VORTRÄGE

Keynote-Lecture

Emotion oder Gefühl: Furcht und Angst im Spiegel der Hirnforschung

Hans-Christian Pape

Institut für Physiologie I, Westfälische Wilhelms-Universität Münster

Aus biologischer Sicht sind Furcht und Angst wichtige Komponenten unseres Verhaltens: sie schützen uns vor Einflüssen, die unangenehm oder sogar schädlich sind. Allerdings können Störungen dieser Verhaltensmechanismen zu exzessiven Reaktionen führen, die durch den Betroffenen kaum kontrollierbar sind: z.B. eine andauernde Angst lange nach Ende der bedrohlichen Situation, oder ein Wiedererleben von Erfahrungen mit extremer Angst. Derartige Zustände können sich zu Angsterkrankungen entwickeln - Panikstörung, Phobie, posttraumatische Belastungsstörung-; sie zählen zu den häufigsten psychischen Störungen. Der Vortrag zeigt, wie die neurophysiologische Grundlagenforschung die Schaltkreise des Gehirns identifiziert, die zum einen die Furcht und die Erinnerung an relevante Erfahrungen begründen („Furchtgedächtnis"), und die zum anderen die Grundlage für eine pharmakologische Intervention schaffen. Darauf bauend werden Prozesse diskutiert, die Angst in eine Angsterkrankung entgleisen lassen, und es wird schlussendlich die Frage nach der Existenz von „Angstgenen" gestellt.

Plen 1

Induced Stem Cells: Disease Modeling and Drug Screening in Hereditary Diseases

Hans R. Schöler

Department of Cell and Developmental Biology, Max Planck Institute for Molecular Biomedicine, Münster, Germany

The reprogramming of mouse and human somatic cells into pluripotent stem cells, designated as induced pluripotent stem (iPS) cells, was first successfully achieved using fibroblasts as the starting population in 2006 by Kazutoshi Takahashi and Shinya Yamanaka. Initially, the introduction of the virally-expressed transcription factor quartet of Oct4, Sox2, c-Myc, and Klf4 was a prerequisite to reprogramming. Alternate methods of inducing mouse pluripotent stem cells involve the use of RNA, recombinant proteins, small molecules and when certain adult unipotent stem cells are used as the starting cell population, introduction of extraneous transcription factors are not needed. Reprogramming adult cells to pluripotency has uncovered exciting new areas in basic and applied research. In my presentation, I will introduce the following three areas as well as discuss the basic principles underlying the induction of pluripotency:

- Potential use of reprogrammed cells capable of supporting cellular transplantation;
- Development of models for human diseases;
- Generation of iPS cells and screening of potential new drug candidates.

Plen 2

Molecular Genetics of Sex Determination and Disorders of Sex Development

Peter Koopman

Institute for Molecular Bioscience, The University of Queensland, Brisbane, Australia

More than 27 years have passed since the discovery of Sry, the Y-chromosomal gene responsible for directing male development in mammalian embryos. This landmark discovery in 20th century molecular genetics opened the door to detailed study of how genetic pathways channel the development of the initially ambiguous embryonic gonads into testes and so generate males, while suppressing ovarian development. I will describe recent progress in understanding the molecular and cellular events that direct gonadal differentiation, using mice as a model, exploiting transcriptomic and other molecular strategies for genome-wide identification of novel sex-determining genes, and functional analysis using CRISPR/Cas9 technology.

Disorders of sex development (DSDs) are a broad group of congenital conditions in which some aspect of reproductive anatomy is atypical. For example, complete sex reversal (XY female or XX male phenotype) is estimated to affect 1 in 20,000 births, genital anomalies 1 in 4,500, and hypospadias (misplacement of the urethral opening) as many as 1 in 250 boys. Individually DSDs tend to be rare, but collectively they require significant healthcare management because they are commonly associated with negative psychosocial

experiences, impaired reproductive capacity, and gonadal cancer. DSDs tend also to be stigmatized, such that relatively little is known about them in the general public.

Many DSDs stem from dysfunction of the molecular pathways of testis or ovary development. Mutation studies in humans and mice have identified some “DSD genes”, but many remain to be discovered; consequently, many DSD children do not receive a definitive diagnosis, and the etiology of their condition remains a frustrating mystery for them and their families.

Much of my research is carried out with a view to identifying new DSD genes, studying how they function at the molecular and cellular levels, and applying this knowledge to the diagnosis and clinical management of DSD, so as to improve outcomes for affected children and adults. Progress in these areas will be described.

Recent reviews by us relevant to the topic of the talk:

Heilker R, Traub S, Reinhardt P, Schöler HR, Sterneckert J. iPS cell derived neuronal cells for drug discovery. Trends Pharmacol Sciences 35(10):510-19, 2014

Tapia N, Schöler HR. Molecular Obstacles to Clinical Translation of iPSCs. Cell Stem Cell 19(3):298-309, 2016

Sterneckert JL, Reinhardt P, Schöler HR. Investigating human disease using stem cell models. Nat Rev Genet 15:625-39, 2014

Ursu A, Schöler HR, Waldmann H. Small-molecule phenotypic screening with stem cells. Nat Chem Biol. 13(6):560-563, 2017

Yamanaka, S., Monteiro, J., and Schöler, H.R. iPS Cells 10 Years Later. Cell. 166:1356-1359, 2016

Talk nach 12

Genom-Editierung an der menschlichen Keimbahn:

eine Zwischenbilanz der ethischen Debatte

Bettina Schöne-Seifert

WWU Münster

Mithilfe der vor wenigen Jahren entdeckten CRISPR-Cas-Genscheren lassen sich Gen-Veränderungen grundsätzlich auch an menschlichen Keimzellen (Spermien, Eizellen und Embryo) durchführen – potentiell deutlich wirksamer und präziser als mit früheren Methoden. Was in den 1990er Jahren noch ein weitgehend hypothetisches Thema und 2015 immerhin noch ein solches der mittleren Zukunft zu sein schien, hat sich inzwischen zu einem höchst umstrittenen Akut-Thema entwickelt: Seit chinesische und zuletzt auch britische und US-amerikanische Forschergruppen verschiedene Genom-Editierungs(GE)-Versuche an der menschlichen Keimbahn bereits unternommen haben, häufen sich die Beiträge zur ethischen Einschätzung solcher Interventionen. Alarmistische, skeptische und vorsichtig-optimistische Stimmen nationaler wie internationaler Provenienz bilden dabei ein höchst kontroverses Spektrum.

Der Vortrag unternimmt den Versuch einer ordnenden Darstellung der vertretenen Positionen sowie einer kritischen Würdigung der wesentlichen Argumente für und wider den Einsatz von GE an menschlichen Keimbahnzellen in der Forschung und später ggf. in der Klinik – aus einer seinerseits vorsichtig-liberalen Perspektive.

Pro & Contra I

Panel versus Exom: Massiv-parallele Sequenzierung zwischen Möglichkeit und Evidenz (in German only)

Moderation: Markus Nöthen (Bonn), Johannes Zschocke (Innsbruck)

Es diskutieren Thomas Meitinger (München), Birgit Zirn (Stuttgart), Martin Zenker (Magdeburg)

Bei der molekulargenetischen Diagnostik mit massiv-parallelen Sequenzierverfahren stellt sich die Frage, wie viele bzw. welche Gene erfasst bzw. ausgewertet werden. Bei der offenen Analyse werden möglichst viele Gene z.B. im Rahmen eines Exoms, mit ggf. semi-automatischer Identifizierung von krankheitsrelevanten Veränderungen durch bioinformatische Verfahren untersucht. Bei einer strikten Auswahl indikationsbezogener Gene werden andere Gene ausgeblendet, möglicherweise auch Gene mit einer bisher nur vermuteten Beziehung zur spezifischen Fragestellung. Argumente, die für die unterschiedlichen Strategien angeführt werden, reichen von der Vermeidung von ungewünschten Nebenbefunden über die Frage nach dem Umgang mit unklassifizierten Varianten bis hin zur Sorge, bei strenger Fokussierung auf einzelne Gene mögliche krankheitsrelevante Veränderungen zu übersehen. Dabei spielen sowohl finanzielle als auch medico-legale

Aspekte eine Rolle. Im Rahmen der pro und contra Sitzung sollen zwischen Podium und Plenum die Vor- und Nachteile der verschiedenen Strategien diskutiert und mögliche Lösungsansätze erarbeitet werden.

Pro & Contra II

Wie sieht die Zukunft der Zytogenetik aus?

Moderation: Thomas Haaf (Würzburg)

Es diskutieren Ron Hochstenbach (Amsterdam), Thomas Meitinger (München), Harald Rieder (Düsseldorf), und Reiner Siebert (Ulm).

Die moderne Humangenetik beginnt im Jahr 1959 mit der Entdeckung der Trisomie 21 als Ursache des Down-Syndroms. Die Erstbeschreibung des Philadelphia-Chromosoms ein Jahr später war ein ähnlicher Meilenstein in der Tumorforschung. Die Einführung pränataler Chromosomenanalysen in den 1970er Jahren hat die Pränatalmedizin mit begründet. Seit den frühen 1990er Jahren haben verschiedene molekularzytogenetische Techniken das Auflösungsvermögen verbessert und auch die Analyse von sich nicht teilenden Zellen ermöglicht. Chromosomenanalysen haben über viele Jahre in der humangenetischen Routinediagnostik die zentrale Position eingenommen. Humangenomprojekt und neue DNA-Sequenziertechnologien haben in den letzten zwei Jahrzehnten die molekulare Humangenetik revolutioniert. Einzelzellanalysen, lange ein Alleinstellungsmerkmal der Zytogenetik, sind heute auf genomischer Ebene möglich. Die zunehmende Ausweitung und immer höhere Aussagekraft molekulargenetischer Diagnostik, aber auch andere Phänomene haben zu einem ständigen Rückgang der Fallzahlen in der Zytogenetik, insbesondere im Bereich der Pränataldiagnostik geführt. Eine gewisse Ausnahme bildet vielleicht noch die Tumorzytogenetik. Für viele humangenetische Institute und Praxen sind die Fälle nicht mehr ausreichend, um das noch vorhandene hochqualifizierte Personal zu finanzieren oder die nächste Generation von Zytogenetikern auszubilden. Das größte Problem von Ärzten und Fachhumangenetikern in Weiterbildung ist heute oft, die geforderten zytogenetischen Fallzahlen zusammenzubekommen. In der neuen Weiterbildungsordnung wird die Bedeutung dieses Bereiches zwangsläufig schrumpfen. Müssen wir jetzt durch qualitätsverbessernde Maßnahmen dafür sorgen, dass es auch in 10 Jahren noch ausreichend Expertise in der Chromosomenbänderungsanalyse gibt? Wie sieht das Methodenspektrum in Zukunft aus? Gibt es eventuell eine Zytogenetik ohne Chromosomen, d.h. nur auf Arrays und NGS basierend? Was würden wir übersehen, wenn nur noch sequenziert wird?

Diese und weitere Fragen und Probleme möchten wir mit Ihnen diskutieren. Die o.g. Referenten werden ihre Standpunkte dazu in kurzen Impulsreferaten (ca. 10 min) darlegen, bevor eine Debatte mit dem Publikum angeregt wird.

EDU 1

Hereditäre Neuropathien

Moderation: Ingo Kurth (Aachen), Sabine Rudnik-Schöneborn (Innsbruck)

Periphere Neuropathien, auch Polyneuropathien, sind häufige Erkrankungen peripherer motorischer, sensibler und autonomer Nerven. Es lassen sich infektiöse, immunvermittelte, metabolische, toxische, vaskuläre, hereditäre und idiopathische Formen abgrenzen. Zu den erblichen Formen zählen die Charcot-Marie-Tooth Erkrankungen (CMT, auch: hereditäre motorisch-sensible Neuropathien, HMSN), die sensibel-autonomen (HSAN), die rein motorischen Neuropathien (HMN, auch: distale spinale Muskelatrophien) und die erblichen Small-Fiber-Neuropathien (SFN). Die klinischen Bilder zeigen Überschneidungen und es gibt Übergänge zu spastischen Paraplegien, den Motoneuronerkrankungen und zu einer Vielzahl zum Teil komplexer erblicher Erkrankungen, bei denen eine periphere Neuropathie ein Teilsymptom ist. Mutationen in deutlich über 100 Genen sind als Ursache hereditärer Neuropathien und entsprechender Differentialdiagnosen beschrieben. Aufgrund des breiteren Einsatzes von NGS-Techniken steigt die Zahl relevanter Genveränderungen und neuer krankheits-assoziierter Gene weiter rasant an.

Die EDU hat folgende Ziele:

Die Einteilung, Klinik und Diagnostik hereditärer Neuropathien wird erläutert. Es soll besprochen werden, wann eine genetische Abklärung sinnvoll ist und welche molekulargenetische (Stufen-) Diagnostik bei Neuropathien sinnvoll ist.

Es zeigt sich für eine Reihe von Neuropathie-assoziierten Genen, dass Mutationen im gleichen Gen zu klinisch distinkten Entitäten führen können und dass mutationsabhängig auch unterschiedliche Erbgänge vorliegen können. Die Komplexität der Interpretation molekulargenetischer Ergebnisse, insbesondere im Rahmen einer breiten NGS-Paneldiagnostik, soll vor diesem Hintergrund diskutiert werden.

Es sollen neu identifizierte Neuropathie-assoziierte Gene exemplarisch vorgestellt werden, die gleichzeitig den rasanten Wissenszuwachs auf dem Gebiet der Neurogenetik und das immer breiter werdende Spektrum der Pathomechanismen widerspiegeln.

Für die meisten hereditären Neuropathien existiert bislang keine kausale Therapie. Bereits verfügbare Therapieansätze für einzelne Formen, klinische Studien und tierexperimentelle Studien sollen im Rahmen der Einzelvorträge diskutiert werden.

13:00 – 13:30
Sabine Rudnik-Schöneborn (Innsbruck)
Hereditäre motorisch-sensible Neuropathien und distale spinale Muskelatrophien

13:30 – 14:00
Katja Eggermann / Ingo Kurth (Aachen)
Hereditäre sensible Neuropathien und klinische Überlappungen

14:00 – 14:30
Jan Senderek (München)
Erbliche Neuropathien: Neue Mutationen, neue Gene, neue Phänotypen

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EDU2

Asymmetrische Wachstumsstörungen – Mosaike

Ute Moog[1], Birgit Zirn[2]

1) Institut für Humangenetik, Universitätsklinikum Heidelberg, Heidelberg; 2) genetikum®, Genetische Beratung und Diagnostik, Stuttgart

Asymmetrische Wachstumsstörungen, besonders ein partieller Großwuchs, sowohl von somatischem als auch zerebralem Gewebe, treten häufig im Rahmen von chromosomalen Mosaiken oder bei im Mosaik vorliegenden Mutationen auf. Der Nachweis gelingt oft nicht an Blut, sondern z.B. an Fibroblasten oder läsionalem Gewebe und erfordert deshalb einen gerichteten Verdacht.

Chromosomale Mosaike können ein wiedererkennbares syndromales Krankheitsbild hervorrufen (z.B. Mosaik-Tetrasomie 12p, Mosaik-Trisomie 8) oder aufgrund allgemeiner Hinweise (Asymmetrie, fokale Veränderungen der Haut oder anderer Organe, Syndaktylien) in den Fokus geraten. Wachstumsstörungen als Folge von postzygotischen Mutationen in Genen des PI3K-AKT-MTOR-Signalwegs (u.a. PROS, PIK3CA-Related Overgrowth Spectrum) können sowohl somatisches Gewebe als auch das Gehirn betreffen und mit kutanen Pigmentauffälligkeiten, kapillären oder anderen Gefäßmalformationen einhergehen. Auch postzygotische Mutationen in Rasopathie-Genen (z.B. HRAS) rufen erkennbare Krankheitsbilder hervor mit richtungsweisenden Haut-, Gefäß- und weiteren Anomalien.

In diesem Workshop möchten wir repräsentative Fälle aus den genannten Gruppen vorstellen und gemeinsam mit den Teilnehmern die richtungsweisenden Symptome und das diagnostische Procedere erarbeiten. Eigene Fälle mit klarer Diagnose können nach vorheriger Anmeldung eingebracht werden (Anmeldung an: ute.moog@med.uni-heidelberg.de).

QW Genetische Beratung

Genetische Beratung

Moderation: Friedmar Kreuz (Dresden) & Dieter Schäfer (Frankfurt)

Vorgesehene Themen

Umfrage des Vereins psychosoziale Aspekte in der Humangenetik e. V. (VPAH) unter den Selbsthilfegruppen zur Genetischen Beratung (Hendrik Berth): Bedeutung der Ergebnisse für die Praxis der Genetischen Beratung

Umfrage der BVDH-Kommission Genetische Beratung und Klinische Genetik zur/zum NIPD/NIPT (Dieter Schäfer): Ergebnisse und Schlussfolgerungen hieraus für die Praxis der Genetischen Beratung

Pilot-Ringversuch „Qualitätssicherung der Humangenetischen Stellungnahme“: Bericht zum aktuellen Stand (Moritz Meins)

QW Molekulargenetik

Molekulargenetik

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

Die NGS-Analysen werden in Zukunft einen Großteil der diagnostischen humangenetischen Untersuchungen darstellen. In diesem Workshop werden mehrere Aspekte der Qualitätssicherung von NGS-Analysen angesprochen und diskutiert. Die S1 Leitlinie der GfH „Molekulargenetische Diagnostik mit Hochdurchsatzverfahren, beispielsweise mit Next-Generation Sequencing“ (www.gfhev.de) wird hier kurz angesprochen. Da mittlerweile viele universitäre und niedergelassene humangenetische Einrichtungen diese Technologie anwenden und jetzt auch akkreditieren möchten, werden aktuelle Fragen zu diesem Thema angesprochen und diskutiert. Wir freuen uns auf eine rege Diskussionsrunde.

DFG-Workshop

Fördermöglichkeiten: Individuelle Förderprogramme der DFG für jede Karrierestufe

Moderation: Astrid Golla (Bonn) und 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.

DFG funding opportunities for every career stage

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.

Oral History Projekt

Zeitgeschichte der Humangenetik

Moderation: Heiner Fangerau
Institut für Geschichte, Theorie und Ethik der Medizin, Heinrich-Heine-Universität Düsseldorf

Vorträge

Zeitgeschichte der Humangenetik in Deutschland: Erste Ergebnisse aus 30 Experteninterviews

Felicitas Söhner, Matthis Krischel und Heiner Fangerau

Institut für Geschichte, Theorie und Ethik der Medizin, Heinrich-Heine-Universität Düsseldorf

In Kooperation mit der und gefördert durch die Deutschen Gesellschaft für Humangenetik (GfH) hat eine Arbeitsgruppe des Instituts für Geschichte, Theorie und Ethik der Medizin der Heinrich-Heine-Universität Düsseldorf zu Beginn des Jahres 2017 ein Forschungsprojekt zur Zeitgeschichte der Humangenetik in begonnen. Ein weiterer Kooperationspartner ist das Institut für Medizingeschichte und Wissenschaftsforschung der Universität Lübeck. Das Projekt wird durch eine Arbeitsgruppe Oral History der

GfH begleitet. Das Ziel ist es, die Entwicklung der Humangenetik in Deutschland ab den 1970er Jahren mit Hilfe von Zeitzeugengesprächen zu dokumentieren und zu analysieren. Durch diese Analyse der Experteninterviews mit Vertretern Faches können Prozesse der Professionalisierung und Institutionalisierung, praktische Auswirkungen wissenschaftlich-technischer Entwicklungen sowie die sozio-politische Einbettung der Humangenetik analysiert werden, welche über die klassische geschichtswissenschaftliche Quellenanalyse schwerer fassbar sind.

Bislang wurden Gespräche mit 30 Zeitzeugen geführt und als Tondokumente archiviert. Zur strukturierten Erschließung und als Auswertungsgrundlage wurden Regeste, also inhaltliche Zusammenfassungen von Struktur und Inhalt der Expertengespräche, erstellt. Angesprochene Themen waren u.a. der soziale Hintergrund der Befragten, ihr fachliches Profil, Motivation für die Fachwahl, Ausbildung und Institutionalisierung, Forschungsnetzwerke, die Stellung der Humangenetik in der Öffentlichkeit, Gestaltungswille/-spielraum, historische Verantwortung, Bilanz der Entwicklung der Disziplin sowie Prognosen für die Zukunft.

Erste Ergebnisse werden vorgestellt und diskutiert. Dabei werden Gemeinsamkeiten und Differenzen zwischen den Antworten der Zeitzeugen herausgearbeitet und im Sinne der Grounded Theory weitergehende Fragestellungen entwickelt. Die Zeitzeugenerinnerungen werden an der Historiographie der Humangenetik sowie an den – teils von ihnen selbst beigebrachten – Primärquellen gespiegelt. So entsteht ein differenzierteres, multiperspektivisches Bild der jüngeren Geschichte des Faches in Deutschland.

Internationale Perspektiven auf Humangenetik, Zeitgeschichte und Oral History

Heike Petermann und Hans-Georg Hofer

Institut für Ethik, Geschichte und Theorie der Medizin, Westfälische Wilhelms-Universität Münster

Im Jahr 2003 fand der 1st Workshop on Genetics, Medicine and History statt, um die Aufarbeitung der Geschichte der Humangenetik anzustoßen. Die Initiative dazu hatte der Humangenetiker Peter Harper aus Cardiff ergriffen. Dies war der Auftakt für eine Reihe von sechs weiteren Workshops, zuletzt 2017 in Kopenhagen, als Satellitensymposium der European Human Genetics Conference. Der Austausch auf den Workshops hat gezeigt, dass europäische, internationale Perspektivierungen ein reichhaltigeres und präziseres Verständnis der Entwicklungsgänge und Wandlungsprozesse ermöglichen. Neben der Aufarbeitung der Geschichte der Humangenetik anhand von Archivalien und der Primär- und Sekundärliteratur bekam die „Oral History“, die Interviews mit Zeitzeugen, mehr und mehr an Bedeutung. Peter Harper hat seit 2003 über 100 Interviews geführt. Auch Tilly Tansey, London, hat in einer Reihe von Interviews mit Medizinern auch Gespräche mit Humangenetikern geführt.

Seit 2015 in Glasgow wurde deutlich, dass Zeitzeugeninterviews eine adäquate Methode für die Geschichte der Humangenetik, besonders seit 1970, sind. Im Vortrag soll unter Berücksichtigung aktueller Forschungsfragen der medizinischen Zeitgeschichte auf die Chancen und Potenziale dieser Methode näher eingegangen werden. Eine besondere Herausforderung stellt etwa die Auswahl der Interviewten dar, da in der Humangenetik sowohl Mediziner in der genetischen Beratung wie auch Naturwissenschaftler in der Forschung tätig sind. Weiterhin sind formale Kriterien, wie die Transkription, Archivierung und Veröffentlichung der Interviews zu klären. Auch sollte der Abgleich der Zeitzeugeninterviews mit schriftlichen Quellen erfolgen, um Aussagen objektivieren und in einen größeren Kontext stellen zu können.

Das Potenzial der Zeitzeugeninterviews liegt u.a. darin, die Relevanz von mündlich tradiertem Wissen darzulegen. Auch ermöglichen die Interviews einen unmittelbaren, wenn auch subjektiv erinnerten Zugang zu den prägenden Entwicklungen und Akteuren des Faches, zu den Themen und Inhalten, die als innovativ angesehen und bestimmend wurden, und nicht zuletzt zu den spezifischen politischen und gesellschaftlichen Rahmenbedingungen, die sich um 1970 wandelten.

Die humangenetische Beratung in der DDR in den 1970er und 1980er Jahren: Legitimierung, Strukturen und Praktiken

Susanne Doetz

Institut für Geschichte der Medizin und Ethik in der Medizin Charité – Universitätsmedizin Berlin

Die Etablierung der humangenetischen Beratung in der DDR kann als ein Beispiel gesehen werden, wie es WissenschaftlerInnen gelang, unter den Bedingungen einer Parteidiktatur ihre Interessen erfolgreich zu vertreten. Dies war auch deshalb möglich, weil die humangenetische Beratung mit ihrer Deklaration als Prophylaxe und ihrer Postulierung, die Säuglingssterblichkeit zu senken, an zentrale Eckpfeiler der DDR-Gesundheitspolitik andockte.

