risk for autosomal recessive disorders

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Genetic childhood disorders leading to prenatal, neonatal or early childhood death are genetically heterogeneous. Many follow autosomal recessive or X-linked modes of inheritance and bear specific challenges for genetic counselling and prenatal diagnostics. Parents are carriers but unaffected and diseases are typically very rare but with recurrence risks of 25 % in the same family. Often, affected children (or fetuses) die before a genetic diagnosis can be established, post-mortem analysis and phenotype descriptions are insufficient and DNA material of affected fetuses or children is not available for later analysis. A genetic diagnosis showing biallelic mutations or mutations on the X-chromosome in male fetuses or children is, however, the requirement for targeted carrier testing in parents, risk calculations, and prenatal and preimplantation diagnostics in further pregnancies.

We employed targeted next-generation sequencing (NGS) for carrier screening of autosomal recessive lethal disorders in 8 consanguineous (C) and 5 non-consanguineous (NC) couples with one or more affected children. We searched for heterozygous variants (non-synonymous coding or splice variants as well as CNVs) in parents' DNAs in a set of 430 genes linked to rare autosomal recessive diseases with poor prognosis. We then compared couples and filtered for variants present in genes overlapping in both partners. Putative pathogenic variants were tested for co-segregation in affected fetuses where material was available and in unaffected siblings. Out of eleven couples of Mediterranean and Arabian ancestry (C:8, NC:3) and two non-consanguineous couples of European ancestry, we found five cases (5/13, 38%, C:4, NC:1) with both parents being heterozygous carriers of rare potentially deleterious variants in one or more overlapping genes. In four of these couples the underlying genetic cause for pre- or early postnatal child death could be established, in two of the families the diagnosis was confirmed by homozygous detection of the parental variant in the available DNA of the affected child. In a consanguineous couple with pathogenic variants for a severe autosomal recessive disorder identified in both parents, the molecular diagnosis for their child that had died at 5 months of age could not be established. Out of 9 couples in whom no causative diagnosis could be achieved 4 consented to undergo further WES analysis.

Identified variants are now used for preimplantation and prenatal diagnostics in all four families in which a causative diagnosis was established. Our data show that NGS based gene panel sequencing of selected genes involved in lethal autosomal recessive disorders is an effective tool for carrier screening in parents and for the identification of recessive gene defects in families that have experienced early child death and / or multiple miscarriages.

P-ClinG-076

Variable presentation of TAF1-related intellectual disability in a German family

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Recently an X-linked syndrome with maternally inherited or de novo mutations in *TAF1* was described with global developmental delay, intellectual disability (ID), delayed speech, characteristic facial dysmorphology, generalized hypotonia and variable neurologic features (MRXS33, MIM: #300966, XLR). There have been only three publications of 14 unrelated families, 11 with single-nucleotide changes and 3 with gene duplications including *TAF1* (Kaya et al., 2012; O'Rawe et al., 2015; Hu et al., 2016). We identified a German family in which two brothers (21 and 5 years) showed severe intellectual disability, absent speech and understanding, and hypotonia but different neurologic and behavioral phenotypes.

Besides severe ID the older brother also had postnatal short stature (-3 SD), a severe Lennox-Gastaut epilepsy and a neurodegenerative course. The younger brother showed autistic behavior and lost his very

limited skills at age 3 to 3.5 years. Both showed mild dysmorphic features (prominent supraorbital ridges, sagging cheeks, long philtrum, long face, thin upper lip, and high-arched palate), oropharyngeal dysphagia and generalized hypotonia. A gluteal crease with a sacral caudal remnant described as a characteristic feature was not seen in our case, and hearing impairment, microcephaly, dystonic movements or tremor were not observed either.

The family history was highly suggestive of X-linked inheritance with an affected maternal uncle, a maternal aunt with multiple miscarriages, and two aunts with learning disability. Since a previous analysis of 107 known X-linked mental retardation genes had not revealed the cause in the older brother, we used a targeted NGS approach (MPIMG1-test: >1200 brain related genes) for the analysis of the younger brother. Following enrichment a 300 bp paired end sequencing was carried out on an Illumina MiSeq system with >95% of target covered >20-fold (Hu et al, 2014). Only *TAF1* fitted the X-linked model and the phenotype. The unreported hemizygous sequence variant c.2833G>A (p.Asp945Asn) in exon 18 of *TAF1* was deemed pathogenic. It affected a highly conserved residue in the central „DUF3591" domain, where 4/11 previously described mutations had clustered. Segregation analysis confirmed hemizygosity in the older brother and heterozygosity in the mother with completely skewed X-inactivation (100:0).

In vivo functional modeling of *TAF1* has already provided evidence for an effect on a neuronal phenotype. The phenotype in patients can be reminiscent of Rett syndrome, but with milder regression and normal movements lacking a specific stereotypic pattern (no hand wringing). While severe neurodegeneration has been described in duplications, the present probands clearly showed developmental regression associated with a missense mutation. The analysis of further family members is pending. Our case adds to the phenotypic spectrum of X-linked syndromic mental retardation type 33.

P-ClinG-077

Targeted enrichment sequencing was successfully applied to identify Greenberg dysplasia as cause of fatal anomalies in one fetus of a dizygotic twin pregnancy.

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In the first pregnancy of a 29 year old Kosovarian woman and her 36 year old husband during the 16th week of gestation one of her dizygotic twins showed a severe skeletal dysplasia with all long bones extremely shortened and partially bended. The thorax was short and narrow. In addition a ventriculomegaly of the brain and an increased nuchal translucency was noticed. A very bad prognosis was expected and an achondrogenesis was suspected clinically. The other twin appeared to be normal. An amniocentesis was performed to potentially identify the genetic basis of the disorder. QF-PCR to rule out common trisomy's and cytogenetics revealed normal results and following a normal Agilent 60k array CGH analysis as a next diagnostic step next generation sequencing by using the TruSight One gene panel focusing on three genes including SLC26A2, TRIP11 and COL2A1 was performed. Since no pathogenic mutation was found by this approach, a more extended bioinformatics study was initiated. By filtering out common variants in the more than 4800 genes of the panel in our own database or in the ExAC- and 1000 genome databases our search was extended to genes with rare homozygous or compound heterozygous variants. By this strategy it was possible to reduce the potentially causative gene mutations dramatically and among those remaining genes for known very severe skeletal phenotypes just in the LBR gene the homozygous missense mutation c.1639A>G, p.Asn547Asp was identified in our fetus. Since this particular mutation is already known to be pathogenic leading to the lethal Greenberg dysplasia (Clayton et al., Nucleus. 2010) the diagnosis could be achieved in the affected fetus of the pregnancy of our patient still before completion of the 23rd week of gestation. Both parents were found to be heterozygous for this mutation in the LBR gene. Recently it was shown that different mutations of the very same gene can also lead to less severe forms of bone dysplasia. The couple was informed about our results and possible consequences were discussed and offered. The couple however came to the decision not to draw any consequences. Both fetuses especially the affected one were well documented sonographically including in a series of 3D images. In the 27th gestational week during a sonographic investigation the affected fetus did not show cardiac function and an oligohydramnios was found. Since development of the second non affected fetus was still within the normal range, we hope the now single pregnancy will carry on normal until birth. From our finding we would propose that our chosen strategy is straightforward and can be applied in a wide range of pregnancies to identify various up to severe and fatal single gene disorders associated with sonographic anomalies within a few weeks which should provide substantial benefits for these families.

Novel DNM2 mutation in a patient with late-onset intermediate Charcot-Marie-Tooth disease

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Charcot-Marie-Tooth disease (CMT), also commonly called as hereditary motor sensory neuropathy, is the most common monogenetic disease of the peripheral nervous system with significant clinical and genetic heterogeneity. The main clinical manifestations of CMT include progressive distal muscle weakness and atrophy, impaired distal sensation, depressed tendon reflexes and high-arched feet. Based upon electrophysiological and histopathological features CMT can be divided into predominantly demyelinating or axonal forms. An intermediate form also exists characterized by evidence of both, demyelinating and axonal, impairments. Genetically CMT can be caused by mutations in over 40 genes, including the DNM2 gene encoding dynamin 2 protein, a large GTPase primarily involved in receptor-mediated endocytosis and membrane trafficking. Only a small number of mutations in DNM2 causing CMT have been described so far.

We report the case of a 48-year-old man presenting with backache, ataxic gait and distal muscle weakness of the lower limb considered to be a consequence of the pre-diagnosed disc prolapse 3 years ago. Over the past months bilateral progressive weakness of ankle dorsiflexion, foot drop and tingling paresthesia in stocking distribution have occurred. Neurological examination disclosed depressed tendon reflexes of the upper and lower limbs. Neurophysiologic investigations revealed an axonal sensible polyneuropathy with normal distal motor latencies and nerve conduction velocities. The sural nerve biopsy indicated single unmyelinated or thinly myelinated axons, loss of myelinated nerve fibers, numerous clusters of regenerating fibers without onion bulb formations suggesting an intermediate form of CMT.

By using Next Generation Sequencing (NGS) and a multi-gene panel, consisting of 751 inherited neurological disease-associated genes, we identified a heterozygous missense mutation c.439G>A, p.Asp147Asn (rs370086632) in the DNM2 gene. This particular mutation is located in a highly conserved nucleotide region encoding the catalytic N-terminal GTPase domain. This evidence suggests a pathogenic phenotype caused by the described mutation, which is being underlined by the following facts: Public 1000 Genome database covering harmless variants of the human genome does not report it. Additionally, the ExAC Browser with exome sequencing data of >60,000 unrelated individuals, lists the mutation and shows a low allele frequency of 0.000008243, corresponding to one known heterozygous mutation carrier. Other online prediction tools like the Mutation Taster, PolyPhen, SIFT and PROVEAN categorize it as pathogenic. In conclusion, the novel DNM2 mutation is responsible with high probability for the late-onset form of intermediate CMT of the investigated patient.

Severe phenotype of PCDH19-related X-linked epilepsy in a girl with Triple X syndrome

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Heterozygous mutations in PCDH19 cause an X-linked female-limited form of an early infantile epilepsy (Juberg-Hellman syndrome). The phenotype of this syndrome is variable, ranging from benign focal epilepsy to severe, serial seizures, repeating up to more than 10 times a day for several consecutive days. The intellectual outcome of affected patients ranges from normal to severe intellectual disability. Psychiatric disturbances are frequent and manifest as autism, schizophrenia or aggressive behavior. Neurological features such as ataxia may also be present. Women with Triple X syndrome usually show a normal physical development. Cognitive deficits and learning disabilities are more common than in the general population and compared to siblings. Their motor skills are likely to be somewhat impaired and coordination problems are frequent. In some patients psychological problems were described. Furthermore, EEG abnormalities are occasionally observed, with clinical seizures present in up to 15% of patients.

Here we report on a 15-year-old girl with a 47,XXX karyotype and early infantile, intractable epileptic seizures, beginning at the age of 9 months. About three years later, she developed severe, serial seizures often related to febrile infectious diseases. In adolescence, the epileptic symptoms became less intense. She additionally showed autistic features, mental deficiencies, hypermobility of the joints and ataxia. Array-CGH, Fragile X-analysis as well as Sanger sequencing and MLPA of the SCN1A and MECP2 genes revealed no additional abnormalities, besides the XXX karyotype. In the PCDH19 gene the heterozygous missense mutation c.695A>G (p.Asn232Ser) was identified, whereby the mutated allele seemed to appear in a 2:1 ratio

compared to the wild type allele. This mutation has been previously described as disease causing. Furthermore, three in silico prediction programs (SIFT, PolyPhen-2, MutationTaster) classified the mutation as pathogenic. The patient's asymptomatic mother had a normal 46,XX karyotype and was not a carrier of the PCDH19 mutation.

PCDH19-related epilepsy exhibits an unusual mode of inheritance in which only heterozygous females are affected and hemizygous males are asymptomatic carriers. Random X-inactivation in the brain of females with PCDH19 mutations causes a cellular mosaicism, which likely accounts for the pathogenesis by altering the cell-cell-interactions ("cellular interference"). However, the precise mechanism is still unknown. Hypothetically, the wide range of phenotypic expressions may be explained by partially skewed X-inactivation and thereby limitation of the cellular interference. Hence, an unequal ratio of mutated to wild type cells should give a milder phenotype compared to the fifty-fifty situation. In contrast to this hypothesis, the phenotype in our patient was rather severe. Nevertheless, we cannot exclude that the Triple X status contributes additionally to the observed phenotypic expression.

P-ClinG-080

Mild manifestations of Proteus syndrome can be challenging to diagnose

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Proteus Syndrome (PS, OMIM 176920) is a highly variable disorder with asymmetric and disproportionate overgrowth of the body, connective tissue nevi, epidermal nevi, dysregulated adipose tissue, and vascular malformations, caused by a somatic activating *AKT1* mutation.

We report on three unrelated individuals (two adults and one 6 year old boy) who showed similar clinical findings that not fulfilled the rigorous clinical criteria for PS (Biesecker, 1999). Beside an asymmetric hyperostosis of the skull or facial bones, all three had an ocular dermoid. Two individuals developed alveolar hyperostoses and intracranial calcifying meningiomas. Only one individual showed skin changes. All three had normal feet and no vascular lesions.

Molecular analyses in individual I performed in blood revealed normal results for array karyotyping and no relevant variant in whole exome sequencing (trio approach).

After the working diagnosis PS had been established, molecular analyses regarding the recurrent *AKT1* mutation (p.Glu17Lys) were performed by Sanger sequencing in available affected tissue specimen of all three individuals. This revealed a high level of mosaic state for the *AKT1* mutation c.49G>A, (p.Glu17Lys) in affected tissues from bone and in meningiomas. Re-evaluation of the NGS data from blood (individual I) confirmed the absence of that mutation in all reads, and no mutation was detected by Sanger sequencing in DNA from blood in individuals II and III.

Thus, a somatic mosaicism leading to a mild Proteus phenotype could be confirmed as the underlying genetic cause in all three affected individuals.

In conclusion, mild forms of Proteus syndrome caused by the recurrent *AKT1* mutation in patients with limited regional involvement may be particularly difficult to diagnose and might be underdiagnosed.

P-ClinG-081

Distal GNE-myopathy: rare differential diagnosis of polyneuropathy

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Here, we report a case of a 37-year-old patient with presumed polyneuropathy and elevated creatine kinase levels (400-800 U/l). Clinical features included atrophic and bilateral paresis of lower legs of the frontal and rear compartment without high arched foot, while sensibility was not affected. Additionally, a myopathic EMG in M. tibialis anterior and a slight axonal damage in the motor neurography was detected.

Due to this overlapping neuromyological phenotype we performed a gene panel including 155 genes associated with neuromuscular diseases using targeted next generation sequencing. Gene panel analysis revealed the homozygous mutation c.829C>T (p.Arg277Trp) in the GNE gene. This mutation is described in

the literature as cause of a distal GNE-myopathy and was also detected in an affected brother (CK 1900 U/l), having consanguineous parents.

The current case emphasizes that a large gene panel analysis is recommended in case of an overlapping neuropathological and myopathological phenotype.

P-ClinG-082

Musculocontractural Ehlers-Danlos syndrome – a complex but distinctive disorder caused by a homozygous CHST14 variant

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Ehlers-Danlos syndrome (EDS) is a heterogeneous group of connective tissue disorders. According to the 1997 Villefranche classification, EDS comprises six major types as well as some rare specific entities. One of these has been referred to as "EDS, musculocontractural type" (MC-EDS), "adducted thumb-clubfoot syndrome" or "EDS, Kosho type". First described in 1959 as an EDS VI subtype, it recently was identified to be caused by biallelic changes in either the *CHST14* or *DSE* genes, resulting in a loss of dermatan sulfate (DS) biosynthesis. Characteristic symptoms are multiple congenital malformations such as contractures (club feet, adducted thumbs), visceral and ocular anomalies, generalized joint laxity, scoliosis, muscular hypotonia, fragile, hyperextensible and bruisable skin, as well as a typical craniofacial appearance. Distinctive features include hypertelorism, down-slanting palpebral fissures, bluish sclerae, micro-corneae, short nose with hypoplastic columella and long philtrum, thin upper lip vermillion, small mouth, retrognathia, low-set and posteriorly-rotated ears. The psychomotor development is delayed. 31 *(CHST14)* and 3 *(DSE)* patients have been reported as yet.

The patient, a 30-year-old woman using a wheelchair, had club feet, surgically corrected ASD II, muscular hypotonia, the characteristic face and hyperextensible skin with atrophic scars; particularly visible were those resulting from bowel surgeries due to colon perforation. Her older sister who died aged 19 following an acute abdomen had club feet and the typical facial appearance, while three healthy sisters seem to be unaffected. Her parents are first cousins of Turkish origin and do not show EDS symptoms either. The two affected sisters had been diagnosed with a syndromic disorder that could represent a rare form of EDS. However, neither a confirmation of the suspected diagnosis nor a classification had yet been achieved.

Due to the distinctive symptom complex and the presumed autosomal recessive inheritance pattern, we strongly suspected this to be a case of MC-EDS. Sequencing of the *CHST14* gene (reference sequence: LRG_600) revealed a formerly undescribed homozygous variant (c.644C>T; p.Pro215Leu). The variant changes the highly conserved Pro215 residue which is located in the critical 3'-phospho-5'-adenylyl sulfate binding site and can be classified as likely pathogenic (ACMG Standards and Guidelines, Richards et al., Genetics in Medicine 2015). The parents are heterozygous carriers of this variant, respectively.

This case represents a unique entity within the umbrella term EDS and illustrates the importance of clinical assessment leading to a diagnosis confirmed by genetic analysis.

P-ClinG-083

*** Molecular mechanisms of mitochondrial DNA disease: pathological and genetic studies in patients with Mendelian disorders of mtDNA maintenance

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The underlying genetic defect in patients with mitochondrial PEO is either a primary mutation of the mitochondrial genome (single, large-scale mtDNA deletion or mtDNA point mutation) or recessively and dominantly-inherited mutations in nuclear genes involved in mtDNA maintenance leading to clonally-expanded multiple mtDNA deletions in muscle. The nuclear disease genes are largely implicated in the replication and stability of mtDNA, and as such a pathogenic mutation leads to secondary instability of the mitochondrial genome. Causal mtDNA deletions can be found in a heteroplasmic (mixture of mutated and wild type mtDNA) state. However, each tissue/cell has its own biochemical threshold of mutant mtDNA load which needs to be exceeded before focal respiratory chain deficiency becomes evident.

To investigate this, muscle biopsies of 17 patients with genetically- and clinically-characterized mitochondrial disease of nuclear origin (9 POLG, 5 TWNK, 2 RRM2B and 1 SLC25A4 (ANT1)) and 4 healthy controls were analysed using quadruple OXPHOS immunohistochemistry, quantifying the biochemical phenotype in individual muscle fibres of patient muscle biopsies. This technique is based on quadruple immunofluorescence to detect structural components of complexes I (NDUFB8) and IV (COXI), as well as

porin (a marker of mitochondrial mass) and laminin (a cell membrane marker to define the boundaries of muscle fibres). Further studies on 7/17 patients (3 POLG, 2 RRM2B, 1 TWNK, 1 SLC25A4 (ANT1)) included the correlation of the biochemical deficiency with the mtDNA abnormality in individual cells, following laser microcapture and determination of the size and level of clonally-expanded mtDNA deletion within fibres by real-time PCR.

Our preliminary data from quadruple immunocytochemical studies show that the muscle biochemical phenotype is different in patients with multiple mtDNA deletions compared to other mtDNA mutations; work is continuing to determine the exact size and level of clonally-expanded mtDNA deletion in individual muscle fibres and correlate this with the observed biochemical defects and disease thresholds.

P-ClinG-084

RNA analysis in incontinentia pigmenti reveals a novel deletion in patient with a mild phenotype

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Introduction: Incontinentia pigmenti (IP) is a rare X-linked male lethal genodermatosis that affects the neuroectodermal tissue and is always associated with a bullous rash of the skin along Blashko lines in female neonates. It is caused by mutations in *IKBKG* which encodes the regulatory subunit of the IkB kinase complex required for NF-kB activation. *IKBKG* has a pseudogene *(IKPKGP)* with identical exons 4 to 10. The most frequent IP mutation is a recurrent exon 4_10 deletion due to non-allelic homologous recombination with the pseudogene. Here, we report a novel deletion of exons 4 and 5 in the *IKBKG* transcript recognized by RNA analysis.

Patient: We investigated a 9 yr old girl with typical erythematous rash after birth which resolved within 2-3 m. Apart from a small hyperpigmented area around the right mammilla there were no skin alterations. She had few conically shaped teeth, normal nail and hair structure, no neurological manifestation and normal intelligence. Only clinical sign were repeated vitreous hemorrhage of the left eye from age 5 m. Family history was negative.

Methods: Analyses on genomic DNA extracted from blood included testing for the common *IKBKG* exon 4_10 deletion by long range PCR and MLPA (P073-A1, MRC Holland), X inactivation analysis in the androgen receptor gene as described, and massively parallel sequencing (MPSeq) of the *IKBKG* gene (TruSightOne, NextSeq, Illumina®; data analysis with NextGENe/Geneticist Assistant [Softgenetics®] and SeqNext [JSI®]; reference sequence GRCh37, hg19). RNA was extracted from cultured blood lymphocytes, and the entire *IKBKG* transcript (NM_003639.4, 10 exons) was Sanger sequenced on cDNA level (the A of the start codon is in exon 2); the results were analyzed with SequencePilot (JSI).

Result: Genomic DNA analyses including MLPA of *IKBKG* and *IKBKGP* specific probes in our patient did not reveal a putative mutation. There was a completely skewed X-inactivation pattern. cDNA sequencing of *IKBKG* demonstrated skipping of exon 4 and 5 (r.400_671del) which is predicted to cause a frame shift starting from codon p.Gln134, a premature stop codon 28 amino acids downstream (p.Gln134Glyfs*29) and complete loss of protein function. The loss of exons 4 and 5 is most likely due to an intronic splice variant in intron 6; investigations regarding the origin of this deletion are ongoing.

Conclusion: The presence of the high homologous pseudogene makes sequence analysis of *IKBKG* challenging. We report a deletion of exons 4 and 5 in the *IKBKG* transcript that required RNA analysis for its identification. Due to the skewed X-inactivation and typical clinical picture causality of the detected deletion is certain. The exact genomic cause of this alteration remains to be clarified. Also in the era of MPSeq, RNA analysis may be necessary for detection of deep intronic mutations or the study of genes with homologous pseudogenes, as shown here in the case of *IKBKG*.

P-ClinG-085

COL1A1 mutations associated with different forms of glaucoma

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Primary congenital glaucoma (PCG) and early onset glaucomas are one of the major causes of of blindness in children and young adults' worldwide. Both autosomal recessive and dominant inheritance have

been described with involvement of several genes including CYP1B1, FOXC1, PITX2, MYOC and PAX6. However, mutations in these genes explain only a small fraction of cases suggesting the presence of further candidate genes. To elucidate further genetic causes of these conditions we performed whole exome sequencing in a patient with PCG and retinal detachment and identified compound heterozygous variants in COL1A1 (p.Met264Leu; p.Ala1083Thr). Targeted COL1A1 screening of 26 additional patients detected three further heterozygous variants (p.Arg253*, p.Gly767Ser and p.Gly154Val) in three distinct subjects: two of them were diagnosed with early onset glaucoma and mild form of osteogenesis imperfecta (OI), one patient had a diagnosis of PCG at age 4 years. All five variants affected evolutionary, highly conserved amino acids indicating important functional restrictions. Molecular modeling predicted that the heterozygous variants are dominant in effect and affect protein stability and thus the amount of available protein, while the compound heterozygous variants act as recessive alleles and impair binding affinity to two main COL1A1 binding proteins: Hsp47 and fibronectin. Dominantly inherited mutations in COL1A1 are known causes of connective tissues disorders such as OI. These disorders are also associated with different ocular abnormalities, although the common pathology for both features is seldom recognized. Our findings expand the role of COL1A1 mutations in different forms of early-onset glaucoma with and without signs of OI. Thus, we suggest including COL1A1 mutation screening in the genetic work-up of glaucoma cases and detailed ophthalmic examinations with fundus analysis in patients with OI.

P-ClinG-086

Titinopathies in human genetics diagnostics

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The gene TTN encodes the largest known protein, titin, which plays a key role in structural, mechanical, developmental and regulatory functions of cardiac and skeletal muscles. Accordingly, titinopathies are characterized by great clinical and genetic heterogeneity. The clinical spectrum ranges from severe phenotypes with cardiac involvement to pure myopathies at the milder end, including autosomal recessive and dominant inheritance patterns (Chauveau et al. 2014, Hum Mutat; 35:1046). Next generation sequencing analysis identifies a large number of variants of unknown clinical significance; the potential clinical relevance of these variants cannot be assessed with certainty without further studies. Three case reports highlight the difficulties in human genetics diagnostics concerning TTN.

The first case is of a 46 year-old woman with proximal muscle weakness, slightly elevated CK, scoliosis, and no family history. A heterozygous known pathogenic variant was identified in Mex1, associated with autosomal recessive congenital core myopathy combined with primary heart disease. Additionally, an unknown variant was detected. Both variants could be clinically relevant with regard to the patient's phenotype, but this can be neither confirmed nor excluded at this time.

The second case is of a 7 month-old Finnish girl who presented with severe muscle hypotonia at birth and mental alertness with normal brain MRI and EEG. Congenital fiber-type disproportion was suspected. A homozygous frame-shift mutation in Mex1 was identified which to our knowledge has not yet been described in the literature. This variant is likely of clinical relevance with regard to the patient's phenotype, but this can be neither confirmed nor excluded at this time.

The third case is that of a 34 year-old woman with suspected myofibrillar myopathy. A known pathogenic homozygous frame-shift mutation in Mex6 was detected which is associated with autosomal recessive congenital myopathy with central nuclei. Segregation analysis revealed that the healthy parents are heterozygous carriers of this variant. The clinical diagnosis of a TTN-associated disease could therefore be confirmed.

TTN variants need to be assessed in combination with detailed clinical and muscle biopsy data. Segregation analysis is necessary but not sufficient for the clinical grading of variants. Identification of a variant in several independent families, segregation of the variant with disease phenotype in these families, and functional studies, together with phenotype descriptions in the literature, are essential for pathogenic grading. However, several difficulties remain, such as the huge size of the TTN gene (>100kb) impeding functional studies, the wide spectrum of phenotypes and variants, the still small patient cohort, and often unspecific immunohistochemical abnormalities in muscle biopsies. The clinical evaluation of TTN variants thus presents a great challenge to the field of human genetics diagnostics.