Wenngleich die innerdeutsche Grenze für die Mehrzahl der HumangenetikerInnen der DDR nicht passierbar war, kam der deutsch-deutsche Wissenschafts- und auch „Warenaustausch“ auf diesem Gebiet keineswegs zum Erliegen: Literatur, Reisekader, das Schmuggeln benötigter Chemikalien sowie der Umweg über die CSSR oder Ungarn trugen dazu bei. Von daher ist es auch nicht verwunderlich, dass sich Zielsetzung

und Legitimierungsdiskurs der humangenetischen Beratung in der DDR und Bundesrepublik nicht wesentlich voneinander unterschieden.

Ziel der humangenetischen Beratung war es, die Geburt behinderter Kinder zu verhindern und die Geburt gesunder Kinder zu fördern. Sie transportierte damit normative Vorstellungen einer gesunden Gesellschaft, in der Behinderung als Leid angesehen wurde, das verhindert werden sollte. Auch wenn die damalige genetische Beratung nicht immer den heutigen Vorstellungen einer non-direktiven, also ergebnisoffenen und möglichst neutralen, Beratung entsprach – die BeraterInnen „empfahlen" und „rieten" stattdessen – so war die Beratung nicht einfach ein top-down-Prozess. Nicht nur, dass sich die BeraterInnen von jeglichem Zwang distanzierten, sie waren auch auf die aktive Teilnahme der Ratsuchenden angewiesen, um das für eine Empfehlung benötigte Wissen zu generieren. Zentraler Bestandteil der Beratungen war nicht die Chromosomenanalyse mittels zytogenetischer Untersuchung, sondern der Stammbaum. Kritik an der humangenetischen Beratung insbesondere im Zusammenhang mit dem Schwangerschaftsabbruch kam von kirchlicher Seite. Limitierender Faktor der humangenetischen Beratung in der DDR waren aber weder ideologische noch ethische Gründe, sondern vielmehr materielle: das Fehlen von Ressourcen, wie Räumen, Laborkapazitäten und Ultraschallgeräten.

SHG-Workshop

Selbsthilfegruppen und Humangenetiker im Dialog – Erwartungen an die Humangenetik.

Moderation: Klaus Zerres, Aachen

Der methodische Fortschritt der Humangenetik hat den Erkenntnisgewinn in unserem Fach enorm beschleunigt. Hierdurch gelang für viele Krankheiten die Aufklärung von Mechanismen ihrer Entstehung. Für einige Erbkrankheiten konnten in der Folge bereits sehr hoffnungsvolle Therapiekonzepte entwickelt werden, die bei einigen Krankheiten bereits Eingang in die Praxis gefunden haben.

Der humangenetische Erkenntnisgewinn hat weiterhin die Möglichkeiten der genetischen Diagnostik für sehr viele seltene Krankheiten entscheidend verbessert. Gentests sind folgerichtig zum festen Bestandteil der Diagnostik vieler Erbkrankheiten geworden und konnten vielfach teilweise invasive konventionelle Verfahren ersetzen. In vielen Fällen konnten auf diesem Wege lange Phasen einer diagnostischen Unsicherheit beendet werden bzw. wurde eine Diagnosestellung überhaupt erst möglich.

Während die Erforschung von Krankheitsmechanismen, die Entwicklung therapeutischer Ansätze und die verbesserten Möglichkeiten der Diagnostik von Vertretern von Selbsthilfegruppen bzw. deren Dachverbänden uneingeschränkt begrüßt werden, werden andere Entwicklungen kontrovers oder kritisch gesehen. Hierzu zählen die sich ständig weiter entwickelnden Möglichkeiten der prädiktiven bzw. Anlageträgerdiagnostik, der Pränatal- und Präimplantationsdiagnostik sowie der Nichtinvasiven Pränataldiagnostik. Das Spektrum der Beurteilung reicht hierbei im Einzelnen von deren positiver Beurteilung bis zu ihrer strikten Ablehnung, wobei selbst bei Vertretern eines Verbandes unterschiedliche Einschätzungen bestehen können.

In der Podiumsdiskussion soll mit Vertretern einzelner Selbsthilfegruppen, die ihre Verbände auf der Jahrestagung in Münster vertreten, über Erwartungen und Befürchtungen unserem Fach gegenüber gesprochen werden. Ziel des Gespräches soll dabei weniger der Austausch offizieller Positionen zu einzelnen Themenbereichen und Entwicklungen sein als vielmehr der Versuch, die persönlichen Erwartungen und Befürchtungen zu reflektieren und zu formulieren.

S1-01

Coupling genomic and phenotypic analysis to diagnose developmental disorders

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Clinically-defined syndrome diagnoses have an excellent record in predicting defined sets of causative genotypes. The Deciphering Developmental Disorders (DDD) project is a UK- and Ireland-wide study that aims to develop and use new genetic technology and statistical analyses to make a definitive diagnosis in individuals with severe or extreme developmental disorders. DNA samples are available from ~13,500 affected individuals have been recruited with 10,000 of these also having samples available from both parents. Using human genetic data alone we have shown a significant excess of damaging de novo variants in many different genes in the DDD probands. We are now developing phenotypic profiles associated with each disease-gene pair using naïve Bayes classification. The phenotype is going to become more important component of the computational approach to the analysis of genome wide sequencing data – particularly in ranking different plausible disease associated variants that come through filtering of whole exome or whole genome sequence

data. A consistent approach to the collection and utilitation of phenotypic information is a vital part of study design

S1-02

Functional characterization and therapeutic targeting of gene regulatory elements

Nadav Ahituv

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Nucleotide variation in gene regulatory elements is a major determinant of phenotypes including morphological diversity between species, human variation and human disease. Despite continual progress in the cataloging of these elements, little is known about the code and grammatical rules that govern their function. Deciphering the code and their grammatical rules will enable high-resolution mapping of regulatory elements, accurate interpretation of nucleotide variation within them and the design of sequences that can deliver molecules for therapeutic purposes. To this end, we are using massively parallel reporter assays (MPRAs) to simultaneously test the activity of thousands of gene regulatory elements in parallel. By designing MPRAs to learn regulatory grammar or to carry out saturation mutagenesis of every possible nucleotide change in disease causing gene regulatory elements, we are increasing our understanding of the phenotypic consequences of gene regulatory mutations. Regulatory elements can also serve as therapeutic targets. To highlight this role, we used CRISPR/Cas9 activation (CRISPRa) of regulatory elements to rescue a haploinsufficient disease (having a 50% dosage reduction due to having only one functional allele) in vivo. By targeting the Sim1 promoter or its 270kb distant hypothalamic enhancer, we were able to rescue the haploinsufficient obesity phenotype in Sim1 heterozygous mice, both using a transgenic and adeno-associated virus approach. Our results highlight how regulatory elements could be used as therapeutic targets to treat numerous altered gene dosage diseases.

S1-03

Early errors in embryogenesis of the growth of the brain

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Holoprosencephaly (HPE) is the most common midline defect of the developing forebrain and the face. The prevalence is 1 in 250 early embryos, 1 in 10,000 live-born infants, and approximately 1 in 100,000 children over 1 year of age. HPE encompasses a phenotypic spectrum that ranges from failure to partition the forebrain in hemispheres and cyclopia to mild midfacial anomalies that occur without forebrain involvement. (Muenke, M., Solomon, B.D., Odent, S. (eds): Holoprosencephaly. Am. J. Med. Genet. Part C: Semin. Med. Genet. Vol. 154C, 2010).

Heterozygous, loss-of-function mutations in components of the sonic hedgehog (SHH) signaling pathway are frequently associated with HPE. However, highly variable clinical presentation is seen in mutation carriers, even within pedigrees. Furthermore, in many apparently “sporadic” cases, mutations in SHH can be inherited from a parent with no clinical manifestation.

In my presentation, I will give an introduction the clinical findings in HPE from early embryo, newborn infant to adolescents and adults with HPE, discuss normal and abnormal brain development during early embryogenesis, and present genetic and environmental causes of HPE, discuss genetic syndromes with HPE. Lastly, I will present data from next generation sequencing of parents and children with HPE and from functional analyses using these data.

However, highly variable clinical presentation is seen in mutation carriers, even within pedigrees. Furthermore, in many “sporadic” cases, mutations in SHH are inherited from a parent with little or no c linical manifestation (Solomon et al., 2012). Statistical analyses have led to an “autosomal dominant with modifier” model, in which the penetrance and expressivity of a predisposing heterozygous mutation is graded by modifiers

S2-01

The Human Cell Atlas

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For the past 150 years scientists have classified cells by their structures, functions, locations, and, more recently, molecular profiles, but the characterization of cell types and states has remained surprisingly limited. This has confined our ability to study fundamental domains in biology and to translate our knowledge to accelerate diagnosis and treatment of disease. But an extraordinary opportunity is emerging because of transformative advances in experimental and computational methods. Massively parallel single-cell genomics assays can now profile hundreds of thousands of cells, and new spatial analysis techniques allow high-resolution analysis of tissues. Computational algorithms have emerged to determine cell types, states, transitions, and locations from these new data, at increasing scale and resolution.

Together, these advances have led the scientific community to launch the Human Cell Atlas (HCA), an international collaborative consortium that aims to create comprehensive reference maps of all human cells—the fundamental units of life—as a basis for both understanding human health and diagnosing, monitoring, and treating disease. The HCA is a foundational open resource charting cells, tissues, organs and systems throughout the body. The HCA should help answer questions in all areas of human biology, from the taxonomy of cells and histological tissue structure, to developmental biology and cell fate and lineage, to physiology and homeostasis and their underlying molecular mechanisms. Because the HCA will be an open resource, it will dramatically accelerate discoveries by biological researchers, data scientists, and translational scientists and clinicians worldwide, inspiring insights in therapeutic discovery, drug development, and diagnosis.

The HCA is an international community effort, open to all scientists who abide by its principles. The HCA is steered and governed by an Organizing Committee, spanning 27 scientists from 10 countries and diverse areas of expertise. The OC is led by two co-chairs, who are members of the OC and it establishes Working Groups in specific key areas and governs the HCA data coordination platform.

The first Draft Atlas v1.0, an effort launched in October 2017, will profile about 100 million cells, both isolated and in their tissue context across major tissues and systems. It will focus primarily on healthy individuals reflecting genetic and geographic diversity, as well as small disease cohorts. This first draft and the lessons learned in building it will serve as the basis for a comprehensive atlas covering all tissues, organs, and systems. We expect that the HCA will have profound impact on every aspect of biology and medicine, propelling translational discoveries and applications and ultimately leading to a new era of precision medicine.

S2-02

Exploring epigenomic landscapes in single cells

Gavin Kelsey

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Profiling epigenetic and transcription information in single cells has emerged as a powerful new capability in recent years, enabling the analysis of rare cells and assessment of cell-to-cell heterogeneity in development and disease. The most recent advances – single-cell multi-omics – enable the parallel capture of gene expression and epigenetic signatures from the same cell. I shall exemplify some of our current methods. We shall explore variability in DNA methylation between individual oocytes and assess the extent to which methylation can be modified by factors such as maternal age or diet. And we shall examine transitions in chromatin state at individual loci during differentiation.

S2-03

FISSEQ: A fully automated massively-multiplex panomic in situ detection platform

Evan R. Daugharthy

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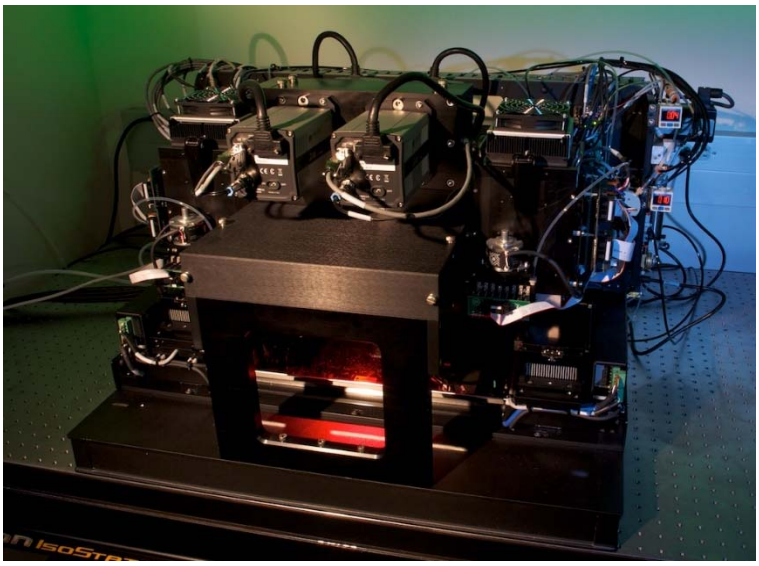

To detect molecules in situ, we use optical detection of fluorescent labels. Because the number of spectrally distinct fluorescent labels is very limited, massively multiplex in situ molecular detection inherently requires cyclic probing and imaging. Molecular labeling can be done additively over k cycles, using N spectrally distinct fluorescent dyes to label k×N distinct molecular species, as in FISH. Fluorescent In Situ Sequencing (FISSEQ) technology, however, uses the more efficient exponential labeling process called sequencing, where the number of distinct species interrogated is limited by Nk using k cycles with N spectrally distinct fluorescent dyes.

To advance whole-transcriptome FISSEQ[1] as well as a wide array of novel in situ sequencing technologies targeting RNA, DNA, and proteins, we have developed the FISSEQ platform for wetware, hardware, and software. For wetware, we are developing, optimizing, and standardizing enzymatic, hydrogel-embedding, and sequencing chemistries. Our hardware platform is based on the I15 Polonator (pictured), with fully automated fluidics and ultra-high-throughput 3D microscopy. Our software platform stores terabytes of FISSEQ data integrating protein, RNA, and genomic sequencing information with standardized APIs enabling novel bioinformatic methods.

Biological systems, such as developing tissues and organoids, are composed of millions of species of biomolecules, including RNA, DNA, and proteins, each with a myriad of configurations and modifications. Using the FISSEQ technology platform to simultaneously measure the spatio-temporal organization of thousands or millions of molecular species within a complex organ, such as the brain, directly enhances our ability to understand, manipulate, and engineer these systems to advance human medicine.

1. Lee* JH, Daugharthy* ER, Scheiman J, Kalhor R, Yang JL, et al. 2014. Highly multiplexed subcellular RNA sequencing in situ. Science 343:1360-3, PMCID: 4140943 (*equal contribution)

S3-01

Genetic disorders of spermatogenesis

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Infertility affects 10-15% of couples and the causes of couple infertility are equally attributed to male and female (co-)factors. Male infertility is a clinically and genetically highly heterogeneous disease. The clinical phenotypes most commonly noted comprise oligozoospermia (reduced sperm count) and azoospermia (no sperm in the ejaculate). The latter can be further classified into obstructive and non-obstructive azoospermia, which denotes the most severe forms of impaired spermatogenesis.

A multitude of genes is involved in spermatogenesis with supposedly more than 800 genes exclusively expressed in the testes. Thus, unravelling the underlying causes and the pathophysiology is challenging and candidate gene approaches, such as picking one or several genes known to cause infertility in mice, so far did not identify novel, clinically relevant genetic causes of infertility in men. However, some genes, such as DMRT1 and NR5A1, have been repeatedly shown to be associated with disorders of spermatogenesis.

Like in many other common diseases, genome-wide analyses have helped to increase the diagnostic yield in the last few years. For example, we have performed genome-wide array-CGH in groups of clinically well-characterised oligo- and azoospermic men. We were the first to report an excess of Copy Number Variants (CNVs) in infertile males especially on the sex-chromosomes. By using high-resolution array-CGH, we identified exon-deletions and nucleotide mutations in TEX11 as the first common X-linked cause for meiotic

arrest in about 15% of men with this phenotype. More recently, whole-exome sequencing (WES) allowed for the identification of several novel genes which, if mutated, are associated with human male infertility.

Taken together, comprehensive screening for small deletions and targeted sequencing of larger numbers of genes will pave the way for the future diagnostics of male infertility. Indeed, we could recently increase causal diagnoses in non-obstructive azoospermic men from ~20% to ~30% by WES. Identifying the causal mutations in an infertile men has direct implications on treatment, especially decisions on performing a testicular biopsy and counselling about risks for offspring.

S3-02

Genetics and Management of Primary Ovarian Insufficiency

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Primary ovarian insufficiency (POI) affects about 1% of women before 40 years of age. This is a highly heterogeneous condition encompassing cases associated with prepubertal onset of the defect and primary amenorrhea together with those associated with post-pubertal onset and secondary amenorrhea. Cancer in young age and familiarity are the main risk factors for POI. Mutations of >50 genes associated with POI have unraveled novel mechanisms of oogenesis, ovarian development and follicular function. The fertility preservation in POI requires early diagnosis in families at risk. The systematic NGS analysis of a panel of candidate genes allows the identification of a predisposing variant in about 30% of idiopathic POI cases. The carriers of these variants have an increased risk to develop POI and are counseled to anticipate conception or cryopreserve oocytes for future fertility. Several of these variants may also predict an increased risk to develop malignancies later in life. The presentation will focus on these aspects and on recently identified molecular mechanisms.

S4-01

Cell-free DNA as a clinical tool in oncology

Nitzan Rosenfeld

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Cancer is driven by genomic alterations, and can evolve in response to selective pressures. Sampling of tumour material however is a limiting factor for both diagnostics and research. Blood plasma contains cell-free fragments of circulating tumour DNA (ctDNA) that can be collected non-invasively. With advanced genomic techniques this becomes an effective source of information. All types of somatic genomic alterations originating from solid tumours can be detected, quantified, and tracked in plasma using locus-specific assays or next-generation sequencing, ranging from very deep sequencing of defined regions to shallow whole genome sequencing. Serially-collected plasma samples can be used to monitor response to treatment, cancer progression and emergence of known or new resistance mechanisms. Methods are constantly being improved to detect trace amounts of tumour DNA present in body fluids. These are applied to study early-stage cancer, and for detection of minimal residual disease after initial definitive treatment. In parallel, the accumulating information allows us to learn more about the biology of cell-free DNA. In the clinical setting, “liquid biopsy” assays for non-invasive molecular profiling of advanced cancers are now being adopted to guide targeted therapy and clinical trials. Initial approvals covered PCR-based assays targeting key host-spot loci. Methods based on targeted next-generation sequencing of gene panels allow screening of multiple actionable mutations, copy numbers and rearrangements. These have undergone rigorous analytical validation showing ability to detect cancer mutations in plasma with high sensitivity and specificity. Clinical evidence for the utility of these methods is rapidly accumulating.

S4-02

Liquid Biopsy for the early detection of cancer: applications in screening and minimal residual disease.

Nickolas Papadopoulos

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Early detection of cancer in the screening and minimal residual disease settings has the potential to significantly reduce cancer deaths. Our goal is to be able to detect cancer earlier than current modalities. Previously, in-proof-of-principle studies we determined the feasibility of liquid biopsy in detecting cancers in blood and other bodily fluids. In a recent study, we have been able to detect minimal residual disease with

exquisite specificity after surgery in patients with stage II colon cancer earlier than recurrence detected by imagining. Recently, we developed a multi-analyte liquid biopsy for the detection and localization of cancers. In a study involving more than 1,000 individuals with cancer and more than 800 controls the combination of ctDNA and other biomarkers showed an additive increase in sensitivity while preserving a specificity of 99%. In certain settings detection of tumor derived DNA in more than one bodily fluid from the same person results in increased sensitivity. For example, by testing both saliva and blood from the same individual, we increased the sensitivity of detecting head and neck cancers. Similarly, testing for mutations in both liquid cytology and blood we increased the sensitivity of ovarian cancer.

S5-01

New developments in the Ehlers-Danlos Syndromes

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The Ehlers-Danlos syndromes (EDS) are a clinically and genetically heterogeneous group of heritable connective tissue disorders (HCTDs) characterized by joint hypermobility, skin hyperextensibility and tissue fragility. Over the past two decades, the 1998 Villefranche Nosology, which delineated six EDS subtypes, has been widely used as the standard for clinical diagnosis. For most of these subtypes, mutations had been identified in collagen-encoding genes (collagen I, III and V), or in genes encoding collagen-modifying enzymes (ADAMTS2, LH1). With the advent of next generation sequencing techniques, molecular defects have recently been identified in a variety of ECM molecules, gradually expanding the list of distinct EDS subtypes. These studies have recently led to a revision of the EDS classification, which now includes 13 distinct clinical EDS subtypes, for which molecular defects have been identified in 19 different genes. Besides defects in fibrillar collagens and their modifying enzymes, defects have now also been found in molecules involved in collagen folding (FKBP22), and in other constituents of the extracellular matrix (e.g. tenascin-X, collagen type XII), enzymes involved in glycosaminoglycan biosynthesis (galactosyltransferase I and II (β4GalT7 and β4GalT6), dermatan 4-O-sulfotransferase-1 (D4ST1), dermatan sulfate epimerase (DSE)), (putative) transcription factors (ZNF469, PRDM5), components of the complement pathway (C1r, C1s) and an intracellular Zinc transporter (ZIP13). Despite advances in our understanding of the genetic basis of EDS, one of the initially recognized and most prevalent EDS subtypes, hypermobile EDS (hEDS), still remains molecularly unexplained.

The recent identification of several novel types of EDS has greatly expanded the clinical and genetic heterogeneity of this group of disorders, and the genomic era promises to provide even more insights into the molecular basis of unresolved types of EDS. At the same time the underlying pathophysiologic mechanisms in these disorders are still poorly understood. Therefore, future research should focus on the elucidation of these mechanisms, and the identification of clinically reliable biomarkers and targetable signaling pathways and cellular processes.

S5-02

Aortic Aneurysm and Dissection: the Translational Journey from Marfan to Sartan and back

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Thoracic aortic aneurysms (TAA) are often asymptomatic but predispose to aortic dissections, which are associated with high mortality rates. Thoracic aortic aneurysms and dissections (TAAD) can be subdivided into syndromic (associated with systemic manifestations) and non-syndromic forms, although recent clinical observations are blurring this distinction. A positive family history occurs in circa 20% of all TAA individuals. Most commonly, familial TAADs segregate in an autosomal dominant manner, but rare autosomal recessive and X-linked families have been described. Non-syndromic forms of TAA are strongly associated with mutations in genes encoding for proteins of the vascular smooth muscle contractile apparatus (ACTA2, MYH11) or its modifiers (PRKG1, MYLK, FOXE3). Syndromic presentations can be linked to alterations in the extracellular matrix (FBN1, FBLN4, MFAP5, LOX) and the transforming growth factor beta signalling pathway (TGFBR1/2, SMAD2/3, TGFB2/3). Marfan syndrome (MFS) is an autosomal dominant connective tissue disorder in which affected individuals present with ocular, skeletal and cutaneous signs besides aneurysm and dissection. Loeys-Dietz syndrome (LDS) is an aneurysmal connective tissue disorder that can be distinguished from MFS by the unique presence of craniofacial, skeletal, cutaneous, and/or vascular manifestations, prominently including hypertelorism, cleft palate or bifid uvula and arterial tortuosity with aneurysms distant from the aortic root. In addition, aneurysms in individuals with LDS tend to dissect at an earlier age and smaller diameter compared to individuals with MFS. Whereas MFS is caused by mutations in FBN1, coding for an

extracellular matrix protein, LDS is caused by loss-of-function mutations in genes coding for components of the transforming growth factor beta signalling pathway. Significant clinical overlap exists between the phenotypes caused by mutations in the currently known LDS genes (TGFBR1/2, SMAD2/3, TGFB2/3). Recent work has demonstrated that both MFS and LDS lead to dysregulation of the TGFbeta signalling pathway. The latter has opened interesting avenues for the application of new therapeutic strategies. Current next generation sequencing based gene panel testing for TAAD individuals yields a molecular diagnosis in up to one third of the patients, suggesting that several genes remain to be discovered.

S6-01

FROM GWAS TO SYSTEMATIC HOST-MICROBIOME ASSOCIATION STUDIES IN COMPLEX IMMUNE-MEDIATED DISEASES

Andre Franke

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Genome-wide association (GWAS) have significantly contributed to our understanding of the etiology of chronic and complex immune-mediated diseases (CID). Inflammatory bowel diseases (IBD) with its main sub phenotypes Crohn's disease and ulcerative colitis, are prototypic CIDs that affect about 2-3 persons out of a 1000 in Western countries. IBD shares part of its genetic and immunological background with diseases like psoriasis, ankylosing spondylitis, and primary sclerosis cholangitis). To this end, over 250 genetic susceptibility loci were identified in the past 10 years through GWAS and candidate-gene association studies. Complex immunogenetics efforts are currently being undertaken to solve CID. Still, the exact cause of most CID has not been identified and components of the gut microbiome are also likely disease-causing environmental factors for CID. In particular the complex interplay between our own immune system and the microbiome needs to be investigated in detail as both a dysbiosised microbiome as well as an inappropriate immune response contribute to CID.