Novel variants in QARS gene causing autosomal recessive progressive microcephaly with therapy refractory seizures and cerebral atrophy - report on a German girl

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Compound heterozygous variants in the *QARS* gene (OMIM 603727) have been identified in only four patients with autosomal recessive progressive microcephaly with seizures and cerebral and cerebellar atrophy (MSCCA), to date. These patients showed severe developmental delay, progressive primary microcephaly, intractable seizures, hypomyelination or delayed myelination, thin corpus callosum, and small cerebellar vermis on brain imaging. Here we report on two unrelated girls with progressive primary microcephaly, epilepsy and brain anomalies. Trio exome analysis in each of the families revealed two different combinations of compound heterozygous variants in *QARS*. All four variants are highly conserved throughout vertebrates, not reported in any database, yet, and in silico analysis predicted the variants as possibly damaging or deleterious. The first patient was born to non-consanguineous German parents. At birth, she was too short (-2.8 SD) and mildly microcephalic (-2.3 SD). She developed intractable seizures within the first hour of life. Her growth continued to be mildly retarded (-2.8 SD at age 9 years) but microcephaly was progressive (-6.5 SD at age 9 years). She did not achieve any of the motor or cognitive developmental milestones, she did not have eye contact. The only interaction with her surrounding was a diffuse reaction to being touched. Cranial MRI showed no myelination of the supratentorial region, corpus callosum agenesis, simplified gyral pattern of frontal lobes, enlarged cerebral ventricles, and normal brain stem and cerebellum. Trio-exome sequencing revealed the compound heterozygous *QARS* variants c.1132C>T, p.(Arg378Cys) and c.1567C>T, p.(Arg523*). Segregation analysis by Sanger sequencing confirmed the heterozygous variants in the parents and two non affected sibling of the index patient. The second patient was initially evaluated at 11 days of age when she exhibited myoclonic seizures, intrauterine growth retardation, microcephaly, and elevated lactic acid. At birth, she was microcephalic (HC 29 cm) and microcephaly was progressive (-5.4 SD at age 19 months). Cranial MRI suggested undersulcation. She has required a gastrostomy feeding tube. Trio-exome sequencing revealed the compound heterozygous *QARS* variants c.40G>A, p.(Gly14Ser) and c.1573C>T, p.(Arg525Trp). Segregation was confirmed by Sanger sequencing analysis. Together with the four previously described patients we conclude that compound heterozygous variants in *QARS* are associated with a primary and progressive microcephaly, early onset of intractable seizures and severe developmental delay. Brain imaging in the neonate can show simplified gyral pattern as an early characteristic feature. Overlapping phenotypes are seen in patients with epileptic encephalopathy, lissencephaly and primary microcephaly. Application of NGS panels or exome technology will allow for early diagnosis and further collection of patients for better delineation of the phenotype.

Mutational spectrum underlying syndromic short stature in an Algerian patient cohort determined by NGS-based approaches

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Next-generation-sequencing (NGS) technology has revolutionized genomic research and has transformed clinical diagnostics. NGS offers enormous potential for providing accurate diagnoses to individuals with previously unresolved syndromes. In the pediatric endocrine clinic, clinicians are often faced with the task of making a diagnosis in children with syndromic short stature. As there may be considerable clinical overlap between short stature syndromes, deriving a clinical diagnosis may prove challenging. Furthermore, even if a clinical differential diagnosis is established, often several genes would need to be tested before a molecular diagnosis is made. As access to genetic testing is limited in Algeria, we conducted a pilot study on 10 Algerian patients with syndromic short stature using a combination of two different NGS modalities, namely whole-exome-sequencing (WES) and Mendeliome sequencing (TruSight One sequencing panel). A molecular diagnosis could be established in 9/10 patients, making the diagnostic rate in this initial cohort 90%. As 7 patients had novel mutations we could expand the mutational spectra of several genes, namely *CUL7, NPR2,*

SOS1, *VPS13B*, and *ZNF81*. We could thus substantiate the clinical utility of WES and the Mendeliome in patients with a diverse array of syndromic short stature syndromes.

Careful family history taking unveils a SMAD4-associated juvenile polyposis in a girl with coinciding Temple syndrome (TS14) in her sister

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Chromosome 14 harbours an imprinted locus at 14q32. Maternal uniparental disomy of chromosome 14, paternal deletions and paternal loss of methylation at the intergenic differentially methylated region (IG-DMR) and the somatic DMR within *MEG3* are associated with **Temple syndrome** (TS14, MIM 616222). The phenotype of TS14 consists of pre- and postnatal growth retardation, early feeding problems and muscular hypotonia, joint laxity, motor developmental delay, premature puberty, and truncal obesity.

Juvenile polyposis syndrome (JPS, MIM 174900) is characterized by predisposition to hamartomatous polyps in the gastrointestinal (GI) tract, specifically in the stomach, small intestine, colon, and rectum, including the risk for gastrointestinal cancer. Pathogenic variants in the *BMPR1A* and *SMAD4* gene are identified in about 40-50% of affected families.

We report on a family with two female children. The index patient, an 8-year-old girl, was diagnosed to have TS14 due to hypomethylation at the somatic DMR within *MEG3* with clinical features reminiscent of Prader-Willi syndrome in early childhood and milder clinical signs at further age (i.e. mild global development delay, muscular hypotonia, suspected central obesity, no prominent facial dysmorphisms). SNP Array-CGH analysis was unsuspicious and no deletion of the imprinting center was observed. Thus, TS14 is caused by a sporadic imprinting defect in our patient.

Her 10-year-old sister was diagnosed with *SMAD4*-associated JPS after an episode of intestinal invagination due to a polyp, histologically diagnosed as Peutz-Jeghers polyp, in early infancy. Sequencing identified a heterozygous pathogenic *SMAD4* variant c.719dupT;p.(Ala241fs) (NCBI Reference Sequence NM_005359.5). Gastric as well as colonic cancer and polyposis was present in the paternal family history.

Conclusions: TS14 and other imprinting disorders are likely underdiagnosed, as the main clinical features (e.g. growth retardation, hypotonia) are distinct but unspecific. As exome sequencing becomes a more frequent diagnostic procedure, imprinting disorders caused by mutations in imprinting centers will presumably be diagnosed more often. Methylation defects, however, will remain underdiagnosed, without a specific clinical differential diagnosis, which would guide to appropriate analysis of the methylation status.

A bowel invagination in early childhood due to a single polyp can be a symptom of JPS, especially in the context of a paternal history of polyposis and intestinal cancer; thus, family history should be carefully obtained.

Interdisciplinary Phelan-McDermid outpatient clinic - a chance for patients and medical professionals

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Phelan-McDermid syndrome is a still underdiagnosed genetic disease, which is usually caused by a terminal or interstitial deletion of 22q13.3 leading to a heterozygous loss of the SHANK3 gene. A wide variety of symptoms contributes to the clinical picture of Phelan-McDermid syndrome. During childhood, the major symptoms are muscular hypotonia, mental retardation, autistic features and speech delay. Not only clinical geneticists, but also child psychiatrists and pediatricians may encounter patients suffering from Phelan-McDermid syndrome, and should therefore know about the main features of the disease and management options.

At Ulm University, an interdisciplinary Phelan-McDermid outpatient clinic has been established in 2013 as a collaboration of the Neurology Clinic, the Institutes of Anatomy and Human Genetics as well as the Department of Child and Adolescent Psychiatry / Psychotherapy. Moreover, workshops and feedback rounds are organized with the Phelan-McDermid parents' organization (Phelan-McDermid Gesellschaft e. V.) and the international PMS foundation. In the outpatient clinic, child psychiatrists as well as neurologists thoroughly work up Phelan-McDermid patients according to a standardized protocol by taking medical history, performing physical examination, and, if needed, organizing further supplementary examinations. In addition, a genetic

analysis and hair/tissue sampling is performed. Since its foundation, a steadily increasing number of so far 70 patients from all over Germany has been seen and treated.

The outpatient clinic aims at facilitating and accelerating the diagnosis of Phelan-McDermid syndrome, improving medical support for affected patients of all ages, and, last but not least, fostering a better understanding of the causes and pathomechanisms leading to the symptoms of the disease.

P-ClinG-091

Molecular characterization of congenital eye malformations

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Congenital eye malformations, such as the microphthalmia-anophthalmia-coloboma (MAC) spectrum, congenital cataracts, anterior segment dysgenesis (ASD), and congenital glaucoma, affect more than 1:8.000 newborns. The phenotypic spectrum of the aforementioned entities is highly variable and partially overlapping. Eye malfomations are very heterogeneous; to date causative mutations have been described in more than 100 genes. Due their heterogeneity, diagnostic testing for congenital eye malformations was limited in the pre-NGS era.

We performed exome analysis in 30 patients with congenital eye malformation (MAC spectrum, ASD, congenital cataract, congenital glaucoma). Primarily, a gene panel comprising 112 genes associated with eye malformations was evaluated. Additionally the exome data was evaluated in selected patients as a second step.

The panel analysis revealed pathogenic sequence variants in 10 patients and 8 genes (MAB21L2, BCOR, NHS, PRSS56, CYP1B1, FOXC1, PITX2, GCNT2). Putatively causative sequence variants were identified additional patients. The diagnostic yield of the panel was highest in patients with non-syndromic microphthalmia / coloboma and congenital cataracts, and lowest in patients with syndromic MAC spectrum (i.e. additional systemic features / malformations).

NGS based panel testing is a strong diagnostic tool to determine the underlying causes of non-syndromic congenital eye malformations. Due to the partially overlapping phenotypes and high heterogeneity it is more sensible to perform large gene panel analysis, as opposed to smaller single phenotype based panels.

P-ClinG-092

Novel PRPS1 mutation in a family with congenital hyperuricemia

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Superactivity of phosphoribosyl¬pyrophosphate synthetase I (PRPPS) is a rare inborn error of purine metabolism that is characterized by increased levels of uric acid in blood and urine (OMIM 300661). The disorder is caused by gain-of-function mutations in the X-chromosomal gene PRPS1. In male patients, disease manifestation is in early childhood. Additional clinical characteristics include intellectual disability, hypotonia, ataxia and hearing loss. Heterozygous female mutation carriers have a later age of onset and a less severe clinical course. Only seven families with PRPS1 gain-of-function mutations have been reported to date.

We report on a 7-year-old boy with congenital hyperuricemia, urolithiasis, developmental delay, short stature, hypospadias and facial dysmorphisms. His mother also had hyperuricemia that was diagnosed at age 17 years but was otherwise healthy. A novel PRPS1 missense mutation (c.573G>C, p.Leu191Phe) was detected in the proband and his mother. Enzyme activity analyses confirmed superactivity of PRPP synthetase.

The family reported here broadens the clinical spectrum of PRPPS superactivity and indicates that this rare metabolic disorder is associated with a recognizable facial gestalt.

Whole-exome sequencing results in the identification of a homozygous SLC18A2 missense mutation and phenotypic amelioration upon treatment with a direct dopamine-receptor agonist in a patient with brain dopamine-serotonin vesicular transport disease

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Brain dopamine-serotonin vesicular transport disease is a rare early-onset neurological syndrome characterized by developmental delay, movement disorders and autonomic dysfunctions. Biallelic mutations in *SLC18A2* encoding VMAT2 have only very recently been described as causal for brain dopamine-serotonin vesicular transport disease in two families with multiple affected children (Rilstone et al., *N Engl J Med* 368, 2013, 543-550; Jacobsen et al., *J Inherit Metab Dis* 39, 2016, 305-308).

The index case presented here is a 7-year-old girl with severe mental retardation and a dystonic movement disorder. She is the tenth child of a consanguineous Arabic couple and was initially referred to neuropaediatric examination at the age of four months due to recurrent oculogyric crises and muscular hypotonia. Blood metabolic testing and cerebrospinal fluid (CSF) analyses were inconclusive. Notably, biogenic amines were within their normal ranges and the differential diagnosis of aromatic L-amino acid decarboxylase (AADC) deficiency could not be confirmed. Conventional cytogenetics, subtelomeric screening, array-CGH and different NGS panel analyses did not identify a causative mutation. Both parents and all eight living siblings are obviously unaffected. A brother with a known hypotonic movement disorder died at the age of three years due to prolonged seizures with hyperthermia and cerebral edema.

By utilizing whole-exome sequencing, we identified a homozygous substitution in the *SLC18A2* gene of the index case causing an amino acid change (c.710C>A; p.Pro237His) in a conserved transmembrane domain of vesicular monoamine transporter 2 (VMAT2). Homozygosity for this missense change could also be verified in a DNA sample of her deceased brother.

An obvious reduction in frequency of oculogyric crises was observed in our index case under therapy with pramipexole already within 4 weeks after start of treatment. Furthermore the patient shows less dystonic movements under therapy. The case presented here highlights the importance of considering brain dopamine-serotonin vesicular transport disease as differential diagnosis for early-onset extrapyramidal movement disorders combined with mental retardation even if neurotransmitters in CSF are normal.

Mutations in CPLX1 in two families with autosomal-recessive severe infantile myoclonic epilepsy and ID

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For a large number of individuals with intellectual disability (ID), the molecular basis of the disorder is still unknown. However, whole exome sequencing (WES) is providing more and more insights into the genetic landscape of ID. In the present study, we performed trio-based WES in 311 patients with unsolved ID and additional clinical features, and identified homozygous CPLX1 mutations in three patients with ID from two unrelated families. All displayed marked developmental delay and migrating myoclonic epilepsy, and one showed a cerebellar cleft in addition. The encoded protein, complexin 1, is crucially involved in neuronal synaptic regulation, and homozygous Cplx1 knockout mice have the earliest known onset of ataxia seen in a mouse model. Recently, a homozygous truncating mutation in CPLX1 was suggested to be causative for migrating epilepsy and structural brain abnormalities. ID was not reported. The currently limited knowledge on CPLX1 suggests that complete loss of complexin 1 function may lead to a complex but variable clinical

phenotype, and our findings encourage further investigations of CPLX1 in patients with ID, developmental delay and myoclonic epilepsy to unravel the phenotypic spectrum of carriers of biallelic CPLX1 mutations.

P-ClinG-095

FOXP2 variants in fourteen individuals with developmental speech and language disorders broaden the mutational and clinical spectrum

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BACKGROUND Disruptions of the FOXP2 gene, encoding a forkhead transcription factor, are the first known monogenic cause of a speech and language disorder. 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. We thus aimed at a further characterization of the mutational and clinical spectrum.

METHODS Chromosomal microarray testing, trio exome sequencing, multi gene panel sequencing and targeted sequencing of FOXP2 were performed in individuals with variable developmental disorders, and speech and language deficits.

RESULTS We identified four different truncating mutations, two novel missense mutations within the forkhead domain and an intragenic deletion in FOXP2 in fourteen individuals from eight unrelated families. Mutations occurred de novo in four families and were inherited from an affected parent in the other four. All index patients presented with various manifestations of language and speech impairment. Apart from two individuals with normal onset of speech, age of first words was between 4 and 7 years. Articulation difficulties such as slurred speech, dyspraxia, stuttering or poor pronunciation were frequently noted. Motor development was normal or only mildly delayed. Mild cognitive impairment was reported for most individuals.

CONCLUSION By identifying intragenic deletions or mutations in fourteen individuals from eight unrelated families with variable developmental delay/cognitive impairment and speech and language deficits, we considerably broaden the mutational and clinical spectrum associated with aberrations in FOXP2.

P-ClinG-096

Brain malformation in a case with Roifman syndrome associated with compound heterozygous mutations of RNU4ATAC

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We report on a 4-year-old boy with microcephaly, arachnoidal cysts, pachygyria, microgyria, and severe intellectual disability. He also had short stature including shortening and deformation of the femora, brachydactyly, and short ribs with costochondral dysplasia. He showed facial dysmorphism with narrow palpebral fissures, a short nose with a depressed nasal bridge, and a broad mouth with full lips. Clinical laboratory investigations demonstrated persistently slightly elevated liver enzymes. Exome sequencing revealed compound heterozygous mutations of the RNU4ATAC gene, g.51G>A;g.16G>A, which has been described in an individual with Roifman syndrome.

Homozygous and compound heterozygous mutations of the RNU4ATAC gene are associated with MOPD1 and Roifman syndrome. MOPD1 is characterized by severe microcephaly with brain malformations including abnormal gyral pattern, corpus callosum agenesis or hypoplasia, vermis hypoplasia and intracranial cysts, psychomotor retardation, short stature, skeletal dysplasia, dry skin, sparse hair, flexion contractures, round face with beaked nose and protruding eyes, and premature death with a majority of the patients who die before the age of 28 months for unknown reasons. Roifman syndrome was first described as a novel association of antibody deficiency, spondyloepiphyseal chondro-osseous dysplasia, retinal dystrophy, poor pre- and postnatal growth, cognitive delay and facial dysmorphism including long eyelashes, downslanting palpebral fissures, a long philtrum and a thin upper lip. All patients with Roifman syndrome reported so far lack

brain malformations. The RNU4ATAC gene encodes a small nuclear RNA (snRNA), which is essential for minor intron splicing. Homozygous (g.51G>A, g.46G>A) and compound heterozygous mutations (g.51G>A;g.55G>A, g.51G>A;g124G>A and g.40C>T;g.124G>A) have been described in MOPD1. All mutations involve the 5′ or 3′ stem loop of the U4atac snRNA. In contrast, all cases with Roifman syndrome investigated so far showed compound heterozygous RNU4ATAC mutations with one allele harboring a mutation in the MOPD1 associated 5′ stem loop and the other allele showing a mutation in the stem II site of the U4atac snRNA, which has not been involved in MOPD1, so far. Thus, the different pattern of the mutations observed in MOPD1 and Roifman syndrome may contribute to the distinct features of both syndromes. However, our patient shows, that features of MOPD1, i.e., brain malformations, may also be present in patients who show Roifman syndrome associated RNU4ATAC mutations. This indicates that both syndromes may represent overlapping features of the clinical spectrum of RNU4ATAC mutations.

P-ClinG-097

Gene panel diagnostics in patients with hereditary retinopathies - three exemplary cases with implications for risk prediction and genetic counseling

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Introduction. Inherited retinal degenerations comprise a genetically heterogeneous group of eye diseases with overlapping clinical presentations. Up to now, more than 200 genes have been associated with different forms of retinal dystrophies (RD) such as retinitis pigmentosa (RP) or cone-rod-dystrophies (CRD) with mutations in 84 and 34 causative genes, respectively. Here, we present the results from three patients with remarkable findings and discuss their implications for risk prediction and genetic counseling.

Methods. Targeted Next-Generation Sequencing (NGS) technology based on Agilent custom designed gene panels (SureSelect) has been established in our diagnostics department to identify causative mutations in a large patient cohort with approximately 200 RD patients. High-throughput sequencing data are routinely analyzed with the CLC Biomedical Workbench. Classification of variants was based on bioinformatic analyses using Alamut visual software, MutationTaster, SIFT and Polyphen-2 prediction programs, allele frequencies, amino acid conservation and literature.

Results. NGS analysis revealed two patients with RP and one patient with CRD, each of whom carry putative causative mutations in several RD genes. First, a male patient with a family history of CRD, is a carrier of a nonsense mutation p.(Arg1144Ter) in RIMS1 and two likely pathogenic missense mutations in AIPL1 (p.(Tyr134Phe)) and GUCA1A (p.(Pro50Leu)), each in a heterozygous situation. Mutations in all three genes can cause adCRD. In addition, the patient carried a hemizygous nonsense mutation p.(Glu1017*) in the X-chromosomal RPGR gene. Secondly, a female patient with simplex RP was found to be homozygous for a frameshift-causing deletion p.(Ser527Leufs*28) in the IMPG2 gene causing arRP. She also carried three heterozygous, likely pathogenic missense mutations in CRX (p.(Tyr142Cys)) causing adRP, in the X-chromosomal RPGR (p.(Ala365Val)) and in USH2A (p.(Ile1621Val)) associated with arRP. Finally, in another female RP patient with no family history of RD, we detected a nonsense mutation p.(Trp558Ter) and a likely pathogenic splice site change (c.6078+3A>G) in the arRP gene EYS, assuming compound heterozygosity. In this patient we also identified two heterozygous, likely pathogenic missense mutations in HMCN1 (p.(Pro2226Thr)) and CEP290 (p.(Arg2210Cys)) underlying dominant and recessive forms of RD. In all three cases, specific mutation(s) could not be uniquely identified as causative. Conclusion. Results in the three RD cases emphasize that NGS can generate unexpected results that are difficult to interpret, particularly in the absence of segregation analysis and functional data on pathogenicity. The implications for genetic counselling and predictive testing will be discussed.

P-ClinG-098

Smart qNIPT study – detection of fetal trisomy 21 based on methylation-specific quantitative real-time PCR

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Objectives
Current non-invasive prenatal testing (NIPT) methods for the detection of fetal trisomy 21 (T21) are primarily based on next generation sequencing (NGS) strategies which are quite costly in clinical application and hence are limited to patients who can afford the testing. Here, we describe the results of a blinded study with respect to the test accuracy of a newly developed NIPT assay based on quantitative real-time PCR (qPCR) for prenatal testing of fetal trisomy 21 (qNIPT).
Methods

In the study maternal plasma samples were collected from 1,044 pregnant women and blinded by an independent Contract Research Organization. After extraction of cell-free DNA using QIAsymphony instrument and methylation-specific digestion of DNA samples a multiplex qPCR was performed. The primary qPCR data were finally evaluated with our CE marked data analysis software. Results from this analysis and from confirmatory NGS testing were compared with NIPT results using NGS.

Results

The study results of successfully analysed maternal plasma samples (n=966) demonstrated a positive percentage agreement (PPA; equates to sensitivity) of 100 % (lower 1-sided 95% confidence interval of 91.8 %; n=35/35) and a negative percentage agreement (NPA; equates to specificity; n=931/931) of 100% compared to NGS-based results. The negative predictive value (NPV) for the novel qNIPT and confirmatory NGS testing was 100 % (lower 1-sided 95 % confidence interval of 99.68 %). The average fetal fraction of the 966 examined blood samples was 8.1%. The qNIPT assay provided reliable test results in 54 blood samples with a fetal fraction below 4% and as low as 2.4%.

Conclusion

Our results suggest that the proprietary qNIPT assay is a very reliable and robust method suitable for clinical routine in accordance with international medical associations. The assay represents a more cost-efficient solution over NGS testing and will also be able to provide results in the shortest possible time. While current NIPT methods require a minimum fetal fraction of 4% in blood samples from singleton pregnancies, we could demonstrate in the study that our smart qNIPT assay can be employed on blood samples with a fetal fraction of as low as 2.4%. In summary, the application of smart qNIPT could have the potential to become a NIPT solution on a global scale for pregnant women of all ages and risk groups. Further studies which aim to include the determination of trisomy 13 and trisomy 18 are currently underway.

Novel SETBP1 mutation in a patient with distinctive craniofacial features and intellectual disability

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We report on a seven-year-old girl, first child of non-consanguineous Italian parents, with developmental delay, muscular hypotonia and distinctive craniofacial features (epicanthus inversus, ptosis, broad nasal bridge, mild retrognathia, low-set posteriorly rotated ears and malpositioned teeth in the mandible).

Because of the tentative diagnosis of blepharophimosis-ptosis-epicanthus inversus syndrome (BPES), conventional cytogenetic analysis, Sanger sequencing and MLPA (multiplex ligation-dependent amplification) of *FOXL2* were initiated and showed unremarkable results.

Microarray-CGH revealed a 414 kb microduplication of genetic material on 15q11.2: arr[hg19] 15q11.2(22765628_23179948)x3 encompassing the genes *TUBGCP5, CYFIP1, NIPA1* and *NIPA2* of maternal origin. Patients with 15q11.2 microduplication have been described to be affected by developmental delay, motor and/or expressive language delay, epilepsy, learning disabilities and/or behavioral problems. However, genotype phenotype correlation is complicated by incomplete penetrance. Healthy and mildly affected carriers are reported in the literature. We speculate that the microduplication might contribute but does not fully explain the phenotype of our patient, in particular concerning the craniofacial features.

Subsequent trio whole-exome sequencing identified a de novo heterozygous mutation in *SETBP1* (c.3909T>A / p.Tyr1303*) leading to a premature stop codon and most probably resulting in a truncated and functionally impaired protein.

Mutations in the SET binding protein 1 gene (*SETBP1*) on 18q12.3 have been identified to cause Schinzel-Giedion syndrome (SGS, OMIM 269150), a rare autosomal dominant disorder characterized by postnatal growth failure, severe developmental delay, seizures, facial dysmorphism, genitourinary, skeletal, neurological, and cardiac defects. Chromosomal deletions in 18q including *SETBP1* have been reported to cause a milder phenotype known as "autosomal dominant mental retardation-29" (MRD29, OMIM 616078). These observations suggest that the severe SGS phenotype might be the consequence of a gain-of-function or dominant-negative effect of the mutations and that *SETBP1* haploinsufficiency results in a different, milder phenotype. So far, the function of the SETBP1 protein is unknown.

The presented case adds up to the yet small number of reported cases of MRD29 and thereby contributes to the clinical spectrum of *SETBP1* haploinsufficiency.

Genetic counselling services are rising steeply in Germany

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The demand for genetic counseling had been constant, in Germany, over many years. From 1996 to 2004 around 47.000 cases per year on the average and with minor fluctuations were reimbursed by the German sickness funds (public health insurance system; Pabst and Schmidtke, Gendiagnostik in Deutschland, BBAW, p. 195-203, 2007). In connection with the "GenBIn2"-project, a new nationwide survey was initiated regarding the annual reimbursement frequencies of the relevant entries in the EBM fee schedule , 08572, 01792, 01837 and 11232, for which only specialists in human genetics and subspecialists in medical genetics can account, from 2009 until 2014. Contrary to the findings in the earlier period the demand for genetic counseling has risen sharply: 41,243 (of a total of 54,360) cases in 2009; 45,525 (58,341) in 2010; 46,691 (59,724) in 2011; 51,316 (63,242) in 2012; 54,739 (69,732) in 2013; and 61,308 (74,780) in 2014. We speculate that the temporal correlation of the rise of genetic counseling demand with the enactment of the German Act on Testing (February 1, 2010) is not coincidental. Further factors that might contribute to the increase in demand are the ensuing guidelines of the German Commission on Genetic Testing and CME activities related to attaining a qualification for genetic counseling for specialties other than human genetics. In the course of these activities the awareness for the importance of genetic counseling delivered by specialists in human genetics and subspecialists in medical genetics may have risen.