Here, I will present our ongoing efforts in host-microbiome association analyses and allude to the methodological challenges of these kind of analyses. Previously, we performed a genome-wide association study (GWAS) of the gut microbiota using two cohorts from northern Germany totaling 1,812 individuals. Comprehensively controlling for diet and non-genetic parameters, we identified genome-wide significant associations for overall microbial variation and individual taxa at multiple genetic loci, including the VDR gene (encoding vitamin D receptor). We observed significant shifts in the microbiota of Vdr-/- mice relative to control mice and correlations between the microbiota and serum measurements of selected bile and fatty acids in humans, including known ligands and downstream metabolites of VDR. Genome-wide significant ($P < 5 \times 10E-8$) associations at multiple additional loci identify other important points of host-microbe intersection, notably several disease susceptibility genes and sterol metabolism pathway components. Non-genetic and genetic factors each account for approximately 10% of the variation in gut microbiota in this study, whereby individual effects were relatively small.

S6-02

Dissecting the genetic contribution to psychiatric disorders: heterogeneity and congruity

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Genome-wide association studies have made strides in identifying common variation associated with psychiatric disorders. These studies have confirmed our hypothesis that psychiatric traits have highly polygenic underpinnings. The substantial progress in recent years has been built on collaborative efforts to build sufficiently large samples sizes for case/control studies to identify variants with only a modest effect on risk. Focussing on major depressive disorder (MDD), I will discuss the progress made in identifying the genetic contribution to this common disorder, and show how the genetics correlates with clinical features.

Detecting the genetic variants associated with MDD has been particularly challenging. An initial meta-analysis of over 9,000 MDD cases and 9,000 screened controls from the Psychiatric Genomics Consortium (PGC) failed to identify any variants at genome-wide significance. More recent studies have successfully applied two different approaches of expanding sample size and reducing the heterogeneity of MDD. By studying severe, recurrent depression in Han Chinese women, the CONVERGE consortium identified two variants at genome-wide significance. At the other end of the spectrum, the direct-to-consumer genetic testing company 23andMe identified 15 loci for depression, based on a single question where customers self-reported diagnosis of, or treatment for, depression; here the lack of clinical information was balanced with extremely large sample sizes.

Finally, in the PCG MDD working group, we have recently identified 44 variants associated with depression in a large meta-analysis. The sample of over 130,000 depression cases was ascertained from diverse sources, with different levels of information on case status and diagnosis. These detected variants overlapped with those previously associated with depressive symptoms, schizophrenia and body mass index; the genetic correlations between these traits, and with other psychiatric disorders, is high. These loci provide invaluable insights into the biology of depression and exciting opportunities to develop new biomarkers and therapeutic targets.

SEL

SEL-01

De novo missense variants in RHOBTB2 cause a developmental and epileptic encephalopathy in humans, and altered levels cause neurological defects in Drosophila

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While the role of typical Rho GTPases and other Rho-linked proteins in synaptic plasticity and cognitive function and dysfunction is widely acknowledged, the role of atypical RhoGTPases such as RHOBTB2 in neurodevelopment has barely been characterized.

We now identified de novo missense variants in RHOBTB2 in ten individuals with a developmental and epileptic encephalopathy. The clinical presentation was highly similar including early onset epilepsy, severe intellectual disability, postnatal microcephaly and movement disorders. In four of the individuals (post-ictal) hemiparesis and secondary MRI anomalies were observed.

The identified variants clustered in the BTB-domain encoding region of RHOBTB2, and three amino acids were recurrently affected. RHOBTB2 interacts with a cullin-dependent ubiquitin ligase complex and thus regulates auto-ubiquitination and ubiquitination of other substrates recruited to the complex. Though direct interaction of mutant RHOBTB2 with CUL3 did not appear to be impaired by co-immunoprecipitation, we observed increased levels of mutant RHOBTB2 compared to wildtype 24 hours after transfection of HEK293 cells. As this effect could be abolished by adding a proteasome inhibitor, our findings indicate decreased degradation of mutant RHOBTB2 in the proteasome, probably due to impaired ubiquitination.

Similarly, elevated levels of the Drosophila ortholog RhoBTB in vivo were associated with seizure susceptibility and severe locomotor defects, while knockdown of RhoBTB resulted in no or only very mild phenotypes. Development and morphology of fly neuromuscular synapses was not altered upon manipulation of RhoBTB dosage. Knockdown of RhoBTB in the Drosophila dendritic arborization neurons, however, resulted in a significantly decreased number of dendrites, thus suggesting a role of RhoBTB in dendritic development.

We establish missense variants in the BTB domain encoding region of RHOBTB2 as causative for a developmental and epileptic encephalopathy and elucidate the role of atypical RhoGTPase RhoBTB in Drosophila neurological function and possibly for dendrite development.

SEL-02

Novel gene and pathomechanism in Cornelia de Lange syndrome

Parenti I.[1], Ruiz Gil S.[1], Strom TM.[2], Brouwer R.[3], Diab F.[4], Dupé V.[4], Gillessen-Kaesbach G.[5], Mulugeta E.[3], van IJcken W.[3], Watrin E.[4], Wendt KS.[3], Kaiser FJ.[1]

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Cornelia de Lange syndrome (CdLS) is a rare multisystem developmental disorder characterized by distinctive facial dysmorphism, intellectual disability and multiple congenital anomalies.

CdLS is caused by mutations in different subunits (*SMC1A, SMC3, RAD21*) or regulators (*NIPBL, HDAC8*) of the cohesin complex. While genetic variants in *SMC1A, SMC3, RAD21* or *HDAC8* can be identified in about 10% of patients, *NIPBL* mutations were described in more than 70% of patients. NIPBL interacts via its N-terminus with MAU2 to form the kollerin complex, which is essential for cohesin's loading onto chromatin.

By genome editing, we generated cell lines expressing an N-terminal truncated form of NIPBL. Molecular characterization of these cells revealed reduced NIPBL protein levels and loss of MAU2, that is degraded as a result of its inability to bind its partner NIPBL. Despite the loss of MAU2, subsequent Chip-sequencing analyses could exclude the presence of significant differences between wild type and edited cells in the total amount of chromatin-bound cohesin or chromatin-bound NIPBL.

Interestingly, by genome sequencing of mutation-negative CdLS patients, we identified the very first de novo variant in *MAU2*. The facial features of the patient are typical for CdLS and include synophrys, broad and arched eyebrows, depressed nasal bridge, anteverted nares, smooth philtrum, thin lips and widely spaced teeth. Up to now, loss of function mutations in *MAU2* have proven to be significantly underrepresented in exome sequences, indicating that *MAU2* is extremely dosage sensitive.

The identified mutation causes an in-frame deletion of seven amino acids within MAU2. In silico analysis of the kollerin structure indicates that the deletion affects a helix-turn-helix motif that is important for wrapping the N-terminus of NIPBL within the MAU2 structure. Thus, the deletion was predicted to affect the interaction with NIPBL with high probability. Accordingly, subsequent in vitro analyses such as mammalian two-hybrid and yeast two-hybrid assays revealed a dramatic reduction of MAU2 heterodimerization with NIPBL in the presence of the in-frame deletion.

It was previously reported that MAU2 is required for the correct folding of the N-terminus of NIPBL and that NIPBL is unstable in the absence of MAU2. Therefore, it is tempting to speculate that the inability of MAU2 to interact with NIPBL results in decreased NIPBL protein levels, thus confirming NIPBL haploinsufficiency as the major pathogenic mechanism of CdLS. In support of this hypothesis, our patient shows phenotypical features characteristic for patients with mutations in *NIPBL*.

In summary, we described the first *MAU2* mutation in a patient with clinical features typical for CdLS. Our functional investigations reveal a new pathogenic mechanism that results in decreased NIPBL protein levels upon functional alteration of its binding partner MAU2.

SEL-03

Mutations in the BAF chromatin remodelling complex subunit DPF2 associated with Coffin-Siris syndrome

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Neurodevelopmental disorders (NDDs) are clinically and genetically highly heterogeneous. In the last years variants in several genes related to chromatin function have been associated with NDDs. These include variants affecting the function of different subunits of the BAF chromatin remodelling complex (SWI-SNF complex) leading to various neurodevelopmental syndromes including Coffin-Siris syndrome (CSS). Furthermore, variants in proteins containing PHD fingers, motifs recognizing specific histone tail modifications, have been associated with several neurological and developmental delay disorders. By exome sequencing in either large studies or diagnostic setting we identified in 8 individuals with NDD heterozygous de novo variants, 1 frameshift, 2 splice-site and 5 missense, in the gene encoding the BAF complex subunit double plant homeodomain finger 2 (DPF2). Affected individuals share common clinical features described in individuals with CSS including coarse facial features, global developmental delay, intellectual disability, speech impairment and hypoplasia of finger and toenails. All variants occur within the highly conserved PHD1 and PHD2 motifs in very close proximity to zinc binding sites and are predicted to disrupt these sites. Recombinant protein and histone peptide pull-down assays in three missense variants revealed abolished or impaired DPF2 binding to unmodified and modified H3 histone tails. By contrast, the interaction with unmodified H4 or acetylated lysines of H4 was not disturbed by these substitutions. These results suggest an impairment of PHD fingers structural integrity and cohesion and likely an aberrant recognition of histone modifications. Furthermore, in HEK293 and COS7 cell lines the expression of these variants was associated with nuclear aggregate formation. In co-transfection experiments both wild-type DPF2 and BRG1 were recruited to the aggregates formed by DPF2 mutants. This recruitment was accompanied by a reduction in cells exhibiting aggregates. Expression analysis of splice-site and frameshift variants found in individuals, indicated that aberrant transcripts escape nonsense-mediated decay. Taken together, we provide compelling evidence that *de novo* variants in *DPF2* cause Coffin-Siris syndrome. The clustering of variants within the PHD fingers as well as the experimental data suggest a dominant negative effect of action. Our study further confirms the crucial role of PHD finger containing proteins in human neurodevelopmental disorders and strengthens the association of variants affecting the function of BAF chromatin remodelling complex subunits with the aetiology of CSS.

SEL-04

CHP1 reduction ameliorates SMA pathology by restoring DNM1 hyperphosphorylation and endocytosis

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Autosomal recessive spinal muscular atrophy (SMA), the leading genetic cause of infant lethality, is caused by homozygous loss of SMN1. SMA disease severity inversely correlates with the number of SMN2 copy genes, which are mainly aberrantly spliced. Recently, the first SMA therapy based on antisense oligonucleotides (ASOs) correcting SMN2 splicing, namely SPINRAZA™, has been FDA- and EMA- approved. Nevertheless, in type I SMA-affected individuals - representing 60% of SMA patients the elevated SMN level may be still insufficient to restore motor neuron function lifelong. Plastin 3 (PLS3) and neurocalcin delta (NCALD) are two SMN-independent protective modifiers identified in humans and proved to be effective across various SMA animal models. Both, PLS3 overexpression and NCALD downregulation protect against SMA by restoring impaired endocytosis, however, the exact mechanism of this protection is largely unknown. Here, we identified calcineurin-like EF-hand protein 1 (CHP1) as a novel PLS3 interacting protein using a yeast-two-hybrid screen. Co-immunoprecipitation and pull-down assays confirmed a direct interaction between CHP1 and PLS3. Although CHP1 is ubiquitously expressed, it is particularly abundant in the central nervous system and at SMA relevant sites including motor neuron growth cones and neuromuscular junctions (NMJs). Strikingly, we found elevated CHP1 levels in SMA mice. Congruently, CHP1 downregulation restored impaired axonal growth in *Smn*-depleted NSC34 motor neuron-like cells, SMA zebrafish and primary murine SMA motor neurons. Most importantly, subcutaneous injection of a low-dose SMN-ASO in presymptomatic mice doubled the survival rate of a severely-affected SMA model, while additional CHP1 reduction by genetic modification, prolonged survival further 3.2-fold. Moreover, CHP1 reduction further ameliorated SMA disease hallmarks including electrophysiological defects, smaller neuromuscular junction size, impaired maturity of neuromuscular junctions and smaller muscle fibre size compared to low-dose SMN-ASO alone. In NSC34 cells, *Chp1* knockdown tripled macropinocytosis whereas clathrin-mediated endocytosis remained unaffected. Importantly, *Chp1* knockdown restored macropinocytosis in *Smn*-depleted cells by elevating calcineurin phosphatase activity. CHP1 is the counterplayer of calcineurin, which collectively dephosphorylates proteins involved in endocytosis, and is therefore crucial in synaptic vesicle endocytosis. Indeed, we found marked hyperphosphorylation of dynamin1 in SMA MNs, which was restored to control level by the heterozygous *Chp1* mutant allele.

Taken together, we show that CHP1 is a novel SMA modifier that directly interacts with PLS3, and ameliorates SMA pathology by improving impaired endocytosis. Most importantly, we demonstrate that CHP1 is a promising SMN-independent therapeutic target for a combinatorial SMA therapy.

W1 AGEING

W1-01

Novel segmental progeroid syndrome is caused by constitutive dysfunction of the Mdm2-p53 axis

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The tumor suppressor p53, a master regulator of the cellular response to stress, is tightly regulated by the ubiquitin ligase Mdm2 via an auto-regulatory feedback loop. In addition to its well established role in tumorigenesis, p53 has also been associated with aging in mice. Several p53 mouse models with aberrantly increased p53 activity display signs of premature aging. However, the relationship between dysfunction of the

Mdm2-p53 axis and human aging remains elusive. We identified an anti-terminating homozygous germline mutation in *MDM2* in a patient affected by a segmental progeroid syndrome. We show that this mutation abrogates MDM2 E3 ligase activity, thereby resulting in enhanced levels and stability of p53. Analysis of various patient's primary cells, followed by genome-edited cells, and in vitro and in vivo analyses confirm the aberrant regulation of p53 activity by this mutation. Interestingly, patient's primary cells display enhanced genomic stability, which however, seemed to occur at the cost of cell viability and premature senescence, compatible with a decreased regenerative capacity and the development of signs of accelerated aging on an organismal level. Further functional data from a zebrafish model further demonstrate that mutant *Mdm2* is unable to rescue a p53-induced apoptotic phenotype. In summary, we show that dysfunctional regulation of p53 by MDM2 can lead to aberrant p53 activity that poses deleterious effects on human aging processes.

W1-02

Recurrent de novo Mutations in SLC25A24 Cause Gorlin-Chaudhry-Moss Syndrome and Mitochondrial Dysfunction

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Gorlin-Chaudhry-Moss syndrome (GCMS) is characterized by coronal craniosynostosis and severe midface hypoplasia, body and facial hypertrichosis, microphthalmia, short stature, short distal phalanges, and a progeroid appearance. We performed exome and genome sequencing on five unrelated girls diagnosed with GCMS, two of them initially diagnosed with Wiedemann-Rautenstrauch syndrome. We identified the recurrent de novo mutations c.650G>A; p.(Arg217His) and c.649C>T; p.(Arg217Cys) in SLC25A24, encoding a mitochondrial inner membrane ATP-Mg/Pi carrier. While in vitro studies have investigated the transport function of this protein, which is activated by Ca2+, its physiological role is not fully understood. We found normal stability and localization of mutated SLC25A24 protein in fibroblasts from affected individuals. However, the mitochondria in patient cells showed mitochondrial swelling, which was exacerbated upon treatment with hydrogen peroxide (H2O2). The same effect was observed after overexpression of the mutant cDNA in HeLa cells. Under normal culture conditions the mitochondrial membrane potential of patient fibroblasts was intact, but significantly increased upon H2O2 exposure compared to control cells. Furthermore, live cell imaging displayed a reduced mitochondrial matrix ATP content in patient cells. These findings demonstrate mitochondrial dysfunction with increased sensitivity to oxidative stress due to the SLC25A24 mutations and point towards and increased opening of the permeability transition pore. Our results suggest that the SLC25A24 mutations induce a gain of pathological function and link mitochondrial ATP-Mg/Pi transport to skeletal and connective tissue development.

W1-03

Constitutively low lamin A/C levels in TOR1AIP1-associated segmental progeria can be partially stabilized by ERK 1/2 inhibition

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Nuclear envelopathies are rare disorders caused by mutations in genes encoding members of the nuclear envelope, which result in a broad variety of human diseases. Biallelic mutations in *TOR1AIP1*, encoding the integral nuclear membrane protein LAP1B, have been found in altogether five patients with a variable degree of muscular dystrophy and cardiomyopathy, and in a single patient affected by cardiomyopathy, dystonia and cerebellar atrophy. Here we studied a patient with a complex form of segmental progeria characterized by congenital bilateral hearing loss, bilateral cataracts, mandibular hypoplasia, scleroderma-like skin changes, Raynaud's phenomenon, progressive muscular atrophy, dilated cardiomyopathy, as well as chronic heart and kidney failure. By exome sequencing and subsequent segregation analysis, we identified compound heterozygosity for mutations in *TOR1AIP1* as the likely underlying genetic cause. Cellular characterization of

patient´s primary fibroblasts revealed absent LAP1B, constitutively low lamin A/C levels, aberrant nuclear morphology as well as premature cellular and replicative senescence, comparable to findings in other segmental progeroid disorders. Low lamin A/C levels are rescued by ectopic expression of wild-type *TOR1AIP1*, providing evidence for the causal role of identified mutations. In addition, we observed an abnormal activation of the extracellular signal-regulated kinase 1/2 (ERK 1/2). Notably, treatment with two ERK1/2 inhibitors, FR 180204 and ulixertinib, ameliorated the observed nuclear abnormalities and resulted in stabilization of lamin A/C levels in fibroblasts of the index patient but also of several healthy control individuals. In summary, we identified a novel genetic cause for segmental progeria, expand the clinical spectrum associated with *TOR1AIP1* mutations and furthermore suggest ERK1/2 inhibition as a novel therapeutic strategy for selected progeroid envelopathies.

W1-04

Genome-wide DNA methylation profiling reveals epigenetic signatures associated with Werner syndrome

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Patients with Werner Syndrome (WS), an autosomal recessive adult-onset segmental progeroid syndrome, show signs of premature aging and an elevated predisposition to cancer. Classical WS is caused by pathogenic variations in the Werner syndrome RecQ helicase like (*WRN*) gene.

Using the Illumina Infinium MethylationEPIC platform we performed genome-wide DNA methylation profiling at single-nucleotide resolution of whole blood and LCL samples of patients with *WRN* mutation and healthy controls.

Applying a DNA methylation based biomarker of aging (the “Epigenetic clock”) we could show that WS is associated with increased extrinsic and intrinsic epigenetic age acceleration, the latter of which is independent of age-related changes in blood cell composition.

In the blood dataset, 659 differentially methylated regions (DMRs) could be identified between controls and WS patients. These regions include 3,656 CpG sites and covered parts of 613 different annotated RefSeq genes. In the LCL dataset, 24 DMRs could be defined encompassing 90 CpG sites. Parts of 20 RefSeq genes were covered. Four regions (31 CpGs) were shown to be differentially methylated in whole blood and LCL samples.

There are a few studies associating expression changes of several genes with WS in fibroblasts derived from WS patients or in WRN-depleted cells. Some of the genes covered by our DMRs were already shown to be differentially expressed in these cell lines.

Pyrosequencing of repetitive elements as surrogate markers of global DNA methylation revealed no global methylation changes in blood samples of WS patients.

The identification of epigenetic signatures associated with WS may contribute to the better characterization of mechanisms and pathways underlying the pathogenesis of premature aging diseases.

W1-05

Molecular features of ageing in sperm and blood DNA in a cohort of healthy ageing men (FAMe)

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Parental age has been steadily increasing in Western societies. In women, the effects of age on reproductive function are well known (e.g. decreased fertility, increase in aneuploidies). In contrast, little is known of the effects of ageing on the male germline. On the other hand, it is difficult to separate the effects caused by ageing from those of associated morbidities. The objective of this study was to investigate molecular parameters of ageing in the germline of a healthy men cohort.

The FAMe (Fertility and Aging in healthy Men) is composed of healthy men (n=197) aged 18 to 84. All participants underwent a thorough selection process, including physical evaluation and andrological

assessment and provided sperm and blood samples. DNA was isolated from blood and swim-up (motile) sperm. Relative telomere length and DNA methylation of *LINE1* transposable elements were analysed by quantitative PCR and pyrosequencing, respectively. Genome wide alterations to DNA methylation were analysed by whole genome bisulfite sequencing (WGBS) in pools prepared from sperm and blood DNA (n=6 each) from young (aged 18-24) and old men (aged 61-71). DNA fragmentation index (DFI) was measured by an acridine orange-based flow cytometric assay.

rTL in blood decreased with age (ρ=-0,41, p< 0,001), while sperm rTL increased (ρ=0,39, p< 0,001). DNA methylation of *LINE1* repetitive elements in blood did not show age-related alterations (mean=79,29±0,14). The mean sperm DFI increased with age (ρ=0,49, p<0,001). WGBS analysis resulted in the detection of 189 differentially methylated regions (DMRs; mindiff=0,3) between sperm of young and old men, which are not present in blood and which are being further explored by deep bisulfite sequencing in the different age groups.

Telomere attrition is a hallmark of ageing and in this cohort we could confirm that, in peripheral blood, rTL decreased with increasing age. In the male germline telomerase has been previously shown to remain active. Our results are in agreement with this, with sperm rTL increasing with age. The effect of ageing on sperm chromatin structure has previously yielded conflicting results, probably due to the heterogeneity of the cohorts analysed. In our cohort, we could detect an increase in the mean DFI and therefore DNA damage with increasing age. Epigenetic drift has been suggested as a consequence of ageing, with some studies showing an overall age-associated decrease of DNA methylation. In this cohort we did not detect changes in *LINE1* DNA methylation in blood with increasing age. Nevertheless, analysis of WGBS data revealed significant changes in DNA methylation in sperm and, to a lesser extent, in blood, suggesting an epigenetic effect of ageing in both somatic and germ cell lineages. In conclusion, this cohort allows the identification of molecular changes caused by ageing itself (telomere attrition in somatic lineages, sperm DNA damage, and DNA methylation changes).

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W1-06

Variant rs2168518:G>A in the seed region of miR-4513 is associated with age-related macular degeneration (AMD) influencing angiogenesis, migration, proliferation and gene regulation

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Purpose: Several studies have highlighted the functional role of microRNAs (miRNAs) in human pathologies and their potential for innovative therapeutic applications. miRNAs could be of particular interest in complex diseases due to their high prevalence, their complex nature of disease risk and pathology, as well as the frequent lack of treatment options. To this end, we have initiated investigations into the function of AMD-associated miRNAs. Recently, the genetic variant rs2168518:G>A within the seed region of has-miR-4513 has been associated with AMD, although not with genome-wide significance. Seed regions of miRNAs play a crucial role in target gene recognition and binding. Variations in these important regions may lead to altered gene expression and therefore may affect corresponding phenotypes. The cellular function of rs2168518:G is unknown, as well as the impact of the variant rs2168518:A.

Methods: We aimed to replicate the association of rs2168518:G>A with late-stage AMD in a dataset of 63,155 individuals, including the currently largest data set of a genome-wide association study for AMD from the International AMD Genomics Consortium (IAMDGC) and in the Genetic Epidemiology Research on Aging (GERA) Cohort. To further study the functional importance of this genetic variant, we transduced human umbilical vein endothelial cells (HUVECs), murine microglia cells (BV2) and retinoblastoma cells (Y79) with hsa-miR-4513 containing the risk and non-risk allele of rs2168518.

Results: We replicated the findings from previous studies and report rs2168518:G>A to be associated with late-stage AMD with genome-wide significance. Furthermore, functional assays to investigate characteristic mechanisms involved in angiogenesis, marking an important feature of the neovascular late-stage form of AMD, revealed significant differences between the risk and non-risk allele of rs2168518 in neovascularization, migration, proliferation and gene regulation.

Conclusion: Taken together, the seed polymorphism rs2168518 is significantly associated with late stage AMD while functional assays revealed differences in important characteristics of angiogenesis between the risk and non-risk allele. Therefore, miR-4513 could be an interesting candidate for treatment options of the pathological vascularizations in AMD.