Small duplication of Down syndrome critical region in a mosaic form

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It is well known that duplications of Down syndrome critical region (DSCR) on chromosome 21q22 can cause Down syndrome whereby the distinct phenotype is associated with the involved genes and the size of duplication. However, in literature are hardly any cases with mosaic duplications of DSCR described.

Here we report on a 6 year old boy with some clinical features of Down syndrome including distinctive craniofacial dysmorphism, simian crease and sandal gap as well as delayed motor and speech development. No other organ abnormalities are known.

Conventional chromosome analysis showed no numerical or structural aberration whereas interphase FISH analysis revealed three signals for DSCR in approx. 40% of lymphocytes and in approx. 80% of buccal mucosa cells. Array-CGH analysis on DNA from peripheral blood confirmed a 2,56 Mb duplication of chromosome 21q22.13q22.2. The duplication involves among others the gene *DYRK1A* which is reported as a candidate gene for Down syndrome.

This case presents one of the smallest known duplications within DSCR which causes even in a mosaic state a mild phenotype of Down syndrome.

FMR1-Duplication in a family with developmental delay and gait ataxia

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A 4-year-old boy was referred to our outpatient clinic due to global developmental delay mainly affecting his speech and his fine motor development. In addition, muscular hypotonia and an abnormal gait were reported by the referring paediatrician. His mother, his maternal grandmother, and numerous relatives are affected by gait ataxia. No causative mutation was detected in the maternal grandmother by means of a multi-

gene panel for spinocerebellar ataxia encompassing 118 genes. Genetic testing for Friedreich ataxia was also without pathological findings.

With respect to the developmental delay of our index patient, chromosome analysis and array-CGH were performed. A microduplication in 3p26.2 (app. 50kb) of unknown significance and a microduplication in Xq27.3 (app. 550kb), which comprises the FMR1-gene, were identified and shown to be of maternal origin (arr[hg19] 3p26.2(2,811,323-2,861,170)x3,Xq27.3(146,663,270-147,212,089)x3).

FMR1 is associated with fragile X syndrome, which is one of the most common causes for X-linked mental retardation. CGG-trinucleotide repeat expansions in the 5' untranslated region (>200 repeats) lead to aberrant hypermethylation of the FMR1-promotor and silencing of FMR1 expression. In contrast, premutations (55-200 repeats) lead to a higher expression of FMR1 and cause a clinical syndrome that is characterised by late progressive cerebellar ataxia (FXTAS). In line with this gain-of-function mechanism, we hypothesize that the Xq27.3 duplication, which could lead to an increased gene dosage of FMR1, causes a Fra(X)-/FXTAS-like syndrome and explain the clinical findings in our family. Vengoechea et al. described a patient with a similar duplication, who was affected by developmental retardation, epilepsy and hyperactivity. They discussed the microduplication, which arose de novo in their patient, as the cause for the boy's symptoms (Vengoechea J. et al., Eur J Hum Genet., 2012 Nov;20(11):1197-200).

In conclusion, we assume a FMR1-duplication syndrome in our family with variable expressivity and a different impact on male and female patients. To further prove this hypothesis, we are planning to perform a segregation-analysis within the whole family.

P-ClinG-103

Paternal uniparental isodisomy of chromosome 17 as cause of a homozygous CHRNE gene mutation in a boy with congenital myasthenic syndrome

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Background: Congenital myasthenic syndromes (CMS) are a genetically heterogenous group of disorders leading to weakness of skeletal muscles - especially ocular, bulbar and limb muscles - with onset mostly at birth or in early childhood. The severity of CMS can vary significantly ranging from death in early childhood due to respiratory insufficiency to only mild muscle weakness in adulthood. More than 25 genes that are highly expressed in the neuromuscular junctions are associated with CMS. Mutations in the *CHRNE* gene on chromosome 17p13.2 are responsible for about one half of genetically solved CMS cases. They can cause different subtypes of CMS with either autosomal dominant or autosomal recessive inheritance.

Results: Here, we report a 3-year-old boy who was born with bilateral eyelid ptosis and congenital vertical talus of the right foot that needed surgical correction. The boy displayed muscular hypotonia with a myopathic facial expression and delayed motor development. Ophthalmologic examination revealed external ophthalmoplegia. A next generation sequencing based gene panel for congenital myopathies detected the homozygous frameshift mutation c.750_769dup (p.Leu257Profs*50) in the *CHRNE* gene in the boy. Gene dosage analysis did not show an exonic deletion in the *CHRNE* gene. Sanger sequencing confirmed the mutation in a heterozygous state in the boy's father. However, his mother did not carry the mutation in the *CHRNE* gene.

Conclusions: These results suggest the rare event of a (partial) paternal uniparental isodisomy of chromosome 17 as cause of the homozygous c.750_769dup (p.Leu257Profs*50) in the *CHRNE* gene in the boy. Further experiments are currently undertaken to confirm this hypothesis.

P-ClinG-104

Phenotypic variability of a SMAD4-mutation within a family

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Our proposita is a 42 years old woman, who was transferred to our genetic counselling department for the suspicion of M. Osler (hereditary hemorrhagic teleangiectasia, HHT). During routine check up an anemia was diagnosed. A tumor search was initiated and unexpectedly the CT of the abdomen showed a suspect coin lesion of about 2.5 cm in diameter localized in the basal part of the right lung. Further investigations revealed a pulmonary arterio-venous malformation which was hemodynamically relevant and already led to chronic right heart overload. A coil embolization was performed. Retrospectively, medical history of the patient included

episodes of severe epistaxis in childhood and a neurosurgical intervention for intracerebral bleeding at the age of 16 years without permanent neurological deficits.

During genetic counselling our proposita mentioned that her 8 years old daughter also suffered from anemia due to multiple polyps of the colon. After polypectomy her hemoglobin values normalized. Although histologically the polyps appeared as juvenile ones a mutation search in the APC-gene was initiated by the gastroenterologists without identifying a pathogenic mutation.

Combining the two pieces of information, we offered a mutation search in the SMAD4 gene and a pathogenic mutation c.1081C>T (p.Arg361Cys) was found in both patients in heterozygosity. Colonoscopy in the mother did not show juvenile polyps or gastrointestinal vascular malformations. Vice versa, no cerebral or pulmonary arteriovenous malformations could be detected in the daughter.

Our family illustrates, that the same mutation within a family may phenotypically appear as different diseases. A careful taking of medical history and the knowledge of all relevant diagnostic findings (in this case e.g. the histology of the polyps) can enable the geneticist to offer a precise differential diagnosis leading to a well-directed molecular testing. To our opinion this is still relevant even in the era of NGS-based panels because the more precise the clinical diagnosis and the choice of the genes to analyse the less problems with unclassified variants will arise.

P-ClinG-105

Unexpected finding of a gross heterozygous MSH6-Deletion in a patient with microsatellite-stable colorectal and synchronous renal cancer

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The Hereditary Non-Polyposis Colon Cancer (HNPCC, Lynch syndrome) is caused by pathogenic germline mutations in mismatch repair genes (MLH1, MSH2, MSH6 and PMS2) causing microsatellite instability (MSI) and decreased or lost expression of the appropriate mismatch repair protein (MMR) in the immunohistochemistry (IHC) on tumor material. Thus, IHC and MSI testing help to identify the MMR gene, which most likely harbors a germline pathogenic variant. MSI and IHC testing prior to germline analysis are specified in the S3 guidelines of HNPCC and if negative normally preclude further genetic analyses.

Here, we present a 51 year old patient with a synchronous colonic (ceacum) and renal cancer at the age of 50 years. At the time of the diagnosis hepatic metastases of the caecal adenocarcinoma have already been present. Histologically, the colonic tumor was a poorly differentiated adenocarcinoma (pT3pN) with lymphangiosis and haemangiosis carcinomatosa. The renal cancer showed histology of a moderately differentiated clear cell renal carcinoma.

In the family history the 50 year old sister and the parents are healthy. The twin sister of the patient´s mother had a collateral breast cancer at the age of 46 years and died two years later. The mother´s grandparents had no cancer, but the patient mother`s grandfather had a cancer of unknown origin and died at the age of 50 years.

Because of the suspicion of having a Lynch syndrome an immunohistochemistry and microsatellite analysis have been performed on the tissue of the colorectal cancer and the hepatic metastasis. All four MMR proteins were properly expressed in immunohistochemistry in colorectal cancer. Just the expression of MLH1 protein in the hepatic metastasis was focally weakened and inhomogeneous. The microsatellite markers BAT25, BAT26, D5S346 (APC), D2S123 and D17S250 (MfD) were all stable, a A146T-KRAS mutation was found.

After performing a multi-gene panel (NGS, next-generation sequencing), a gross heterozygous deletion of exon 1 in MSH6 gene has been found in the CNV analysis of the NGS data. This mutation was confirmed with a MLPA and quantitative real time PCR analyses. Furthermore, RNA expression of MSH6 was reduced to 50% in blood lymphocytes in comparison to control samples pointing to a potential role of MSH6 loss in the patient´s tumor development.

We are observing more and more patients with probably pathogenic and pathogenic mutations in one of the MMR genes with normal immunohistochemistry and microsatellite analysis. Therefore, we propose that the criteria for performing a molecular genetic analysis of HNPCC/Lynch syndrome should be revised.

Exome sequencing reveals GATA1 mutation in a patient with partial delta-storage pool deficiency and mild thrombocytopenia

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Objectives. We report about a 35-year-old male patient of Russian background with severe and frequent epistaxis and hematoma since infancy. He presented with mild thrombocytopenia and increased mean platelet volume. Von Willebrand's disease and subhemophilia had been excluded. Previously, he was diagnosed with immune thrombocytopenic purpura. He never underwent elective surgery. His parents were asymptomatic. However, his 4-year-old daughter also suffers from severe bleeding symptoms (multiple, light red hematoma in consequence of minimal trauma).

Methods. Whole exome sequencing (WES) was carried out for the patient, his asymptomatic wife, his symptomatic daughter and her asymptomatic 8-year-old brother.

Platelet function was assessed by light transmission-, lumi-aggregometry and flow cytometry. Lysates of gel-filtered platelets were analyzed for total granule P-selectin, CD63 and von Willebrand factor (VWF) content by Western blotting and for serotonin levels by ELISA, respectively.

Results. Platelet function and characterization of the patients granula suggested a delta-storage pool disease (SPD). In most cases delta-SPD occurs as part of a syndrome, e.g. combined with albinism, immunodeficiency or a thalassemic-like blood disorder. As the patient and his daughter did not show any conclusive phenotype, their DNA was subjected to WES.

Exome sequencing revealed a not yet described GATA1- mutation close to two zinc finger domains (ZnF1 and ZnF2) in a highly conserved region of the GATA1 gene in the 4-year old daughter (c.886A>C, p.T296P, heterozygous) and her father (c.886A>C, p.T296P, hemizygous). This mutation was absent in 150 wildtype-controls but could also be demonstrated in the indexpatients´ asymptomatic mother. Only a few mutations are known to be located in this C-terminal region to date. Mutations in GATA1 may lead to different clinical presentations, depending on their location within GATA1 (e.g. Diamond-Blakfan anemia (exon2), X-linked thrombocytopenia (ZnF1), transient myeloproliferative disorder (Intron 1, exon 2, exon 3) and acute megakaryoblastic leukemia (Intron 1, exon 2, exon 3) in case of Down-Syndrome). Significantly increased HbF-levels (reference level: ≤0,8%) in the affected family members of 13,5% (4-year-old daughter) and 2,8% (35-year-old indexpatient) suggested dyserythropoiesis, although thalassemic features of the blood count were lacking.

Conclusion. We describe a GATA1 mutation as the cause of a delta-storage pool disorder. Imbalanced X-chromosome inactivation might explain the different phenotypes of the GATA1 mutation carriers and will be investigated through allele-quantification based on RNA isolated from whole blood and from platelet rich plasma in case of the index patient and different family members.

A familial Case of Patterned Macular Dystrophy originally diagnosed as Stargardt's Disease

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We saw three siblings (aged 62, 61, 54; two female, one male) with variable signs of retinal disease. All three developed night blindness till the 3rd decade, furthermore near-sightedness and deterioration of the visual acuity to a various degree at age 35-45. The clinical diagnosis was given as Stargardt's disease, Fundus flavimaculatus or unspecified retinal degeneration respectively.

Two more siblings (aged 59 and 57) and the mother (died age 62) were clinically unaffected. The father (died age 69) was reported to have had bad eyesight beginning in the 5th decade, his father similarly (no further information available). The three affected siblings have a total of five children (age 25-39), none of them showing clear signs of retinal disease up to now.

Considering the clinical diagnosis Stargardt's disease we analysed the genes ABCA4, ELOVL4, PROM1 and CNGB3 by Sanger sequencing. No pathogenic mutation could be detected. Afterwards we performed next generation sequencing of several genes associated with retinal dystrophies and found a novel splice site mutation in the PRPH2 gene (MIM #179605). The mutation was confirmed in all three affected siblings by Sanger sequencing. Considering that mutation pathogenic we could re-diagnose our patients with Patterned

Macular Dystrophy type 1 (MIM #169150). That disease is inherited in an autosomal dominant fashion, corresponding to the pattern of inheritance evident in our family.

Severe early-onset epileptic encephalopathy due to a novel GABRG2 missense mutation

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The etiology of epileptic encephalopathies, characterized by severe, early-onset seizures accompanied by developmental delay or regression, is highly heterogeneous. In recent years, next-generation sequencing approaches have led to the discovery of numerous causative genes; however the spectrum of associated phenotypes still needs to be further explored for many of these genes. We performed multi-gene panel analysis in a little boy of German non-consanguineous parents who showed severe early-onset infantile epileptic encephalopathy and almost absent neurological development. In this patient we identified a novel heterozygous missense mutation in the GABRG2 gene which was absent in the parents. In silico analyses strongly suggested a pathogenic relevance of this sequence variation which resides within a highly conserved region. So far, GABRG2 mutations have mainly been associated with milder types of epilepsy such as febrile seizures and childhood absence epilepsy. Therefore, our findings extend the phenotypic spectrum associated with mutations in this gene at the severe end.

Pitfalls in molecular genetic diagnostics

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Probably every clinical laboratory geneticist may look back on at least one case in his career which has caused him quite a headache. This means those cases with completely unexpected and at a first glance implausible results which could be interpreted correctly only after intensive enquiry and additional testing. Here, we report on three of such pitfall cases from our routine diagnostics.

Case 1:

A 30-year-old woman, pregnant with monozygotic twins, was referred to prenatal cystic fibrosis diagnosis. She and her partner were carriers of mutations in the CFTR gene (621+1G>T and G542X, respectively). Prenatal molecular diagnosis demonstrated that both fetus had inherited only the paternal mutation. Routinely, we performed maternal cell contamination analysis by comparing polymorphic microsatellite loci between the maternal and fetal DNA. Surprisingly, 12 of 16 tested microsatellite loci revealed a discrepancy between the maternal and fetal genotypes, meaning neither of both maternal alleles was present in fetal DNAs. A potential confusion of samples was excluded. Moreover, the presence of paternal mutation in fetal DNAs indicated a correct genetic relationship between awaited children and the partner of pregnant woman. The only one plausible interpretation of the obtained result was a pregnancy by egg donation. Afterwards, this suspicion was confirmed by the couple.

Case 2:

We present a 38-year-old man with infertility resulting from azoospermia. Conventional chromosomal analysis and an additional FISH analysis using Y probes indicated a 46,XX karyotype with no detectable SRY. In parallel, a molecular AZF (Azoospermia factors) diagnostic was performed by a standard multiplex PCR. By this method the absence of SRY region and a deletion of regions AZFb and AZFc, was identified, explaining the observed azoospermia. Interestingly, the PCR showed that the AZFa region was still present in patient chromosomes, contradicting cytogenetic and FISH results. Thus, a complementary FISH analysis was performed in order to reveal a low-grade Y-mosaicism and SRY material was detected in 3% of the cells (a result under the threshold level). Based on this observation, PCR conditions for the AZF diagnostic were modified and a very weak SRY-specific PCR product detected.

Case 3:

A molecular diagnostics for FraX (fragile X syndrome) was requested for a 4-year-old boy with a slight delay in speech development. His brother was already molecular-genetically diagnosed as having FraX. The analysis by Southern Blot hybridisation in the patient revealed a smear of methylated fragments characteristic for an expanded allele in the full mutation range. Surprisingly, two fragments of normal length, methylated and non-methylated, could also be detected in patient's DNA. A subsequent aneuploidy MLPA confirmed a

supernumerary X-chromosome in the patient consistent with a Klinefelter syndrome. These results were verified by an independent cytogenetic analysis.

P-ClinG-110

An atypical form of congenital symmetric circumferential skin creases or a new syndrome?

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The syndrome of congenital symmetric circumferential skin creases (CSCSC1 and CSCSC2) replaces the old term Michelin-tire-baby syndrome (MIM 156610) and is characterized by congenital circumferential skin folds, primarily of the limbs, facial dysmorphism, cleft palate and intellectual disability. Mutations in the ß-tubulin encoding gene TUBB or in the microtubule end binding family member MAPRE2 are the underlying genetic cause.

Here, we present two girls from two non-consanguines families showing clinical aspects of the syndrome of congenital symmetric circumferential skin creases. Additional features are present in both girls.

Patient 1 is the first child of healthy parents. She was born at 39+6 weeks of gestation with a weight of 2660 g, a length of 49 cm and a head circumferences of 34 cm. Respiratory distress, a cleft palate, a heart defect (atrial septum defect), an anogenital malformation and facial dysmorphism were diagnosed after delivery, additionally to the skin creases phenotype. Conventional karyotyping performed on blood lymphocyte cultures and CGH-Array analysis showing normal results.

Patient 2 is the second child of unrelated healthy parents. Her older sister is healthy as well. Because of an intrauterine growth retardation an amniocentesis with chromosomal analysis was performed, showing a normal karyotype of 46,XX. Patient 2 was born at 38+3 weeks of gestation by caesarean section. The birth weight was 2450 g, the birth length was 47 cm and the head circumferences was 34 cm and the APGAR score was 1/3/5. Due to respiratory distress and hypoxia a tracheotomy was initiated. She also presented with cleft palate, feeding difficulties, a heart defect (atrial and ventricular septum defect), asplenia and facial dysmorphism. The skin phenotype was remarkably similar to that of patient 1 with a prominent neck fold and skin creases mainly on the back part, but also at the limbs.

In both children the skin folds gradually diminish over the time without any intervention like it was described for Michelin-tire-baby syndrome/CSCSC1 and CSCSC2 patients. For these reason, a disease-causing mutation in the genes MAPRE2 and TUBB were excluded in both children. To identify a genetic cause, we performed a trio whole-exom sequencing, including the healthy parents and the affected child, in both families. A de novo stop mutation was detected in patient 1, while no promising results could be detected in patient 2. Further studies, especially functional in vivo studies and analysis of further patients with a similar clinical presentation will answer the question if the above described phenotype is an expanded variant of the congenital symmetric circumferential skin creases or a unique new syndrome.

P-ClinG-111

Clinical findings in a family with X-linked hypohidrotic ectodermal dysplasia due to a duplication of exon 2 in the EDA gene - a case report

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We want to summarize the phenotypic spectrum in one affected three generation family with X-linked hypohidrotic ectodermal dysplasia. We report on one strongly affected male and 2 slightly affected female carriers, carrying a duplication of exon 2 in the EDA gene.

Reason for genetic assessment was the request of a female for evaluation of a possible risk for occurrence of the familial disorder in a further planned pregnancy. The woman reported an inability to breastfeed and she and her daughter showed conical teeth, a dry skin and sparse hair. The affected male showed absent deciduous teeth, hypodontia of permanent teeth, missing regulation of the temperature due to a lack of sweat glands, bilateral nipple hypoplasia, a dry and wrinkled skin, missing eye lashes, eyebrows and scalp hair and sparse body hair. The fingernails were inconspicuous, whereas the nails of the toes, particularly the nails of the hallux were yellowish and thickened. The affected male had an operation due to a gallstone and cysts were found in one kidney. There was no increased predisposition to infections.

The suspected diagnosis of X-linked hypohidrotic ectodermal dysplasia was confirmed by genetic analysis of the EDA gene (sequencing and MLPA analysis). A duplication of exon 2 was detected in the affected male patient and was confirmed in the two mildly affected female relatives by Realtime-PCR.

Referring on a case report by Al Marzouqi et al. (2014), who reported on a girl with bilateral amastia in the context of skewed X-inactivation, we want to underline the importance of MLPA analyses in the case of negative sequencing of the EDA gene, as whole exon duplication can be the cause of hypohidrotic ectodermal dysplasia. To our knowledge, the report of Al Marzouqi et al. has been the only case about this genetic alteration in the literature so far.

P-ClinG-112

Novel POGZ mutation in a patient with intellectual disability, microcephaly, strabismus and sensorineural hearing loss

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Mutations in the pogo transposable element with zinc finger domain (POGZ) gene have recently been reported as a cause of syndromic autosomal dominant intellectual disability. POGZ encodes a heterochromatin protein 1 alpha-binding protein localized to the nucleus in neurons. It plays an important role in mitotic progression through heterochromatin formation and chromosomal segregation. Additional clinical features of the patients including short stature, hypotonia, microcephaly and/or brachycephaly, visual abnormalities and characteristic facial dysmorphisms had enabled the delineation of a new clinical entity termed White-Sutton syndrome (OMIM 616364).

We report on a 3-year-old male patient with severe intellectual disability, microcephaly, sensorineural hearing loss, ocular abnormalities (strabismus and hyperopia), congenital heart defect (atrial septum defect and pulmonary stenosis) and minor facial abnormalities (thin upper lip, frontal upsweep). Next-generation sequencing analysis revealed a novel heterozygous de novo mutation in POGZ: c.2703_2710del, p.(Thr902Serfs*39).

The clinical problems of this patient are in accordance with the findings in the previously reported POGZ mutation carriers. Reports of additional patients with POGZ mutations will be needed to establish detailed phenotype-genotype correlations of this novel and probably underdiagnosed syndrome.

P-ClinG-113

Novel clinical and molecular aspects in two patients with Kleefstra syndrome

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The phenotype of Kleefstra syndrome is clinically variable but characterized by facial dysmorphism, intellectual disability, childhood hypotonia and variably associated other malformations. Haploinsufficiency of EHMT1, caused by either heterozygous microdeletions in 9q34.3 or sequence mutations in EHMT1, has been identified to be the underlying causal mechanism.

Here we present two girls from two unrelated families with clinical signs of Kleefstra syndrome. Besides the main features such as facial dysmorphism, intellectual disability/developmental delay and childhood hypotonia, the 17 years old girl presented with additional accelerated growth whereas the other girl, 2 years old, showed failure to thrive. Both children have no heart defect, renal or urogenital anomalies or severe respiratory infections.

We identified two rare variants likely to be causal : a novel heterozygous splice site EHMT1 variant and a heterozygous microdeletion in chromosome 9q34.3, including exons 26 an 27 of the EHMT1-gene .

These patients broaden the spectrum of Kleefstra – associated EHTM1 causes, contribute to novel aspects of genotype- phenotype correlations and a better understanding of the clinical variability of the disorder.

Sequence analyses of EMX2 in 142 female patients with anomalies of the Müllerian ducts

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The mammalian female and male reproductive tracts develop from the paramesonephric ducts (Müllerian ducts, MD) and mesonephric ducts (Wolffian ducts, WD), respectively. In the absence of testicular differentiation and anti-Müllerian hormone, the WD regress and the MD give rise to the fallopian tubes, uterus, cervix and the upper part of the vagina.

Disorders of normal MD development in females can manifest as fusion anomalies of the uterus such as septate uterus, bicornuate uterus, unicornuate uterus and uterus didelphys, or more complex malformation patterns like Mayer–Rokitansky–Küster–Hauser syndrome (MRKH) or Herlyn-Werner-Wunderlich syndrome. MRKH is characterized by congenital absence of the uterus and the upper two-thirds of the vagina in individuals with a normal female karyotype, most of whom show normal ovarian function. MRKH can further be associated with additional malformations e.g. of the kidneys and the skeletal system. Herlyn-Werner-Wunderlich syndrome is characterized by uterus didelphys, obstructed hemivagina and ipsilateral renal agenesis.

Despite anomalies of the MD occur quite frequently, the etiology of most cases with these disorders remains unknown. The homeodomain transcription factor EMX2 (empty spiracles homeobox 2) was found to be critical for urogenital and central nervous system development. Previous studies showed that in Emx2 mutant mice, the kidneys, ureters, gonads and genital tracts were completely missing. In order to elucidate whether mutations in EMX2 are causative for MD anomalies in humans, we performed sequence analyses of EMX2 (GenBank NM_004098.3) in our study group of 142 female patients with clinically characterized disorders of the MD including 62 patients with MRKH and 7 cases with Herlyn-Werner-Wunderlich syndrome. We found the heterozygous intronic mutation c.592-17C>A twice in this cohort. This variant has been described earlier (rs41308651) and is listed in the Exome Aggregation Consortium ExAC variant with a minor allele frequency of 0.23%. In silico analysis revealed no significant changes for the correct splicing of EMX2. We therefore consider this variant to be a benign polymorphism. We conclude that mutations in EMX2 are not causative for disorders of the MD in our cohort.

Deep intronic variants introduce DMD pseudoexon in patient with dystrophinopathy

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Dystrophinopathies are X-linked muscle diseases caused by mutations in the *DMD* gene (OMIM: 300377). Due to the huge size of this gene, the detection of mutations is sometimes challenging. Despite multiplex ligation-dependent probe amplification (MLPA) and sequencing of all 79 exons, about 7% of patients do not show any mutations in coding regions and therefore remain without molecular diagnosis. We assume that the majority of these patients have deep intronic variations (DIV) which are not detectable by standard diagnostic techniques.

The index patient analysed is a twelve-year-old boy who was by chance diagnosed with elevated CK levels (up to 15,000 IU/I) at eight weeks of age. Today he is still able to walk without walking aids but needs assistance when climbing stairs. In 2008, a muscle biopsy revealed complete absence of dystrophin which established the diagnosis of DMD. For the molecular diagnosis, standard diagnostics ascertained no causative mutation. Therefore we decided to search for deep intronic mutations. We isolated mRNA from muscle tissue of the patient and amplified overlapping cDNA fragments using RT-PCR. The fragments were analysed by gel electrophoresis for size differences compared to an unaffected control. The cDNA product comprising exons 6-12 revealed an augmented fragment size compared to the control and the expected product size (about 1100 bp instead of the expected 1007 bp). We sequenced the altered cDNA product using BigDye Terminator sequencing mix v1.1 on an ABI3130xl Genetic Analyzer (Applied Biosystems) and detected an insertion of 77 bp between the exons 7 and 8. We located the insertion's sequence in intron 7 of the *DMD* gene and sequenced flanking sequences of gDNA to find the underlying mutation causing the insertion. Two hemizygous single nucleotide variants (SNVs) surrounding the inserted fragment could be identified. The first variant (c.650-39575 A>C) is a common polymorphism (MAF according to 1000 Genomes Project: 14.97 %) at the position of an existing acceptor splice site. The second variant (c.650-39498A>G) is novel and creates a new cryptic donor splice site with high probability. These two cryptic splice sites create the formation of a 77 bp pseudoexon which produces a frameshift and a premature stop codon (p.Asp217Alafs*) in the *DMD* gene.

In summary, we could genetically confirm the clinical diagnosis of a dystrophinopathy by two DIVs in *DMD*. Although the insertion of the pseudoexon creates a premature stop codon the patient's clinical phenotype

indicates a milder type of Becker muscular dystrophy. This contradiction could be explained by the remaining existence of DMD wild type mRNA most likely due to a not constantly active cryptic splice site. Most interesting about the present case is the fact that a common SNV facilitates the creation of a pseudoexon. This makes the region a potential hotspot for DIVs in the *DMD* gene which would be worthwhile further investigations.

P-COMPLEX DISEASES, POPULATION & EVOLUTIONARY GENETICS AND GENETIC EPIDEMIOLOGY

P-Compl-116

Impact of the androgen receptor CAG-repeat length on handedness

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With more than 90% of all humans preferring to use their right hand, handedness is the most noticeable functional expression of cerebral lateralization in humans. However, the precise molecular mechanisms that regulate handedness and other related forms of cerebral lateralization remain largely elusive. Therefore, the question which genetic, epigenetic and environmental factors contribute to human handedness is one of the central questions in research on lateral asymmetries. Handedness is a complex, heritable trait, for which polygenic inheritance is assumed, meaning that a large number of genetic factors with a small additive effect contribute to the observed variance in hand preference. To date, genetic association studies have implicated only a few specific genes influencing handedness. Particularly interesting is the association between the human androgen receptor (AR) gene and different aspects of handedness, since the interrelationships constitute a conceptual bridge between the theories that invoke testosterone as a factor in the development of cerebral asymmetries with theories proposing that the X chromosome contains a locus that influences the direction of hand preference. In an initial large association study in 1057 samples of healthy adults we already demonstrated that handedness in both sexes is associated with the AR CAG-repeat length, with longer repeats being related to a higher incidence of non-right-handedness. In addition, we have performed a second association study in an independently collected healthy cohort of more than 1000 test persons with comprehensive data on the handedness phenotype. We were able to replicate the association with longer CAG repeats being related to a higher incidence of non-right-handedness, especially in females. Since longer CAG-repeat blocks have been linked to less efficient AR function, these results implicate that differences in AR signaling in the developing brain might be one of the factors that determine individual differences in brain lateralization.

P-Compl-117

Whole exome sequencing to identify genetic causes of Congenital Prosopagnosia

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Congenital prosopagnosia (CP), also known as developmental prosopagnosia or face blindness, describes the inability to recognize faces. Cognitive functions such as intelligence as well as the sensory visual capabilities are usually not impaired but people with Prosopagnosia are negatively affected in their social life because individuals with the disorder have difficulty in recognizing family members, close friends or colleagues. Although the prevalence of CP is estimated at 2.5% and it appears to run in families, the contexts, which genetic, epigenetic and environmental factors contribute to this trait, are largely unknown. Therefore we started to establish a large, well-characterized CP cohort for genetic studies. We hypothesize that rare highly penetrant non-synonymous genetic variants could explain some cases of CP. As part of a larger genetic study of patients with CP, we performed family based whole-exome sequencing and targeted re-analysing on four individuals from two families with multiple affected members. By obtaining samples from affected and unaffected members of the same family, we hope to effectively identify de novo and inherited variations. Variations are considered on the basis of allele frequency, mutation type, literature and mutation prediction tools, thus generating a list of candidate variations/genes for each patient that is amenable to interpretation and further analyses in the

extended cohort. Through this approach, we hope to identify causal variations/genes in families and isolated patients with CP.

P-Compl-118

CARD14 mutations in patients with an "EKV-like" phenotype

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Erythrokeratodermia variabilis (EKV) is a rare, autosomal dominant genetic skin disorder with a highly heterogeneous phenotype. To date, three causative genes (*GJB3, GJB4 and GJA1*) are described but further genetic heterogeneity is expected. *CARD14* mutations are only described for psoriasis vulgaris, generalized pustular psoriasis and pityriasis rubra pilaris.

For the first time, we present disease causing *CARD14* mutations in patients with an "EKV-like" phenotype. It refers to one familial case with two affected individuals, with an autosomal dominant transmission from the mother to the daughter and one independent sporadic case. All patients present typical EKV symptoms. A rash of well-demarcated erythematous and scaly plaques interspersed with distinct islands of uninvolved skin or small reddish papules coalescing into large reticulated scaly plaques and palmoplantar keratoderma. To confirm the suspected diagnosis of EKV, we analyzed a custom designed multi-gene panel by next generation sequencing with 74 genes associated to hereditary skin diseases (Agilent Haloplex Technology). The sequencing results did not reveal any mutation in the genes *GJB3*, *GJB4* and *GJA1*, but we found two pathogenic mutations in *CARD14*. In the familial case *c.467T>C* p.Leu156Pro (rs387907240, ENST00000570421.5) was detected, while the sporadic case carries *c.371T>C* p.Leu124Pro.

We hypothesize that different genetic and environmental factors are involved in the evolution of the phenotype in patients with *CARD14* mutations. Our cases show that classification of unusual skin phenotypes can be challenging without genetic testing. Therefore, gene panel sequencing is a cost-efficient and time-saving solution for solving difficult cases with sometimes unexpected genetic background.

P-Compl-119

Generalized pustular psoriasis as an oligogenic disease and further evidence for significant younger age of disease manifestation in carriers of IL36RN mutations

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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 identified in ~25-40% of patients with generalized pustular psoriasis (GPP), a rare, severe pustular variant of psoriasis. In addition, heterozygous missense variants in *CARD14* and *AP1S3* have been associated to pustular psoriasis as well and shown to be functionally relevant.

In order discover how relevant those genes were in our large GPP cohort of 61 patients recruited all over Germany, we screened them for coding variants in *IL36RN*, *CARD14* and *AP1S3* by Sanger sequencing and for quantitative aberrations by MLPA. We identified homozygous or compound heterozygous IL36RN

mutations in 15 of 61 GPP patients (25%) and single heterozygous mutations in 5 patients (8%). The most common mutations were c.338>C>T/p.Ser113Leu and c.227C>T/p.Pro76Leu, present on 49%/ 20% of mutated alleles, respectively. We also identified three so far unreported mutations: c.338C>A/p.Ser113X, c.295-300delACCTTC/p.Thr99_Phe100del and c.130G>A/p.Val44Met. According to molecular modeling, c.338C>A/p.Ser113X resulted in a shortened, de-stabilized protein analogous to c.280G>T/p.Glu94X. The other two mutations were also predicted to result in destabilized, likely disease-relevant, *loss-of-function* proteins.

Heterozygous *AP1S3* mutations were detected in two GPP patients, both of whom carried additional homozygous or compound-heterozygous *IL36RN* mutations. 4 GPP patients were heterozygous carriers of rare missense variants in *CARD14* (7%); of note, two of these patients carried additional mutations in *IL36RN*.

Our genotype-phenotype correlation revealed a similar gender distribution in carriers of *IL36RN* mutations and wildtype carriers, but a strong association between bi-allelic mutations in IL36RN and early age of manifestation (p=7.4x*10-04). As in other autosomal recessively inherited mutations, the frequency of parental consanguinity was significantly higher in patients with two *IL36RN* mutations compared to non-carriers.

Overall, our genetic studies suggest a lower impact of variants in *AP1S3* and *CARD14* in pustular psoriasis than of those in *IL36RN*. Interestingly, the combination of *IL36RN* mutations with either *AP1S3* or *CARD14* variants in several patients indicated an oligogenic inheritance rather than a purely monogenic one. Moreover, the oligogenic basis of this group of inflammatory diseases might currently be underestimated, as our study suggests that genetic risk factors other than *IL36RN* mutations remain to be identified in the majority of GPP patients.

P-Compl-120

Exome sequencing of 46 multiply affected schizophrenia families provides new insights into the pathogenesis of the disorder

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Schizophrenia (SCZ) is a multifactorial psychiatric disorder with a lifetime risk of ~ 1% and a heritability of about 60-80%. Analysing multiply affected families using whole exome sequencing (WES) is a very promising approach to identify new SCZ risk factors. In these families, individuals are affected with SCZ over several generations. It is likely, that in multiply affected families genetic variations with particularly strong effect co-segregate with the disorder and contribute to the development of the psychiatric symptoms. To our knowledge, the present study is the largest study analysing multiply affected SCZ families using WES worldwide so far.

We included 46 families with at least 3 affected members each. From each family, 3-5 individuals were exome sequenced on an Illumina HiSeq 2500 and analysed using the Varbank pipeline of the Cologne Center for Genomics (http://varbank.ccg.uni-koeln.de) and the CLC bio Biomedical Genomics Workbench. We included rare (allele frequency ≤ 0.1% in the Exome Aggregation Consortium dataset) variants that were predicted to be pathogenic (Combined Annotation Dependent Depletion Score ≥ 15; cadd.gs.washington.edu), confirmed by Sanger Sequencing and co-segregating with the disorder.

In total we identified potentially pathogenic mutations in ~ 880 genes. A substantial proportion of these will not contribute to the pathogenesis of SCZ. In order to further tease out the most promising candidate genes we applied multiple strategies: (i) Screening our mutations in independent patient and control cohorts through international cooperations (access to more than 3,000 SCZ patients), (ii) gene-based tests, (iii) pathway- and network-analyses, (iv) gene expression analyses and (v) sequencing of the candidate genes in 2,500 SCZ patients and 2,500 controls. Analyses are ongoing and will be presented at the upcoming conference.

Exome sequencing of multiply affected bipolar disorder families and follow-up resequencing implicate rare variants in neuronal genes contributing to disease etiology

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Bipolar disorder (BD) is a complex psychiatric disorder affecting more than 1% of the world's population. The highly heritable disease is characterized by recurrent episodes of manic and depressive symptoms.

As the cumulative impact of common alleles with small effect may only explain around 38% of the phenotypic variance for BD, rare variants of high penetrance have been suggested to contribute to BD susceptibility.

In the present study we investigated 226 individuals of 68 large multiply affected families of European origin using whole exome sequencing (WES). In each family, two to five affected individuals with BD or recurrent major depression were selected for sequencing. WES was performed on the Illumina HiSeq2500 platform and the Varbank pipeline of the Cologne Center for Genomics was used for data analysis. All identified variants shared within each family were filtered for a minor allele frequency <0.1% and potentially damaging effects predicted by at least four of five different bioinformatics tools.

We identified a total of 1214 rare, segregating and functional variants implicating 1122 different genes, of which 903 were brain expressed. Subsequently, we applied the Residual Variation Intolerance Scores (RVIS, Petrovski et al., 2013) and identified 294 genes that were ranked among the 20% most "intolerant" genes in the genome. Gene enrichment analysis of these genes showed a significant enrichment for a total of 18 pathways (p<0.001) including neuron projection, axon development and cell adhesion.

For follow up analyses, we prioritized genes that were either found in at least two unrelated families in the present study or that were previously reported in next generation sequencing or GWAS studies of BD. In addition, we enclosed the genes that were predominantly driving the significant pathways in the above mentioned gene enrichment analysis.

The different approaches of prioritization yielded 42 candidate genes that are currently being followed up by resequencing in cohorts of about 2500 independent BD cases and 2500 controls of European ancestry. The candidate genes include *CDH22* that encodes a calcium-dependent cell adhesion protein that may play an important role in the morphogenesis of neural cells during the development and maintenance of the brain.

For resequencing we use the single molecule molecular inversion probes (smMIPs) technology that enables multiplex targeted resequencing in large cohorts. The smMIPs sequences were designed with an empirically trained design algorithm MIPgen (Boyle et al., 2014) and sequencing is currently performed on the Illumina HiSeq2500 platform.

Our preliminary results strongly suggest that rare and highly-penetrant variants in neuronal and cell adhesion genes contribute to BD etiology. The results of resequencing of a large case/control sample will provide further evidence for an involvement of particular pathways.

P-Compl-122

The use of zebrafish as model system to quantitatively assess the impact of risk variants in non-coding regions in vivo

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Most human malformations occur early in embryonic development and are present immediately after birth. One common human birth defect is nonsyndromic cleft lip with or without cleft palate (nsCL/P), affecting about 1 in 1,000 newborns. NsCL/P has a multifactorial background with a strong genetic component. Recent genome-wide association studies identified several loci as risk factors for nsCL/P. Notably, most of them map to non-coding regions and are expected to have a functional impact through regulatory mechanisms. Given the early developmental time point of facial development and the resulting lack of accessible human tissue, follow-up analyses of risk variants are challenging. We are hypothesizing here that we might be able to quantitatively detect differences in regulatory activity between wildtype and risk variants located in predicted enhancers by using the zebrafish as model organism. The advantages of using the zebrafish are (1) *ex utero* development, (2) transparency of the fish, (3) easy manipulation and (4) relatively short generation times. We applied a dual-luciferase assay plasmid system which is based on a sequential measurement of two luciferases (firefly and sea pansy luciferase) in fish lysates upon injection of a single plasmid. This plasmid, which contains a minimal promoter (minP) and the enhancer region of interest, is microinjected into zebrafish eggs of one-cell stage. After three days, fish are collected, lysed and luciferase activity is measured using a luminometer.

For our *proof-of-principle* analysis we analyzed an nsCL/P risk locus on chromosome 13q31 (Ludwig et al. 2012). Through database research one enhancer was predicted that contained two strongly associated risk variants. *In vivo* fluorescence analysis using EGFP in zebrafish embryos revealed this enhancer to be active in craniofacial development, but qualitative differences in activity were not observed by eye. Upon cloning of the enhancer in the dual-luciferase system, our injection results *in vivo* indicate that the system is working in principle. However, a high standard deviation between single replicate measurements was observed, probably due to variability in transfection efficiency. We therefore are planning to adapt the protocol in order to screen for positively injected fish embryos. We are currently investigating the functionality of these screening constructs in zebrafish embryos. Results will be presented at the meeting. Once successful, our approach represents a practical method to quantify the activity of regulatory elements in real time *in vivo*. This will be of particular importance in the functional follow-up of genetic findings in non-coding regions for the majority of birth defects.

P-Compl-123

*** Discovery of selfish Mutations with Ultra-sensitive Sequencing (USS)

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New mutations in the germline are directly transmitted to our children and have therefore profound consequences in future generations. Surprisingly, despite the importance of de novo mutations (DNM) in heritable disease and our evolution, we know very little about the different mutagenic processes in our germline. Of particular interest are a handful of highly recurrent DNM associated with congenital disorders and/or rasopathies, that have been described as driver mutations expanding in the male germline. The mutation itself causes a change in the tyrosine kinase receptor/Ras/MAPK pathway, which in turn confers the spermatogonial stem cell a proliferative advantage. Selfish or driver mutations are quite common in cancer, but we still know very little about the selfish expansion in the male germline. The reason might be that mutations in the human germline are very rare, and it is rather difficulty to directly measure such rare events. Most of our knowledge on germline mutagenesis comes from indirect sequence comparisons or whole genome sequencing of pedigree families, but it renders little information about individual mutagenic events. For this reason, we have adapted an ultrasensitive, next generation sequencing (USS) technology for the measurement of rare mutations to study the expansion of selfish genes in the male germline. As a proof-of-principle, we have sequenced at an extremely high coverage exon 10 and 15 of the FGFR3 gene in young and old sperm donors. We found an increased mutation frequency for the loci associated with achondroplasia and thanatophoric dysplasia II in sperm of older donors. Our results also show that we can distinguish ultra-rare mutations occurring at a frequency of one in hundred thousand wild type; thus, making this method ideal to discover potential driver DNM that might be expanding with paternal age.

P-Compl-124

Genome-wide DNA methylation analysis of probands with genetic and environmental risk factors for affective disorders.

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Introduction: Affective disorders such as major depressive disorder (MDD) and bipolar disorder (BD) are genetically complex and heterogeneous disorders. Both genetic and environmental risk factors contribute to the etiology of the diseases. However, the neurobiological correlates by which these risk factors influence the disease development are hardly understood. Increasing evidence suggests that epigenetic modifications such as DNA methylation have important implications on the development of psychiatric disorders including MDD and BD. Several studies revealed that alterations in the DNA methylation can modulate gene expression in response to the environment. To investigate this, genome-wide DNA methylation analysis of 66 female individuals from three extreme groups (genetic-, environmental risk and healthy controls) was performed.

Methods: For the genome-wide DNA methylation analysis we selected: (i) 22 individuals with genetic risk (at least one 1st degree relative with a life-time diagnosis of MDD or BD), (ii) 22 individuals with environmental risk (maltreatment in the childhood trauma questionaire) and (iii) 22 matched healthy controls. All individuals were of European origin. Processing was done according to the manufacturer's protocol using the Infinium MethylationEPIC BeadChip (Illumina, San Diego, CA, USA) covering more than 850.00 methylation sites at the Life & Brain Center (Bonn, Germany). State-of-the-art data processing protocols, including correction for blood cell type heterogeneity, color correction, eliminating probes containing SNPs and cross reactive probes were used. After quality control and normalization 780,467 CpG-sites were tested for genome-wide DNA methylation by a linear regression model while accounting for biological as well technical covariates.

Results: The genome-wide DNA methylation analysis of the three extreme groups revealed 39 CpG sites ($p < 1 \times 10^{-04}$) in the subgroup analysis "environmental risk vs. controls" and 35 CpG sites ($p < 1 \times 10^{-04}$) in the analysis "genetic risk vs. controls". In addition, we identified 48 CpG sites ($p < 1 \times 10^{-04}$) in the comparative analysis of "genetic risk vs. environmental risk". None of these CpGs showed significant differential DNA methylation after correction for multiple testing. However the hierarchical clustering of the differentially methylated sites provided some evidence for differentially methylated patterns between the subgroups.

Discussion: Our genome-wide DNA methylation analysis of the extreme groups provided some evidence for differentially methylated CpG sites which unfortunately did not withstand correction for multiple testing. This may reflect at least in part that the sample size of the present study was too small to detect differentially methylated CpGs at the genome-wide level.

P-Compl-125

*** Immunochip meta-analysis and follow-up study in alopecia areata

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Alopecia areata (AA) is a common hair loss disorder that occurs in both sexes and all age groups. AA is thought to be a tissue-specific autoimmune disease directed against the hair follicle. Both, gene-based and genome-wide association studies have identified more than 10 susceptibility loci for AA; however, a large percentage of the overall heritable risk still awaits identification. To provide further insight into the immune related nature of AA, we and our US collaborators had each performed an Immunochip-based analysis. We

recently performed a meta-analysis, combining the data from both studies, and are now aiming to follow-up the best results in an additional German cohort by use of a Sequenom assay to identify novel susceptibility loci. We conducted the meta-analysis by using data from the above mentioned two studies on Illumina BeadChip arrays including a total of 1,096 cases and 3,176 controls of Central European origin. Method of synthesis of regression slopes (MSRS) was used for the analyses which are implemented in METAINER software package. For follow-up step, we chose the most promising candidate SNPs. These will be examined with the Sequenom MassARRAY iPLEX Platform in an independent AA sample comprising 1,459 cases and 970 controls. By use of the meta-analysis combining data from the US and our sample, we identified 49 novel loci with a suggestive p-value of PBecker-Wu ≤ 10-3 (PHet ≥ 0.01). Among them, NFkB is the most significant locus (PBecker-Wu = 1.5 x 10-07). In order to achieve genome-wide significance, we plan to follow-up the most promising SNPs in an independent German sample. We considered the 19 most significant loci (lower P-Becke-Wu value) for the replication step. The experiments are ongoing and results will be presented at the meeting.Despite the recent identification of susceptibility loci for AA, our understanding of the genetics of AA is incomplete. Identification of new loci may provide novel insights into biological pathways and a better elucidation of disease pathophysiology.

P-Compl-126

ISL1 is a major susceptibility gene for classic bladder exstrophy and a regulator of urinary tract development

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Previously genome-wide association methods in patients with classic bladder exstrophy (CBE) found association with *ISL1*, a master control gene expressed in pericloacal mesenchyme. This study sought to further explore the genetics in a larger set of patients following-up on the most promising genomic regions previously reported. Genotypes of 12 markers obtained from 268 CBE patients of Australian, British, German Italian, Spanish and Swedish origin and 1,354 ethnically matched controls and from 92 CBE case-parent trios from North America were analysed. Only marker rs6874700 at the *ISL1* locus showed association (p = 2.22 x 10^{-08}). A meta-analysis of rs6874700 of our previous and present study showed a p value of 9.2 x 10^{-19}. Developmental biology models were used to clarify the location of *ISL1* activity in the forming urinary tract. Genetic lineage analysis of *Isl1*-expressing cells by the lineage tracer mouse model showed *Isl1*-expressing cells in the urinary tract of mouse embryos at E10.5 and distributed in the bladder at E15.5. Expression of *isl1* in zebrafish larvae staged 14 hpf to 24 hpf was detected in the developing pronephros region. Our study supports *ISL1* as a major susceptibility gene for CBE and as a regulator of urinary tract development.

P-CYTOGENETICS AND CNVS

P-CytoG-127

Clinical and Molecular Characterization of a Novel Patient with a 8q22.2-q22.3 Microdeletion

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To date, seven patients with interstitial deletions at chromosome 8q22.2-q22.3 have been described in the literature. All patients reported had moderate to severe intellectual disability and a characteristic facial phenotype including blepharophimosis, telecanthus, epicanthus, flat malar region, and a thin upper lip vermillion. Six of the seven patients had epileptic seizures. By analyzing the deletion's overlaps, two distinct critical regions have been suggested for the facial phenotype as well as for intellectual disability and seizures.

Here we present another patient with a de novo 3.6 Mb deletion in 8q22.2-q22.3. The patient shows moderate mental retardation. He has an abnormal EEG, however, only one episode of clinical seizures has been observed so far. The facial gestalt resembles the typical dysmorphic features of the patients with 8q22.2-q22.3 deletions reported previously. Minor anomalies were short fingers and toes, and a single palmar crease. Our report supports the assumption that deletions in 8q22.2-q22.3 cause a distinctive and clinical recognizable microdeletion syndrome with characteristic facial features and intellectual disability. Since the patient's deletion overlaps with most of the critical region for the dysmorphic phenotype but only with parts of the intellectual disability critical region, the molecular data presented here further narrow down the critical region for the intellectual disability seen in patients with 8q22.2-q22.3 microdeletions.

P-CytoG-128

*** Cooks syndrome is associated with a duplication of the potassium channel KCNJ4

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Cooks syndrome (MIM106995) is a rare autosomal dominant disorder classically characterized by onychodystrophy, and anonychia, with absence or hypoplasia of the distal phalanges of the hands and feet. Large duplications including KCNJ2 were shown to be causative for Cooks syndrome. Recently mouse studies revealed that tissue specific misregulation of KCNJ2, a potassium channel of the subfamily J, cause hypoplasia of nail beds and abnormal distal phalanges, thus resembling the Cooks phenotype. Here we report on a three generation pedigree with typical features of Cooks syndrome that was negative for KCNJ2 testing. We performed high resolution array-CGH and identified 80 kb duplication on chromosome 22q13.1 encompassing only one gene: KCNJ4. The duplication segregates with the phenotype in the family. KCNJ4 belongs to the same subfamily of potassium channels as the known disease gene for Cooks syndrome KCNJ2 and both share several biological functions. Recent data show that gain of function mutations in another potassium channel KCNH1 cause Zimmermann-Laband syndrome, a congenital malformation syndrome also associated with hypoplasia or aplasia of nails and terminal phalanges. Therefore we propose that duplications of KCNJ4 may also cause tissue specific misregulation resulting in digit and nail defects. Taken together we show in a three generation pedigree that Cooks syndrome is associated with a duplication of KCNJ4. Our data further highlight the emerging role of potassium channels in congenital digit and limb anomalies.

P-CytoG-129

Case report: Deletion of the terminal short arm of chromosome 5 (Chromosome 5p deletion syndrome) without 5q-duplication with a familial history of a large pericentric inversion of chromosome 5

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We report on a male patient referred as a newborn with typical clinical features of Chromosome 5p deletion syndrome. Conventional karyotyping of lymphocyte cultures confirmed a deletion of the terminal short arm of

chromosome 5 with breakpoint in 5p14. The size of the deletion (22Mb) could be refined by microarray analysis and assigned to Pos 5:16497-22278242.

Parental cytogenetic investigations showed a normal karyotype in the mother whereas the father was revealed to be carrier of a large pericentric inversion of one chromosome 5. Odd crossing-over in the inverted segment of a pericentric inversion in a parent can lead to unbalanced offspring caused by gametes with a terminal deletion of the p-arm together with a duplication of the q-arm or gametes with a duplication of the p-arm together with a deletion of the q-arm.

In order to find out, whether the 5p-deletion in the child is the result of an independent event or if it is related to the structural chromosomal aberration of the father a microsatellite analysis is going to be performed

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P-CytoG-130

A small supernumerary marker chromosome of the pericentric region of chromosome 8 in a child with intellectual disability: case report and literature review

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Small supernumerary marker chromosomes (sSMC) are reported

in 0.043% of newborn infants. We report on a girl, which was born preterm at 28 weeks of gestation via cesarean section due to pathological CTG. The pregnancy was complicated by gestational diabetes. She presented with muscular hypotonia, multiple hemangiomas, dysmorphic features and feeding problems. The body measurements were in normal range. The feeding problems made a tube feeding necessary until the age of four months. Facial features consisted in epicanthus, high palate, broad nasal tip and broad nasal root. A brain MRI showed periventricular leukomalacia and hypoplasia of corpus callosum. Drug-resistant seizures with hypsarrhythmia started at the age of ten months.