W2 BASIC MECHANISMS

W2-01

Genomic predetermined breaking points - "FANCD2 binding identifies conserved fragile sites at large transcribed genes"

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Fragile sites are regions within the genome that are particularly prone to exhibit aberrations on metaphase chromosomes following replication stress. Chromosomal rearrangements at fragile sites are often associated with cancer or the development of neurological disorders. It is known that FANCD2, a Fanconi anemia protein involved in DNA repair, localizes to fragile sites in human cells. We established a new approach to map common chromosomal fragile sites (CFSs) genome-wide. This mapping method enables an unbiased analysis of chromosomal instability not relying on traditional cytogenetic analysis, using by eye-defined structures to classify broken chromosomes on metaphase spreads. But instead, using a FANCD2 ChIP-seq (Chromatin Immunoprecipitation followed by massively parallel sequencing) high-throughput approach opens the way for standardized mapping of CFSs by the definition of objective parameters that can predict breakage events genome-wide. We have been using the avian cell line DT40, a well-established B cell line derived from chicken lymphocytes, to address questions of genome stability at CFSs. We validated FANCD2 enrichment sites as bona fide CFSs in DT40 cells by traditional metaphase spread analysis (for macrochromosomes). The advantage of using DT40 cells for pioneering the genome-wide ChIP-seq mapping of FANCD2 is that avian cells - other than human cells - are characterized by a karyotype containing lots of microchromosomes. These small chromosomes are not suitable for traditional breakage analysis, denying also breaks close to telomeres or centromeres, respectively. However, our novel FANCD2 ChIP-seq approach is covering putative breakage events genome-wide irrespective of the originating tissue or quality of the sample, even separating two fragile sites in close vicinity to each other. It thereby opens the way for whole genome mapping also in human cells and every cell line that fulfills the criteria of FANCD2 protein localization.

To further elucidate genomic consequences of CFS expression, we established a site-specific mutation assay based on a fluctuation analysis that is driven by counter-selection of the fusion gene Hygromycin Thymidine kinase (HyTK). The site-specific integration of this fusion gene enables tracking of mutational events close to or within ChIP-seq defined FANCD2 enrichment sites, thereby reflecting genetic consequences of fragility. Interestingly, the HyTK counter-selection assay demonstrated a correlation between increased mutational events and FANCD2 enrichment sites. The HyTK mutation assay can be used as a universal tool for the measurement of mutation rates in various cell systems, particularly in human cells.

Besides methodological approaches, our study reveals for the first time a genome-wide evolutionary conservation of CFSs at large transcribed genes beyond the mammalian lineage. It opens the way for speculations on the beneficial existence of CFSs throughout the animal kingdom.

W2-02

Development of an in vitro model for the induction of artificial imprints

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Genomic imprinting is an epigenetic process which marks specific gene regions by DNA methylation in only one of the parental germ lines. In humans about 100 imprinted genes are known which occur in clusters on chromosome 6, 7, 11, 14, 15 and 20. Germline imprints are erased in primordial germ cells and are newly established during germ cell maturation by *de novo* DNA methyltransferase *DNMT3A* and its cofactor *DNMT3L*. The majority of imprints occur during oogenesis and a minority during spermatogenesis. Transcriptional read-through has been implicated in the establishment of DNA methylation in germ cells and somatic cells. Understanding of this underlying mechanism in humans is hampered by restricted access to human germ cells. To investigate the role of transcription in the induction of DNA methylation at imprinted promoters, we have developed an inducible Flp-In T-Rex-HeLa cell culture model. We will determine if

transcription from the upstream promoter silences selected test promoters followed by induction of DNA methylation in somatic cells (induction of artificial imprints). The long-term goal is to identify factors necessary and sufficient for the induction of artificial imprints using this *in vitro* system.

We have chosen promoter fragments of *MSH2*, for which induction of DNA methylation by transcriptional read-through from the neighbouring *EPCAM* gene has been demonstrated in rare human patients with Lynch syndrome, and of the imprinted gene *SNRPN*. The targeting vector pcDNA5/FRT/TO was modified to include exon 2, intron 2 and exon 3 of the rabbit *β-globin* (*ocHBB2*) gene under control of the inducible CMV promoter. The promoter and exon 1 of *MSH2* and *SNRPN*, respectively, were then cloned into intron 2 of the *ocHBB2* gene downstream of the CMV promoter, which acts as interfering upstream promoter, in tandem orientation. The test promoters drive expression of a transcript consisting of exon 1 of *MSH2* or *SNRPN* and exon 3 of *ocHBB2* gene, labelled as exon 1/3 transcript. So far, 14 and three stable inducible cell lines containing the *MSH2* and the *SNRPN* promoter, respectively, have been generated. Induction of the CMV promoter by doxycycline for 72h in three independent *SNRPN* promoter cell lines resulted in an average of 12-fold upregulation of the CMV promoter driven *ocHBB2* transcript exon 2/3 (varies from 6- up to 17-fold) and in an average of 5-fold downregulation of exon 1/3 transcript. This indicates that transcriptional read-through suppresses *SNRPN* promoter activity. We currently investigate if this is accompanied by the induction of DNA methylation and the generation of an artificial imprint in somatic cells.

W2-03

Inhibition of histone deacetylation up-regulates the repressed paternal allele of the imprinted Kcnk9 gene towards improving the behavioral phenotype of a mouse model of Birk-Barel syndrome

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Kcnk9/*KCNK9* is a maternally expressed imprinted gene, whose mutations are causative for the maternally inherited Birk-Barel mental retardation syndrome. It encodes a member of the superfamily of K^+-channel and is involved in the modulation of the resting membrane potential and excitability of neurons.

We first set out to characterize the allele-specific expression of *Kcnk9* in various regions of the mouse brain and correlate this with several parameters of brain function in homozygous (*Kcnk9*KO^{hom}) and heterozygous *Kcnk9*KO mice with inactivation of the maternal *Kcnk9* allele (*Kcnk9*KOmat).

Using Quantification of Allele-Specific Expression by Pyrosequencing (QUASEP) and Allele-Specific RT-qPCR in wildtype (WT) in (C57BL/6xCast/Ei)F1 hybrid mice, biallelic *Kcnk9* expression with low mRNA amounts (1-6% of the transcripts) from the repressed paternal allele was observed in all analyzed brain regions. In subsequent QUASEP analysis of *Kcnk9*KO^{mat} (C57BL/6xCast/Ei)F1 hybrid mice, some brain regions displayed levels of paternal *Kcnk9* expression higher than those detected in WT mice.

By analyzing WT, *Kcnk9*KO^{mat} and *Kcnk9*KO^{hom} mice in a behavioral test battery, a clearly impaired working memory of the *Kcnk9*KO^{mat} and *Kcnk9*KO^{hom} mice in comparison to WT mice was seen. In addition, spontaneous alternation in the Y-Maze test was significantly reduced by approximately 10-20%. In acoustic startle response and prepulse inhibition tests, *Kcnk9*KO^{hom} mice showed a significantly reduced acoustic startle response (ASR). *Kcnk9*KO^{mat} and *Kcnk9*KO^{hom} further a reduced pre-pulse inhibition compared to B6-WT. Investigations of the circadian rhythm revealed an increased locomotor activity during the dark phase in *Kcnk9*KO^{hom} and, to a significantly smaller extent, in *Kcnk9*KO^{mat} mice. RNA in situ hybridization stainings of the brain, as well as RT-qPCR expression and electrophysiological data of locus coeruleus cells in *Kcnk9*KO^{mat} mice support the hypothesis that non-canonical *Kcnk9* imprinting in brain regions associated with the sleep-wake cycle leads to the observed intermediate pictures of circadian and other behavioral phenotypes in *Kcnk9*KO^{mat} mice.

Upregulation of the paternal allele in several brain regions and the observation of intermediate phenotypes in *Kcnk9*KO^{mat} animals led us to the hypothesis that epigenetic manipulation could further stimulate paternal gene expression and thereby improve the phenotype. Indeed, epigenetic drug treatments with CI994, a specific histone deacetylase inhibitor, induced a significant up-regulation of the paternal *Kcnk9* allele in primary cortical neurons and in several analyzed brain regions after intraperiteonal injection in *Kcnk9*KO^{mat} mice. Preliminary results indicate a better behavioral performance of *Kcnk9*KO^{mat} mice after CI994 treatment. Further analyses will show if a significant improvement of cognitive deficits in these animals can be achieved and new avenues for the treatment of Birk-Barel syndrome may be opened.

W2-04

The mTOR pathway regulates mRNA stability of synaptic cell adhesion proteins in neurons.

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Dysfunction of the mTOR signaling pathway is thought to be the root cause for several neurodevelopmental disorders such as autism spectrum disorder (ASD), intellectual disability (ID) and epilepsy. Although it has been known for many years that mTOR regulates local mRNA translation in neuronal dendrites, few targets have been identified and specific mechanisms remain largely unexplored. Identifying these targets and mechanisms will be important to understand neuronal function as well as the pathogenesis of neurodevelopmental disorders associated with changes in mTOR activity.

Here, we identified the synaptic cell adhesion molecule N-cadherin (Ncad) as a new candidate target of mTOR. Ncad is a homophilic cell adhesion protein that plays important roles during neuronal development and function and may therefore be an important downstream effector of mTOR.

Our studies suggest that mTOR positively regulates expression of Ncad. Surprisingly, inhibiting the mTOR pathway led to a decrease in Ncad expression at both mRNA and protein level. After transcriptional inhibition using Actinomycin D the Ncad mRNA decreased more rapidly in mTOR inhibited than in control neurons suggesting that mTOR regulates Ncad posttranscriptionally at the level of mRNA stability. The stability of a certain mRNA is regulated by microRNAs or RNA binding proteins (RBPs) that typically interact with short sequence motifs in the mRNAs 3'UTR. Alternative polyadenylation shortens the 3'UTR thereby removing microRNA or RBP binding motifs and influencing mRNA stability. By carrying out 3'Race experiments we could show that mTOR regulates alternative polyadenylation of the Ncad mRNA. Inhibiting the mTOR pathway resulted in an increased production of Ncad mRNA containing a short 3'UTR. Currently we are searching for RBPs that bind to the Ncad 3'UTR and regulate Ncad mRNA stability.

Alternative polyadenylation is emerging as a widespread and important mechanism to regulate mRNA expression in the brain where 3'UTRs are in general longer than in other tissues. Regulation of gene expression by influencing alternative polyadenylation and RNA stability is a new and so far undescribed mechanism of regulation by the mTOR pathway. We expect that many more transcripts in addition to Ncad are regulated by this mechanism and that a misregulation of alternative polyadenylation may contribute to mTOR related diseases including ASD and ID. To identify all of these mTOR targets we are currently performing 3'end RNA-sequencing experiments.

W2-05

Understanding (in)fertility - A broad single-cell transcriptome view of the male mouse germ line

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An estimated 15% of couples worldwide are affected by infertility, with approximately equal contributions from both partners. Genetic abnormalities are thought to account for 15%–30% of male factor infertility and affect changes in spermatogenesis and sperm quality. Mouse mutants have been invaluable in obtaining information on all aspects of normal and abnormal spermatogenesis and are therefore an excellent model for the systematic evaluation of male infertility factors. Spermatogenesis takes place in the seminiferous tubules of the testis, which are almost exclusively comprised of germ cells. Besides undifferentiated spermatogonial stem cells (SSCs) and mature spermatozoa, all other germ cells in the adult testis represent transitional stages in the continuous process of germ cell differentiation. This has made gene expression studies challenging and hampered understanding of the molecular basis of infertility.

Here, we present the first comprehensive, unbiased single-cell transcriptomic view of mouse spermatogenesis. In order to obtain single-cell expression profiles for a large number of testicular cells, we prepared cell suspensions from the testes of two 8 week old C57Bl/6J mice and obtained transcriptomes for roughly 1250 cells each. The mice were virtually indistinguishable in any QC statistic, and yielded identical distributions after t-stochastic neighbor embedding (t-SNE). The continuity of expression changes could be confirmed using unsupervised pseudotime analysis. The present data impressively demonstrate the continuous, dynamic and heterogeneous differentiation process during mammalian spermatogenesis. We show that single-cell RNAseq (scRNA-seq) is a powerful tool for the investigation of differentiation networks, reveals new insights into the regulation of sex chromosomes during spermatogenesis, and improves upon stage marker detection and validation, capturing the continuity of differentiation.

In addition, we obtained single-cell expression profiles from 2821 cells from *Phf13*$^{-/-}$ testis tissue. PHF13, a nuclear protein associated with chromatin during mitosis, is an epigenetic reader and transcriptional co-

regulator and involved in chromosome condensation, DNA damage response and cell division. Male *Phf13*-deficient mice show a progressive loss of germ cells, leading to testis hypoplasia and infertility. Comparative analysis of the scRNA-seq data revealed highly interesting expression changes in the *Phf13*$^{-/-}$ germ cells shedding light on the regulatory function of PHF13 during spermatogenesis and demonstrating the suitability of single-cell transcriptomics in the systematic evaluation of mouse infertility mutants.

W2-06

Germline de novo mutation clusters arise during oocyte aging in genomic regions with increased double-strand break incidence

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Clustering of mutations has been observed in cancer genomes as well as for *de novo* mutations (DNMs) in the germline. We identified 1,796 clustered DNMs (cDNMs) within whole-genome sequencing data from 1,291 parent-offspring trios to investigate their patterns and inferred a mutational mechanism. We found that the number of clusters on the maternal allele was positively correlated with maternal age and that these consist of more individual mutations with larger inter-mutational distances compared to paternal clusters. More than 50% of maternal clusters were located on chromosomes 8, 9 and 16, in regions with an overall increased maternal mutation rate. Maternal clusters in these regions showed a distinct mutation signature characterized by transversions. Finally, we found that maternal clusters associate with processes involving double-stranded-breaks (DSBs) such as meiotic gene conversions and de novo deletions events. These findings suggest accumulation of DSB-induced mutations throughout oocyte aging as an underlying mechanism leading to maternal mutation clusters.

W3 NEUROGENETICS

W3-01

Biallelic mutation in CHP1 causes human autosomal recessive ataxia by impairing NHE1 membrane targeting

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Autosomal recessive cerebellar ataxias (ARCAs) are a complex group of neurodegenerative disorders that affect the cerebellum, brain stem and spinal cord. Approximately 40% of the ARCA patients remain genetically unresolved. By using a combination of WES and linkage analysis, we identified a biallelic 3-bp deletion (p.K19del) in *CHP1* (Calcineurin like EF-hand protein 1) that co-segregates with motor neuropathy, cerebellar atrophy and spastic paraparesis in two siblings of a consanguineous family. Focused screening for *CHP1* variants in two cohorts (ARCA: N=319 and NeurOmics: N=657) and GeneMatcher interrogation did not yield additional variants, thus revealing the scarcity of *CHP1* mutations.

CHP1 plays a crucial role in neuronal pHi regulation and ion homeostasis, by controlling the function of the Sodium/Hydrogen Exchanger 1 (NHE1, encoded by *SLC19A1*). Reduced CHP1 expression as well as NHE1 depletion cause Purkinje cell degeneration and ataxia in mouse. Moreover, a loss-of-function mutation

in NHE1 causes ataxia in human. Here we demonstrate that mutant CHP1 fails to integrate into functional protein complexes and is prone to aggregation, thereby leading to diminished levels of soluble CHP1 and reduced membrane targeting of NHE1. Moreover, we show that morpholino-mediated *chp1* knockdown in zebrafish resembles the phenotype of ARCA-affected humans, leading to spastic trunk movements, cerebellar hypoplasia and motor axon abnormalities. These defects were ameliorated by co-injection with WT, but not mutant, human *CHP1* mRNA. Collectively, our results identified *CHP1* as an ataxia-causative gene in humans, further expanding the spectrum of ARCA-associated loci, and corroborate NHE1 mistargeting as a key event underlying neuronal degeneration in the context of inherited cerebellar ataxias.

W3-02

Biallelic mutations in VPS13D are a novel cause of ataxia with spasticity and lead to mitochondrial dysfunction

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Ataxia is a neurological sign characterized by loss of voluntary coordination of muscle movements and follows a dominant, X-linked, or recessive mode of inheritance. Recessive forms of ataxia are clinically and genetically highly heterogeneous and only in a minor fraction of cases a mutation in a known ataxia gene can be identified, suggesting that much of the genetic heterogeneity remains to be discovered.

Exome sequencing in a German family with two affected sisters with spastic ataxia with onset in the third decade of life revealed compound-heterozygous variants in *VPS13D* ([c.5409C>A [Tyr1803Ter] and c.12629C>T [Ala4210Val]) in both patients. Through an international collaboration, six additional ataxia patients were independently identified carrying biallelic mutations in *VPS13D* including a large family. Of note, the majority of these patients carried a loss-of-function variant on one and a missense variant on the other allele. Symptoms were slowly progressive and led to loss of independent ambulation in five of 12 patients.

Sanger sequencing of cDNA in our family revealed lower expression of the nonsense mutated allele compared to the missense mutated allele, resulting in a ~50% reduction in the total mRNA level of *VPS13D* in the patient compared to two non-mutation carriers. This reduction was caused by nonsense mediated mRNA decay as demonstrated by stabilization of the nonsense mutated allele by cycloheximide treatment.

VPS13D encodes a large protein with 4388 amino acids, of which the paralogs cause other neurological disorders including chorea-acanthocytosis (*VPS13A*), Cohen syndrome (*VPS13B*), and Parkinsons's disease (*VPS13C*). It was shown that *Vps13d* disruption in Drosophila is lethal and caused mitochondrial defects. In light of these fly data, we also investigated mitochondrial function and morphology in humans. While the typical mitochondrial network of elongated organelles and branches was observed in two controls, patient-derived, cultured fibroblast cells showed more roundly mitochondria and less branches as quantified by form factor analysis. Further, Western blot analysis demonstrated overall less mitochondrial GRP-75 protein in the patient´s cells. These structural mitochondrial changes were accompanied by a reduced ATP production rate compared to control fibroblasts. Interestingly, mitochondrial dysfunction has been associated with several other ataxias and spastic paraplegias such as Friedreich's ataxia, *SCA28*, and *SPG7* as well is in ~25% of unexplained ataxias.

In conclusion, our study demonstrated that biallelic mutations in *VPS13D* cause a movement disorder along the ataxia-spasticity spectrum, making *VPS13D* the fourth VPS13 paralog involved in neurological disorders. Analysis of a Drosophila knock-out model and patient-derived fibroblasts suggest that mutations in this new ataxia/spasticity gene impact on mitochondrial structure and function.

W3-03

Tubulin polyglutamylation defects cause infantile-onset neurodegeneration

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Tubulin polyglutamylation is a prominent posttranslational modification in neurons. Defective tubulin glutamylation was first linked to neurodegeneration in the Purkinje cell degeneration (pcd) mouse which displays massive cerebellar atrophy, lacks deglutamylase CCP1 and accumulates hyperglutamylated tubulin in neurons that subsequently degenerate. We found CCP1 biallelic rare and damaging variants in ten individuals with infantile-onset neurodegeneration and confirmed absence of functional CCP1 along with dysregulated deglutamylation. The human disease affects the cerebellum, spinal motor neurons and peripheral nerves. Revisiting the pcd phenotype, we demonstrate previously unrecognized peripheral nerve and spinal motor neuron degeneration, thus largely recapitulating the human disease. The demonstration that CCP1 deficiency causes neurodegeneration in humans implicates tubulin hyperglutamylation in other forms of neurodegenerative illness and entails potential targets for drug development to treat these conditions.

W3-04

Distinct functional consequences of SCN2A mutations determine the phenotype

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Objective:

Mutations in the voltage-gated sodium channel type 2 ($Na_v1.2$) lead to a broad spectrum of phenotypes ranging from self-limited familial neonatal-infantile epilepsy (BFNIE) to very severe developmental and epileptic encephalopathy (EE) to intellectual disability without seizures (ID). Since the underlying mechanisms determining this phenotypic variability are incompletely understood, we investigate the functional effects of six mutations (2 novel and 4 previously described).

Methods and patients:

Pathogenic *SCN2A* mutations of five patients with either EE or ID and one BFNIE mutation from the literature were selected for functional studies. Biophysical properties of recombinant wildtype and mutant $Na_v1.2$ channels were measured using voltage-clamp of transiently transfected HEK293T cells co-expressing auxiliary β-subunits and EGFP. In-silico protein modeling was used to gain insight into the structural effects of the mutations.

Results:

We report on five patients with heterozygous de novo mutations in *SCN2A*, including two novel mutations, and additionally on one BFNIE mutation already functionally characterized in the literature. The two missense mutations causing EE showed profound gating changes in patch-clamp experiments, whereas all ID mutations, nonsense and missense, exhibited no relevant current. The BFNIE mutation showed a small change of channel inactivation resulting in a small gain of function. The protein modeling suggested structural aberrations for all studied missense mutations consistent with the electrophysiological findings.

Discussion:

By examining the functional consequences of *SCN2A* mutations causing epileptic encephalopathy, self-limited familial neonatal-infantile epilepsy or intellectual disability this study contributes to the elucidation of mechanisms leading to the broad phenotype variability reported for *SCN2A* mutations. Our findings support the hypothesis that complete loss-of-function mutations lead to intellectual disability without seizures, small gain-of-function mutations cause BFNIE and epileptic encephalopathy mutations exhibit variable but profound $Na_v1.2$ gating changes.

W3-05

A homozygous activating ATAD1 mutation impairs postsynaptic AMPA receptor trafficking and causes a lethal encephalopathy with congenital stiffness

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Members of the AAA+ superfamily of ATPases are involved in the unfolding of proteins and disassembly of protein complexes and aggregates. *ATAD1*, encoding the ATPase family AAA+ domain containing 1-protein Thorase, plays an important role in the function and integrity of mitochondria and peroxisomes. Postsynaptically, Thorase controls the internalization of excitatory, glutamatergic AMPA receptors (AMPAR) by disassembling complexes between the AMPAR-binding protein, GRIP1, and the AMPAR subunit GluA2. Using whole-exome sequencing, we identified a homozygous frameshift mutation in the last exon of *ATAD1* [c.1070_1071delAT; p.(His357Argfs*15)] in three siblings of consanguineous parents who presented with a severe, lethal encephalopathy associated with stiffness, arthrogryposis, and respiratory distress. Transcript analysis of fibroblast-derived mRNA from one affected sibling revealed that the *ATAD1* mRNA is not degraded and harbors the 2-bp deletion leading to deletion of the last five codons and addition of 14 unrelated *ATAD1* codons at the 3' end. ATAD1 protein with a novel C-terminus was found to be expressed in patient cells. The protein level of a subset of Golgi, peroxisomal, and mitochondrial proteins was slightly reduced in patient fibroblasts compared to control cells, but morphology and respiratory chain performance of mitochondria were normal. By size-exclusion chromatography we demonstrated that mutant ATAD1 elutes at a higher molecular weight as wild-type ATAD1 suggesting that the mutant protein is locked in an oligomeric state. *In-vitro* experiments confirmed that the p.(His357Argfs*15) mutation impairs normal disassembly of Glu2A-GRIP1 complexes and Thorase oligomers. *Atad1*$^{-/-}$ neurons expressing Thorase mutant^{His357Argfs*15} displayed reduced amount of GluA2 at the cell surface suggesting that the Thorase mutant may inhibit the recycling back and/or reinsertion of AMPARs to the plasma membrane following endocytosis resulting in a decrease in the steady-state levels of these receptors at the cell surface. Taken together, biochemical and cellular analyses show that the C-terminal end of Thorase mutant gained a novel function which strongly impacts its oligomeric state and affects disassembly of GluA2 and Thorase oligomer complexes. A homozygous loss-of-function mutation (p.Glu276*) in *ATAD1* has been previously reported to underlie hypertonia, seizures, respiratory failure, and early death in multiple individuals in a highly consanguineous family. Collectively, these data demonstrate that *ATAD1* is a new disease gene for severe encephalopathy and congenital stiffness, and the distinct combination of functional alterations arising from either gain- or loss-of-function *ATAD1* mutation may provide an explanation for the discrete although overlapping phenotypes observed in the siblings reported here and the previously described family.

W3-06

Exome-wide copy number variant analysis in multiply affected families provides new insight into the pathogenesis of schizophrenia

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Schizophrenia (SCZ) is a severe neuropsychiatric disorder with an estimated heritability of 80%. Among the established risk factors are both common and rare variants which explain approximately 30% of the variance. It is therefore reasonable to assume that a large number of SCZ relevant genes await identification.

Specific copy number variants (CNVs) in 11 chromosomal loci are well established genetic risk factors for SCZ. Due to technical limitations (resolution of DNA-arrays), the known SCZ risk-associated CNVs are large in size and span numerous genes. Therefore, it is difficult to pinpoint which gene/genes within these CNV loci are relevant for the pathogenesis of SCZ.

The analysis of whole exome sequencing (WES) data facilitates the detection of smaller, single gene affecting CNVs and therefore allows for the implication of specific genes in the development of SCZ.