The affected girl was the only child of healthy non-consanguineous parents. The father also presented with a few small hemangiomas in the face. There was no history of intellectual diasability in the extended family.

Karyotyping showed sSMC in mosaicism. Molecular characterization by array-CGH showed that the sSMC consists of pericentric chromosomal material derived from chromosome 8 (arr[hg19]8p11.1p21.3(22,816,527-43,396,776)x2~3,8q11.1q11.21(47,673,716-52,164,874)x2~3 dn

(GRCh37/hg19).

A review of the literature showed that the few patients with a sSMC of the pericentric regions of chromosome 8 described in literature show early feeding problems, developmental delay, delay in acquired language, difficulties in social skills, difficulties in attention and activity levels or autistic-like behavior. The facial dysmorphic features were not specific. Multiple hemangiomas have been reported in some cases. West syndrome or seizures have not been reported so far.

The phenotype of our patient is consistent with the literature data, however there are no patients with a sSMC showing the same breakpoints reported so far. Especially due to the mosaicism and the rare number of patients described in the literature, no precise clinical prognosis for this patient was possible.

P-CytoG-131

Copy number variation in integrating and non-integrating reprogrammed human induced pluripotent stem cells

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The generation of patient-specific induced pluripotent stem cells (iPSCs) by reprogramming of adult somatic cells (e.g. skin fibroblasts) represents a novel technology for studying disease mechanisms. For generation of iPSCs several reprogramming methods, including the use of integrating vectors and non-integrating vectors, have been developed. Since genetic abnormalities can arise during the reprogramming process, genetic quality assurance is indispensable.

The aim of this analysis was to compare the genetic stability of iPSCs generated using either a DNA-integrating or a DNA-non-integrating reprogramming technique in order to reveal possible method-specific differences.

We studied iPSCs derived from patients with Parkinson's disease (PD), the second most common neurodegenerative disease worldwide. To monitor the genetic stability, we investigated copy number variants (CNVs) in addition to conventional chromosome analyses in iPSCs of ten PD patients and six healthy control

individuals. Using high-resolution chromosomal microarray analysis (CMA), we monitored the formation of relevant CNVs during reprogramming to pluripotency by comparing fibroblasts and iPSCs of the respective individual.

To date, fibroblast cultures of all 16 selected probands as well as 47 retroviral reprogrammed iPSC clones and eight Sendai-viral reprogrammed iPSC clones were analyzed. Aneuploidies were detected in three fibroblast cultures (19%), in five retroviral iPSC clones (11%), and in none of the Sendai iPSC clones. *De novo* CNVs were identified in 33 retroviral iPSCs (70%) – 19 clones showed one (40%), nine clones showed two (19%), four clones showed three (9%), and one clone showed four newly arisen CNVs (2%). In seven of eight Sendai clones, *de novo* CNVs were detected (88%). Two *de novo* CNVs were identified in three clones (38%), three CNVs in two clones (25%), four in one clone (13%), and even six *de novo* CNVs were detected in one clone (13%). Additionally, a mosaic gain of the whole long arm of one chromosome 9 was identified in one Sendai clone. Only 14 of the retroviral reprogrammed clones (30%) as well as one Sendai reprogrammed clone (13%) showed no newly arisen CNVs. The CNVs were between 106 kb and 6.4 Mb in length in retroviral clones and between 106 kb and 926 kb in Sendai clones. Because almost all CNVs contained genes, including genes involved in neurodevelopment and transcriptional regulation, a biological relevance seems highly probable and changes in cell phenotype cannot be excluded.

Since iPSCs and cells derived thereof – independent of reprogramming methods – often contain aberrations not detected by standard chromosomal analysis, our findings highlight the importance of high resolution CMA procedures. In spite of differences in the analyzed number of clones, our results indicate more and larger CNVs in Sendai clones than in retroviral reprogrammed clones.

P-CytoG-132

Chromosome 17q23.1-q23.2 deletion syndrome – an additional case with sensorineural hearing loss.

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The Chromosome 17q23.1-q23.2 deletion syndrome (MIM #613355) is a contiguous gene syndrome caused by a deletion encompassing the chromosome region 17q23.1-q23.2. Initially, it has been described by Ballif et al. (2010). Up to now, only a few cases with a microdeletion 17q23.1 q23.2 have been reported. Most of them carry a microdeletion with recurrent breakpoints and similar size of about 2.2 Mb. The common clinical features of cases with the chromosome 17q23.1-q23.2 deletion syndrome comprise mild-to-moderate developmental delay, microcephaly, postnatal growth retardation, heart defects, limb anomalies, and hearing loss.

We present an additional male patient with a 2.2 Mb deletion of chromosome 17q23.1-q23.2, detected by array CGH (CytoChip ISCA 4x180K v1.0, Illumina). Our index patient shows typical symptoms of the chromosome 17q23.1-q23.2 deletion syndrome like developmental retardation (in particular speech delay), a head circumference at the 3rd centile, postnatal growth retardation with a body height at the 4th centile, heart anomalies (right-sided aortic arch, patent ductus arteriosus), and sensorineural hearing loss on both sides.

Our data improve the characterization of the typical phenotype caused by a chromosome 17q23.1-q23.2 deletion and reinforce the suspicion that this region might be associated with sensorineural hearing loss.

P-CytoG-133

Partial, homozygous deletions of AHI1 gene causes Joubert syndrome type 3

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We describe a patient who is second child of consanguineous healthy parents from Turkey. He was born after 37 weeks of gestation with normal birth parameters (2850 g, 51 cm, OFC 32 cm). At the age of three months atypical eye movements became apparent. Psychomotor development was delayed from the beginning, he presented with hypotonia and later developed ataxia. An abnormal breathing pattern was not noticed. An abnormal MRI with hypoplasia of the cerebella vermis at the age of 21 months and the clinical signs described above led to a clinical diagnosis of Joubert syndrome. At that time no diagnostic testing was available. He returned to the Outpatient Clinic of the Institute of Human Genetics at the age of 22 years having developed retinopathy in the meantime. His height was below average (~3 cm, <P3) but still within his family's range. Aside from the known irregular eye movements he showed nail hypoplasia of the thumbs, and small hands and feet. He was working at a sheltered workshop. The year before, the Pediatric Department of the University Hospital Heidelberg had initiated testing of 7 of the by then known genes causative for Joubert with no mutation being found (NPHP1, TMEM216, CC2D2A, MKS3, RPGRIP1L, OFD-1 and MKS4). The gene

associated with Joubert syndrome type 3 (OMIM #608629) was not included though. With the external MRI not being available at that time we decided to first perform chromosome and array analyseis. The conventional chromosome analysis was without pathological findings. Subsequent array analysis by Affymetrix® Cytoscan HD Oligo/SNP showed an 11 kb homozygous deletion in 6q23.3 within the AHI1 gene, the gene for Joubert syndrome type 3. The deletion encompasses exon 10 to 13 which includes parts of the WD-repeats (tryptophan-aspartic acid (W-D) dipeptide) on the protein level. The homozygous deletion was confirmed by MLPA in the patient and both parents identified as heterozygous carriers. So far, alterations in AHI1 leading to Joubert syndrome comprise homozygous point mutations or small heterozygous deletion/duplications in combination with a second pathogenic mutation. So we here present the first case of a large homozygous deletion in the AHI1 gene, being causative for Joubert syndrome type 3. We conclude that in unclear cases of a Joubert phenotype an array-CGH is recommended besides sequencing to clarify the molecular basis of the phenotype.

P-CytoG-134

A microduplication encompassing TBL1XR1 causes a genomic sister-disorder for the 3q26.32 microdeletion syndrome

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Recently, a new syndrome of intellectual disability (ID) with dysmorphisms due to deletions within the chromosomal band 3q26.32 harboring the TBL1XR1 gene was described. Additionally, a specific point mutation in TBL1XR1 causes Pierpont syndrome, a distinct mental retardation syndrome. We report four patients in which array-CGH analysis and real-time quantitative PCR of genomic DNA revealed TBL1XR1 microduplication. Adjacent genes were not affected. The microduplication was verified as de novo by real-time PCR of parental DNA in one patient. The other three cases occurred in two generations of a second, unrelated family.

We compare the remarkably similar clinical findings in TBL1XR1 microdeletion, point mutation and microduplication cases and expand the TBL1XR1-associated phenotypic spectrum. Among others, intellectual disability, hearing loss and autism spectrum disorders are common features of TBL1XR1-associated diseases. Our clinical observations add to the increasing evidence of TBL1XR1's role in physiological brain development and simultaneously demonstrate that opposing genetic disease mechanisms affecting TBL1XR1 can lead to similar ID phenotypes.

In conclusion, the observed phenotypic overlap indicates that this novel microduplication is a genomic sister-disorder to the 3q26.32 microdeletion syndrome.

P-CytoG-135

Supernumerary marker chromosome with putative neocentromere formation within 12q2?3q24.33 after complex rearrangement involving chromosome 4 and chromosome 12

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Neocentromere formation has been reported in more than 100 small supernumerary marker chromosomes (sSMC). Here we report the formation of a putative neocentromere within a supernumerary ring chromosome derived from chromosome 12 in a five year old patient with generalized developmental delay, microcephaly and hexadactyly. Chromosomal analysis (GTG-banding, resolution 550 bands) of the boy showed an aberrant karyotype with a derivative chromosome 12 demonstrating additional chromosome 4 specific material inserted into the distal part of the long arm (ins(12;4)(q24.33;q24q28)), a simultaneous deletion of the chromosomal region 12q2?3-q24.33 on the same homolog and an additional supernumerary small marker chromosome, presumably a ring chromosome in all metaphases tested. In parallel, Array-CGH was performed and revealed a gain of 22.6 Mb in chromosomal region 4q24-q28.1 without a detectable imbalance of chromosome 12 material within the limits of the method (400k array). FISH analysis with wcp probes and telomeric probes for chromosomes 4 and 12 confirmed the interstitial insertion of chromosome 4 material into the long arm of the derivative chromosome 12. The marker chromosome stained completely with a wcp12 probe but centromere probes for chromosomes 4 and 12 were both negative suggesting the formation of a neocentromere within region 12q2?3q24.33. In total, the complex rearrangement resulted in a partial trisomy 4q in the boy as a presumable explanation for the phenotype. The mother has experienced one abortion and the parents have two more children who show a normal development so far. Both parents were karyotyped. The analysis of the father was normal (46,XY). Konventional chromosomal analysis of the mother`s lymphocytes showed a

balanced form of the aberrant karyotype found in her son. The healthy mother possesses the insertion ins(12;4)(q24.33;q24q28), the simultaneous resection of the 12q2?3-12q24.3 material and generation of the additional ring chromosome with this material. During meiosis a normal chromosome 4, the derivative chromosome 12 and the ring chromosome were passed on to the son leading to the unbalanced karyotype as described above. Further analysis with pan-centromeric probes and immunohistochemistry with an antibody against CENP-A might confirm the existence of a neocentromere on the ring chromosome which was meiotically transmitted from the mother to the son.

P-MONOGENIC DISEASES - FROM GENE IDENTIFICATION TO MOLECULAR MECHANISMS

P-MonoG-136

*** Early shift of E/I balance in a heterozygous Tsc2KO mouse model leads to a tuberous sclerosis phenotype

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The mTOR (mechanistic target of rapamycin) kinase is the most important regulator of local dendritic and presynaptic protein translation in the brain. It has been shown to fundamentally influence AMPA receptor activity by shifting GluA1 to GluA2 balance and to control the synthesis of several proteins involved in synaptic function and plasticity. Both, loss and gain of mTOR activity lead to significant disturbances of brain homeostasis and neuronal function resulting in intellectual disability (ID), epilepsy, autism and behavioural alterations. In order to analyse mechanisms of homeostatic regulation of mTOR-dependent synaptic function we are using a heterozygous Tsc2 knockout (KO) mouse model for tuberous sclerosis (TS). TSC2 (together with TSC1) is part of a complex that inhibits the mTOR kinase. Mutations in either of the two genes result in increased mTOR activity and upregulated downstream signalling and are causative for autosomal dominant TS.

It had been shown previously that mTOR hyperactivity leads to a set point shift and significantly influences neuronal homeostasis by altering cell excitability and E/I balance in heterozygous Tsc2KO animals. According to these studies we hypothesize that this shift significantly leads to synaptic connectivity and plasticity as well as network alternations that result in progressive development of cognitive dysfunction, epilepsy, autism and behavioural alterations over time in TS.

We have established a behaviour battery, which consists of a novel object recognition test (NORT), a morris water maze test (MWM) and an evaluation of nest building and social interaction to study social behaviour and cognitive performance in heterozygous Tsc2KO mice in different age groups.

Interestingly we found that, while two months old animals do not show any alterations, three to four months old animals develop significant disturbances in social behaviour in the social interaction test and in nest building. Furthermore, we could show significant changes in cognitive performance of seven to eight months old heterozygous Tsc2KO mice in the NORT compared to three to four months old heterozygous Tsc2KO animals.

These data show that the TS phenotype builds up on shifts of the E/I balance that develop at a very early stage. Pharmacological intervention with E/I balance is a promising therapeutic strategy to prevent brain damage caused by congenital hyperexcitability and homeostatic dysfunction.

P-MonoG-137

The challenge to insert Costello syndrome causing HRAS mutations into human keratinocytes using the CRISPR/Cas9 editing technology.

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Germline missense mutations in the *HRAS* gene cause Costello syndrome, a rare developmental disorder characterized by a typical facial gestalt, postnatal growth deficiency, intellectual disability, and predisposition to malignancies as well as skeletal, cardiac and dermatological abnormalities. The molecular pathophysiology caused by heterozygous *HRAS* gain of function mutations has been analysed in various tissues and cell types, however, up to date the molecular basis for cutaneous manifestations in Costello syndrome is largely unknown. To address this question in an appropriate model system, permanent human keratinocyte (HaCaT) cells carrying Costello syndrome-associated mutations in the endogenous *HRAS* gene should be generated by

using the CRISPR/Cas9 technology. Double strand breaks induced by Cas9 can be repaired in two ways: the error-prone non-homologous end-joining pathway for the generation of knockout models or the homology directed repair pathway, which allows precise editing. The latter enables the introduction of specific point mutations into a cell line by using a single stranded DNA (ssDNA) as repair template. However, we found that this is a very rare event and its efficiency depends on various factors including used cell line, selected guide RNA, length and amount of ssDNA and also Cas9 variant. Nonetheless, by using Cas9 wildtype, we could insert the disease-associated c.35G>T (p.G12V) mutation into genomic *HRAS* both in HaCaT cells (8 positive clones out of 800) and HEK cells (3 positive clones out of 15). In contrast, using the Cas9 nickase protein variant that prevents off target effects, did not result in positive clones. Taken together, in 10 months of working with CRISPR/Cas9 we gradually gained experience with many problems and pitfalls of this technology and, finally, now we are able to introduce point mutations in cell lines. Next, we will use the mutant HaCaT cell line to gain deeper insight into the function of HRAS for epidermal homeostasis and its deregulation in Costello syndrome.

P-MonoG-138

Mutations in the Heme Exporter FLVCR1 Cause Sensory Neurodegeneration with Loss of Pain Perception

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Pain is necessary to alert us to actual or potential tissue damage. Specialized nerve cells in the body periphery, so called nociceptors, are fundamental to mediate pain perception and humans without pain perception are at permanent risk for injuries, burns and mutilations. Pain insensitivity can be caused by sensory neurodegeneration which is a hallmark of hereditary sensory and autonomic neuropathies (HSAN). Although mutations in several genes were previously associated with sensory neurodegeneration, the etiology of many cases remains unknown. Using next generation sequencing in patients with congenital loss of pain perception, we here identify bi-allelic mutations in the *FLVCR1* (Feline Leukemia Virus subgroup C Receptor 1) gene, which encodes a broadly expressed heme exporter. Different FLVCR1 isoforms control the size of the cytosolic heme pool required to sustain metabolic activity of different cell types. Mutations in *FLVCR1* have previously been linked to vision impairment and posterior column ataxia in humans, but not to HSAN. Using fibroblasts and lymphoblastoid cell lines from patients with sensory neurodegeneration, we here show that the *FLVCR1*-mutations reduce heme export activity, enhance oxidative stress and increase sensitivity to programmed cell death. Our data link heme metabolism to sensory neuron maintenance and suggest that intracellular heme overload causes early-onset degeneration of pain-sensing neurons in humans.

P-MonoG-139

Analysis of the STAP1-gene in patients with familial hypercholesterolemia

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Autosomal-dominant familial hypercholesterolemia (FH, OMIM 143890) is one of the most common genetic diseases with an estimated prevalence of 1: 200 to 1: 500. Three major genes have been associated with the disease: low density lipoprotein receptor (LDLR), apolipoprotein B (APOB) and proprotein convertase subtilisin / kexin 9 (PCSK9). We have recently published data on the mutation spectrum in these three genes in about 200 FH patients from Germany, however, this screen for mutations was negative in about 40% of the cases. STAP1 (signal transducing adaptor family member 1) was recently suggested to be the fourth FH gene. We therefore screened the STAP1 gene for disease causing variants in a group of 70 FH patients negative for mutations in the three established FH genes by direct DNA sequencing (Sanger). The newly identified variants will be presented including a phenotype-genotype correlation.

P-MonoG-140

MIDAS Case Report: Molecular Diagnostics of Helsmoortel-Van der Aa syndrome by Exome Trio Sequencing

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Phenotype: We present a 13-year-old girl with developmental delay, craniofacial anomalies, congenital eye anomalies (4.5 dpt), short stature and behavioral disorder with auto-aggressions. Preliminary examinations (array-CGH analysis, karyotyping, diagnostic of metabolism, EEG, cMRT, sequencing of *SRCAP*-gene) revealed normal results.

Methods: In course of the MIDAS-Project, we sequenced the index patient and her healthy, non-consanguineous parents on a NextSeq500 platform (Illumina, San Diego, CA, USA) to perform a trio analysis. For library preparation we used an enzymatic fragmentation approach. Exome capture was performed with a SureSelect Human All Exon kit V6 (Agilent, Santa Clara, CA, USA), targeting coding exons and conserved splicing sites of over 20.000 genes. The library of the index patient was sequenced with a 285-fold mean coverage as 151-bp paired-end reads. 94% of the target region were covered 20-fold or higher. Data analysis and variant evaluation was done using the CLC Genomic Workbench 9.0 (Qiagen, Hilden, Germany) and annotations from commercial as well as public databases.

Results: The trio analysis of whole-exome sequencing data from the index patient and her parents revealed 58 variants. We identified the disease-causing *de novo ADNP* mutation c.2188C>T (p.Arg730*, ADNP), resulting in the introduction of a stop codon in exon 5/5 and truncation of the corresponding protein. Both of the parents did not carry this mutation. ADNP is part of the ATP-dependent BAF chromatin-remodeling complex, which is involved in the regulation of gene expression. An influence on neuronal cell differentiation is postulated. Mutations in the *ADNP* gene were previously reported in 10 patients with autism and mild to severe intellectual disability. All reported *ADNP* mutations are characterized by *de novo* mutations that are clustering in exon 5 and severely disrupting the protein-coding sequence, either by the introduction of a stop codon or a frameshift induction. By additionally considering the clinical symptoms, we were able to diagnose the Helsmoortel-Van der Aa-Syndrome (HVDAS; OMIM ID: 615873).

Discussion: The challenge of next-generation sequencing (NGS) approaches in diagnostics is no longer the technical feasibility or costs but the medical interpretation of the large sequencing data sets. Besides a robust genetic analysis with focus on disease-associated genes, family anamnesis and detailed information of clinical symptoms in a standardized scheme are important for a reliable diagnosis. Here, we present one of over 50 cases that will help to establish the Multiple Integration of Data Annotation Software (MIDAS), which will enable phenotype-genotype-correlations to enhance the validity of results of genetic analysis, minimize the risk of misinterpretation and enable faster diagnosis in routine patient care.

P-MonoG-141

NGS in peripheral neuropathies – experiences and conclusions

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Peripheral neuropathies are a group of clinically and genetically heterogeneous disorders, mutations in a large number of genes have been described so far. Among patients with Charcot Marie Tooth disease (CMT) / Hereditary Motor and Sensory Neuropathies (HMSN), a common duplication of the PMP22 gene on chromosome 17p12 is by far the most frequent mutation; sequence variants in the genes GJB1, MPZ and MFN2 account for a considerable proportion of patients but all other associated genetic variants are extremely rare. Deletions of the PMP22 gene lead to Hereditary Neuropathy with Liability to Pressure Palsies (HNPP) but transition between the different phenotypes may be rather fluid in some instances.

Next Generation Sequencing (NGS) has revolutionized CMT diagnostics as a large number of genes can be investigated at a time but it also leads to challenging questions. Use of NGS has certainly improved the diagnostic yield in CMT diagnostics and it has repeatedly resulted in unexpected findings. Broad molecular testing has already disclosed overlaps between clinical conditions and more surprising results are yet to come. Here we report our recent experiences with the diagnostics of neuropathies. Our results, different testing

strategies, implications for clinical management and genetic counselling will be discussed and possible further steps suggested.

P-MonoG-142

Ccdc66/ CCDC66 expression and localization during embryonic development in the mouse

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Introduction:

The *Ccdc66*-deficient (*Ccdc66*^{-/-}) mouse model exhibits slow retinal degeneration similar to a human Retinitis pigmentosa (RP) phenotype (Gerding *et al.*, *Hum Mol Genet.*, 2011). Retinal CCDC66 protein expression in the wildtype (WT) mouse demonstrates highest levels shortly after birth. Moreover, CCDC66 protein can also be detected early postnatally in mouse brain tissue. Therefore, there is strong evidence that CCDC66 could also play a role in prenatal development of the mouse retina and brain. Embryonic stages of interest comprise <E10 at the initial development of the eye, E13 after formation of nasal pits and prominent brain growth, when brain vesicles are initially visible and E18 where the eye and nasal brain develops. This study aims at characterizing *Ccdc66* RNA and CCDC66 protein expression during key steps of prenatal development in the WT mouse and respective *Ccdc66* reporter gene expression in the *Ccdc66*^{-/-} model.

Methods:

Ccdc66 RNA expression was analyzed by quantitative *Ccdc66* qRT-PCR and CCDC66 protein expression was determined by SDS page and Western blot in eye and whole brain homogenates of the mouse at selected embryonic stages (<E10, E13 and E18). In addition, 15µm sagittal cryosections of mouse embryos were Hematoxylin and Eosin stained and analyzed for *Ccdc66* reporter gene expression by X-gal staining or antibody staining followed by light or fluorescence microscopy.

Results:

Ccdc66 RNA and CCDC66 protein is detected in the WT mouse at different developmental time points in the eye and brain tissue. Accordingly, *Ccdc66*^{-/-} reporter gene expression is evident at prenatal developmental stages in cryosections of whole embryos.

Conclusion:

Expression studies of *Ccdc66* gene and products in the eye and brain in WT and *Ccdc66*^{-/-} mice in different developmental embryonic stages are of crucial interest, and the role of *Ccdc66* gene and products in prenatal development has to be evaluated in detail in further studies.

P-MonoG-143

*** Splice-site variant validation by NGS based amplicon sequencing of rtPCR products

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The application of next generation sequencing technologies (NGS) has been extremely successful in the identification of the underlying causes of genetic disorders. Whereas, most variants are located in coding regions, variants within splice-sites that might lead to alternative splicing affecting protein function or causing mRNA decay are less well characterized. Even for canonical splice-sites, prediction of the splicing effects for an identified variant is cumbersome. This becomes even more apparent as NGS based exome and genome sequencing tremendously increase the number of variants potentially affecting splice-sites.

In our study to identify the underlying causes of idiopathic short stature by whole exome sequencing in more than 200 patients, we observed 44 variants in consensus splice-sites defined as up to 12 bases from the 5' and 5 bases from the 3' end of an exon. We used several computational methods (splice site finder, MaxEntScan, NNSplice) to calculate their potential effect on splicing. These results were often inconclusive and did not predict the definite effect on transcripts. Therefore we performed rtPCR amplification of the respective regions, electrophoretic evaluation of fragments and Sanger sequencing. In case of a suspected alternative splicing the evaluation of the distinct resulting isoform, though, was often hampered by the overlap of sequences. This was especially severe when multiple naturally occurring alternative transcripts were present.

To overcome these issues, we established a deep sequencing approach (targeted rtPCR-Seq) to identify all present isoforms in the affected patients compared to controls. For this method the 44 rtPCR fragments were normalized to generate an equimolar mixture. Libraries for patients and controls, respectively, were

generated by ligation of distinct index adaptors for both amplicon pools and subsequently sequenced on an Illumina MiSeq platform. The resulting reads were aligned and visualized as sashimi plots. Observed splice-junctions in patients were compared to the controls and public databases.

We found concordant results between standard rtPCR evaluation and our rtPCR-Seq approach for 40 variants (alternative splicing for 8 variants). For these confirmed variants the specific effect was more obviously ascertained by the NGS based approach. For 1 variant only rtPCR-Seq was able to separate multiple co-occurring distinct isoforms, both in controls and patients. Discordant results for 4 variants proposed a higher specificity of the rtPCR-seq.

Thus, we established a simple amplicon based deep sequencing approach for standard rtPCR fragments to ascertain the effects of specific splice site variants. This technique has proven to be highly scalable, fast and efficient to analyze splice-site variants.

P-MonoG-144

EIF2S3 mutations associated with severe X -linked intellectual disability syndrome MEHMO

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MEHMO (MIM %300148), is a rare X-linked syndrome characterized by profound intellectual disability, epileptic seizures, hypogonadism, hypogenitalism, microcephaly, and obesity. In 1998 Steinmüller and colleagues described a large family with MEHMO syndrome with five affected males in two generations and assigned the disease locus to the short arm of chromosome X (Xp11.3-22.13). We took advantage of massively parallel sequencing in four families with MEHMO syndrome, including the family reported by Steinmüller et al. to identify the underlying genetic cause if this severe disorder. We here show MEHMO syndrome is associated with mutations in the X chromosome gene EIF2S3. In three families we identified a C-terminal frameshift mutation (p.Ile465Serfs) and in an unrelated boy who is less severely affected, we identified a novel maternally inherited missense mutation (p.Ser108Arg) in EIF2S3. EIF2S3 encodes the gamma subunit of the eukaryotic translation initiation factor 2 (eIF2). eIF2 is essential for eukaryotic translation initiation and regulation of the integrated stress response (ISR). Subsequent studies in patient fibroblasts (p.Ile465Serfs) showed increased ISR activation due to the mutation and functional assays in yeast demonstrated that the p.Ile465Serfs mutation impairs eIF2 gamma function to a greater extent than tested missense mutations, consistent with the more severe clinical phenotype of the affected males with Ile465Serfs mutation. Our results suggest that more severe mutations in EIF2S3 cause the full MEHMO syndrome, while less deleterious mutations are associated with a milder form of the syndrome with only a subset of the symptoms.