We performed an exome-wide CNV analysis in 55 multiply affected SCZ families. To reduce false-positives, CNVs were *in silico* predicted with eXome-Hidden Markov Model (XHMM), Copy Number Inference From Exome Reads (CoNIFER), and ExomeDepth. Rare deletions and duplications that were detected by all CNV callers and that were co-segregating with SCZ within each family were experimentally verified.

In order to obtain additional evidence for an involvement of the newly identified candidate genes in the pathogenesis of SCZ, a multi-tier approach was applied. We combined the CNV data with gene expression data derived from brain tissues (both from publically available datasets and our own inhouse SCZ brain samples), and data from complex protein-protein-interaction (PPI) analyses. We were able to identify several new SCZ candidate genes. Among our top candidate genes is *SMARCC1* on chromosome 3p21.31. In one family we identified a co-segregating, partial duplication in *SMARCC1* (SWI/SNF related, matrix associated, actin dependent regulator of chromatin subfamily c member 1). In addition, the PPI analyses showed that *SMARCC1* rank among the top four genes interacting with other previously reported SCZ candidate genes. Mutations in the SWI/SNF complexes have been reported to play a role in neurodevelopmental disorders.

To our knowledge, this is the first exome-wide CNV analysis in multiply affected SCZ families. With our data we provide new insight into the pathogenesis of SCZ.

W4 COMPLEX GENETICS

W4-01

Genome-wide analysis of transcriptional and cytokine response variability in activated human immune cells

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The immune system plays a major role in human health and disease. Understanding variability of immune responses on the population level and how it relates to susceptibility to diseases is vital. In this study, we aimed

to characterize the genetic contribution to interindividual variability of immune response using genome-wide association and functional genomics approaches.

For this purpose, we studied genetic associations to cellular (gene expression) and molecular (cytokine) phenotypes in primary human cells activated with diverse microbial ligands. We isolated monocytes of 134 individuals and stimulated them with three bacterial and viral components (LPS, MDP, and ppp-dsRNA). We performed transcriptome profiling at three time points (0 min/90 min/6 h) and genome-wide SNP-genotyping. In addition, we profiled five cytokines produced by peripheral blood mononuclear cells activated by five components from the same individuals to perform a genome-wide association study.

Comparing expression quantitative trait loci (eQTLs) under baseline and upon immune stimulation revealed 417 immune response specific eQTLs (reQTLs). We characterized the dynamics of genetic regulation on early and late immune response, and observed an enrichment of reQTLs in distal cis-regulatory elements. Analysis of signs of recent positive selection and the direction of the effect of the derived allele of reQTLs on immune response suggested an evolutionary trend towards enhanced immune response. Furthermore, multivariate GWAS analysis of cytokine responses to diverse stimuli revealed 159 genome-wide significant loci; however, only a small number of these could be reliably linked to potentially causal eQTLs in monocytes. Finally, given the central role of inflammation in many diseases, we examined reQTLs as a potential mechanism underlying genetic associations to complex diseases. We uncovered novel reQTL effects in multiple GWAS loci, and showed a stronger enrichment of response than constant eQTLs in GWAS signals of several autoimmune diseases. These results indicate a substantial, disease-specific role of environmental interactions with microbial ligands in genetic risk to complex autoimmune diseases. While tissue-specificity of molecular effects of GWAS variants is increasingly appreciated, our results suggest that innate immune stimulation is a key cellular state to consider in future eQTL studies as well as in targeted functional follow-up of GWAS loci.

W4-02

Androgenetic alopecia – functional annotation of GWAS findings using tissue-specific gene expression and the FUMA platform.

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Androgenetic alopecia (AGA) is a heritable trait and the most common form of hair loss in men. Over the past year, large scale genetic association studies have made decisive progress in the identification of the contributing genetic factors and have identified >100 genome-wide significant risk loci. The majority of the identified loci are located in noncoding genomic regions and our understanding of the underlying biological mechanisms is incomplete. Therefore, studies that enable the functional annotation and identification of causal genes and pathways are highly warranted to improve our understanding of AGA-pathobiology. Here, we used (i) a comprehensive data set on mRNA- and miRNA-expression in human hair follicle and immortalized human dermal papilla cell lines and (ii) the FUMA web application (http://fuma.ctglab.nl) to functionally annotate all 109 reported genome-wide significant risk loci from our recently published genome-wide meta-analysis (Heilmann-Heimbach et al., 2017) and two additional large-scale genetic studies on AGA (Hagenaars et al., 2017; Pirastu et al., 2017). Of 1,257 genes located at the 109 risk loci, a total of 649 (52%) revealed expression in human hair follicle and/or dermal papilla cells, thus prioritizing these genes as likely candidate genes in AGA. This included the biologically plausible androgen receptor gene, *FGF5* and *TGFB1*. Moreover, hair follicle expressed candidate genes showed an enrichment in several pathways, including WNT- and hormone signaling pathways, apoptosis signaling and melanocyte degradation. The latter two are likely to contribute to the observed gradual transformation of pigmented terminal hair into unpigmented vellus hair follicles during AGA. While we identified at least one hair follicle expressed gene at the majority of loci, there were other loci, where none of the nearby genes showed expression in hair follicle cells. These loci may evolve their functional effects in AGA-pathogenesis in cell-populations outside the hair follicle. We therefore used the SNP2GENE function in FUMA that incorporates biological data from 18 different repositories to identify additional candidate genes at the AGA loci. The query resulted in the mapping of 257 independent significant SNPs to 157 genes. Among them 45 genes, that were not implicated by our expression analyses. This included the immune cell expressed genes *IRF4* and *NLRC4*. Subsequent GENE2FUNC analysis in FUMA revealed an enrichment of AGA genes in the GO-processes hair cycle and hair molting, suggesting that perifollicular cells contribute to the AGA-typical changes in hair follicle cycling (anagen shortening/ premature catagen entry). Taken together, our data enable an increasingly detailed understanding of the genes and pathways that play a role in AGA-

pathogenesis and aid the generation of mechanistic hypotheses that can be further tested in functional experiments.

W4-03

Comprehensive Genetic Analysis Of Whole Genome Sequencing Data From 108 Individuals Of 8 Multigenerational Spanish Families Affected With Bipolar Disorder

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Bipolar Disorder (BD) is a common, genetically complex neuropsychiatric disorder. Affected individuals suffer from recurrent, severe mood swings ranging from mania to depression. The heritability of BD has been estimated to be approximately 70%, indicating a strong involvement of genetic factors in the development of this disorder. Former studies reported that the cumulative effect of common alleles explain only 25-38% of the phenotypic variance for BD. Therefore, rare variants with medium to large effects are thought to play a substantial role in the genetic architecture of BD. A research strategy to identify such rare variants is Whole Genome Sequencing (WGS) of large multigenerational families multiply affected with BD.

For the present study we conducted WGS of 108 individuals in a set of 8 extended multigenerational and multiply affected families of Spanish origin. On average we sequenced 14 affected and unaffected individuals per family. With this extended family model we increase the power to detect rare variants with higher penetrance which might be located in coding and non-coding regions. We also included WGS data of unaffected family members in order to evaluate whether segregating variants show reduced penetrance. WGS was performed at 30X on a Illumina HiSeq2500v4 system. Variant calling files (VCF) were created for all individuals using an in-house developed pipeline based on GATK´s best practice guidelines. For the visualisation and data handling we set up an analysis workflow based on the CRAN package vcfR and in-house developed R-scripts.

In our analysis we focused on rare variants with a minor allele frequency (MAF) below 1% (according to the ExAC database). We applied a narrow analysis model to identify overlapping, rare, co-segregating variants in the affected individuals in one family which are not shared by healthy individuals within the same family. In addition, we also applied a broad analysis model in which we take into account incomplete penetrance. Various prediction algorithms (MutationTaster, SIFT, PolyPhen2, Provean) were used to evaluate possible damaging/disease causing impacts of the identified variants. In a further analysis step we plan to conduct pathway analysis with our findings using Ingenuity Pathway Analysis and Consensus Path DB, perform Polygenic Risk Score Analyses as well as to analyse functional and regulatory elements with the ENCODE database.

We completed WGS for all 108 individuals and created VCFs using our in-house developed pipeline. So far, we have completed the analysis of a 2 generation family comprising 8 affected and 1 unaffected individual

in which we identified 40 rare, segregating variants. The results of the remaining families as well as segregation, pathway and polygenic risk score analyses will be presented at the congress.

W4-04

Exome sequencing with subsequent multi-tier analyses in a large multiply affected family cohort provides new insight into schizophrenia

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Schizophrenia (SCZ) is a severe psychiatric disorder with a prevalence of ~ 1% and a heritability of ~ 60-80%. Analysing multiply affected families using whole exome sequencing (WES) is a very promising approach to identify new SCZ risk factors as in these families genetic variants with particularly strong effect might co-segregate with the disorder. The present study is the worldwide largest SCZ family study using WES.

We sequenced 2-5 individuals from 55 families, each, on an Illumina HiSeq 2500 and analysed the WES data using the Varbank pipeline of the Cologne Center for Genomics and the CLC bio Biomedical Genomics Workbench. We focused our analyses on rare (minor allele frequency ≤0.1%), single nucleotide variants and small indels that were *in silico* predicted to be pathogenic, and that were shared between all affected individuals in one family. To prioritize the implicated genes for the follow-up analyses, large publically available and inhouse WES datasets (> 3,000 SCZ patients) were analysed for additional pathogenic mutations in the newly identified candidate genes.

In a subsequent, multi-tier approach we combined the WES data with gene expression data derived from brain tissues (both from publically available datasets and our own inhouse SCZ brain samples), and complex protein-protein-interaction analyses. Using this multimodal data analysis, we have built a SCZ network implicating promising new genes in the pathogenesis of the disorder, e.g. *MECP2* and confirming already established SCZ risk genes, e.g. *DGCR2* (risk locus 22q11.2) and *CSMD1* (genome-wide significant in worldwide largest genome-wide association study).

With this worldwide largest family-based WES study, we have identified new candidate genes for the disorder and with that have gained new insight into the pathogenesis of SCZ.

W4-05

Genome-wide association study of panic disorder reveals genetic overlap with depression and neuroticism

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Background: Panic disorder (PD) is one of the most common anxiety disorders with a lifetime prevalence of about 4%. PD is characterized by recurrent episodes of abrupt intense fear accompanied by additional physiological or cognitive symptoms. Although PD shows moderate heritability estimates of around 40%, the genetic variants contributing to PD are largely unknown, with only few and inconsistent loci reported to date. To address the challenge of underpowered individual studies, we conducted the largest genome-wide association study of PD to date comprising more than 10,000 individuals.

Methods: We generated genome-wide SNP data from 2,260 patients with PD and 7,988 ethnically matched controls originating from four different European countries (Denmark, Estonia, Germany, Sweden). Standard GWAS quality control procedures were performed on each dataset individually. Imputation was performed using the 1,000 Genomes Project reference panel. Then a meta-analysis was performed using the Ricopili

pipeline (https://sites.google.com/a/broadinstitute.org/ricopili/). 311 SNPs with p<1e-04 were followed up in independent European cohorts comprising 2,400 PD patients and 228,000 controls.

We used the LD Score regression method to investigate the SNP-based heritability for PD and genetic correlations between PD and other neurological/psychiatric disorders. The FUMA framework (fuma.ctglab.nl) was applied to perform gene-set analyses.

Results: In our PD GWAS we did not identify any genome-wide significant locus. 36 linkage disequilibrium independent SNPs had a p-value <1e-05. Using LD Score regression the estimated heritability for PD was 34.2%. In addition, we found significant genetic correlations between PD and major depressive disorder, depressive symptoms and neuroticism (p<0.0026). Gene-set analyses with FUMA revealed a total of 29 significantly enriched gene-sets including GABAergic neuron differentiation. In the follow up analysis none of the 311 investigated SNPs reached genome-wide significance with rs144783209 showing the strongest association (p=3.1e-07).

Discussion: In this collaborative study with sample sizes being larger than any other PD GWAS published to date, we did not identify any genome-wide significant locus. However, our PD meta-analysis shows highly similar characteristics as similar sized GWAS previously conducted in other psychiatric disorders. LD Score regression analysis provides the first SNP-based heritability estimate for PD based on genotype data of more than 10,000 individuals. Furthermore, our results provide evidence for a genetic correlation of PD with depression and neuroticism indicating that PD is not an isolated trait. Gene-set enrichment analyses suggest that GABAergic signaling is involved in PD development. Interestingly, GABA is the key inhibitory neurotransmitter in the cortex and benzodiazepines which are frequently used for the treatment of panic attacks bind to GABA receptors.

W4-06

Phenome-wide association studies (PheWAS) across large “real-world data” population cohorts support drug target validation

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Phenome-wide association studies (PheWAS), which assess whether a genetic variant is associated with multiple phenotypes across a phenotypic spectrum, have been proposed as a possible aid to drug development through elucidating mechanisms of action, identifying alternative indications, or predicting adverse drug events (ADEs). Here, we evaluate whether PheWAS can inform target validation during drug development. We selected 25 single nucleotide polymorphisms (SNPs) linked through genome-wide association studies (GWAS) to 19 candidate drug targets for common disease therapeutic indications. We independently interrogated these SNPs through PheWAS in four large “real-world data” cohorts (23andMe, UK Biobank, FINRISK, CHOP) for association with a total of 1,892 binary endpoints. We then conducted meta-analyses for 145 harmonized disease endpoints in up to 697,815 individuals and joined results with summary statistics from 57 published GWAS. Our analyses replicate 70% of known GWAS associations and identify 10 novel associations with study-wide significance after multiple test correction (P<1.8x10-6; out of 72 novel associations with FDR<0.1). By leveraging directionality and point estimate of the effect sizes, we describe new associations that may predict ADEs, e.g., acne, high cholesterol, gout and gallstones for rs738409 (p.I148M) in PNPLA3; or asthma for rs1990760 (p.T946A) in IFIH1. We further propose how quantitative estimates of genetic safety/efficacy profiles can be used to help prioritize candidate targets for a specific indication. Our results demonstrate PheWAS as a powerful addition to the toolkit for drug discovery.

W5 MOLECULAR BASIS OF DISEASE

W5-01

Identification of EDIL3 sequence variants as genetic risk factors for intracranial aneurysms and subarachnoid hemorrhage

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Objective: Familial occurrence of intracranial aneurysms (IA) and subarachnoid hemorrhage (SAH) suggests a genetic predisposition for this disease; however, genetic risk factors are poorly understood. In this study, we aimed to identify susceptibility genes by stringent bioinformatics analyses of whole-exome data from familial cases.

Methods: Our study cohort consists of 68 substantially affected individuals with a history of unruptured intracranial aneurysms (UIA) or SAH. From this cohort, we selected 17 individuals from 7 families having at least 2 affected family members and performed whole-exome sequencing (WES). To identify dominant, putative disease causing sequence variants we searched for unknown, heterozygous variants that are shared by affected family members. Sequence variants were prioritized by genetic impact, predicted pathogenicity, functional relevance and documented disease association. 2 top candidate genes were Sanger sequenced in the remaining cohort.

Results: We identified unknown sequence variants in *STIM2*, *NKX2-4*, *FBLN2*, *TAGLN*, *EDIL3*, *FOCAD*, *SYNC*, *TIE1*, *TMPRSS12*, *ACTN1*, *MAP7D3*, *PLXD1*, *PFKP*, *MYH3*, *THY1* and *EHD2*. All of these, missense and protein truncating variants, are predicted to be highly damaging and the respective gene products are functionally relevant for vascular biology. Sequencing of *EDIL3* and *TAGLN* in the remaining cohort revealed a further very rare *EDIL3* sequence variant in 2 unrelated sporadic patients. The documented function in vascular wall integrity and the crucial localization of affected amino acids underscore *EDIL3* as a valid candidate disease gene for IA/SAH.

Conclusions: By thorough clinical characterization and a family-based gene identification approach, we found *EDIL3* mutations both in familial and sporadic UIA/SAH cases. The identification of various family-specific candidate disease genes emphasizes that IA/SAH is a distinctly heterogeneous, complex disease.

W5-02

Characterization of Glycosylphosphatidylinositol Biosynthesis Defects by Clinical Features, Flow Cytometry, and Automated Image Analysis

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Background: Glycosylphosphatidylinositol Biosynthesis Defects (GPIBDs) cause a group of phenotypically overlapping recessive syndromes with intellectual disability, for which pathogenic mutations have been described in 16 genes of the corresponding molecular pathway. An elevated serum activity of alkaline phosphatase (AP), a GPI-linked enzyme, has been used to assign GPIBDs to the phenotypic series of Hyperphosphatasia with Mental Retardation Syndrome (HPMRS) and to distinguish them from another subset of GPIBDs, termed Multiple Congenital Anomalies Hypotonia Seizures syndrome (MCAHS). However, the increasing number of individuals with a GPIBD shows that hyperphosphatasia is a variable feature that is not ideal for a clinical classification.

Methods: We studied the discriminatory power of multiple GPI-linked substrates that were assessed by flow cytometry in blood cells and fibroblasts of 39 and 14 individuals with a GPIBD, respectively. On the phenotypic level, we evaluated the frequency of occurrence of clinical symptoms and analyzed the performance of computer-assisted image analysis of the facial gestalt in 91 individuals.

Results: We found that certain malformations such as Morbus Hirschsprung and Diaphragmatic defects are more likely to be associated with particular gene defects (PIGV, PGAP3, PIGN). However, especially at the severe end of the clinical spectrum of HPMRS, there is a high phenotypic overlap with MCAHS. Elevation of AP has also been documented in some of the individuals with MCAHS, namely those with PIGA mutations. Although the impairment of GPI-linked substrates is supposed to play the key role in the pathophysiology of GPIBDs, we could not observe gene-specific profiles for flow cytometric markers or a correlation between their cell surface levels and the severity of the phenotype. In contrast, it was facial recognition software that achieved the highest accuracy in predicting the disease-causing gene in a GPIBD.

Conclusions: Due to the overlapping clinical spectrum of both, HPMRS and MCAHS, in the majority of affected individuals, the elevation of AP and the reduced surface levels of GPI-linked markers in both groups, a common classification as GPIBDs is recommended. The effectiveness of computer-assisted gestalt analysis for the correct gene inference in a GPIBD and probably beyond is remarkable and illustrates how the information contained in human faces is pivotal in the delineation of genetic entities.

W5-03

Loss-of-function Mutations in C11ORF70 Cause Primary Ciliary Dyskinesia with Outer and Inner Dynein Arm Defects

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Severe, recurrent respiratory infections, laterality defects and infertility characterise primary ciliary dyskinesia (PCD), a genetically heterogeneous, autosomal recessive disorder. The defective assembly of dynein arms result in disturbed ciliary motility and therefore disturbed muco-ciliary clearance of the airways. Up to now, ten genes (*PIH1D3*, *DNAAF1*, *DNAAF4*, *DNAAF2*, *SPAG1*, *C21orf59*, *DNAAF3*, *DNAAF5*, *ZMYND10* and *LRRC6*) are known to be involved in the cytoplasmic preassembly of dynein arms. Mutations in these genes result in a combined defect of outer and inner dynein arms.

We identified loss-of-function mutations in the open reading frame *C11ORF70* in five PCD individuals from five distinct families. Expression analyses of *C11ORF70* showed that *C11ORF70* is expressed in ciliated respiratory cells and that the expression of *C11ORF70* is upregulated during ciliogenesis, similar to other previously described cytoplasmic dynein arm assembly factors. Transmission electron microscopy analyses and high resolution immunofluorescence microscopy in respiratory epithelia cells and sperm flagella reveal an absence of both outer and inner dynein arms causing ciliary and flagellar immotility and indicating that C11ORF70 is involved in cytoplasmic assembly of dynein arms. Furthermore, C11ORF70 shows an interaction with the cytoplasmic assembly factor DNAAF2, supporting our hypothesis that C11ORF70 is a novel preassembly factor involved in the pathogenesis of PCD.

Therefore *C11ORF70* is a new gene causing PCD and male infertility and playing an important role in the cytoplasmic preassembly of dynein arms. The identification of novel genetic defects that cause PCD and male infertility is of great clinical importance as well as for genetic counselling.

W5-04

The ClC-K2 Chloride Channel is Critical for Salt Handling in the Distal Nephron

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Bartter syndrome is a group of renal tubulopathies with autosomal recessive inheritance, which is characterized by marked secondary hyperaldosteronism caused by renal salt-wasting with normal or low blood pressure, hypokalemia and metabolic alkalosis. The syndrome typically presents during the neonatal period and is frequently associated with hypercalciuria and nephrocalcinosis. It is caused by inactivating mutations in one of at least four independent genes, i.e. SLC12A1, KCNJ1, CLCNKB, and BSND. These genes encode for the Na+/K+/2Cl- co-transporter NKCC2, the potassium channel ROMK, the chloride channel ClC-K2, and Barttin, an essential subunit for ClC-Ka and ClC-Kb Cl- channels, respectively. These findings suggest a transport model, in which NaCl and K+ are taken up apically via NKCC2. To maintain a high luminal K+ concentration, which is required for the sustained activity of NKCC2, K+ has to be recycled across the apical membrane via ROMK. NaCl exits the cell basolaterally via the Na+/K+-ATPase and ClC-Kb/Barttin. However, the expression pattern of each ClCK homolog has not been fully resolved because of the close homology between ClC-Ka and -Kb and the lack of isoform specific antibodies. To dissect the role of this channel in detail, we generated a mouse model with a targeted disruption of the murine orthologue ClC-K2. Mutant mice developed a phenotype characterized by renal salt loss, marked hypokalemia, and metabolic alkalosis resembling Bartter syndrome. Patch-clamp analysis of tubules isolated from knockout mice suggested that ClC-K2 is the main basolateral chloride channel in the thick ascending limb and in the aldosterone-sensitive distal nephron. Accordingly, ClC-K2 knockout mice did not exhibit the natriuretic response to furosemide and

exhibited a severely blunted response to thiazide. We conclude that ClC-Kb/K2 is critical for salt absorption not only by the thick ascending limb, but also by the distal convoluted tubule.

W5-05

Mutation of Serine/Threonine Protein Kinase 36 (STK36) Causes Primary Ciliary Dyskinesia with a Central Pair Defect

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Primary ciliary dyskinesia (PCD) is a genetic condition of impaired ciliary beating, characterized by chronic infections of the upper and lower airways and progressive lung failure. Defects of the outer dynein arms are the most common cause of PCD. In about half of the affected individuals, PCD occurs with *situs inversus* (Kartagener syndrome). A minor PCD subgroup including defects of the radial spokes (RS) and central pair (CP) is hallmarked by the absence of laterality defects, subtle beating abnormalities and unequivocally apparent ultrastructural defects of the ciliary axoneme, making their diagnosis challenging.

In order to identify novel disease-causing genetic variants, nNO measurement and high-speed video microscopy were used as screening tools for PCD individuals suspected with a CP defect. Immunofluorescence (IF) staining of known components of the ciliary axoneme and transmission electron microscopy (TEM) are routinely performed. To identify disease-causing mutations, next-generation whole-exome sequence, PCD panel diagnostic, and Sanger sequencing were performed.

We identified homozygous loss-of-function mutations in *STK36* (c.1399delG, p.Glu467Argfs12Ter) in one PCD-affected individual with *situs solitus*. Transmission electron microscopy analysis demonstrates that *STK36* is required for cilia orientation in human respiratory epithelial cells. To analyse the localization of STK36 on the sub-cellular level, we performed IF microscopy of respiratory epithelial cells from the affected individual. STK36 appears to be not essential for recruiting RS head proteins to the axoneme since RSPH1, RSPH4A and RSPH9 are normally distributed in *STK36*-mutant cilia. However, an intact RS head is essential for axonemal recruitment of STK36 as demonstrated by the absence of STK36 from RS-mutant axonemes.

We propose that STK36 is not only essential for the assembly of components of the CP apparatus but also contributes to the functional integrity of the CP/RS-interaction, which provides an important step forward in understanding the complex biology of the CP/RS-interaction. *STK36* screening can now be included for this rare and difficult to diagnose PCD subgroup.

W5-06

Exome sequencing in human bladder exstrophy and developmental biology studies in zebrafish implicate SLC20A1 as a major regulator of urinary tract development

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Introduction: Exome sequencing in patients with bladder exstrophy-epispadias complex (BEEC) identified two novel de novo variants (p.G237R; p.V298A) and one novel maternaly transmitted variant (p.K441Q) from an affected mother to her affected son in SLC20A1. All three variants were predicted to be disease causing. The BEEC represents the severe end of human congenital anomalies of the kidney and urinary tract (CAKUT). To follow up on our genetic findings we investigated the developmental function of slc20a1a in developing zebrafish larvae (zfl) performing Morpholino (MO) knockdown (KD) experiments.