*** Individuals with hereditary trichilemmal cysts present a combination of an inherited high-risk PLCD1 allele (including two sequence variants) and additionally acquired somatic PLCD1 variants in the cysts.

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Trichilemmal cysts are benign tumors derived from the outer root sheath of the hair follicle. They occur most often solitary or multiple on the scalp but can also be found on other body parts. The cysts can develop sporadically or in an autosomal dominant mode of inheritance with incomplete penetrance. In 2005, the hereditary cyst form was mapped to the *TRICY1* locus on chromosome 3 p24-p21.2 in a familial genome-wide linkage study.

We analyzed 5 affected individuals from 4 different Tunisian families by whole-exome sequencing. The bioinformatic evaluation revealed no rare, disease causing mutations but the sequence variant *c.1379C>T*, p.S460L (MAF = 0.025, rs75495843, ENST00000334661.4) in the phospholipase C delta 1 (*PLCD1*) gene within the *TRICY1* locus. Furthermore, all five individuals present a second variant *c.903A>G*, p.P301P (MAF = 0.27, rs9857730) in the same gene. *PLCD1* is a member of the phospholipase C family. The enzyme is involved in calcium-dependent intracellular signal transduction and catalyzes the hydrolysis of phosphatidylinositol 4, 5-bisphosphate into the second messenger diacylglycerol and inositol triphosphate. Homozygous knockout-mice have hair defects and show aberrant skin development with increased progression of skin tumors and intradermal hair-follicle derived cysts. In humans, *PLCD1* is highly expressed in the hair follicle but so far only nonsense mutations have been described causing hereditary leukonychia totalis without any skin or hair abnormalities.

A segregation analysis of *PLCD1* in a Tunisian family cohort and one German family (13 families, 64 individuals, 38 affected) showed that all affected individuals contain the same two sequence variants. Based on these results, we propose that *PLCD1* is responsible for the cyst phenotype. cDNA sequencing from three different cysts revealed additional acquired somatic sequence variants in *PLCD1*. We found *c.2234C>T* p.S745L in two cysts and the two variants *c.2129C>T*, p.S710F and *c.2132C>T* p.S711F in the third one. All three somatic variants are not described in the ExAC database and the 1000 Genomes Project. Allele-specific RT-PCRs were performed with cyst cDNA and we could show that the somatic variants are on the same allele as the inherited variants. The acquired somatic cyst sequence variants lie within or respectively near the C2 domain of the PLCD1 protein. The C2 domain is involved in the calcium-dependent binding to membrane-integrated phospholipids. Depletion of the domain leads to decreased membrane association and protein activity. We assume that the allele with the variants *c.1379C>T* and *c.903A>G* is a risk factor for hereditary trichilemmal cysts and that additional acquired rare sequence variants are the genetic trigger for the development of the cysts.

Detection of deletions during diagnostic massive parallel NGS gene panel sequencing

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Introduction

Larger structural genomic duplications or deletions (copy number variations = CNVs) are routinely detected by array comparative genomic hybridization (aCGH). While aCGH has been established as a robust and effective approach for CNV screening, it remains expensive and is limited by resolution. In addition, commercial MLPA testing today allows the identification of exon deletions or duplications for a limited number of core genes.

More recently, massive parallel sequencing of multi gene panels (MGPS) has been introduced as a fast and cost-effective tool in routine genetic diagnostic testing to identify causal intragenic sequence alterations not only in core genes but also in those with small contribution to the respective phenotypes. The obtained

MGPS data may also be bioinformatically assessed to detect exon deletions or duplications within the analyzed genes panels and thus can be expected to further improve the diagnostic yield.

Methods

More than 100 patients were sequenced with phenotype specific gene panels on a MiSeq platform (Illumina) and analyzed with our bioinformatic diagnostic workflow including a quantitative data assessment using our *in house* JAVA based bioinformatic script to search for gene or exon deletions. Detected deletions were confirmed by an independent method (e.g. MLPA, PCR amplification of the junction fragment or linkage analysis), if available.

Results

We here report details for six suspected deletions in seven patients detected by our in house bioinformatic workflow: heterozygous gene deletions of *PAFAH1B1*, *Spastin* or *ARFGEF2*, respectively; two heterozygous *CFTR* deletions of exon 2 and 3, one homozygous partial *SFTPB* deletion and a homozygous *ISPD* exon deletion. The complete gene deletions of *PAFAH1B1* and *Spastin* as well as the *CFTR* deletion of exon 2 and 3 could be confirmed by MLPA. For the partial *SFTPB* deletion both breakpoints could be precisely located within the readout, allowing determining correct deletion size and design of primers to amplify the junction fragment. The homozygous deletion of exon 6 in the *ISPD* gene could be confirmed by PCR and linkage analysis.

Discussion

Diagnostic multi gene panel sequencing after Nextera enrichment allows sufficient homogeneity of the obtained patient and control data per target to quantitatively search for constitutional deletions covering two or more adjacent targets.

Combined data assessment considering the individual clinical data will not only further increase the diagnostic yield but can also be expected to further delineate the mutational spectrum for specific phenotypes by the simultaneous detection of clinically relevant sequence variants as well as CNVs. MGPS sequence assessment may also allow to gain new insights into the genomic architecture and origin of target regions and haplotypes, involved in common structural variations.

P-MonoG-147

Novel mutation in HOXC13 expand the mutation spectrum of pure hair and nail ectodermal dysplasia

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Introduction: Ectodermal dysplasias (EDs) are a large group of heterogeneous genetic disorders characterized by abnormal development in ectoderm-derived tissues and organs including skin, hair, and nails. Among the EDs, pure hair and nail ectodermal dysplasia (PHNED) is a rare genodermatosis characterized by nail dystrophy and sparse or absent hair on the scalp.

Materials and Methods: A family of Iranian origin was enrolled in this study. Two children from a consanguineous marriage are affected from PHNED. In addition, the father has alopecia areata (AA) but does not show any nail dysplasia. The mother is unaffected. The paternal and maternal grandfathers had nail dysplasia almost similar to the siblings but did not manifest any hair loss or AA. Homozygosity mapping and Genedistiller analysis were performed to identify candidate genes. Sanger sequencing was used to localize the mutations.

Results: In total, we identified 10 homozygous regions with almost 700 candidate genes. Among these genes were also *KRT85* and *HOXC13*, already known to be related to the phenotype of our patients. Therefore, we focused our additional analyses on *KRT85* and *HOXC13*. Sanger sequencing showed a so far unknown homozygous insertion of 28 bp in exon 2 of *HOXC13*.

Conclusion: We identified an unknown mutation for PHNED which expands the spectrum of mutations for PHNED.

The hair loss of the father seems rather be due to a distinct type of hair loss, namely AA, which is quite common in the general population. However, the nail dysplasia from both grandfathers is unclear and cannot be examined anymore. It still remains unclear if the nail dysplasia in the grandfathers was due to the same mutation in *HOXC13* or is based on a different mutation.

Generation of a cell culture model for epidermodysplasia verruciformis by knock-out of EV3, a novel gene involved in this genodermatosis

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Epidermodysplasia verruciformis (EV) is a rare hereditary skin disease leading susceptibility to certain types of cutaneous human papilloma viruses, mainly β-HPV, and a high risk for development of cutaneous squamous cell carcinoma. Homozygous or compound heterozygous loss of function mutations either in *TMC6* or in *TMC8* have been described in several EV patients, but more than 1/3 of affected families do not have mutations in one of them. A third gene (*EV3*) has recently been identified to be mutated in EV patients without *TMC6* or *TMC8* mutation. Investigations on the function of this gene may elucidate pathomechanisms of EV. We aim to generate an *EV3* deficient model cell line to study the effects of *EV3* despite the scarcity of patient material.

For this purpose we used the CRISPR/Cas9 system to delete the *EV3* coding sequence in an immortalized keratinocyte line. After isolation by FACS, single cell clones have been expanded. We screened 41 clones for deletion of the whole gene as well as for the expression of *EV3*. Three clones showed no detectable gDNA sequence or expression of the *EV3* gene. We found only one wildtype clone without deletion of *EV3* or alterations near the Cas9 cut sites. All clones have been characterized by a SNP-array as well as sequencing of the knockout site and the most probable offsite targets. These *EV3* deficient keratinocyte lines are the first cell culture model for EV. It will be a valuable tool to identify cellular pathomechanisms of the disease and allow insight into the control of β-HPV in the general population.

*** iPS cell models for X-linked disorders

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Hemizygous loss of function of single X-chromosomal genes is the most frequent cause for genetic intellectual disability (ID). While a long list of gene mutations have so far been described to be responsible for the disease phenotype, little is known about the underlying neuronal mechanisms. Reprogramming of somatic cells into stem cells (iPSCs) followed by differentiation into neuronal precursors (NPCs) is an important tool for translating research allowing an understanding of network dysfunction in ID patients.

Opitz BBB/G syndrome (OS) is characterized by a number of ventral midline defects combined with learning disability, developmental delay and intellectual disability. It is caused by mutations in the X-linked MID1 gene, which, as we have shown previously, regulates mTOR dependent local protein synthesis. In a mouse model, loss of MID1 function leads to significant disturbance of axonal outgrowth.

We have generated iPS cells from several patients with OS and from one mother that carries a loss of function mutation in the MID1 gene. By sorting cell clones after reprogramming we have established iPS cell clones from this female carrier of a 4bp deletion in the MID1 gene (c.1801_1804delCTCC) either expressing from the mutated X-chromosome or the non-mutated X-chromosome and have shown that indeed the generated iPSC-clones express either 0% of the mutated or 0% of the wildtype MID1. We determined this directly using an allele-specific MID1-RT-PCR and indirectly by comparing the methylation of HUMARA-alleles.

Comparison of mutation expressing iPSC-clones with non-mutation expressing iPSC-clones showed that the MID1 mutation results in significantly smaller cells with reduced S6 phosphorylation supporting aberrations in the mTOR/PP2A signaling cascade. When differentiating iPSCs into neuronal precursor cells, significantly

bigger Embryoid Bodies (EBs) can be detected in the mutation expressing clones, while the total EB number is higher for the non-mutated MID1 expressing clones. When further differentiated, EBs from all iPSC-clones form neuronal rosette structures expressing beta-III-tubulin, with a lower rosette structure count in the mutated MID1 expressing cells. These data clearly hint a defect in neurogenesis in cells with hemizygous MID1 mutations.

Interestingly, while iPSCs stably kept the X-incatvation pattern of the original fibroblast, during differentiation X-inactivation was lost leading to biallelic expression from both X-chromosomes in the NPCs pointing towards an as yet unknown reactivation mechanism of the inactive X-chromosome. We are currently analyzing X-inactivation throughout the differentiation line in order to see if or if not X-inactivation is restabilized during neuronal differentiation at a later time point.

P-MonoG-150

A novel mutation c.4A>G, pSer2Gly in DES gene in a family with catecholamine polymorphic ventricular tachycardia

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Background: Myofibrillar myopathy (OMIM 601419) is a heterogeneous neuromuscular disease characterized by progressive muscle weakness, partially with cardiomyopathy and/or arrhythmia. Pathohistological examinations show desmin-positive protein aggregations. The mutations in *DES*, *CRYAB*, *BAG3*, *MYOT*, *LDB3*, *FLNC*, *FHL1*, and *DNAJB6* have been identified in patients with myofibrillar myopathy. Desmin is a intermediate filaments and composed of non-helical N- and terminal head domain and a central -helical Rod domain. Till now more than 67 mutations myofibrillar myopathy were identified in patients with and a genotype-phenotype was reported: mutations in Rod domain tend to cause neuromuscular symptoms, and mutations in head domain is associated with cardiac symptoms. The head-domain is a serine rich domain and phosphorylated by protein kinase c. Almost all reported mutations in head-domain in *DES* are at Serine residues (e.g. S2I, S7F, S13F, S46F, S26Y, S26T). These findings suggested that the Serine residues in head-domain could play an important role in biological function of desmin. Here we report a novel mutation c.4A>G, pS2G in *DES* in a family with catecholamine polymorphic ventricular tachycardia.

Case: A 58 years old patient developed a syncope in a cold winter. The cardiological investigations revealed a diagnosis of catecholamine polymorphic ventricular tachycardia and the implantation of permanent pacemaker was indicated. His older brother, father and paternal uncle suffered from arrhythmia and all of them received a permanent pacemaker implantation at around 60 years of age. His cousin was died at age of 24 years due to sudden cardiac arrest. We performed a genetic analysis for ryanodin-rezeptor 2-gen and lamin A gene and no mutation in these gene was identified. Next, we performed an exome sequencing. Here a novel heterozygous mutation c.4A>G, pS2G in *DES* was identified. This mutation was also identified in uncle of index patient. The c.4A>G, pS2G in *DES* was not reported neither in 1000 human genome project, nor in Exome Aggregation Consortium. A mutation at Serine 2 (p.S2I) was previously reported in patients with myofibrillar myopathy with cardiac symptoms. Based on these findings, it was strongly supposed that the p.S2G in *DES* gene is a causative mutation for myofibrillar myopathy with arrhythmia in our patients.

Discussion: Recent studies suggest that the mutations in *DES* influence not only on muscle stability and myocardial force generation, but also impaired ubiquitin proteasome system, which could be caused by aggregated desmine. Generally, phosphorylation of Serin and Threonine in head-domain of desmin is related to disassembly of filaments. Therefore the p.Ser2Gly mutation in *DES* could cause altered phosphorylation of desmin. The functional abnormalities caused by pSer2Gly in *DES* gene need to be studied.

P-MonoG-151

Gene dosage manipulation of the chromatin organizer CTCF in the nervous system of Drosophila melanogaster results in neurological and morphological phenotypes

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Three-dimensional organization of eukaryotic genomes is crucial for temporal and spatial regulation of gene expression. Architectural proteins, like the CCCTC-binding factor CTCF are responsible for establishing and maintaining this organization. CTCF is involved in virtually all chromatin regulating processes including enhancer function, insulation, alternative splicing, imprinting, V(D)J recombination, chromatin loop formation and defining topologically associated domains (TADs).

Recently, we identified de novo mutations in CTCF in patients with a surprisingly mild phenotype of variable developmental delay or intellectual disability, mild short stature and microcephaly, and behavioural anomalies. Apart from observing brain malformations and early lethality or learning deficits in two conditional knockout

mouse models, little is known about the role of CTCF in neuronal development and preservation so far. Therefore, we utilized the model organism Drosophila melanogaster to further explore the role of CTCF in CNS development and function.

Similar to observations in knockout mice, complete knockout or ubiquitous knockdown of Ctcf is embryonic lethal in Drosophila. We therefore utilized the UAS/GAL4 system to induce tissue specific knockdown or overexpression of Ctcf in the fly nervous system. We first investigated development and morphology of the larval neuromuscular junctions (NMJs), an established model for synaptic development. While pan-neuronal overexpression of Ctcf showed no morphological NMJ alterations, pan-neuronal knockdown resulted in fewer NMJ branches than in a specific control. Additionally, we observed a reduced number of active zones in a hypomorphic mutant line compared to a wildtype control.

Using the negative gravitaxis assay to examine gross neurological function, we found a highly significant impairment of geotaxis behavior in flies with Ctcf knockdown in neurons, motoneurons and muscle and in flies with overexpression of Ctcf in glia cells, muscle and motoneurons. Currently we are testing learning and memory behavior with the courtship conditioning paradigm.

Our findings of various neurological and morphological anomalies upon manipulation of Ctcf dosage in the fly nervous system emphasize the role of Ctcf in nervous system development and function and provide a basis to further study the molecular mechanisms underlying cognitive dysfunction caused by CTCF-deficiency.

P-MonoG-152

Targeted sequencing in patients with hypogonadotropic hypogonadism

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Congenital hypogonadotropic hypogonadism (CHH) is a rare and clinically and genetically heterogeneous disorder. CHH is characterized by incomplete or absent puberty caused by the lack or deficient number of hypothalamic gonadotropin-releasing hormone (GnRH) neurons, disturbed secretion or action of GnRH, or both. CHH is often associated with anosmia and is then termed Kallmann syndrome (KS), as well as with other phenotypes like unilateral kidney agenesis, skeletal abnormalities, midline malformations, and hearing loss. X-linked, autosomal-dominant, and autosomal-recessive, as well as di- and oligogenic inheritance has been described for CHH. In the meantime a multitude of genes has been reported to be associated with CHH. Actually, in fewer than 40% of the CHH cases the underlying genetic cause can be identified.

We analyzed a total of 19 patients with CHH (4 female/15 male) by using targeted sequencing of 16 CHH-associated genes KAL1, CHD7, FGF8, FGFR1, FSHB, GNRH1, GNRHR, HS6ST1, KISS1, KISS1R, NSMF, PROK2, PROKR2, SPRY4, TAC3 and TACR3 and identified pathogenic mutations in 8 CHH patients of our cohort. Mutations were detected in KAL1 (3 patients), TACR3 (2 patients), PROKR2 (1 patient), HS6ST1 (1 patient) and GNRHR (1 patient). Furthermore, we found in two patients with described pathogenic mutations (one patient with PROKR2 mutation and the other with KAL1 mutation, respectively) additional mutations in NSMF gene and TACR3 gene, respectively, suggesting digenic inheritance in these cases.

In conclusion, targeted sequencing represents an effective, fast, cost-efficient and flexible method, since new candidate genes can be added easily to the panel, for the sequential analysis in CHH patients. Furthermore, panel sequencing alleviates the uncovering of oligogenic inheritance in genetic traits like CHH.

P-MonoG-153

Mutations in the aminoacyl-tRNA-synthetase genes SARS and WARS2 are associated with autosomal recessive intellectual disability

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Intellectual disability (ID) is the common feature of a very heterogeneous group of disorders, which comprises a broad variety of syndromic and non-syndromic phenotypes. Here we present mutations in two aminoacyl-tRNA synthetases that are associated with ID in two independent Iranian families. In the first family, we found a missense mutation (c.514G>A, p.D172N) in the cytoplasmic seryl-tRNA synthetase (SARS) gene that affects the enzymatic core domain of the protein and impairs its enzymatic activity. This probably leads to

reduced tRNASer concentrations in the cytoplasm. In silico analyses predicted the mutant protein to be unstable. This prediction could be experimentally substantiated by results obtained through studies with ectopic mutant SARS in transfected HEK293T cells.

In the second family, we identified a compound heterozygous genotype of the mitochondrial tryptophanyl-tRNA synthetase (WARS2) gene, consisting of a nonsense mutation (c.325delA, p.Ser109Alafs*15), which very likely leads to nonsense-mediated mRNA decay, in combination with a missense mutation (c.37T>G, p.W13G). The p.W13G mutation affects the mitochondrial localization signal of WARS2, leading to mislocalization of the mutant protein. Thus, when taking AIMP1 into account, which we have recently implicated in the aetiology of ID as well, there are now three genes with a role in tRNA-aminoacylation that are associated with this condition. Hence we propose that the functional integrity of t-RNAs in general is an important constituent in the development and maintenance of human cognitive functions.

P-MonoG-154

Whole Exome Sequencing reveals a novel deletion in SCN1A gene :A case report of Dravet Syndrom

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Dravet syndrome is a rare autosomal dominant genetic disorder with early-onset epileptic encephalopathy is mainly caused by different de novo mutations of the SCN1A gene encoding the type 1 subunit of voltage-gated sodium channel.

Whole exome sequencing(WES) enables scanning a large number of genes which not only can confirm the diagnosis but also helpful in understading for possible relationship between clinical manifestations and mutaion. The authors investigate WES in a 18 months term boy with hypotonia and convulsion of normal and relative parent. She had uncontrolled sizure without fever, and developmental delay from 6 months.In treatment protocol anti covulsants changes to valorate, clonazepam and stiripentol as well. A deleterious novel heterozygous splice site mutation in SCN1A gene(Chr.2:166895931,AC,DEL) were identified which had not been previously reported. This finding expands the SCN1A mutation spectrum and highlights the importance of WES in the emerging field of pediatric neurogenetic.

P-MonoG-155

*** Hepatocyte-like Cells for Drug Assessment in Familial Amyloid Polyneuropathy and Wilson Disease

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Various types of somatic cells have been reprogrammed to induced pluripotent stem cells (iPSC) followed by differentiation into hepatocyte-like cells (HLC). Recently, cells that shed from the renal epithelial system were shown to be a suitable and convenient source for iPSC generation. In the current study, urine-derived cells (UCs) were isolated from urine donations of patients having familial amyloid polyneuropathy (FAP), a neurodegenerative disease caused by mutation of the transthyretin (TTR) gene and Wilson disease (WD), a genetic disorder of ATP7B causing copper accumulation, predominantly in liver and brain. Patient-specific HLCs were differentiated in order to study disease-specific mechanisms and to investigate the efficacy of novel compounds.

For isolation of renal epithelial cells, urine of FAP and WD patients was processed. UCs were reprogramed into iPSCs using plasmids resulting in transient expression of factors Sox2, Oct3/4, Klf4, and c-myc. After characterization of iPSC that expressed high levels of pluripotency markers, like OCT4 and NANOG, a 3-step hepatocyte differentiation protocol was performed. iPSCs were subjected to a treatment with growth factors (activin A, Wnt3a, FGF2, HGF) for 14 days. The hepatic, patient-specific character of differentiated HLCs was assessed by functional analysis, gene expression profiling, genotype analysis, and immunostainings. Therapeutic oligonucleotide efficacy targeting TTR was determined by immunocytochemistry, qRT-PCR and western blot analysis. TTR-stabilizing activity of tafamidis was investigated by means of thermal shift assay and western blot analysis. Copper chelation by methanobactin was determined by atomic absorption spectroscopy.

Reprogramming of UCs resulted in stable iPSC lines with characteristic pluripotent marker expression. Differentiated HLCs showed high similarity to human hepatocytes in terms of genetic profile and functional activity. Small-interfering RNAs (siRNAs), antisense oligonucleotides (ASO) (Niemietz et al. 2016, PlosOne 11(9):e0161455), and the TTR stabilizing compound tafamidis that are currently assessed in clinical studies were studied in HLCs derived from FAP patients. A novel chelator was used to determine intracellular copper

accumulation in HLCs derived from WD patients (Lichtmannegger et al. 2016, JCI 126(7):2721-35). FAP-specific HLCs revealed differently expression of key regulators of the protein quality control (PQC) system.

Our results demonstrate that iPSC derived from urine are excellently suited to study hereditary liver diseases. HLCs could be investigated in the patient-specific genetic background. The efficacy of novel compounds was assessed and individual responses were monitored.

P-MonoG-156

Whole exome sequencing to rapidly identify genetic variants associated with congenital nystagmus.

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Congenital or early-onset nystagmus (CN) is characterized by involuntary eye movements and shows enormous clinical and genetic heterogeneity. CN may be an ambiguous sign of many different diseases, including retinal dysfunction / degeneration, ocular / oculocutaneous albinism, and severe central nervous system disorders, such as Pelizaeus-Merzbacher or Pelizaeus-Merzbacher-like diseases (PMLD).

Due to enormous heterogeneity found among the diseases leading to CN, whole exome sequencing (WES) and panel-based bioinformatics was considered as an approach to rapidly identify disease-associated genetic sequence variants.

We have analyzed 9 families with 16 CN-affected patients. Herein, we present three clinically different patients who were initially affected with CN, but developed further clinical symptoms of various severities. One of these patients showed features of retinal dysfunction, including night blindness and myopia, while two other patients developed severe phenotypes including mental retardation or PMLD.

WES identified four genetic variants in genes associated with CN. The first patient showed a hemizygous splice-donor variant (c.2576+1G>A) in the calcium channel voltage-dependent alpha-1f subunit (CACNA1F) gene, the second patient carried a hemizygous variant (c.1403G>A, p.R468H) in the ferm domain-containing protein 7 (FRMD7) gene, and the third patient showed two heterozygous variants (c. 291C>G, p.Y97* and c.716T>C, p.V293A) in the gap junction protein gamma-2 (GJC2) gene. Sanger sequencing confirmed the identified variants in the index patients and verified co-segregation in several family members.

Our results suggest a beneficial role of WES to identify the molecular causes of CN and to rapidly confirm an initially unclear clinical diagnosis. Especially, patients with rare and severe disorders (e.g. PMLD) will benefit from a WES analysis performed in the early stage of the disease.

P-MonoG-157

*** A novel ARL13B mutation impairs its guanine nucleotide-exchange factor activity in patients with Joubert syndrome

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Abstract

ARL13B encodes for the ADP-ribosylation factor-like 13B GTPase, which is required for normal cilia structure and Sonic hedgehog (Shh) signaling. Disruptions in cilia structure or function lead to a class of human disorders called ciliopathies. Joubert syndrome is characterized by a wide spectrum of symptoms, including a variable degree of intellectual disability, ataxia, and ocular abnormalities. Here we report a novel homozygous missense variant (c.223 G>A; p.G75R) in the ARL13B gene, which we identified by whole Exome sequencing

of a trio from a consanguineous family with multiple affected individuals suffering from intellectual disability, ataxia, ocular defects, and epilepsy. The same variant was also identified in a second family. We saw a striking difference in the severity of ataxia between affected male and female individuals in both families. Functional analysis demonstrated that dihydrotestosterone treatment of SH-SY5Y cells induced a down regulation of ARL13B expression. Both ARL13B and ARL13B-p.G75R expression rescued the cilia length and Shh defects displayed by Arl13bhennin (null) cells, indicating that the mutation did not disrupt either ARL13B function. In contrast, ARL13B-p.G75R displayed a marked loss of ARL3 guanine nucleotide-exchange factor activity, despite retention of its GTPase activities, highlighting the correlation between its loss of function as an ARL3 guanine nucleotide-exchange factor and Joubert syndrome.

P-MonoG-158

Molecular pathomechanisms of TAAD

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TAAD (Thoracic Aortic Aneurysm and Dissection) is a heterogeneous disease that often remains silent until a life-threatening complication occurs. It belongs to the connective tissue disorders and causes 1% of death in industrial countries. Several disease genes have been already identified; however, about 50% of patients with TAAD-associated syndromes do not show a mutation in these genes. Thus, further heterogeneity is obvious. Since individual risk stratification and therapeutic options highly depend on the individually mutated gene, it is very important to identify more disease genes which are aimed to be found by Whole Exome Sequencing (WES).

Many of the known disease genes encode for proteins that are important for the structure and stabilization of the extracellular matrix as well as for the contraction of Vascular Smooth Muscle Cells. One central pathway is the TGF-beta signaling which functions among other proteins via the TGF-beta receptor, its ligand and its downstream target SMAD2/3.

Our project plan includes (i) exome sequencing both in affected individuals within families as well as in sporadic patients, (ii) filtering of raw data and prioritization of sequence variants by using a bioinformatic in house pipeline, (iii) verification of novel putative disease genes in a cohort of 200 mutation-negative patients with TAAD spectrum disease and (iv) functional analyses to gain deeper insight into the pathobiology of TAAD. In a first round of WES analysis and variant prioritization, we identified a highly conspicuous sequence variant in three family members with TAAD. Screening of the respective gene in a large cohort of mutation negative patients revealed another variant in two siblings with TAAD. Structural and functional considerations strongly support deleterious effects for both identified putative pathogenic missense variants that affect a novel cell cycle- and/or apoptosis regulating protein. Functional analyses did not show an involvement of this protein in TGF-beta signaling. In ongoing experiments, we focus on mutation-induced consequences on cell proliferation, cell cycle progression and apoptosis. Indeed, we found inhibitory effects of the missense variants on proliferation by affecting the cell cycle key protein CDKN1A. We hypothesize that dysregulation of proliferation and/or apoptosis of specific cells, e.g. smooth muscle cells, underlies TAAD.

P-MonoG-159

Neurodegeneration in the olfactory bulb and olfactory impairment in the Ccdc66 -/- mouse model for retinal degeneration

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Background: The *Ccdc66*-deficient (*Ccdc66-/-*) mouse model exhibits slow retinal degeneration similar to a human Retinitis pigmentosa (RP) phenotype (Gerding *et al.*, *Hum Mol Genet.*, 2011). In order to determine whether *Ccdc66* gene expression might also play a role outside the retina, this study aimed at characterizing CCDC66 protein expression during early postnatal development of the mouse brain. Furthermore, morphological and behavioral impact of *Ccdc66* deficiency in the mouse brain was analyzed.

Methods: CCDC66 protein expression was determined by SDS page and Western blot in whole brain homogenates and in selected brain regions of interest (olfactory bulb, hippocampus, cortex, striatum, cerebellum, brain stem) during early postnatal development and in adult wildtype (WT) mice. In addition, cryosections of the *Ccdc66-/-* olfactory epithelium and bulb (during postnatal development) and the rostral migratory stream (in adult) were analyzed for *Ccdc66* reporter gene expression by X-gal staining. Selected brain regions were additionally analyzed by electron microscopy. In order to correlate anatomical with behavioral data, olfactory performance was studied in aged *Ccdc66-/-* mice compared to *Ccdc66+/+* controls

by an olfactory habituation/dishabituation test (Yang and Crawley, *Curr Protoc Neurosci.*, 2009), where olfactory exploration-time during the presentation of neutral and social odors is examined.

Results: CCDC66 protein was detected throughout the early postnatal development of the WT mouse brain, decreasing after birth. Amongst analyzed brain regions, highest expression of CCDC66 protein was detected in the olfactory bulb exhibiting similar CCDC66 levels to retinal expression. Accordingly, *Ccdc66* reporter gene expression was demonstrated in the mature olfactory bulb glomeruli, the adjacent olfactory epithelium and along the rostral migratory stream in the *Ccdc66-/-* mouse brain. Interestingly, strong *Ccdc66* reporter gene expression in glomeruli of the *Ccdc66-/-* olfactory bulb was correlated with signs of degeneration in the *Ccdc66-/-* mouse, but not in controls. The degeneration was also reflected by olfactory impairment in *Ccdc66-/-* mice, which spent significantly less time for sniffing at initial presentation of unknown, neutral odors and barely responded to social odors.

Conclusion: Besides the retina, CCDC66 protein plays a crucial role in the olfactory system as shown by its expression there as well as by *Ccdc66* deficiency resulting in neurodegeneration and alteration of olfaction-related behavior in the *Ccdc66-/-* mice. As impairment of the olfactory sense in multiple neurodegenerative disorders is a common finding, the *Ccdc66-/-* mouse model is not only restricted to study retinal degeneration but possibly also degeneration of the central nervous system.

P-MonoG-160

Ccdc66 RNA and protein studies in a mouse model for retinal degeneration

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Background:

The *Ccdc66*-deficient (*Ccdc66*-/-) mouse model exhibits slow retinal degeneration similar to a human Retinitis pigmentosa (RP) phenotype (Gerding et al. 2011). In order to gain insights into the molecular mechanisms that govern *Ccdc66*-deficient degeneration, a detailed evaluation is performed in order to reveal processes that contribute to retinal degeneration during early postnatal development and adulthood.

Methods:

The functional role of CCDC66 deficiency is investigated by two independent approaches: 1) gene expression profiles at P10 and P28 are analyzed in retinal tissue of *Ccdc66*-/- and wildtype mice (GeneChip Mouse Gene 2.0 ST Array, Affymetrix) followed by quantitative real-time PCR (qRT-PCR) and 2) potential retinal interaction partners of CCDC66 protein are identified by yeast-two hybrid screening in the wildtype mouse and further analyzed by immunohistochemistry.

Results:

Using two screening methods (RNA-expression profiles and protein interaction partners), our results indicate that 1) the *Ccdc66* deficient mouse model reveals early changes in retinal RNA gene expression already at P10 and the highest number of genes differential expressed at P28. Most expression differences were related to genes associated with the extracellular matrix. Further genes are involved in retinal degeneration, angiogenesis, transcription factors and proteolysis. 2) The CCDC66 protein interacts with the proteins EPS8 (epidermal growth factor receptor kinase substrate 8) and MPDZ (multiple PDZ-domain protein). Both proteins are expressed in several retinal layers of the retina, confirmed by immunohistochemistry. Moreover, mutations in the *MPDZ* gene were already identified in patients with Retinitis pigmentosa/ Leber Congenital Amaurosis.

Conclusion:

Expression profiles reveal expression changes at an early time point of retinal degeneration in the *Ccdc66*-/- mouse model enabling further studies on the role of genes and processes involved in early retinal degeneration. In addition, the interaction partners of CCDC66, EPS8 and MPDZ, are the basis for further studies examining the pathways of retinal degeneration in the mammalian retina including man – and possibly contribute to future studies in man and human disease.

Ccdc66 null mutation causes retinal degeneration and dysfunction. Gerding W. M., Schreiber S., Schulte-Middelmann T., de Castro Marques A., Atorf J., Akkad D. A., Dekomien G., Kremers J., Dermietzel R., Gal A., Rülicke T., Ibrahim S., Epplen J. T., Petrasch-Parwez E.

Hum Mol Genet. 2011, 20(18):3620-31. doi: 10.1093/hmg/ddr282

P-MonoG-161

MIDAS Case Report: Identification of a de novo insertion in the ARID1B gene causing the rare congenital disease Coffin-Siris Syndrome

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Phenotype: In a young undiagnosed patient with developmental delay, intellectual disability and craniofacial dysmorphic anomalies, whole-exome sequencing (WES) identified a *de novo* insertion in the gene *ARID1B*, known to cause the rare congenital Coffin-Siris syndrome and Nicolaides-Baraitser syndrome, respectively.

Methods: As part of the MIDAS Genotype-Phenotype-Correlation project, we sequenced the index patient and his healthy parents on a NextSeq500 platform (Illumina, San Diego, CA, USA) and performed a trio analysis. For library preparation we used an enzymatic fragmentation approach. Exome capture was performed using the SureSelect Human All Exon kit V6 (Agilent, Santa Clara, CA, USA) to target most of the over 20.000 genes. The libraries were sequenced to approximately 190-fold mean coverage as 151bp paired end reads. 92% of the target region was covered 20-fold or higher. Data analysis and variant evaluation was performed using the CLC Genomic Workbench 9.0 (Qiagen, Hilden, Germany) and annotations from commercial as well as public databases (dbSNP, HGMD, CLINVAR, ExAC).

Results: We identified a *de novo* 1bp insertion in the *ARID1B* gene, causing a frameshift mutation that leads to the truncated protein. ARID1B is part of the ATP-dependent chromatin remodeling BAF-complex, which is involved in gene regulation. By parent-patient trio whole-exome sequencing, we were able to characterize the unterlying genetic cause in the patient, who has undergone multiple diangostic test before without receiving a diagnosis.

Discussion: Although for many congenital syndromic diseases the disease-associated genes are known it remains difficult for physicians to interpret the highly variable phenotypes as well as their variable nomenclature in order to request the appropriate molecular analysis. Here we present one of more than 50 cases that will help to establish a database connecting specific phenotypes, using the Human Phenotype Ontology terms, with the each corresponding disease-causing genes (MIDAS).

P-MonoG-162

Novel compound heterozygous NALCN variants in two brothers with muscular hypotonia and global development delay

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We report on two brothers aged two and three years, with muscular hypotonia, global development delay, abnormal respiratory rhythm, mild facial dysmorphism, recurrent respiratory infections, and failure to thrive. Sequencing of 3089 disease related genes identified compound heterozygosity for two novel mutations in *NALCN*: c.4281 C>A (p.Phe1427Leu) and c.4103+2 T>C. *NALCN* encodes a voltage-independent, non-selective cation channel, which is involved in regulation of neuronal excitability. The missense variant c.4281 C>A affects the highly conserved amino acid position Phe1427 which is located in segment S6 of domain IV in the pore-forming unit of NALCN. The variant c.4103+2 T>C alters the donor splice site in intron 36 and is predicted to cause skipping of exon 36, resulting in loss of function of NALCN. Biallelic mutations of *NALCN* are associated with infantile hypotonia, psychomotor retardation and characteristic facies (IHPRF1), whereas heterozygous de novo mutations cause congenital contractures of the limbs and face, muscular hypotonia, and global developmental delay. The clinical features of our patients resemble mild IHPRF1, caused by a biallelic missense mutation in segment S3 of domain IV. It has been suggested that variants in or close to S5 and S6 of the pore-forming domains lead to the above mentioned autosomal dominant condition whereas variants in other regions or loss of function mutations result in autosomal recessive inheritance. This is the first report of a mutation in a S6 segment, inherited in autosomal recessive manner. Our findings indicate that phenotype-genotype correlations in *NALCN* are more complex than suggested so far.

Identification of a critical key player in the nucleocytoplasmic shuttling of ataxin-3

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Spinocerebellar ataxia type 3 (SCA3) or Machado-Joseph disease (MJD) is an autosomal-dominantly inherited neurodegenerative disorder caused by a CAG expansion in the ATXN3 gene leading to a polyglutamine expansion in the encoded ataxin-3 protein. One hallmark of SCA3 and other polyglutamine diseases is the formation of the so called neuronal intranuclear inclusion bodies (NII). As ataxin-3 is predominantly located in the cytoplasm, the formation of protein aggregates in the nucleus require a nucleocytoplasmic shuttling of ataxin-3. We already demonstrated in vivo using transgenic mouse models that the toxicity of expanded ataxin-3 depends on its intracellular localization: While nuclear ataxin-3 gave rise to a strong phenotype with a high number of protein aggregates, purely cytoplasmic ataxin 3, however, even with a highly expanded polyglutamine repeat (148 glutamines), was not able to induce a phenotype and even did not aggregate. We further identified and characterized intracellular transport signals (two nuclear export signals, NES, and one nuclear localization signal, NLS) within the coding sequence of ataxin-3. Therefore, it is evident that proteins involved in the nucleocytoplasmic transport machinery recognize these localization signals, control the intracellular localization of ataxin-3, thereby influence the toxicity and aggregation of ataxin-3 and, thus, the pathogenesis of SCA3.

We now screened a library of transport proteins in order to identify the transport protein which is critically involved in the nucleocytoplasmic shuttling of ataxin-3. We indeed identified a transport protein which modifies both the formation of aggregates and the intracellular localization of ataxin 3. While the overexpression of this protein moved ataxin-3 into the nucleus, its downregulation kept it out of the nucleus. We replicated this correlation in vivo in drosophila and observed in addition to this again a clear link between the intracellular localization of ataxin-3 and its toxicity i.e. its ability of induce neurodegeneration and a behavioral phenotype. Likewise we even confirmed in a mouse model of SCA3 the importance of the identified transport protein as its knockout largely prevented ataxin-3 from aggregating and alleviated behavioral and movement deficits. Understanding the mechanisms behind the intracellular transport of ataxin-3 could give us clues into the pathogenic functions of expanded ataxin-3 and ways to mediate the progression of neuronal degeneration in SCA3.

Evidence for genetic factors outside CARD14 influencing the phenotype of a family with familial pityriasis rubra pilaris and psoriasis

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Familial pityriasis rubra pilaris (PRP) is an erythematous inflammatory skin disease caused by heterozygous activating mutations in *CARD14*, a known activator of the NF-κB pathway. Different genetic variants within *CARD14* have been associated with psoriasis.

The purpose of our study was to clinically and genetically investigate affected as well as unaffected members of a family with PRP in order to determine the mutation responsible for this severe skin disease in the three affected family members.

A father, three of his adult children as well as the mother of one child affected by PRP were investigated clinically. In addition we extracted genomic DNA from the blood of each individual and performed whole exome sequencing as well as direct sequencing of single genes.

Clinical investigation confirmed that the father and two of his children were affected by familial PRP, with the skin showing the characteristic pattern of PRP, early onset and chronic course. A third child was unaffected by PRP, suffered however from psoriasis. The mother of one child affected by PRP showed no sign of skin disease. Genetic investigation revealed a heterozygous missense mutation in exon 4 of *CARD14*, c.[371C>T], p.[Leu124Pro] present in all investigated individuals with PRP or psoriasis. The same mutation has been described before as being pathogenic in a different family with PRP. Regarding genetic variants associated with psoriasis, we found the risk alleles of three coding variants in *CARD14*: rs2066964, c.[1641G>C] (risk allele G), p.[R547S]; rs34367357, c.[1753 G>A] (risk allele A), p.[V585I] and rs11652075, c.[2458 C>T] (risk

allele C), p.[P820W] heterozygous in all individuals with PRP or psoriasis. Furthermore, the patient affected by psoriasis carried the surrogate marker SNP rs4406237–A for the PSORS1 risk variant HLA-Cw 0602 haplotype homozygously; and the same SNP was found heterozygous in his PRP-affected father. Neither the pathogenic variant in *CARD14*, nor the risk variants for psoriasis described above were found in the healthy mother. Whole exome sequencing revealed genetic variants, predicted to have serious consequences in further genes involved in the NF-κB as well as the NOTCH pathway. These variants either segregate with PRP or are present in the psoriasis affected individual only.

The presence of an individual carrying the same *CARD14* mutation as his PRP-affected relatives but suffering from psoriasis instead strengthens the relation between PRP and psoriasis, which has been repeatedly suggested in literature. We propose a balance between familial PRP and psoriasis in the family investigated in this study and present genetic variants, which might influence this balance in addition to variants in *CARD14*.

P-MonoG-165

ATP7B mRNA expression, which is likely regulated by MTF1, is altered in Wilson's disease

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Wilson's disease (WD) is an autosomal recessive disease resulting from copper (Cu) excess due to mutations in the ATP7B gene coding for a Cu-transporting ATPase. WD pathogenesis, however, can not only be explained by gene coding mutations since phenotypes exhibit strong variations despite the same exonic DNA makeup in the gene. Also in several patients with clinical WD symptoms no gene coding variants are detectable. Our former studies revealed decreased liver ATP7B mRNA expression in some WD patients. This decrease was not only observed in patients with nonsense ATP7B mutations leading to rapid mRNA decay, but also in patients with missense mutations and also in some patients with suspected WD without ATP7B mutations. Patients with low ATP7B expression presented with a more fulminant disease progression. However, we could not detect mutations in the ATP7B promoter region (c.-900 to ATG) in those patients. There are possibly other deregulating mechanisms responsible for decreased ATP7B mRNA expression. Up to now, ATP7B transcriptional regulation is only poorly characterized. It is known, that four metal responsive elements (MRE a, c, d and e) are located within the ATP7B promoter. Gene regulation through MREs is often metal-dependent. Liver ATP7B mRNA expression revealed also to increase under Cu addition in several species by an unknown mechanism. Up to now, only one ATP7B transcription factor (TF), the MREa binding Ku protein, is known. The aim of our work was to further analyze the regulation of the ATP7B gene, especially through MREs.

To screen for TF-MRE interactions and to narrow down the binding site of TF, we performed electrophoretic mobility shift assays (EMSA) by incubating nuclear extracts of the liver cell line HLE with probes corresponding to ATP7B MREc, d and e. To identify MRE-binding TF MatInspector analysis was performed. Identified candidate TF were coexpressed with ATP7B promoter-driven reporter gene to evaluate their impact on reporter gene expression. One in the reporter assay positively tested TF was validated by different EMSA experiments. Further it was overexpressed with and without addition of metal ions in HLE to investigate the impact on endogenous ATP7B expression.

We showed that TF MTF1 is able to bind to MREe within the ATP7B promoter and significantly increases ATP7B promoter driven reporter gene expression. MTF1 binding was primarily mediated by the first three bases of the MRE consensus sequence. Also for MREc and MREd specific protein interaction could be shown and the protein binding site was narrowed down by EMSA with protein identification still pending. Furthermore, we found the endogenous ATP7B mRNA expression to be significantly increased in HLE after Cu treatment or Cu treatment and concurrent MTF1 overexpression. Sole MTF1 overexpression did not alter ATP7B expression.

We newly identified MTF1 to bind MREe within the ATP7B promoter. Its in vivo role in the pathogenesis of WD needs to be further elucidated.

Modifying Genes of the Monogenic Disease Cystic Fibrosis – Beyond Gene Identification to Molecular Mechanism

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Modifying genes have been identified for lung function in cystic fibrosis [1], disease severity [2] and several comorbidities [3]. Within the European CF Twin and Sibling Study, we focus on genes that modify the basic defect, assessed as defective chloride conductance in CFTR-expressing epithelia, which we could describe by an association study on patient cohorts selected for informative endophenotypes among F508del-CFTR homozygous patients [2,4,5]. As a first example, rs7901656 in FAS modifies FAS gene expression (P = 0.0009, data from 16 intestinal biopsies [6]), CF disease phenotype (Praw = 0.0039, comparing 13 concordant mildly affected F508del homozygous sib pairs and 12 concordant severely affected sib pairs; Praw = 0.0047, comparing 20 unrelated F508del homozygous index cases without residual chloride secretion by nasal potential difference measurement (NPD) to 13 patients with CFTR-mediated chloride secretion; [2]) and alters binding affinity for the transcription factors NF-KBp50, NF-KBp65 and HIF1a [7] which govern the cellular response to infection and hypoxia. As a second example, the transcription factor EHF, derived as a positional candidate from a North American genome wide scan [1], is associated with 2 NPD-defined phenotypes (Praw = 0.0082, comparing 17 index cases with high response to amiloride in NPD to 13 index cases with low response to amiloride in NPD; Praw = 0.0268, comparing 14 index cases without chloride secretion in intestinal current measurement (ICM) to 9 index cases with CFTR-mediated residual chloride secretion in ICM [5]) and affects the transcriptome of CF patients' intestinal biopsies in favor of a better processing of F508del-CFTR [5] which we could confirm in epithelial cell lines as siRNA provided against EHF results in a downregulation of MGAT2 and MGAT4, both of which are key enzymes for the complex glycosylation of proteins such as CFTR. These examples indicate that small, albeit carefully selected subpopulations facilitate the identification of genetic variants by an association study that can be validated in functional assays. Furthermore, we suggest that while the selection of subsamples within a population with a rare disease such as cystic fibrosis results in a loss of power, findings obtained for more than one endophenotype are indicative for a true-positive finding of a modifying gene. Finally, transcriptional regulation influences the CF basic defect. Interference with these pathways may results in better F508del-CFTR maturation, leading to better CFTR function in patient's tissue and thereby promoting health in cystic fibrosis.

References:
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Clinical relevance of deep phenotyping and exome sequencing in patients with short stature

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Short stature is a common condition of great concern to patients and their families. In most cases it is genetic in origin but the underlying cause often remains elusive due to clinical and genetic heterogeneity. In an unbiased approach we carefully phenotyped 565 patients and randomly selected 200 for whole exome

sequencing. Sequence variants were analyzed for pathogenicity and the affected genes characterized regarding their functional relevance for growth.

All patients received extensive clinical and endocrinological examinations, careful clinical genetic phenotypic evaluation followed by targeted diagnostic assessment for suspected diagnoses. We identified a known disease-cause in only 14 % of patients, the most common causes being CNVs found in 7 %, followed by syndromic monogenic causes in 5 % and Turner syndrome in 2 %. Whole exome sequencing identified additional mutations in known short stature associated genes (27) in 17 % of patients who manifested only part of the symptomatology precluding an early clinical diagnosis. Here, heterozygous carriers of recessive skeletal dysplasia alleles (ACAN, NPR2) were a surprisingly frequent cause of idiopathic short stature found in 3.5 % of cases.

We next selected known short stature genes with mutations for pathway analyses of the affected proteins and found that 54 % are involved in the main functional categories cartilage formation, chromatin modification and Ras-MAPK signaling. In addition we identified 37 further strong candidate genes, of which seven had deleterious mutations in at least two families. Interestingly, 48 % of these candidate genes are involved in the 10 main functional categories already identified for the known short stature associated genes further supporting their pathogenicity.

Finally, in 16 % of the 200 sequenced individuals our findings were of significant clinical relevance regarding preventive measures, symptomatic or even targeted treatment. Besides evaluation for orthopedic or developmental issues especially screening for neoplasias (TRIM37, PTPN11, NF1), symptomatic treatment for chronic kidney disease (CLCN5) and targeted treatment for severe hypertension (PDE3A) were of clinical relevance for the affected individuals.

These results demonstrated that deep phenotyping combined with targeted genetic testing and whole exome sequencing is able to increase the diagnostic yield in short stature up to 31 % with concomitant improvement in treatment and prevention. Rigorous variant analysis considering phenotypic data further led us to the identification of further 37 probable novel candidate genes.

P-MonoG-168

The role of de novo mutations in the development of amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a late-onset progressive, neurodegenerative syndrome. Most ALS cases are sporadic (≈90%). In familial forms, mutations in several different genes have been identified with a repeat expansion in *C9orf72* and mutations in *SOD1* being the most prevalent. No non-genetic cause of ALS has been identified. Since there are no overt clinical or pathoanatomical differences between sporadic or familial cases, *de novo* mutations have been suggests in disease pathogenesis and two previous studies provided some evidence for this hypothesis.

We present data of 82 patient-parents trios from an international collaborative study. By whole exome sequencing we identified 69 non-synonymous *de novo* mutations in the patients, however all of them occurred in different genes. There was no concordance between the mutated genes found in our trio set and the two earlier smaller trio studies. *In silico* analyses suggest that none of the here identified mutations are part of any of the previously postulated molecular pathways. Also, gene-gene-interaction analyses failed to find an enrichment of interacting genes. Lastly, we demonstrate that the *de novo* mutations in ALS patients in this and the two earlier studies are located in genes prone for *de novo* mutations in general.

Our results thus indicate that, in contrast to previous reports, *de novo* mutations do not seem to be a major contributor for ALS.

Disproportioned short stature and multiple anomalies in a patient with a homozygous PIK3C2A mutation – a new ciliopathy?

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We present a 21 year old female with disproportional growth retardation, eye and renal anomalies. She was born small for gestational age as the first child of healthy, consanguineous Tunisian parents at 37 weeks after an uneventful pregnancy (2470 g, 47 cm). During her first year of life, there were severe feeding problems with regurgitation and she developed postnatal growth retardation. Developmental milestones were normal. During the second year the girl developed strabismus in both eyes. Later on, a cataracta polaris anterior, anomalies of the cornea, hyperopia and progressive retinal degeneration led to profound visual impairment. Furthermore, a right kidney agenesis and a complex tubulopathy, as well as increased echogenicity of the single left kidney were diagnosed.

At presentation at age 21 years her head circumference was on the 75th centile, her height below the 3rd centile, and her weight on the 3rd centile. She showed a disproportional short stature and mild skeletal signs like clinodactyly of the 5ths fingers. Minor facial dysmorphisms included an oval face, epicanthic folds, upslanting palpebral fissures and a bulbous nose. Karyotype was normal and copy number variants were excluded by high-resolution CMA. As no aetiological diagnosis could be made clinically we performed whole exome sequencing (WES) and detected a homozygous splice site mutation (c.1640+1 G>T) in PIK3C2A (phosphatidylinositol-4-phosphate 3-kinase catalytic subunit type 2 alpha)(NM_002645). Sequencing of RT-PCR products from cDNA of patient's fibroblasts showed in-frame skipping of exon 5 and 6, equally affecting all known isoforms.

Phosphatidylinositol 3-kinases (PI3Ks) are lipid kinases involved in a large set of biological processes, including membrane receptor signaling, cytoskeletal organization, and endocytic trafficking. PI3KC2A is ubiquitously expressed and has been proposed to play an important role in clathrin-mediated endocytosis and regulation of Phosphatidylinositol 3-phosphate (PtdIns3P) levels . Furthermore PIK3C2A has been implicated in the biology of the primary cilium.

The patient's distinct phenotype resembles the previously described phenotype in Pik3c2a hypomorphic mice with pre- and postnatal growth retardation and a broad spectrum of renal abnormalities. The complete knockout of mouse Pik3c2a showed embryonic lethality. Ongoing studies on the exact consequences of the splicing defect will determine if and how much residual wild-type transcript is retained and if this is a hypomorphic variant. This case is to our knowledge the first description of a PIK3C2A human phenotype.

P-TECHNOLOGY AND BIOINFORMATICS

Adaption of the CRISPR/Cas genome editing system as a platform for the mutation of NIPAL4 as a representative of ARCI-associated genes in HeLa cells

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Autosomal recessive congenital ichthyosis (ARCI) is a rare genetic disorder with known disease-causing mutations in 9 genes. Functional implementations of identified mutations are in most cases still unknown, which is amongst others due to the limited amount of skin biopsies of ARCI-patients. Thus, suitable cell culture models for the investigation of keratinocyte differentiation are highly needed.