The urinary tract in zfl consists of two fused glomeruli and their corresponding pronephric ducts. The segmentation of the pronephros is similar to human nephrons. There is no urinary bladder described in zf, but the terminal parts of the collecting ducts, as most distal part of the pronephros, fuse and build a caudal opening at the cloaca of zf larvae. SLC20A1 encodes for a Na+/PO4 co-transporter known to play a role in proliferation and TNF-induced apoptosis. It is ubiquously expressed in mammals. It's zf orthologue slc20a1a is frequently used as a pronephric in situ hybridization (ISH) marker.

Methods: Slc20a1a KD in zfl was done by injecting antisense oligonucleotides with a morpholino® backbone in 1-4 cell staged eggs, blocking the gene's translation. Specificity of the MO was shown by Western Blot and rescue experiments, co-injecting human mRNA transcripts of SLC20A1. We characterized the MO phenotypes using different assays such as sulforhodamine 101 excretion, ISH with several urogenital marker

probes, immunohistochemistry (IHC) staining and the transgenic zf reporter line Tg(wt1b:GFP). IHC was performed on paraffin sections using proliferation and apoptosis markers.

Results: MO KD of Slc20a1a in zfl results in a severe lethal phenotype that affects development of different organ systems. We see a grading of lethality and severity of the phenotypes depending on the strength of KD, identifying SLC20A1 as a major regulator of zf development. Focusing on urinary tract, we see formation of pronephric cysts and disorganization of the cloaca. Sulforhodamine 101 assay uncovers that there are severe opening defects of the cloaca for the intestine in KD. IHC shows defects in proliferation and apoptosis compared to control zfl.

Discussion: Our data suggests slc20a1a as a major player in zf development. Mild MO phenotypes present with urinary tract anomalies such as pronephric cysts and disorganization of the cloaca. Severe MO phenotypes present with hydrocephalus, vertebral defects, disorganization of the eyes and cardiovascular anomalies with a high lethality among zfl - underlining the overall importance of slc20a1a during embryonic development. In conclusion, exome sequencing in human bladder exstrophy and developmental biology studies in zfl implicate SLC20A1 as a candidate gene for human BEEC and as a major regulator of urinary tract development.

W6 CANCER GENETICS

W6-01

Rare ADAR and RNASEH2B variants and a type I interferon signature in glioma and prostate carcinoma risk and tumorigenesis

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In search of novel germline alterations predisposing to tumors, in particular to gliomas, we studied a family with two brothers affected by anaplastic gliomas, and their father and paternal great-uncle diagnosed with prostate carcinoma. In this family, whole-exome sequencing yielded rare, simultaneously heterozygous variants in the Aicardi-Goutières syndrome (AGS) genes *ADAR* and *RNASEH2B* co-segregating with the tumor phenotype. AGS is a genetically induced inflammatory disease particularly of the brain, which has not been associated with a consistently increased cancer risk to date. By targeted sequencing, we identified novel *ADAR* and *RNASEH2B* variants, and a 3- to 17-fold frequency increase of the AGS mutations *ADAR*,c.577C>G;p.(P193A) and *RNASEH2B*,c.529G>A;p.(A177T) in the germline of familial glioma patients as well as in test and validation cohorts of glioblastomas and prostate carcinomas versus ethnicity-matched controls, whereby rare *RNASEH2B* variants were significantly more frequent in familial glioma patients. Tumors with *ADAR* or *RNASEH2B* variants recapitulated features of AGS, such as calcification and increased type I interferon expression. Patients carrying *ADAR* or *RNASEH2B* variants showed upregulation of interferon-stimulated gene (ISG) transcripts in peripheral blood as seen in AGS. An increased ISG expression was also induced by *ADAR* and *RNASEH2B* variants in tumor cells and was blocked by the JAK inhibitor

Ruxolitinib. Our data implicate rare variants in the AGS genes *ADAR* and *RNASEH2B* and a type I interferon signature in glioma and prostate carcinoma risk and tumorigenesis, consistent with a genetic basis underlying inflammation-driven malignant transformation in glioma and prostate carcinoma development.

W6-02

BRIP1- A high risk gene for late-onset familial ovarian cancer, but not for familial breast cancer

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Purpose: Germline mutations in the *BRIP1* (BRCA1-interacting protein C-terminal helicase 1) gene were mostly associated with ovarian cancer (OC)[1]. The impact of *BRIP1* mutations on breast cancer (BC) risk remains elusive. A case-control study with familial index cases was performed to assess the role of deleterious *BRIP1* germline mutations in BC/OC predisposition.

Methods: 5,668 well-characterized index patients with familial BC, 611 index patients with familial OC and 2,189 geographically-matched female controls (GMC) were screened for loss of function mutations (LoFs) via Next Generation Sequencing (NGS) and Sanger Sequencing. All patients were tested negative for pathogenic *BRCA1/2* mutations. The results were compared with FLOSSIES, ExAC and GMCs.

Results: *BRIP1* LoF mutation carriers show a high OC risk for familial cases (OR=20.97, 95%CI=12.02-36.57, P<0.0001). A mean age of first diagnosis (AAD) of OC for *BRIP1* mutation carriers of 61 years was observed (range 26-76 years), which tended to be older than in the overall sample of familial OC index patients (mean AAD 54 years, range 20-93 years). No association between *BRIP1* LoFs and the BC phenotype was observed in 4,641 familial BC index cases without a family history of OC (OR=1.42, 95%CI=0.70-2.90, P=0.3030). In 1,027 familial BC index patients with a family history of OC, the *BRIP1* mutation prevalence was significantly higher than that observed in controls (OR=3.59, 95%CI=1.43-9.01; P=0.0168).

Conclusion: We suggest that *BRIP1* represents a high risk gene for late-onset OC. To avoid ambiguous results, studies aimed at assessing the impact of candidate predisposing gene mutations on BC risk should differentiate between BC index patients with a family history of OC and those without. We conclude that the elevated mutation prevalence in familial BC index patients with a family history of OC is caused by the occurrence of OC in these families.

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W6-03

Germline loss-of-function variants in the BARD1 gene are associated with familial breast cancer

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Purpose: Recent studies revealed an association of *BARD1* (BRCA1 associated RING domain 1) germline loss-of-function (LoF) variants with an increase in breast cancer (BC) risk.[1,2] Here, we aim to determine the mutation prevalence of *BARD1* in a sample of well-characterized familial BC index patients of German descent and analyze potential associations with familial BC to validate *BARD1* as a diagnostic target gene. Additionally, we characterize copy number variations (CNV) in the *BARD1* gene in a new diagnostic setting to estimate the role of deleterious *BARD1* germline mutations in BC predisposition.

Methods: We screened a sample of 3,373 *BRCA1/2* negative, female BC index patients, which met the inclusion criteria of the German Consortium for Hereditary Breast and Ovarian Cancer (GC-HBOC), and 2,743 geographically matched female controls for LoF variants in *BARD1* by next generation sequencing employing customized gene panels (Agilent). Additional control datasets (ExAC, FLOSSIES) were included for the calculation of odds ratios (OR). For CNV detection, the SOPHiA DDM® platform (version 4.7.2.2, SOPHiA GENETICS®) was applied. Identified CNVs were verified by multiplex ligation-dependent probe amplification (MLPA) assay covering all 11 exons of the *BARD1* gene.[3] Real-time PCR and subsequent junction fragment PCR was carried out for breakpoint analysis.

Results: We identified 14 LoF variants (excluding CNVs) in 3,373 female BC index patients (carrier frequency (CF) = 0.42%) compared with 43 in a total of 37,241 controls (CF=0.12%, OR=3.61, 95%CI=1.88-6.81, p=0.00018). The CF of each control dataset was as follows: GMCs 0.07%, FLOSSIES 0.11% and ExAC 0.12%. For *BARD1* mutation carriers the mean age of first diagnosis was 41.7 years (24-60) compared with 47.3 years (17-92) in the overall sample. Furthermore, three CNVs including their breakpoints were characterized in a smaller cohort of 2,810 familial BC index patients.

Conclusion: Our study of BC index patients showed a significant association of LoF variants with an increased BC risk and confirms *BARD1* as a moderate/low penetrant risk gene. Since approximately 20% of LoF variants are CNVs, their inclusion is crucial for a comprehensive genetic screening of BC patients.

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W6-04

Analysis of chromatin architecture and DNA-methylation in paediatric B-cell lymphoblastic leukaemia

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Background: The lymphoid leukaemias are the most common neoplastic diseases in childhood constituting around 30% of cancers before the age of 15 years. During recent years a continuous increase in incidence has been observed. The causes of childhood leukaemia are not sufficiently understood and novel treatment targets are needed to improve survival rates of aggressive leukaemias. Although the genetic alterations in paediatric ALL are well characterised, the epigenome of paediatric ALL is less understood.

Material & Methods: To characterize the epigenome of paediatric ALLs we analysed so-called reference epigenomes defined by the International Human Epigenome Consortium (IHEC) consisting of chromatin immunoprecipitation-sequencing of 6 histone marks, RNA-sequencing data and whole-genome bisulfite-sequencing data. Complete datasets of 15 paediatric ALL patients of the following subgroups were included: 3 of each: BCR-ABL, ETV6-RUNX1, KMT2A rearranged and 2 of each: hyper- and hypodiploid, and TCF3-PBX1. Beyond epigenomic data, also clinical data, comprehensive genomic data, characterised by means of molecular cytogenetic analyses, SNP-array and whole exome sequencing data will be integrated in the analysis. In addition to the comparison between the different subgroups, we analysed epigenomic data of benign B-cell progenitors as controls.

Results: Cohort-wide analyses of chromatin determinant regions revealed 18,692 paediatric subtype specific chromatin determinant regions. Homer and KEGG pathway enrichment analysis revealed significant enrichment of terms like proteoglycans in cancer, hedgehog signaling, signaling pathways regulating pluripotency of stem cells and lymphoid neoplasms. Moreover, each ALL subtype segregates into distinct clusters by Multiple Correspondence Analysis (MCA), even if the MCA is performed not genome wide, but for the individual autosomes.

In addition, analysis of average genome-wide CpG methylation showed all paediatric ALL subgroups to be hypomethylated in comparison to benign precursor B-cells. We identified in average 1,064 differentially methylated regions (DMRs) with ten or more CpGs between the different ALL subgroups and 117 DMRs between the ALLs and the non-neoplastic controls. We observed an enrichment of DMRs in distinct regulatory elements of the genome.

Conclusions: In summary, our primary focus on chromatin architecture and DNA-methylation analysis already provides novel insights into the epigenomic regulation of various subtypes of pediatric ALL. The integration of epigenomic, transcriptomic, genomic and clinical analysis in pediatric ALL will decipher subtype-specific modifications and deregulated pathways, which can potentially be used as prognostic markers or elude treatment options.

W6-05

Identification of prognostic genetic markers via transcriptomics in children with precursor-B acute lymphoblastic leukemia (pB-ALL)

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Background: Acute lymphoblastic leukemia (ALL) is the most frequent leukemia in children. Chromosome aberrations leading to gene fusions are an important factor used for prognostification, risk stratification and therapy decision in precursor B-ALL (pB-ALL). However, in about 33 % of pB-ALL cases – the so-called B-other ALL -, no stratification marker can be identified with standard diagnostics. Within this subgroup, around 10 % of cases have been shown to carry genetic lesions leading to rare but recurrent fusions of cytokine-receptor-genes and tyrosine-kinase receptor-genes. Recent studies describe these aberrations as 'targetable lesions' because of the promising responsiveness e.g. towards tyrosine-kinase-inhibitors.

It was the aim of our study to investigate a large cohort of children with pB-ALL using RNA sequencing (RNASeq), for the detection of gene fusions.

Methods: RNASeq was performed on 99 consecutive patients (n=41 female, n=58 male) with pediatric pB-ALL in parallel to standard diagnostics using classical karyotyping and fluorescent in-situ hybridization (FISH) and evaluated for informative output. We used the TruSight RNA Pan-Cancer Panel (Illumina), which allows detection of 1385 cancer-associated genes as well as their fusion partner. Sequencing was performed on a MiSeq sequencer (Illumina). Bioinformatic analysis for the detection of fusion genes was performed via Base Space Apps (Illumina): RNA-Seq Alignment und TopHat Alignment.

Results: Within this cohort of 99 patients, RNASeq was able to detect all fusion transcripts (ETV6-RUNX1, PBX1-TCF3) that have been detected by conventional FISH analysis (n = 20). Moreover, RNASeq was able to further characterize chromosomal translocations that had been detected by classical cytogenetic analysis (n=7). Here, unknown fusion gene partners involved in these translocations could be identified using RNASeq (e.g. JAK2-TERF2, PICALM-MLLT10, KMT2A-AFF4). In 82 cases no genetic marker used for stratification according to the criteria of the ALL BFM study group had been detected using standard cytogenetics. In these cases RNASeq was able to identify fusion transcripts in 19 (23.2 %). The identified included JAK2-MPRIP, CRLF2-P2RY8, MYO1G-PAX5 - fusion transcripts that had been associated with pB-ALL and that may serve as therapeutic targets with established tyrosine kinase inhibitors.

Conclusions: Our aim was to evaluate the benefit of RNASeq in ALL diagnostics compared to current diagnostic methods. This study shows that stratification relevant genetic markers in pB-ALL, e.g. ETV6-RUNX1, detected via cytogenetics were reliably detected using RNASeq (17.2 % of cases). Moreover, new fusion genes that may have prognostic or therapeutic relevance were identified. Thus, RNASeq seems to be as reliable as karyotyping and FISH in the detection of fusion genes and may even replace these techniques in the future.

W6-06

IG-MYC-positive leukemia and lymphoma with precursor B-cell phenotype are genetically and epigenetically distinct from Burkitt lymphomas

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Burkitt lymphoma (BL) is a mature aggressive B-cell lymphoma which is the most common lymphoma in childhood. BL involves extranodal sites and/or bone marrow as acute leukemia. A rare BL-subset, called B-lymphoblastic leukemia/lymphoma (B-ALL/LBL), has been described showing a different immunophenotype to BL. B-ALL/LBL is characterized by expression of B-cell markers such as CD19 and CD79A, and immature cell markers including terminal deoxynucleotidyl transferase (TdT) and CD34, and lacking CD20 and surface immunoglobulin expression. Nevertheless, this subset harbors the hallmark event of BL, the IG-MYC translocation leading to deregulation of the MYC oncogene. The reason for the immature phenotype is unclear. The differential diagnosis between BL and B-ALL/LBL is important with regard to treatment strategies. Hence, the aim of the project was to analyze the genetic and epigenetic profile of this rare subset of MYC+B-ALL/LBL (MYC+LBL).

We recruited 12 MYC+LBL patients expressing TdT and harboring an IG-MYC translocation. Of these, we could extract DNA from 11 patients which were analyzed for genetic alterations by whole–exome sequencing (WES, N=5 patients) and OncoScanTM CNV FFPE arrays (N=11 patients). In addition, a cell line model for MYC+LBL was analyzed using whole-genome sequencing (WGS) and OncoScanTM CNV FFPE array. Using DNA methylation analysis, the cell of origin of 2 MYC+LBL was assessed by HumanMethylationEPIC BeadChip array in comparison to 5 BL samples, 209 published acute lymphatic leukemia (ALL) samples and 93 benign B-cell samples from various differentiation states.

In a first step, we screened the WES and WGS data for reads containing the IG-MYC breakpoint junctions. These could be retrieved in 2 cases and the cell line and were validated by PCR and Sanger sequencing. The breakpoints showed features of aberrant VDJ recombination which indicates that the IG-MYC translocation arose in precursor B-cells. Using WES, recurrent mutations in NRAS and KRAS genes were identified. These mutations were located in hotspot regions recurrently altered in ALL. After screening of the 6 additional MYC+LBL without WES data for NRAS and KRAS mutations, we identified in a total of 6/11 cases RAS mutations. By analysis of the copy number profile of the cases, we detected a gain in 1q21.1-q44 in 7/11 MYC+LBL which is also recurrently gained both in ALL and BL. Finally, we performed a phylogenetic tree analysis on loci becoming differentially methylated during B-cell development. We could show that MYC+LBL and ALL cluster together with the precursor B-cells whereas the BL cluster towards the mature B-cells.

Taken together, based on the IG-MYC breakpoint analysis, the mutational landscape and the epigenetic profiling we could show a precursor B-cell origin of the MYC+LBL. Hence, we can conclude that MYC+LBL is a B-cell precursor disease which likely acquires a L3 morphology by the IG-MYC translocation and should not be classified as Burkitt lymphoma.

W7 BONE AND SKIN

W7-01

Genetic and phenotypic dissection of a neglected patient population with early onset osteoporosis reveals a significant contribution of monogenic disorders

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Osteoporosis is a common clinical problem affecting 22% of females and 7% of males above the age of 55 years. On the other hand, there are rare pediatric disorders with bone fragility, most commonly osteogenesis imperfecta. In between these categories are adults with increased bone fragility before the age of 55 years/before menopause (early-onset osteoporosis, EOOP) for whom neither guidelines nor approved drugs exist. We aimed at a clinical, radiological, and genetic characterization of a cohort of 300 EOOP patients recruited by the National Bone Board. Gene panel sequencing revealed clearly pathogenic mutations in known genes associated with bone fragility in 20% of the patients. The majority of mutations were found in the genes encoding type 1 collagen, followed by genes involved in WNT signaling, and several other genes for rare bone disorders. In addition, a significant overrepresentation of SNPs associated with postmenopausal osteoporosis was detected in our cohort. Mutation-negative and -positive patients showed differences in their bone phenotype. Using statistical phenotype-genotype correlation we defined criteria to predict whether an EOOP patient is likely to suffer from a rare bone fragility disorder and therefore should undergo genetic testing. The next steps are an analysis of the correlation between genotype and treatment response and genome sequencing of mutation-negative cases. Our study demonstrates that rare disorders can be identified among patients with a common diagnosis as one strategy of putting the principles of precision medicine into practice.

W7-02

Identification of microRNAs in human neural crest cells and their potential implication in nonsyndromic cleft lip with/ without cleft palate

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Nonsyndromic cleft lip with or without cleft palate (nsCL/P) is a common complex genetic malformation. While the application of GWAS partially explains the underlying genetics, molecular interpretation of these findings is still scarce. So far, 40 risk loci for nsCL/P have been identified, 38 of which are located in noncoding regions. This suggests that regulatory elements contribute to nsCL/P development. One possible mechanism by which these regions might exert their regulatory effect on gene expression is the control of microRNA (miRNA) expression in disease relevant tissue. MiRNAs are small, noncoding RNAs that alter the abundance of their expressed target genes via binding and subsequent degradation or translational repression. Previous studies have shown that changes in miRNA sequences are involved in the development of orofacial clefts. In the present study, we sought to identify candidate miRNAs in human neural crest cells (hNCCs), a precursor cell population of facial tissues that might be involved in nsCL/P.

First, we performed miRNA profiling in four independent hNCC samples, generated from induced pluripotent stem cells, on an Affymetrix miRNA 4.0 chip. All samples showed high inter-sample correlations. MiRNAs were considered as likely relevant candidates if showing (i) a detection P value $P<0.01$ in all samples, or (ii) a prior report in craniofacial development plus detection in at least one sample at $P<0.01$. In total, 152 miRNAs were identified (125 miRNAs according to (i), and 27 according to (ii)). Among the first group was e.g. miR-4668-5p that showed consistently high expression. *MiRWalk 2.0* was used to identify target genes of all candidate miRNAs. These targets were filtered for expression in hNCCs using RNA-Seq data obtained from 3‘Lexogen transcriptome analyses in the same four hNCC samples. This resulted in identification of 7,137 expressed target genes. To prioritize miRNAs for functional follow-up we have started to integrate the genetic data of an in-house GWAS on nsCL/P, with the miRNA loci. Within 1kb of the candidate miRNAs, we identified

25 variants that showed a P-value <0.01 in the GWAS data, including rs41280157 at 9q31.3 (P= 7.32E-05) at the miR-4668 locus. To identify whether these are false-positive findings or true signals with small effect sizes, we are currently performing a replication analysis in additional 223 patients and 968 controls. Based on these results, which will be presented at the conference, functional work-up of selected miRNAs will be performed *in vivo* using zebrafish as model system.

Our study will help to reveal regulatory interactions of noncoding regions in a precursor cell population of facial tissues relevant to nsCL/P pathology. By integrating those findings with the genetic information of patients we hope to get insight into the functional correlation of risk alleles and their potential connection with miRNAs.

W7-03

Identification of novel candidate genes for idiopathic short stature using whole exome sequencing

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Short stature is a common medical concern in childhood and affects 3 % of the general population. We recently demonstrated that the application of exome sequencing in patients with idiopathic short stature is able to identify disease causing mutations in genes associated with short stature in 19 % of patients. After recruitment and clinical characterization of a large study group of 565 families with idiopathic short stature, we performed exome sequencing in 211 patients without mutations in known short stature associated genes for potential candidate genes. Variants were assessed for a potential effect on the gene and its product using multiple lines of evidence including expression in chondrocytes.

We identified 21 genes mutated in at least two patients. Six were especially strong candidates (*CPZ*, *EDEM3*, *FBRS*, *RASA3*, *SLC7A8* and *USP45*) as at least two patients carried likely pathogenic or pathogenic variants. Gene Ontology analyses showed that the resulting proteins participate in protein degradation, transcriptional regulation and protein transport. One outstanding candidate gene, *RASA3*, is a member of the RAS-MAPK pathway. Mutations in other members of this pathway are known to cause diseases of the RASopathy complex, with short stature as a main symptom. The two patients carrying *de novo* mutations in *RASA3* share some clinical characteristics with other RASopathy patients, especially short stature, a barrel shaped chest, and café au lait spots in one patient. In collaboration, we recently identified a third patient with a *de novo* mutation in *RASA3*. As GH treatment is discussed for some RASopathy patients, successful application in one of our patients suggested that GH might be indicated in the treatment of these patients. The identification of one nonsense mutation proposes a loss of function mechanism. Consequently, we are performing functional analyses on CRISPR-Cas9 mediated knockout cells to confirm a proposed hyperphosphorylation of genes of the RAS-MAPK pathway.

In conclusion, using exome analyses in patients with idiopathic short stature, we found 21 strong candidate genes in 40 patients. Thus, our data highlight genes with growth related function. Exome sequencing can therefore be of great value to identify the underlying genetic cause in these individuals.

W7-04

Missense and splicing mutations in the retinoic acid catabolizing enzyme CYP26C1 in idiopathic short stature

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Height is a complex quantitative trait with a high heritability. Short stature is diagnosed when height is significantly below the average of the general population for that person´s age and sex. We have recently found that the retinoic acid degrading enzyme CYP26C1 modifies SHOX deficiency phenotypes towards more severe clinical manifestations. Here, we asked whether damaging variants in CYP26C1 alone could lead to short stature. We performed exome and Sanger sequencing to analyze 706 individuals with short stature where SHOX deficiency was previously excluded. Four different damaging missense variants and one splicing variant were identified and their functional significance tested in vitro or in vivo using Zebrafish as a model. Although incomplete penetrance was observed in one case, the genetic and functional data reported here strongly suggest that CYP26C1 represents a novel gene underlying growth disorders and that damaging variants in the absence of SHOX mutations can lead to short stature.

W7-05

Periodontal Ehlers-Danlos syndrome is related to aberrant posttranslational processing and/or secretion of mutated complement 1 subunits C1r and C1s

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Periodontal Ehlers–Danlos syndrome (pEDS) is a connective-tissue disorder characterized by early and severe periodontitis and various joint and skin manifestations. Other EDS subtypes are caused by mutations in connective tissue proteins such as collagens or protein-modifying enzymes. In contrast, periodontal subtype is caused by heterozygous missense or in-frame insertion/deletion mutations in *C1R* or *C1S* which code for subunits C1r and C1s of complement 1. This finding opens a previously unknown connection between the inflammatory complement pathway and connective tissue homeostasis. Pathogenesis of pEDS involves a gain-of-function effect as *C1R/C1S* null mutations are asymptomatic when heterozygous and cause a lupus-like phenotype when homozygous. We now report extensive functional studies of all known pEDS mutations. *In vitro* overexpression in HEK cells show that all pEDS mutations retain enzymatic function in the complement cascade but show domain-specific abnormalities of intracellular processing and secretion. For instance, mutations within the C1r CCP1 domain lead to retention inside the cell, whereas mutations located in the CUB2 domain are in part secreted as aggregates. Normal secretion was observed for one *C1R* missense mutation that affects a C1q binding site. Immunofluorescence analyses indicate a non-classical secretion pathway for several C1r/s mutant proteins: whereas C1r WT co-localizes with the trans Golgi Network (TGN), mutant C1r proteins studied are diffusely distributed within TGN and the cytosol. Moreover, we observed vesicle formation exclusively for C1r WT proteins so far. Patient fibroblasts show mild enlargement of the endoplasmic reticulum compatible with a derangement of intracellular processing. We hypothesize that the unique connective tissue and periodontal inflammatory phenotype reflects abnormal interaction of the mutant complement subunits with collagens or other ECM proteins and an abnormal function of the classical complement pathway. This effect may be most prominent in the oral region characterized by a high bacterial load. Clarifying the effects of ER stress due to misfolded C1r/s as well as the impact of alternative interaction partners of mutated C1r/s will support understanding the disease pathogenesis of both pEDS and other connective tissue pathologies.

W7-06

Mutations in SULT2B1 cause autosomal recessive congenital ichthyosis in humans

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Ichthyoses are a clinically and genetically heterogeneous group of genodermatoses characterized by abnormal scaling of the skin over the whole body. To date, mutations in nine genes are known to cause non-syndromic forms of autosomal-recessive congenital ichthyosis (ARCI). However, not all genetic causes for ARCI have so far been discovered. Using whole exome sequencing (WES) and multigene panel screening we identified six ARCI individuals from three unrelated families with mutations in Sulfotransferase family 2B member 1 (*SULT2B1*), showing their causative association with ARCI.

Cytosolic sulfotransferases form a large family of enzymes that are involved in the synthesis and metabolism of several steroids in humans. Overall, we identified four distinct mutations including missense, nonsense and splice site mutations. We demonstrated the loss of SULT2B1 expression at RNA and protein levels in keratinocytes from individuals with ARCI by functional analyses. Furthermore, we succeeded in reconstructing the morphologic skin alterations in a 3D organotypic tissue culture model with *SULT2B1*-deficient keratinocytes and fibroblasts. By thin layer chromatography (TLC) of extracts from these organotypic cultures we could show the absence of cholesterol sulfate, the metabolite of SULT2B1, and an increased level of cholesterol indicating a disturbed cholesterol metabolism of the skin upon loss of function mutation in *SULT2B1*.

In conclusion, our study reveals an essential role for SULT2B1 in the proper development of healthy human skin. Mutation in *SULT2B1* leads to an ARCI phenotype via increased proliferation of human keratinocytes, thickening of epithelial layers, and altered epidermal cholesterol metabolism.

W8 NEURODEVELOPMENT

W8-01

Abnormal protein glycosylation in AAMR-syndrome

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Recently we identified mutations in GMPPA underlying a rare autosomal recessive disorder characterized by achalasia (swallowing difficulties), alacrima (reduced/absent tear secretion) and mental retardation often in combination with other neurological symptoms (AAMR-syndrome) such as gait abnormalities. GMPPA is a homologue of GMPPB (GDP-Mannose-Pyrophosphorylase-B), which catalyzes the formation of GDP-mannose from mannose-1-phosphate. GDP-mannose is an essential substrate for glycosylation reactions. Mutations in GMPPB underlie congenital myasthenic syndromes (CMS), inherited disorders with mutations in genes encoding proteins essential for the integrity of neuromuscular transmission. These disorders often show involvement of motor endplates and muscular dystrophy. A major subgroup of CMS is associated with glycosylation defects of alpha-dystroglycan (α-DG). GMPPA itself does not have catalytic activity and we hence proposed that GMPPA might be an allosteric inhibitor of GMPPB. Along this line we observed increased GDP-Mannose levels in lymphocytes of GMPPA-patients. To further characterize the pathophysiology of AAMR-syndrome we have now established a mouse model with a targeted disruption of GMPPA. These mice display a progressive neurological phenotype with impaired gait and learning deficits but lack alacrima and achalasia. Consistent with our assumption that GMPPA serves as an allosteric inhibitor of GMPPB we find increased GDP-Mannose levels in different tissues from knockout mice. Moreover, we can show a hyperglycosylation of selected proteins with functional consequences for synaptic transmission. Thus our data add AAMR to the growing list of congenital disorders of glycosylation, in which dysregulation rather than mere enzyme deficiency is the basal pathophysiological mechanism.

W8-02

Identification of further individuals with de novo CTCF mutations refines the phenotypic spectrum, and altered dosage causes locomotor defects in Drosophila

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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 essential for establishing and maintaining this organization. CTCF is involved in virtually all chromatin regulating processes including enhancer blocking, mediating enhancer-promotor interactions, 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 four patients with a surprisingly mild phenotype of variable developmental delay or intellectual disability, mild growth delay and microcephaly, and behavioral anomalies. Therefore we aimed at expanding the phenotypic spectrum associated with CTCF mutations and identified nine further individuals with de novo aberrations in CTCF. In a total of now 13 cases we identified five missense and four truncating mutations and one single exon deletion, all but two located in one of the 12 zinc-finger domains, and one larger deletion. The phenotype is highly variable with mild to severe intellectual disability (13/13), autistic features (6/13), microcephaly (8/13), short stature (6/13), congenital heart defects (5/13), palatal anomalies (3/13), and hearing loss (3/13).

Apart from two conditional knockout mouse models with brain malformations and early lethality or learning deficits, little is known about the role of CTCF in neurodevelopment, so far. Therefore, we utilized *Drosophila melanogaster* as a model to explore the role of CTCF in CNS development and function. Similar to observations in mice, ubiquitous depletion 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.

Using the negative gravitaxis assay to examine gross neurological function, we found a highly significant impairment of locomotor behavior in flies with Ctcf knockdown in neurons, motoneurons and muscle and in flies with overexpression of Ctcf in glia cells, muscle and motoneurons. We observed only minor alterations in synaptic morphology upon Ctcf dosage alteration utilizing larval neuromuscular junctions, an established model system for synaptic development.

We further delineate the mutational and clinical spectrum of CTCF-associated developmental phenotypes. Our findings of neurological anomalies upon manipulation of Ctcf dosage in the fly nervous system emphasize the role of Ctcf in nervous system development and function.

W8-03

The genetic etiology of early-onset epileptic encephalopathies: a combined whole-exome sequencing and high-resolution copy number study

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Epileptic encephalopathies (EE) represent a rare group of difficult-to-treat early-onset epilepsies accompanied by intellectual disability. They were long perceived as acquired and recent advances in genomic technologies evidenced a variety of mostly de novo germline mutations as underlying causes in a fraction of studied patients. To further uncover the etiology of EE we performed a comprehensive single center study applying high-resolution chromosomal microarray analysis and whole-exome sequencing in 63 independent

mostly non-consanguineous families. Assessment of pathogenicity included molecular modelling of missense variants and untargeted plasma-metabolomics in selected patients. We identified causative copy number variants in 6 patients (about 10%), (likely) pathogenic sequence variants of 14 known and 4 newly confirmed disease genes in 21 patients (about 33%), and compound heterozygosity for causative sequence and copy number variants in another patient (about 2%). 32% of diagnosed cases were caused by recessive genes, albeit with one allele occurring de novo in two instances. About 11% of patients were shown to harbor a secondary genetic disorder. Additionally, we delineated 18 novel high-level candidate genes for EE, which followed a recessive inheritance pattern in 50% of cases. Our comprehensive genetic study demonstrates a diagnostic yield of about 45% in otherwise undiagnosed epileptic encephalopathies and suggests a genetic cause in at least 67% of cases. We also illustrate the major role of recessive inheritance and consequently high recurrence risk even beyond consanguineous families.

W8-04

Chances and challenges associated with an exome-based diagnostic algorithm: a single center experience from 1,500 cases

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Advances in sequencing technologies revolutionized molecular diagnostics of patients with clinically and genetically heterogeneous rare diseases. While whole exome sequencing (WES) has proven its cost- and time-efficacy and has become standard of care as a first line diagnostic tool for many indications, in Germany, its broad application in clinical routine is still under debate. Reasons for this include political, financial, technical, and ethical aspects.

We here report on our experiences of exome sequencing across a cohort of 1,500 index cases with various different inclusion criteria analyzed by exome sequencing in a research as well as routine diagnostics context. We established a firm diagnosis in about 35 % of the patients. In another 15 % we identified variants of unknown significance and confirmatory follow-up studies are currently pending. In about half of the patients we failed to identify defects in known disease genes. However, in about 5 % we prioritized variants in candidate genes which have so far not been associated with human disease and which are currently under further investigation in a research context. Reporting of incidental findings was a highly discussed topic and was decided on at an individual basis by group decision in about 5 % of the cases. Prenatal exome trio-sequencing was performed in 8 cases and we established a fast-track pipeline including a multidisciplinary decision board.

The implementation of semi-quantitative copy number analysis as well as variant calling in mitochondrial DNA sequences further contributed to the diagnostic yield.

Unresolved cases are currently being included in follow-up trio-analysis, genome, and transcriptome sequencing. While the bioinformatic tools to take full advantage of combinatory genome and transcriptome datasets are still under development, trio sequencing with a filter for de novo changes lead to the prioritization of several predictively low-impact (e.g. nearsplice) variants in known disease genes.

W8-05

Large-scale systematic analysis of recessive neurodevelopmental disorders in consanguineous families

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Consanguineous marriages are common in about one-fifth of the world's population. In children from these marriages, homozygous mutations are frequent and lead to a doubling of birth defects. To analyze the contribution of homozygous recessive mutations to developmental brain diseases, we assembled a cohort of more than 2,500 families with neurodevelopmental disorders (NDDs) from the Greater Middle East. These families have been extensively phenotyped, have high rates of consanguinity, and have disorders consistent with an autosomal recessive inheritance pattern. We performed whole-exome sequencing across these families to systematically discover putatively causal variants in both known and novel disease genes. For about half of our families, we identified likely deleterious variants in previously published disease genes. These mutations affected more than 400 genes, highlighting the extreme genetic heterogeneity of

neurodevelopmental disorders. Roughly 70 of these genes have previously been implicated in only a single case, and our study provides additional evidence to confirm that these are true disease genes. Current work is ongoing to identify recurrently mutated novel disease genes by comparing mutational burden between our cohort and ethnically matched unaffected individuals. Our solve rate was strongly influenced by consanguinity rates and phenotypic features of affected individuals. Cases with high levels of parental relatedness and with pathognomonic brain abnormalities had the highest solve rates. Analyzing recessive disease genes as a whole showed that in contrast to dominant/sporadic/haploinsufficient disease genes, recessive disease genes experience weaker evolutionary constraint. Most pathogenicity prediction scores perform worse on recessive disease genes, particularly those based predominately on evolutionary constraint. About half of the identified recessive mutations were predicted to produce a truncated protein, and half were missense variants that affect highly conserved amino acids. We predict that these recessive missense variants have a loss-of-function phenotype, as they did not cluster to the same degree as missense variants from dominant disorders. As to be expected, recessive disease genes shower higher brain expression levels compared to genes not associated with NDDs, particularly during prenatal development. Furthermore, recessive and dominant disease genes differed significantly in predicted functions and biological processes. Recessive genes were constrained to distinct processes, which can tolerate dosage reduction.

W8-06

Spatial Clustering of de Novo Missense Mutations Identifies Candidate Neurodevelopmental Disorder-Associated Genes

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Haploinsufficiency (HI) is the best characterized mechanism through which dominant mutations exert their effect and cause disease. Non-haploinsufficiency (NHI) mechanisms, such as gain-of-function and dominant-negative mechanisms are often characterized by the spatial clustering of mutations, thereby affecting only particular regions or base pairs of a gene. Variants leading to haploinsufficency might occasionally cluster as well, for example in critical domains, but such clustering is on the whole less pronounced with mutations often spread throughout the gene. Here we exploit this property and develop a method to specifically identify genes with significant spatial clustering patterns of de novo mutations in large cohorts. We apply our method to a dataset of 4,061 de novo missense mutations from published exome studies of trios with intellectual disability and developmental disorders (ID/DD) and successfully identify 15 genes with clustering mutations, including 12 genes for which mutations are known to cause neurodevelopmental disorders. For 11 out of these 12, NHI mutation mechanisms have been reported. Additionally, we identify three candidate ID/DD-associated genes (*ACTL6B, GABBR2, PACS2*) of which two have an established role in neuronal processes. We further observe a higher intolerance to normal genetic variation of the identified genes compared to known genes for which mutations lead to HI. Finally, 3D modeling of these mutations on their protein structures shows that 81% of the observed mutations are unlikely to affect the overall structural integrity and that they therefore most likely act through a mechanism other than HI.

W9 TECHNOLOGIES AND BIOINFORMATICS

W9-01

Long-read sequencing – for detecting clinically relevant structural variation

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Current whole genome sequencing approaches are extremely successful in identifying disease causing mutations. Nonetheless, these methods yield data that is incomplete, and fall short of detecting all clinically

relevant genetic variants present in an individual human genome. Specifically, short-read technologies are not optimal for the identification of structural variation (SV) and of variation in repeat regions.

We anticipate that long-read sequencing techniques may augment genetic diagnosis for a clinically well-characterized patient population with intellectual disability that we have studied over the years. These samples have been previously analyzed extensively with CNV-microarrays, whole exome sequencing (de Ligt, NEJM 2012) and by short-read whole genome sequencing (Gilissen, Nature 2014). All previous analyses failed to detect a causal variant. We performed long-read SMRT-sequencing in five such patient-parent trios with coverages up to 45-fold.

Per individual genome we identify up to 25,000 SVs. Preliminary comparison shows that about >60% of SVs are novel compared to high-depth short-read sequencing. These previously hidden SVs include repeat expansions and contractions; segmental duplications; centromere and telomere near regions; and gaps in the reference genome.

Using the trio approach we systematically identify de novo SVs for each patient. Validations of up to 88 candidate de novo SVs per case are ongoing. Next to identifying novel SVs our data also confirm that long-read sequencing data provide coverage in previously uncovered genome regions. In total, we identify 20Mb of sequence that was not covered reliably by short-read data. Furthermore, long-read sequencing data facilitate phasing of de novo SNVs and compound heterozygous variants.

In summary, long-read sequencing identifies a significant number of previously hidden SVs; allows sequencing previously inaccessible regions of the human genome and allows phasing of genetic variants. The de novo mutation rates as well as the clinical relevance of these previously hidden genetic variants can now be further explored.

Conflict of interest:

A.M.W., P.B., L.H., J.K., K.C. and J.K. are employees and shareholders of Pacific Biosciences, a company commercializing DNA sequencing technologies. The other authors declare no conflict of interest.

W9-02

Long-read sequencing goes clinical

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Next generation sequencing has revolutionized the field of human genetics by offering new possibilities to unravel human diseases. Due to limitations of short read sequencing however, various more traditional tests including single gene sequencing, MLPA or genescan are still performed, leading to a complex landscape of different available genetic tests running on a variety of platforms. The ideal scenario would be to have one generic test. We think that such a ‘one test fits all’ strategy will be an efficient and cost-effective way to perform genetic testing.

Long-read sequencing is still too expensive to be offered routinely for genome analysis. However, long-read sequencing does provide an opportunity to move a step closer towards this objective. To get a feeling for both the platform and underlying chemistry we started with (long-read) amplicon-based sequencing on the Sequel (PacBio). We experimented with different amplicon-based enrichment strategies (up to 16kb), and learned that long-read sequencing works well for HLA typing, mtDNA, repeat-disease regions or long range amplicons designed to avoid pseudogene regions (i.e. PMS2). We also reasoned that small amplicons (< 1kb) can be sequenced accurately on a long-read sequencer and therefore started to transfer our amplicons based workflows from the IonTorrent PGM towards the Sequel. Ultimately, we aim to combine three different workflows into one amplicon based sequencing run (i.e. Sanger amplicon based (0.5-1kb), long read amplicon based sequencing (2-8kb), and repeat expansion detection using amplicons). A LIMS-based automated workflow and an automated bioinformatic pipeline thereby facilitate streamlined sample processing and data analysis, and assure highest flexibility.

We will show how long-read sequencing provides new potential to current diagnostics. By combining different targeted approaches in a single test, we move away from the complexity of running multiple platforms in a clinical setting. We believe that long-read amplicon sequencing is a first step to make use of the advantages of long reads in NGS-based diagnostics. Once the price for (long-read) genomes is at a range acceptable for routine diagnostics, targeted sequencing approaches will ultimately be replaced by genome sequencing.

W9-03

Advances in computer-assisted syndrome recognition and differentiation in a set of metabolic disorders

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Significant improvements in automated image analysis have been achieved over the recent years and tools are now increasingly being used in computer-assisted syndromology. However, the recognizability of the facial gestalt might depend on the syndrome and may also be confounded by severity of phenotype, size of available training sets, ethnicity, age, and sex. Therefore, benchmarking and comparing the performance of deep-learned classification processes is inherently difficult.

For a systematic analysis of these influencing factors we chose the lysosomal storage diseases Mucolipidosis as well as Mucopolysaccharidosis type I and II, that are known for their wide and overlapping phenotypic spectra. For a dysmorphic comparison we used Smith-Lemli-Opitz syndrome as a metabolic disease and Nicolaides-Baraitser syndrome as another disorder that is also characterized by coarse facies. A classifier that was trained on these five cohorts, comprising 288 patients in total, achieved a mean accuracy of 62%.

The performance of automated image analysis is not only significantly higher than randomly expected but also better than in previous approaches. In part this might be explained by our large training sets. We therefore set up a simulation pipeline that is suited to analyze the effect of different potential confounders, such as cohort size, age, sex, or ethnic background on the recognizability of phenotypes. We found that the true positive rate increases for all analyzed disorders for growing cohorts (n=[10...40]) while ethnicity and sex have no significant influence.

The dynamics of the accuracies strongly suggest that the maximum recognizability is a phenotype-specific value, that hasn't been reached yet for any of the studied disorders. This should also be a motivation to further intensify data sharing efforts, as computer-assisted syndrome classification can still be improved by enlarging the available training sets.

W9-04

Deep Phenotyping for Deep Learning (DPDL): A searchable knowledge base which integrates genotype-phenotype information for clustering analysis in the phenotype space

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Motivation:

Most of databases that combine phenotype and genotype information are disease-oriented. For all clinical features with reduced frequency, it is difficult to distinguish whether they are linked to other symptoms. Furthermore, genotype-phenotype correlations cannot be studied by a database design that focuses on disorders. There is now a crucial need for a database which is case-based and does not aggregate phenotypic data.

Methods:

To build a computer-searchable database of case reports, in which genotype and phenotype information is curated on an individual's level. Clinical phenotypes are annotated with terminology of the Human Phenotype Ontology (HPO). In addition, raw image data, such as photos are collected and tagged with meta information such as the age when a picture was taken. On the molecular level, disease-causing mutation from a diagnostic report or variant calls from an entire exome are stored per individual. This database collects phenotype and genotype information from curated patient data and calculates phenotype and genotype scores by different scoring approaches such as Phenomizer and Combined Annotation Dependent Depletion (CADD). Image similarity score is calculated via CLINIC app form Face2Gene. These similarity scores are further integrated into the database.

Results:

We present the knowledge base Deep Phenotyping for Deep Learning (DPDL) as a public resource that aims at compiling phenotypic similarity scores for syndromes in a collection of molecularly diagnosed patients.

DPDL is a case-oriented database. Hence, all queries for co-occurring features or for correlations between mutations and the expressivity can be performed. By focusing on individual cases, our database is also robust to perform disease classification and can be used to evaluate incidences or rare disorders. Moreover, we visualize known molecular pathways in the phenotype space and proposing new hypothesis about potential molecular interactions by the clustering analysis.

W9-05

eDiVA – Classification and Prioritization of Variants for Clinical Diagnostics

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Whole-exome and whole-genome sequencing accelerated the identification of causal variants in Mendelian diseases, helping to link genetic variation and de novo mutations to disease phenotypes. Multiple tools are available for calling, annotating, and prioritizing of genetic variants. However, the low diagnostic rates between 25-50% reported in multiple studies indicate the need for improved pathogenicity classification and causal variant prioritization methods. Here we present eDiVA (exome disease variant analysis platform, http://ediva.crg.eu), a machine learning based framework for identification, annotation, pathogenicity classification and prioritization of genetic variants in WES-based parent-child trio and family analysis.

eDiVA integrates genomic, evolutionary and clinical features into a machine-learning based variant pathogenicity classifier (eDiVA-score). By comparison to state-of-the-art classification methods (e.g. CADD, Revel, M-Cap, Eigen) using thousands of pathogenic and benign variants from ClinVar, HGMD, VariBench and HumVar we demonstrate the favourable performance of eDiVA-score. Causal variant prioritization performance of eDiVA was benchmarked against pheno-db and PhenGen using thousands of semi-synthetic parent-childe trios generated by integrating ClinVar variants into real exome data (CEPH family from 1000GP). eDiVA consistently outperformed other methods on all tested segregation types (autosomal recessive homozygous, autosomal recessive compound heterozygous and autosomal dominant de novo) in terms of precision, recall and pathogenicity ranking.

In summary, the fully automated workflow, high precision and recall, and ease of use of eDiVA facilitate rapid diagnostics as well as periodic reanalysis of unsolved cases using new disease knowledge.

W9-06

Whole Exome Sequencing as a universal first line diagnostic test

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Whole Exome Sequencing (WES) has been adopted by many laboratories to detect single nucleotide variants (SNVs) in a diagnostic setting and was recently shown to be more efficient, less costly and less time consuming if performed as an 'Exome First Approach'. This requires WES to detect the vast majority of clinically relevant SNVs and indels that are currently being detected using Sanger sequencing and panel-based NGS approaches. The capability of WES detecting all such known diagnostic relevant variants is however understudied.

We here set out to determine the sensitivity of WES to identify 147.555 exonic and splice site variants previously reported to be of diagnostic relevance (class 3-5) in 3.623 genes genes. These variants were selected from HGMD professional and our in-house variant classification database. The detectability of SNVs and indels was determined based on the median sequence coverage at the corresponding genomic position (= nucleotide coverage) as well as on the median coverage at exon level (= exon coverage), using in 50 representative exomes.

Based on a nucleotide coverage of at least 20X, WES allows detection of 97% of previously identified diagnostically relevant variants. Importantly, with 97% of all protein-coding bases of diagnostically relevant genes covered well, WES is able to replace Sanger sequencing. A parallel (NGS-based) technology should however be considered to fill those WES coverage gaps highly prone to disease mutation. Our results show

that WES can be used as a first-line genetic diagnostic test for all genetic disorders , thereby facilitating a more efficient and uniform process for all genetic disorders.

POSTER

P-BASIC MECHANISMS AND EPIGENETICS

P-BasEpi-001

The contribution of maternal effect gene variants to the etiology of Imprinting Disorders

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While genomic causes of congenital imprinting disorders (deletions, duplications, upd, point mutations in single genes) are increasingly better understood and their relevance is obvious for genetic counseling, the causes of isolated epimutations (i.e. altered methylation marks at imprinted loci) are basically unclear. Although single cases with genomic alterations in cis and trans have been reported, resulting in secondary changes in methylation at imprinted loci and thus leading to the manifestation of an imprinting disorder (e.g. small deletions in 11p15.5, ZFP57), in the majority of cases the underlying cause of the epimutation remains unclear. The recent identification of a NLRP2 variant in a family with recurrent BWS was the first description of a mutation in a member of the NLRP gene family as the underlying genetic cause for an altered imprinting pattern. Recently, further variants could be identified in the NLRP5 and NLRP7 genes. Interestingly, not the patients themselves but their mothers were carriers of the mutations, therefore the variants are called “maternal effect” mutations. The pathological function of NLRP mutations is still unknown, but it has been suggested that these maternal effect genes influence the epigenetic reprogramming during oocyte development. The mothers themselves are clinically normal but can experience a history of pregnancy loss and liveborn offspring often present with multi-locus imprinting disturbance (MLID). The association between MLID and NLRP genes has been suggested recently, while the predisposition of NLRP7 mutation carriers for an increased risk of pregnancy complications and loss is well known.