During the last years the prokaryotic clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) system has been turned into a potent tool in the field of genome engineering. The Cas9 endonuclease is directed by a short guide RNA (gRNA) to its target sequence, where it generates a DNA double strand break (DSB). As cellular repair mechanisms often fail in reconstituting the original sequence, insertion/deletion (indel) mutations occur potentially leading to a complete gene knock-out (KO). Consequently, the CRISPR/Cas system offers a simple RNA-programmable tool for in vitro mutation of ARCI-associated genes.

Hence, this system was applied in HeLa cells to target NIPA Like Domain Containing 4 (*NIPAL4*), the second most frequently mutated gene in ARCI patients.

In this context, functional studies were used to validate *NIPAL4* as suitable target in HeLa cells.

Successful application of the CRISPR/Cas system lead to the generation of a new clonal HeLa cell line carrying a one basepair insertion in *NIPAL4* exon 1 (c.106insG (CCDS 47328.1)). This mutation was further characterized and potentially results in a complete KO of *NIPAL4*.

This system can now be used as a basis for targeting further ARCI-associated genes and for transferring the system into keratinocytes, which are the primarily affected cell type in the skin of ARCI patients. Furthermore, it is only a small step to expand the system from generating gene KO to gene editing allowing the introduction of patient-specific mutations in non-patient derived keratinocyte cells. Hence, setting-up the CRISPR/Cas system to target an ARCI-associated gene is an important starting point for future studies to investigate pathogenic effects of ARCI-causing mutations and to understand ARCI pathogenesis on a molecular level. In this context, the transfer of the established protocol into keratinocytes offers the possibility to generate 3D cell culture models of mutations in ARCI-associated genes and allows in vitro investigation of their implications on the differentiation process.

This system would further represent an organotypic model of ARCI-disease with potential application in screening and identification of chemical compounds for the complementation of existing clinical therapies.

P-Techno-171

Comparison of different methods for telomere length measurement (TLM) in whole blood and blood cell subsets: Recommendations for TLM in hematological diseases

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Background: Telomeres cap and protect chromosome ends from degradation and fusion, and are therefore essential for maintaining chromosome stability and genomic integrity. They have a length of 5 to 15 kb (depending on age, sex and cell populations). Due to the end-replication problem there is a continuous telomere loss of 50 to 150 bp/cell cycle and thus throughout lifetime.

In laboratory practice different methods of telomere length measurement are used to identify patients with bone marrow failure syndromes (e.g. dyskeratosis congenita, aplastic anemia), hematological diseases, or other telomeropathies. Each telomere length measurement method has its advantages and disadvantages regarding material required the complexity and feasibility of the method and other parameters.

Methods: In this study we compared and validated four different methods for telomere length measurement, i.e. Southern blot analysis, quantitative PCR (qPCR), quantitative fluorescence in situ hybridization (T/C-FISH) and flow cytometry-FISH (FlowFISH). Whenever possible, EDTA and/or heparin blood samples were collected from a population of 154 healthy individuals of different age groups (newborn – 81 years). Depending on the method DNA (Southern blot and qPCR), metaphases (T/C-FISH) or rather vital cells (FlowFISH) were analyzed.

Results: Comparison and validation of the telomere length measurement methods allowed us to calculate percentiles for all age groups. Percentile curves could be used in diagnostic to identify patients with short telomeres. All methods showed acceptable accuracy, but equally imply the necessity of validation and appropriate controls in each experiment. Here, FlowFISH was the most precise, accurate and reproducible method compared to the other methods.

Discussion: Our study emphasizes the influence of expertise and experience that is required in order to produce robust and reliable telomere length analyses. Here, we provide advice on how to choose the appropriate method in general and for individual cases to safely discriminate between natural variability and pathological telomere shortening in individual cases.

P-Techno-172

MIDAS – Multiple Integration of Data Software

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Next-generation sequencing may lead to a significant improvement in diagnostic yield for rare, heterogeneous disorders through the ability to simultaneously sequence all genes contributing to a certain indication at a cost and speed that is superior to traditional sequencing approaches. On the other hand, the practical implementation of NGS in a clinical diagnostic setting involves a variety of new challenges which need to be overcome. Among these are the generation, analysis and storage of unprecedented amounts of data, strict control of sequencing performance, validation of results, interpretation of detected variants and reporting. Especially the variant interpretation emerges as current bottleneck of the diagnostic workflow.

Here we present MIDAS (Multiple Integration of Data Annotation Software), a central software system for data integration in a diagnostic laboratory. The goal of MIDAS is to construct a modular software system to integrate data from Laboratory Information Management System (LIMS), data from the routine Sanger sequencing workflow as well as NGS sequencing results and correlate the identified variants with the patients' phenotypical features to aid in variant interpretation and accelerate reporting. The phenotype is systematically recorded using the Human Phenotype Ontology standard nomenclature. In particular, Genotype-Phenotype correlations identified in one patient are made available for all other cases, to aid the interpretation and build a comprehensive knowledge base. The MIDAS software may thus serve as central information system all diagnostic patient data.

MIDAS is implemented in Java using direct database access via JDBC, and JavaFX as graphical user interface. Its architecture is designed modular including a dynamic module loader, a user management with LDAB connection and basic search functionality. According to available modules the user management and search form are adjusted; granting access for module specific views. As an advantage of this architecture, other molecular diagnostic data, such as arrayCGH or MLPA results can easily be integrated by implementing new modules.

MIDAS aims to aid molecular diagnostics by simplifying and accelerating data analysis and interpretation, improving patient care. MIDAS is being developed as part of a prospective multicentric study including clinical, diagnostic and software development partners. A grant by the Bavarian Ministry of Economy, Media, Energy and Technology is used to fund this effort.

P-Techno-173

Optimus Primer, a free new web-based tool for primer design

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With the implementation of Next Generation Sequencing (NGS) based assays as key tool in DNA-sequencing, conventional Sanger-sequencing has become a standard method to verify single nucleotide polymorphisms (SNPs) of interest. However, designing specific PCR- and Sanger-sequencing primers has always been a rather time consuming task in terms of searching the right genomic sequence, considering known SNPs that could result in allele-specific amplification, and converting selected primer-sequences to upload them to web-based services. To circumvent this, we developed Optimus Primer, a Python script, that automatically designs respective primers. The script uses the database of the UCSC genome browser to download positional information of genomic sequence (refSeq Gene: hg38) and common SNPs (SNP147common: hg38) in the region of interest. The genomic sequence is downloaded via the UCSC API and common SNPs are annotated. In the following step primers are picked using primer3 (Whitehead-Institute). The target sequence is either the SNP-containing exon in case of exons smaller than 300 bp, or, the genomic region flanking the SNP of interest. The positional information of the SNP is gathered using the Mutalyzer API (LUMC). The last two bases of each primer are checked for all SNPs in the 3' End (SNP147: hg38). The script will be integrated into a website for free easy access and usability (www.optimus-primer.com). The only information needed is the refSeq ID, the exon, and the cDNA position (eg: NM_000249.3:c.464T>G). The script Optimus Primer will provide the user with primers in the widely used primer3 format. The users are also able to download the source from our website, and to run it on a local UNIX system as a command line tool.

P-Techno-174

MutationTaster3: Moving towards a comprehensive evaluation of disease causing mutations

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MutationTaster is a free and user-oriented application for comprehensible evaluation of non-synonymous and synonymous as well as non-coding DNA sequence variants. As 1500+ citations show, the software has been strongly embraced by the clinical and research community. However, its capacities for assessing the functional consequences of non-coding variants are still limited. While MutationTaster is able to predict the variant's effect on major intragenic regulatory features such as splice sites or polyadenylation signals, many other potential effects of intronic or UTR variants are not covered by the software yet.

Variants in the non-coding region of the genome are frequent and thought to cause a substantial part of yet unsolved Mendelian diseases. These variants can occur either in extragenic regulatory regions (see abstract on RegulationSpotter) or in the untranslated regions, including introns, of a gene. Unfortunately, their

effects are much harder to predict than those of non-synonymous variants. Therefore, only few disease mutations outside the coding sequence have hitherto been found and experimentally confirmed.

To close this gap, we are advancing the current version of MutationTaster to improve the analysis of intragenic non-coding variants. We do so by implementing additional tests for variants in the 5' and 3'UTR to determine their effect on AU-rich elements, microRNA binding sites and the influence of secondary structure on gene expression. Moreover, we plan to shift from the analysis of exclusively monogenic to complex diseases with cancer being the first disease model to be integrated into MutationTaster3.

Already implemented enhancements of MutationTaster3 comprise the improved splice site analysis using MaxEntScan and the integration of mitochondrial polymorphisms from the Human Mitochondrial Genome Database. The addition of links to the Integrative Genomics Viewer and the LoF-Metrics of the ExAC browser (Exome Aggregation Consortium) streamline the usage of the MutationTaster3 results and enable researchers to get a detailed view of the relevant variants and associated genomic sequences.

Taken together, MutationTaster3 will offer much better capabilities to predict the disease-causing potential of intragenic variants than its predecessor.

The software is freely available at http://www.mutationtaster.org

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P-Techno-175

*** MutationDistiller: User-driven prioritisation of disease mutations

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In the Next Generation Sequencing age, the strongest challenges have shifted from genotyping to handling the myriad of variants detected. Whole Exome Sequencing yields tens of thousands of coding and non-coding variants, a number increased to millions if the whole genome is sequenced.

To master this mountain of data, computer-based identification of potential disease mutations is absolutely indispensable.

However, current computational strategies are usually generated by computer scientists without integrating human geneticists into software development. This often leads to tools which fulfil their main purpose but are not ideally suited to the needs of human geneticists. To assess the relevance of a suggested disease mutation, geneticists need detailed information about the effect on the protein and about the gene or protein itself.

To close this gap, we have developed MutationDistiller, a web-based tool for user-driven variant prioritisation based on biological disease properties. Its analysis includes the potential role of a mutated gene in pathogenesis as well as the estimated effect of a variant on gene/protein function. Thus, MutationDistiller allows human geneticists to use every piece of information they consider relevant. Unlike similar tools, its input goes beyond the Human Phenotype Ontology and can include complete diagnoses, biological pathways, gene expression, and gene function. Potentially harmful variants are identified by MutationTaster, which provides a deleteriousness score together with data on the actual effect of the variants. MutationDistiller is not restricted to non-synonymous variants but can also handle intragenic non-coding or synonymus variants. Moreover, the program incorporates the known modes of inheritance of disease genes and the genotype of the queried variants (including compound heterozygosity).

The output page provides all information on one site: The core component is a concise overview table of the most likely disease genes and variants. Here, the most crucial information such as the variants' effect on the protein, frequencies in polymorphism databases and known diseases caused by mutations within this gene and their mode of inheritance are listed. More detailed gene and disease information and hyperlinks to external data are provided below, hence offering a comprehensive overview of the available knowledge. This allows geneticists to draw their own conclusions without any tedious collection of relevant information from the Internet.

A beta-version of the program is freely available at http://www.mutationtaster.org.

P-Techno-176

Alternate-Locus Aware Variant Calling in Whole Genome Sequencing

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With the GRCh37 human genome release in 2009 the Genome Resource Consortium extended the previous linear, "golden-path" paradigm of the human genome and introduced a more graph-like model in the sense of regions with alternate loci, representing common alterations in sequence and structure. In whole-genome sequencing (WGS) these stretches of sequence are largely but not entirely identical between the primary assembly and an its corresponding alternate locus can result in multiple variant calls against regions of the primary assembly. This results in characteristic and recognizable patterns of variant calls at positions that we term alignable scaffold-discrepant positions (ASDPs).

We developed an algorithm (ASDPex) that analyzes these patterns in the 178 structurally variable regions of the current GRCh38 genome assembly. A heuristical approach then infers whether the pattern of variant calls of a sample contains sequences from the primary assembly, an alternative locus, or their heterozygous combination at each of these 178 regions.

We investigate 121 in-house WGS datasets and found that on average 51.8 ± 3.8 of the 178 regions correspond to an alternate locus rather than the primary assembly sequence. Filtering these genomes with our algorithm identified around 7900 variant calls per genome that colocalized with ASDPs.

Our findings suggest the potential of fully incorporating the resources of graph-like genome assemblies into variant calling. Our algorithm already uses the information contained in the 178 structurally variable regions of the GRCh38 genome assembly to avoid spurious variant calls in cases where samples contain an alternate locus rather than the corresponding segment of the primary assembly.

P-Techno-177

Updates on the Human Phenotype Ontology Project

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The Human Phenotype Ontology project provides three resources, the ontology of clinical features, disease-phenotype associations and algorithms that enable analysis of data that is described using HPO. This resource is being used for computational deep phenotyping and precision medicine. It enables clinical data integration for translational research. The HPO is being increasingly adopted in software, research projects and companies world-wide. We will discuss the progress and recent developments that the HPO project has made since 2008. This will include the expansion of HPO for common (complex) diseases, novel algorithms for phenotype-driven analysis of genomic variation, cross-species mapping of phenotype data, translation of HPO into several languages and also the addition of a more patient-friendly terminology for HPO terms.

P-Techno-178

Finding regulatory DNA variants with RegulationSpotter

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Millions of patients worldwide suffer from a rare genetic disease. To date, the OMIM database lists more than 8.000 diseases with proven or suspected Mendelian basis, but the molecular cause is known for less than 60%. Although Next Generation Sequencing has drastically facilitated the discovery of disease mutations, a substantial fraction of Whole Exome Sequencing (WES) projects fail to identify the causal variant. This may be due to the fact that disease-causing mutations are not always located within the coding sequence. Whole Genome Sequencing (WGS) could solve this problem, but we are not yet readily prepared to handle the vast amount of variants which are generated by this technique, as intuitive and reliable software solutions for variant evaluation are missing. The currently existing approaches are not well-suited for a diagnostic setting, because they output a battery of different scores whose interpretation is left to the users. However, human geneticists are specialists for the assessment of symptoms of genetic diseases – not for the analysis of numerical scores indicating different likelihoods of the occurrence of regulatory elements. This is especially true when it comes

to the hundreds of thousands of extragenic variants found by WGS, for which effect predictions always face a high level of uncertainty.

We consider it crucial to provide geneticists with the information they need to determine the significance of a variant, not to flood them with scores whose meanings are difficult to grasp. To facilitate the interpretation of extragenic variants, we are developing RegulationSpotter. In contrast to other approaches such as Genomiser or CADD, we integrate as much knowledge as possible in a user-friendly fashion to pinpoint the variants which are most likely to disturb the expression of candidate genes.

RegulationSpotter includes different data on regulatory DNA elements such as DNA methylation, transcription factor binding sites, histone modifications, or genome-wide interactions. All variants which have the potential to influence one or several candidate genes are presented in a graphical interface and ranked according to their predicted effect on the target gene(s). Instead of giving scores, we present the potentially affected regulatory features in an intuitive graphical matrix.

The software can freely be used at http://www.mutationtaster.org

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P-Techno-179

GeneCascade2017 – a one-stop shop for finding disease mutations

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In the last years, our GeneCascade software suite for the elucidation of rare diseases has seen some major extensions, mainly for the discovery of non-coding mutations. The website contains a number of tools focusing on the different steps in studying genetic disorders. All applications are web-based, have easy-to-use interfaces and are aimed directly at human geneticists, without any need to install software, use the command line or to try to explain clinical features to IT specialists. We think that software should adapt to the user – not the other way around. And, unlike other web shops, its use is completely free.

HomozygosityMapper finds disease-linked regions in consanguineous families. Users can upload genotypes from SNP chips as well as WES and even WGS data. We display likely disease loci in intuitive graphical interfaces. The results can also be used in our 'downstream' applications such as GeneDistiller, MutationTaster, or MutationDistiller to identify the actual disease mutation.

GeneDistiller provides a user-driven way to find the best candidate gene for a genetic disorder. Users can specify various aspects of the patients' phenotypes and the results are presented in a comprehensive way without the need to manually query other Internet resources to collect further information relevant to asses the disease potential.

MutationTaster evaluates the disease potential of non-synonymous as well as of non-coding and synonymous DNA variants within genes. It does not only display a prediction but also detailed information on the variants' likely effect on mRNA and protein. It can either analyse single variants as found by Sanger sequencing or complete VCF files from WES or WGS projects.

RegulationSpotter lists information about potentially regulatory DNA variants outside of genes.

Variants are ranked according to their predicted effect on gene regulation. In addition to a score, we provide a user-friendly summary of the functional effects a variant may have.

MutationDistiller combines GeneDistiller and MutationTaster in a single application for convenient variant and gene prioritisation. It offers human geneticists much more freedom to enter the phenotype than similar applications and provides deeper information about the variant and the gene.

CNVinspector is aimed at the study of copy number variants. Users can upload their patients' (or cohorts') CNVs and compare them with their own healthy controls or public data stored in our database. We include DECIPHER to indicate known diseases caused by CNVs at the same position.

ePOSSUM examines the effect of DNA variants on transcription factor binding. As TF binding site prediction is notoriously unreliable, we also give an indication of the statistical relevance of a result.

The GeneCascade website can be accessed at http://www.mutationtaster.org.

P-Techno-180

Best of both world´s: a novel, rapid capture protocol that overcomes drawbacks associated with DNA fragmentation in established methods

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Rapid capture protocols are an attractive proposition for clinical sequencing labs, as they enable quicker sample-to-sequencing turnaround times. The fragmentation of input DNA for the construction of pre-capture libraries is a bottleneck in established protocols. Mechanical shearing is the gold standard, but is laborious

using single-tube Covaris instruments; and higher-throughput instrumentation is cost-prohibitive to many smaller labs. "Tagmentation"-based methods (e.g. the Nextera Rapid Capture system from Illumina, or Agilent's SureSelect QXT system) employ transposases for fast ond simple library construction. However, these protocols are associated with significant sequence bias, especially with low-quality FFPE samples and are extremely sensitive to DNA input - thus requiring meticulous quantification
of viscous, high-molecular weight DNA.

Here we describe a newly-developed rapid capture protocol that combines the KAPA HyperPlus Kit (Kapa Biosystems) with integrated, low-bios enzymatic fragmentation, and Agilent´s proven SureSelect XT Target Enrichment Technology. The streamlined method follows for the preparation of high-quality, sequencing-ready libraries in one working day. The novel enzymatic fragmentation reagent does not require careful quantification of input DNA, yielding reproducible fragmentation profiles optimal for capture over the 5fold range-tested (50 - 250 ng) The single-tube KAPA HyperPlus protocol results in very efficient conversion of input DNA to precapture library thereby decreasing duplication rates and increasing the complexity of the library going into the modified, 90min SureSelect Fast XT hybridization protocol.

Our protocol represents a significant improvement for fast routine diagnostics, where robust and reproducible pipelines ore needed to support timely treatment decisions.

P-THERAPY FOR GENETIC DISEASES

P-Therap-181

Chemical compound screening in Best vitelliforme macular dystrophy (BVMD)

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Purpose:

Human Bestrophin-1 (BEST1) is a chloride channel controlled by Ca^{2+} and cell volume and is localized at the basolateral membrane of the retinal pigment epithelium (RPE). So far, there is no therapy for the BEST1-associated diseases, of which the most common is Best vitelliforme macular dystrophies (BVMD). In this study, we developed an assay applicable for high and small-scale compound screening targeting BEST1 localization and function.

Methods:

To assess BEST1 channel function we developed a halide assay. Briefly, MDCKII cell lines were established, stably expressing wildtype BEST1 or BVMD-associated BEST1 mutants together with a yellow fluorescent protein (YFP)-based halide sensor. In polarized MDCKII cells, wildtype BEST1 and BEST1-R218C localize regularly at the basolateral plasma membrane (PM) while BEST1-L224M and BEST1-Y227N appear significantly reduced in quantity and grossly mislocalized to cytosolic compartments. Cells were stimulated with extracellular addition of iodide known to pass the PM through anion cannels and, as a consequence, intracellularly quench YFP fluorescence. Variations in YFP fluorescence levels as a marker for BEST1 function were recorded in 96 well plates by a plate reader setup. A small-scale 2,560 compound library, commercially available as Spectrum Collection (MicroSource Discovery Systems, Gaylordsville, USA) was used for screening. Positively tested compounds were reanalyzed by whole-cell patch clamp recordings and cell volume measurements.

Results:

The halide assay revealed reproducible halide permeability across wells and, as a control, reliably detected MDCKII cells overexpressing wild type BEST1 by a decrease of YFP fluorescence to 70% following 60 seconds iodide stimulation. Cells harboring mutant BEST1 showed 85% of default YFP fluorescence after the same time interval.

Conclusion:

The current study established an assay appropriate for high and small-scale compound screening targeting BEST1 localization and function. This assay will be used to screen for compounds in mutant cells lines BEST1-T6P and BEST1-Y227N for their ability to improve trafficking to the PM or correcting protein folding to enhance ion permeability.

Intranasal application of NPY and NPY13-36 ameliorate disease pathology in R6/2 mouse model of Huntington's disease

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Background

Neuropeptide Y-Y2 receptor (Y2 receptor), an auto-receptor of Neuropeptide Y (NPY) and attractive guanine nucleotide (G) protein-coupled receptor target, has been implicated as a potential therapeutic target for many clinical conditions, including epileptic seizure, depression, pain, and alcoholism. In Huntington's disease (HD) patients and animal models of HD, NPY-expressing striatal interneurons are selective preserved and increased with advancing disease. However, the potential role of Y2 receptor in HD pathology remains under-explored.

Aims

To investigate whether activation of Y2 receptor using NPY and selective Y2R ligands could ameliorate behavioral deficits and neuropathology in R6/2 mouse model of HD.

Methods/Techniques

NPY and selective Y2 receptor agonist NPY13-36 were intranasally administered to R6/2 mice, five days in a week, beginning from 4 weeks of age until 12 weeks of age. In the second study, R6/2 mice received daily intraperitoneal administration of selective non-peptide Y2 receptor antagonist (SF-31) to selectively block Y2 receptor.

Results/Outcome

Intranasal application of NPY showed significant increase in rotarod performance compared to the saline and SF-31 treated R6/2 mice (*$p < 0.05$ and **$p < 0.01$ at 8 and 12 weeks of age respectively, n=12). However, treatment with NPY13-36 showed a clear trend towards increased rotarod performance at 12 weeks of age compared to the saline and SF-31 treated R6/2 mice but the difference did not reach significance. Also, treatment with NPY and NPY13-36 showed no significant effect on body weight loss in R6/2 mice, contrasting with previous data obtained with single intracerebroventricular (ICV) injection of NPY in R6/2 mice. Furthermore, intranasal application NPY or NPY13-36 led to decrease in mutant Huntingtin (Htt) aggregation and mediated increase in dopamine-and cAMP regulated phosphoprotein (DARPP-32) and brain derived neurotrophic factor (BDNF) levels. Additionally, we found that NPY and NPY13-36 attenuate microglial activation, inducible nitric oxide synthase (iNOS) expression, and proinflammatory cytokines production in R6/2 mice compared to the saline and SF-31 treated R6/2 mice.

Conclusion

Taken together, our findings suggest that targeting NPY-Y2 receptor might be a potential neuroprotective therapy for HD and other neurodegenerative diseases.

Allele-specific suppression of dominant-negative Bestrophin 1 mutations

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Purpose: Retinal pigment epithelium (RPE) differentiated from human induced pluripotent stem cells (hiPSC) demonstrated degradation and mislocalization of mutant bestrophin 1 (BEST1) protein in autosomal dominant Best disease (BD). Importantly, mutated alleles revealed a dominant-negative effect leading to an impairment of volume-regulated chloride transport, the basic function of the homo-pentameric BEST1 channel. Here, our study aimed for a proof-of-concept to treat BD by selectively eliminating BEST1 mutant transcripts in patient-derived hiPSCs prior to RPE differentiation via the CRISPR/Cas9 genome editing technology.

Methods: Adult human dermal fibroblast were obtained from skin biopsies of BD patients and reprogrammed into hiPSCs. Single guide RNA sequences (sgRNA) targeting 6 BEST1 mutations were selected by the "Optimised CRISPR Design Tool" (Zhang Lab, MIT 2015). Editing efficiency and specificity of designed sgRNAs were tested in HEK293 cells using an established fluorescence-based assay. After transfection of hiPSCs the percentage of indel formation of on- and off-targets was determined by a PCR-based CRISPR/Cas Cleavage Detection Kit. Cas9-treated stem cell populations were analyzed for pluripotency and selected for full genome sequencing before differentiation to RPE cells.

Results: Computational design of 6 disease-causing BEST1 variants (N11K, V86M, S108R, Q238R, A243V, and I295del) offered at least one sgRNA with predicted high quality per mutant allele. The guide sequences were cloned into the Cas9-expressing plasmid PX459 and co-transfected with pCAG-EGxxFP

plasmids containing genomic fragments of ~500 bp of either the mutated or wildtype sequence to the corresponding sgRNA. As targeted Cas9 cleavage results in reconstitution of the EGFP expression cassette by homology dependent repair, the efficiency and specificity of Cas9 cleavage was evaluated on a plate reader by quantifying EGFP fluorescence after 48h of transfection. As a result, 5 out of the 6 sgRNAs tested showed high allele-specificity and are now used for targeted genome editing in hiPSCs.

Conclusion: So far, there is no treatment for BD although the molecular pathology of BEST1 has recently been established. Our proof-of-concept study aims to determine whether haploinsufficiency of normal BEST1 protein is sufficient to fully or partly restore cellular function in cells of primary BD pathology, namely the RPE. To this end, we will determine the degree of rescue (i.e. reconstitution of volume-regulated chloride conductance) by whole-cell patch-clamp analysis. If successful, our CRISPR/Cas9-driven approach will be useful to treat other diseases with dominant negative effects of the mutated allele.

P-Therap-184

*** Metformin rescues early cognitive symptoms in the HdhCAG150 mouse model and is therefore a promising candidate for treatment of HD patients.

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder that is caused by an unstable glutamine (CAG) trinucleotide repeat expansion within exon 1 of the Huntingtin gene and 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 step for the development of a causal therapy. 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 h 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.

We have shown previously that in HD patients, mediated through mTOR signaling, translation of mRNA carrying expanded CAG repeats is elevated (Krauss et al., 2013). We have also seen that the biguanid Metformin antagonizes mTOR signaling in neurons in-vitro and in-vivo (Kickstein et al., 2010).

We show here that metformin, by interfering with the mTOR kinase and its opposing phosphatase, PP2A, regulates local protein synthesis in the brain and is able to suppress the production of disease making protein in early HD. Furthermore Metformin leads to a significant improvement of movement abilities in a C-elegans model for HD and to a rescue of early cognitive symptoms in the HdhCAG150 animal model. These data suggest that metformin is a very promising candidate for early phase treatment of HD patients.

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