We were able to identify further families with rare NLRP variants which were identified mainly because of altered imprinting marks of their offspring associated with clinical features, or because of recurrent pregnancy loss. We analyzed 12 families with an imprinting disorder/MLID by Whole Exome Sequencing, and we will present the clinical and molecular hallmarks of five families with NLRP gene mutations. Furthermore, we analyzed a cohort of >20 mothers of Silver-Russell syndrome patients with ICR1 hypomethylation for variants in maternal effect candidate genes to elucidate whether these factors also contribute to non-MLID imprinting disturbances. In conclusion, we could show that maternal effect mutations in NLRP genes are associated with MLID and/or reproductive failure, but they are not responsible for isolated epimutation

P-BasEpi-002

*** Genome-wide methylome and transcriptome analysis of embryos fathered by obese male and the corresponding sperm in a murine model of diet-induced obesity

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The prevalence of obesity is rising dramatically worldwide, leading to increased mortality rates from obesity-related health complications. Obesity is a multifactorial disease. In addition to an imbalance of calorie intake and calorie expenditure, genetic predispositions and epigenetic changes, based on the interaction of environment and genes, contribute to its etiopathogenesis. Obesity-relevant epigenetic modifications can be induced by adverse environmental exposures (i.e. to gestational diabetes) or be inherited inter-generationally through the germline. Previous studies already reported an association between paternal body weight and metabolic phenotype of the offspring.

The aim of this study is to elucidate the effect of paternal obesity on sperm methylation and development of the resulting embryos, and to identify candidate genes associated with possible long-term consequences in the next generation (transgenerational epigenetic inheritance). A murine model of diet-induced obesity was used to analyze sperm and the corresponding 8-cell embryos fathered by either obese (HFD) or control (CD) mice by Reduced Representation Bisulfite Sequencing (RRBS), Whole Genome Bisulfite Sequencing (WGBS) and "single-embryo" RNA sequencing (seRNAseq) to detect differential methylation and expression patterns in both cohorts. To avoid potential confounders, all obese male mice were mated with normal-weighted females. We analyzed sperm samples from 16 different mice (8 HFD vs. 8 CD) via RRBS as well as 6 embryos with WGBS and a total of 38 embryos (18 HFD vs. 20 CD) with seRNAseq.

A first analysis of almost five million CpG sites sequenced in total and 29,638 genes in the RNASeq dataset showed an overall weak effect on both sperm DNA methylation and gene expression in resulting embryos. Nevertheless, regional analysis of genome-wide methylation patterns revealed several differently methylated regions (DMRs), including one associated with *BCL11B*, which is known to be involved in adipogenesis. Among the top candidate genes differentially expressed between HFD and CD is *SAMD4B*, which has previously been described to play a role in body weight regulation in mice.

P-BasEpi-003

Transcription is the key – further evidence for the necessity of KCNQ1 transcription through the ICR2 in the maternal germline for imprint establishment in humans

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The chromosomal region 11p15.5 harbours two imprinted domains each regulated by its own imprinting control region (ICR). The parent-of-origin-specific expression of several imprinted genes of the centromeric domain (e.g. *KCNQ1* and *CDKN1C*) is regulated by the ICR2 (*KCNQ1OT1*:TSS-DMR). The ICR2 is methylated on the maternal allele only. This methylation imprint is set in the maternal germline, but the mechanism behind it is poorly understood. Based on a family with disruption of *KCNQ1* and ICR2 hypomethylation and experimental evidence for other imprinted loci our group previously proposed that transcription of *KCNQ1* on the maternal allele is necessary to establish the methylation imprint in the maternal germline. This is in line with a recently described mouse model.

We here report another familial case of ICR2 hypomethylation where extensive cytogenetic and molecular genetic studies led to the identification of the underlying genetic cause, thus yielding further evidence for this hypothesis. The couple investigated here presented with a history of four early miscarriages. Both are healthy and non-consanguineous and the family history was otherwise unremarkable. Cytogenetic analysis was carried out in a chorionic villus sample from the last pregnancy and revealed a complex rearrangement involving chromosomes 11 and 13. The same rearrangement was identified in blood lymphocytes of the mother. As one of the breakpoints was located within 11p15.5, MS-MLPA (methylation specific multiplex ligation dependent probe amplification) of this region was performed in the mother and the fetus. The mother showed a normal gene dosage and a normal methylation at the ICR1 and the ICR2, which indicates that she has the rearrangement on her paternal allele. We could prove this by studying the segregation of a rare variant in the family. The fetus also showed a normal gene dosage and a normal methylation for the ICR1 but a hypomethylation for the ICR2. As no deletion was detected by MLPA, we conducted whole genome sequencing to determine the breakpoints of the complex rearrangement. We identified a balanced translocation between the telomeric parts of chromosomes 11p and 13q together with an inversion of the biggest part of chromosome 11. The translocation disrupts *KCNQ1* and relocates exons 1-9 of this gene to the derivative chromosome 13, while exons 10-15 including the ICR2 (*KCNQ1OT1*:TSS-DMR) remained on chromosome 11.

The family presented here strongly supports the hypothesis that transcription of *KCNQ1* in the maternal germline is necessary to establish the methylation imprint at the ICR2. Furthermore it emphasises the need of detailed molecular analyses in families with recurrent early pregnancy losses where an involvement of chromosome 11p is assumed.

P-BasEpi-004

*** Single CpG hypermethylation, allele methylation errors, and decreased expression of multiple tumor suppressor genes in normal body cells of mutation-negative early-onset and high-risk breast cancer patients

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To evaluate the role of constitutive epigenetic changes (in normal body cells) in *BRCA1*/*BRCA2*-mutation negative patients, we have developed a deep bisulfite sequencing assay targeting the promoter regions of 8 tumor suppressor (TS) genes (*BRCA1*, *BRCA2*, *RAD51C*, *ATM*, *PTEN*, *TP53*, *MLH1*, *RB1*) and the estrogene receptor gene (*ESR1*), which plays a role in tumor progression. We analyzed blood samples of two breast cancer (BC) cohorts with early onset (EO) and high risk (HR) for a heterozygous mutation, respectively, along with age-matched controls. Methylation analysis of up to 50,000 individual DNA molecules per gene and sample allowed quantification of epimutations (alleles with >50% methylated CpGs), which are associated with epigenetic silencing. Compared to *ESR1*, which is representative for an average promoter, TS genes were characterized by a very low (<1%) average methylation level and a very low mean epimutation rate (ER; <0.0001% to 0.1%). With exception of *BRCA1*, which showed an increased ER in BC (0.31% vs. 0.06%), there was no significant difference between patients and controls. One of 36 HR BC patients exhibited a dramatically increased ER (14.7%) in *BRCA1*, consistent with a constitutive epimutation. Approximately one third (15 of 44) EO BC patients exhibited increased rates of single CpG methylation errors in multiple TS genes. Both EO and HR BC patients exhibited global underexpression of blood TS genes. We propose that epigenetic abnormalities in normal body cells are indicative of disturbed mechanisms for maintaining low methylation and appropriate expression levels and may be associated with an increased BC risk.

P-BasEpi-005

CNV Detection from Targeted NGS Data: A Case Report on a Family with Hereditary Spherocytosis

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Diagnostic CNV (copy number variation) analysis in molecular genetics with exon level resolution is performed mainly by hybridization techniques (e.g. multiplex ligation-dependent probe amplification). Therefore, the examinable genomic region is in most cases restricted to commercially available test kits. The amount of clinically relevant CNVs located beyond the examinable region of these kits remains elusive. Here we present a case report on a family with hereditary spherocytosis (HS) caused by a heterozygous deletion in SCL4A1, a gene located in a region for which no commercial CNV detection kit is available. A 32-year-old male presented in the Department of Human Genetics of the Ruhr-University Bochum, with a medical history of multiple attacks of hemolytic crisis, jaundice and fatigue. In his teenage years, the symptoms were misdiagnosed as Gilbert syndrome. Years later, following the diagnosis of spherocytosis in his father, further investigations were initiated. The diagnosis of spherocytosis was confirmed at the age of 28 based on reticulocytosis, hyperbilirubinemia, spherocytes on the peripheral blood smear, increased osmotic fragility and positive eosine-5-maleimide binding (EMA) test. A curative splenectomy was performed two years after diagnosis.

For clarification of the genetic cause of HS, DNA samples from the index patient were submitted for further NGS panel diagnostics including the genes ANK1, SLC4A1, SPTB, SPTB and EPB42. In addition to routine NGS testing, binary alignment mapping files (BAM) were further analyzed via the MIDAS CNV pipeline. Coverage of targeted regions was normalized with control samples. CNV calls were performed using CoNVaDING, ExomeDepth and XHMM. Results were further investigated if CNVs were called from two out of three callers. Testing revealed a heterozygous deletion of six exons of SCL4A1 (chr17:g.(42330780_42331853)_(42335083_42335542)del, hg19). The deletion was confirmed with quantitative PCR (qPCR) and classified pathogenic (Richards et al. 2015). The symptomatic father was also tested, confirming the cosegregation of the deletion with the HS phenotype.

P-BasEpi-006

Mitochondrial dysregulation in an animal model of Parkinson´s disease: breakdown of a system or degeneration?

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Parkinson´s disease (PD) is a neurodegenerative disorder characterized at the molecular level by the accumulation of insoluble alpha-synuclein (a-syn) in inclusion bodies. Sporadic outbreaks of MPTP-induced Parkinsonism (neurotoxin inhibiting mitochondrial complex I) highlighted the importance of mitochondrial dysfunction in the etiopathogenesis of the disease. The relevance of both mechanisms is supported by the identification of mutation in genes coding for a-syn or mitochondria-related proteins in rare hereditary PD-forms. In this context, the observation in cell models and in post mortem brain tissue that a-syn inhibits the complex I activity suggests a potential interplay between a-syn and mitochondrial dysfunction which can occur in vivo. In our study, we observed in an animal model overexpressing human a-syn a time-dependent mitochondrial deficit. We used transcriptomics of affected brain regions and measured an overexpression of mitochondrial-related genes in young animal which decreased with aging. Our data suggest that in vivo compensatory mechanisms exist to reduce mitochondrial stress and that these mechanisms are age dependent. Therefore, we propose that upregulating mitochondrial genes might be a promising target to cope with mitochondrial stress and to slow the evolution of synucleinopathy.

P-BasEpi-007

Methylation Profiling of Human Spermatogonial Stem Cells (hSSCs) at Single Allele Resolution

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Introduction:

The characterization of hSSCs is crucial for clinical applications like fertility treatment of the male adult or re-initiation of spermatogenesis after cancer treatments in prepubertal boys. Thus, the determination of spermatogonial subpopulations based on mRNA and protein expression allows the establishment of cell culture conditions to enable reproductive interventions, like transplantation in the testis for the development into mature spermatozoa or in vitro-spermatogenesis. In our previous work, we identified the membrane protein fibroblast growth factor receptor 3 (FGFR3) as a specific marker for a subpopulation of undifferentiated spermatogonia/potential hSSCs that possess self-renewal capacity and maintain the supply of stem cells for spermatogenesis. Furthermore, we established an approach to selectively isolate FGFR3-positive (FGFR3+) single cells. In this study, we focus on the dynamics of DNA methylation patterns at single DNA molecule resolution after isolation of distinct subpopulations of FGFR3+ and FGFR3- spermatogonia from a cell suspension of human testicular cells, using the limiting dilution (LD) bisulfite pyrosequencing method.

Patients and Methods:

Testicular biopsies were obtained from patients with normal spermatogenesis and patients with meiotic arrest who presented to the Department of Andrology, University Hospital Hamburg-Eppendorf, Germany, or the Fertility Center Hamburg, Germany. We isolated single cells and pools of 10 cells that were FGFR3+ or FGFR3-. After DNA extraction and bisulfite conversion, DNA of single cells and 10-cell pools was diluted to a distinct degree to obtain one DNA molecule per well for subsequent PCR reactions (LD method). Here, the distribution of the target molecules across the partitions can be seen as a Poisson process. In the next step, multiplex-PCR followed by gene-specific single nested-PCR was performed with primer pairs binding in regulatory regions of potential hSSC marker genes (FGFR3, L1TD1, PLZF, GFRA1), pluripotency genes (OCT4, NANOG), paternally imprinted genes (GTL2, H19) and maternally imprinted genes (LIT1, SNRPN, PEG3). For each assay, we also analyzed sperm DNA as well as somatic DNA for comparison of methylation patterns. Pyrosequencing of amplicons was performed and methylation levels were analyzed.

Results and Conclusions:

Our approach allowed us to analyze methylation profiles of multiple genes in single and pools of 10 FGFR3+ and FGFR3- spermatogonia at single allele resolution. In both FGFR3+ and FGFR3- cells, we found hypomethylation at the promoters of hSSC marker genes and control regions of maternally imprinted genes

as well as hypermethylation at the promoters of pluripotency genes and control regions of paternally imprinted genes. These methylation profiles were very similar to those of mature sperm DNA. Therefore, we speculate that the transcriptional program underlying human spermatogenesis is early primed already at the level of SSCs.

P-BasEpi-008

Biallelic Intragenic Deletion in MASP1 in an Adult Female with 3MC Syndrome

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Biallelic mutations in *MASP*1, *COLEC11* and *COLEC10* cause 3MC syndrome characterized by craniofacial dysmorphism and multiple structural anomalies. The genes encode components of the lectin complement pathway. Pathogenic *MASP1* mutations affect the serine protease domain specific to its MASP-3 isoform. This domain is encoded by exon 12 of *MASP1*. We identified a novel, homozygous 2kb deletion encompassing exon 12. The affected individual was an adult female with the characteristic appearance typical of 3MC syndrome in addition to hearing loss. She, however, does not show intellectual disability, short stature or cleft lip/palate. This variant was incorrectly called by the software and was only identified by scrutinous manual evaluation. This is the first report of a pathogenic CNV in *MASP1* resulting in 3MC syndrome. We propose that some of the unsolved 3MC cases are caused by this CNV.

We describe novel clinical features not previously observed in 3MC patients and the patient's clinical development from birth to the age of 21 years.

P-BasEpi-009

Comparative expression analysis of Shox2-deficient embryonic stem cell-derived sinoatrial node-like cells

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The homeodomain transcription factor Shox2 represents one of the major genes in the developing sinoatrial node (SAN) and its proper function is of crucial relevance regarding the origin of arrhythmogenic phenotypes. In humans, heterozygous mutations in the *SHOX2* gene have been linked to early-onset atrial fibrillation, which often coexists with sinus node dysfunction in patients. However, the underlying molecular mechanisms are complex and still not fully understood. To provide a cell model for unravelling these mechanisms in health and disease, we established an embryonic stem cell (ESC)-based cardiac differentiation model using Shox2 as a molecular tool. *Shox2*$^{+/+}$ and *Shox2*$^{-/-}$ ESC clones were isolated from *Shox2*-deficient mice and differentiated according to five different protocols in order to evaluate the most efficient enrichment of SAN-like cells. Expression analysis of cell subtype-specific marker genes revealed most efficient enrichment after CD166-based cell sorting. Comparative cardiac expression profiles of *Shox2*$^{+/+}$ and *Shox2*$^{-/-}$ ESCs were examined by nCounter technology. Among other genes, we identified *Nppb*, a well-studied marker in cardiovascular disease, as a novel putative Shox2 target during differentiation in ESCs. Differential expression of *Nppb* could be confirmed in heart tissue of *Shox2*$^{-/-}$ embryos. In summary, heterozygous mutations in the *SHOX2* gene have been linked to atrial fibrillation, the most common arrhythmia in humans. In turn, elevated BNP levels and higher *NPPB* mRNA expression have been detected in subjects with atrial fibrillation. These and other data suggest that our generated ESCs represent an important tool for the investigation of underlying molecular mechanisms under physiological and pathophysiological conditions and for evaluating novel therapeutic approaches.

P-BasEpi-010

Genome-wide Analysis of the Nucleosome Landscape in Individuals with Coffin-Siris Syndrome

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Coffin-Siris syndrome (CSS) is a rare congenital disorder characterised by facial dysmorphism and digital anomalies, most prominently hypoplasia of the fifth nail, as well as mild to severe intellectual disability. CSS-affected individuals have causative mutations in various genes encoding subunits of the SWItch/Sucrose Non-Fermenting (SWI/SNF) complex, a majority of them in the subunit *ARID1B*.

Chromatin remodellers, such as the SWI/SNF complex, slide or evict nucleosomes by utilising ATP and thereby regulate the spacing of nucleosomes and control gene expression. The presence and absence of nucleosomes can be displayed by genome-wide Nucleosome Occupancy and Methylome sequencing (NOMe-seq), which introduces artificial methylation of accessible cytosines in GpC contexts.

We hypothesised that mutations in genes encoding ubiquitous subunits of the SWI/SNF complex may lead to alterations of the nucleosome profiles in many cell types. First, we analysed cell-free DNA isolated from whole blood and found that CSS-individuals do not display a grossly altered nucleosome profile around transcription start sites. Next, we analysed $CD14^+$ monocytes from patients and normal controls by NOMe-seq and detected few genomic regions with significantly different nucleosome occupancy. We further investigated a possible correlation with gene regulation by differential expression analysis with RNA-seq. The significantly deregulated genes were not in proximity to any of the identified differential nucleosome-depleted regions and did not cluster to specific pathways. Our results confirm that most subunits of the SWI/SNF complex show functional redundancy, and few genomic regions might be particularly sensitive to the failure of one subunit in blood cells. This sensitivity may play a greater role in disease-relevant tissue.

P-BasEpi-011

X-inactivation in female human iPSCs- a tool for research on brain development

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The process of X-chromosomal silencing is of utmost importance for regulating gene dosage in females. The X-chromosome plays an important role in the development of the human brain and of human intelligence, containing about 30% of genes related with mental retardation. Furthermore, many X-chromosomal genes are known regulators of autosomal gene expression. Thus changes in X-inactivation not only influence expression of X-chromosomal genes, but also of autosomal genes.

In humans about 20% of X-chromosomal genes outside the pseudoautosomal region escape X-inactivation in females, which are likely to play a role in neuronal differentiation, directly or by influencing other (autosomal) genes. So far, however, detailed research into this has only been possible in mice and with non-invasive methods in patients suffering from Turner syndrome. iPSCs are a promising tool for modelling gender differences during human brain development.

We have successfully reprogrammed two different fibroblast cell lines of female carriers of MID1 frameshift mutations into iPSCs and could identify clones that either express MID1 from the mutated or the non-mutated X-chromosome. We planned to use these cells as isogenic controls and differentiate them into NPCs and neurons, to get a better understanding of Opitz BBB/G syndrome. Interestingly, while X-inactivation was stable in the iPSCs, we observed a re-activation upon differentiation into NPCs of the second MID1 allele, leading to a biallelic expression of MID1 in NPCs. When having a closer look at the different stages of the differentiation process, we found that the re-activation happened after FGF2 was added to the media. At the same time however we could show that other genes kept their inactivated status, which led us hypothesize that FGF2 causes a partial re-activation of the inactivated X-chromosome during neuronal differentiation, a mechanism that would allow fine-tuned up-regulation of selected genes in female brain.

To get a better understanding of the underlying mechanism, we are currently further analyzing FGF2 effects on iPS cells and fibroblasts and study the expression pattern of over 100 X-chromosomal genes using QUASEP-assays and RNAseq. We furthermore will establish brain organoids to study X-re-activation in tissue.

P-BasEpi-012

*** Induced pluripotent stem cell clones from a patient with multilocus imprinting disturbance (MLID) show marked heterogeneity of the DNA methylation at ubiquitously imprinted CpG

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Patient specific induced pluripotent stem cells (iPSC) are widely used for functional modelling. However, the reprogramming process can affect DNA methylation at imprinted loci, thereby influencing modelling of imprinting syndromes. Thus, we aimed at analyzing variability of DNA methylation at ubiquitously imprinted CpGs in iPSC clones of a patient with a multi locus imprinting disturbance (MLID).

We here report on the DNA methylation analysis of iPSC clones reprogrammed with foot-print free episomal vectors from fibroblasts (Okita et al., 2011) of a patient with MLID in the framework of the BMBF-funded network project “Diseases caused by imprinting defects: clinical spectrum and pathogenetic mechanisms”. DNA methylation was measured using the Infinium HumanMethylation450k BeadChip.

We focused the analysis on the 714 CpGs previously published to be ubiquitously imprinted (Court et al., 2014). We arbitrarily defined the normal imprinted (hemimethylated) state as DNA methylation (beta value) of 0.3-0.7, with <0.3 being hypo- and >0.7 being hypermethylated.

Of the 282 CpGs hemimethylated in the fibroblasts, 50%, 57% and 64% of CpGs remained hemimethylated in the individual iPSC clones. A total of 39%, 33% and 26% of these CpGs changed to hypomethylation and 11%, 10% and 10% CpGs changed to hypermethylation. Only 63/282 CpGs remained hemimethylated in all three iPSC. In total 91/282 CpGs changed the original DNA methylation pattern in one iPSC clone, with 82 being hypomethylated. Moreover, 39% CpGs changed the original DNA methylation pattern in two iPSC clones, with 86 CpGs being uniformly hypo- and 19 CpGs being uniformly hypermethylated in both iPSC clones. Four CpGs showed opposite DNA methylation changes in both clones.

Of the 365 CpGs hypomethylated in the fibroblasts 4%, 4% and 3% changed to hypermethylation in the iPCS clones and 35%, 48% and 19% changed to hemimethylation. Of the 61 CpGs hypermethylated in fibroblasts 64%, 34% and 21% were hemimethylated in the iPSC clones and 13%, 41% and 54% became hypomethylated. Overall, of 708 CpGs ubiquitously imprinted only 231 showed a uniform DNA methylation pattern in all three iPSC clones. For seven CpGs, all three iPSC clones showed a different DNA methylation state.

We conclude that DNA methylation of imprinted CpGs changed to a variable degree during the reprogramming process in the analyzed MLID patient. A high interclonal variability is observed at imprinted loci with only 33% of CpGs showing a uniform DNA methylation in all three analyzed clones. Whether this is due to mosaicisms of the imprinting disturbance, the yet unknown disease mechanisms underlying MLID or random effects of the reprogramming needs to be further investigated. The present analysis indicates that studies of imprinted loci in patient derived iPSC need to be considered with caution in diseases caused by epimutations.

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P-BasEpi-013

The genetic spectrum of West syndrome

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West syndrome belongs to the heterogeneous spectrum of epileptic encephalopathies. It is characterized by distinctive clinical and electrophysiological features comprising repetitive epileptic spasms, hypsarrhythmia and developmental delay. We designed a sequencing panel targeting 131 genes associated with epilepsy or epileptic encephalopathy and screened a cohort of 45 index cases with clinical diagnosis of West syndrome. Furthermore, we performed CNV-analysis (copy number variants) based on the NGS data. We identified disease-causing SNV (single nucleotid variants) in 10 out of 45 patients in genes described earlier with West syndrome (e.g. CDKL5, ARX) or other epileptic disorders expect West syndrome (e.g. DEPDC5, MECP2, SCN1A). CNV were identified in 2 additional cases, comprising a 6,7 Mb Deletion on chromosome 2 including SCN2A and SCN3A and a supernumerary marker chromosome 15. SNV and CNV together provided a diagnostic yield of 27%, which is a higher than previous studies. However, our study illustrates an etiologic

overlap of West syndrome with other epileptic encephalopathies, such as Rett syndrome and Dravet syndrome. With a diagnostic yield of 27% our approach proved to be useful in diagnostics of West syndrome, especially when combined with NGS-based CNV-analysis.

P-BasEpi-014

Exploring the molecular causes of the obesity risk factor POMC hypermethylation variant

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The *POMC* gene encodes for proopiomelanocortin, a preproprotein that is processed to yield different peptide hormones. One of these hormones (MSH, melanocyte-stimulating hormone) mediates the anorectic functions of leptin. Variants in its coding region have been linked to the development of early-onset severe obesity. Moreover, a *POMC* variably methylated region located at the intron 2-exon 3 border is more frequently hypermethylated in peripheral blood cells of obese individuals compared to normal controls, and it has been proposed to be a metastable epiallelle that leads to increased risk for obesity development. Hypermethylation of this region might be triggered by adjacent Alu elements in intron 2. It has remained unclear whether the variation in DNA methylation is linked to the parental origin, genetic variation and/or environmental factors.

First, we analysed whole genome bisulfite sequencing data of monocytes from five healthy donors and observed reads derived from mainly methylated molecules and reads derived from mainly unmethylated molecules at the *POMC* intron 2-exon 3 border and spanning through most of exon 3, indicative of allele-specific methylation (ASM) at this region. This was confirmed by deep bisulfite amplicon sequencing. We ruled out *POMC* being an imprinted gene by analysing methylation data from germ cells, which showed that this region is unmethylated in oocytes and sperm. Next, we focused on finding a genetic variant within or outside the *POMC* intron 2-exon 3 region that might be responsible for haplotype-dependent ASM. We performed genome-wide association studies (GWAS) using ~500,000 SNP genotypes and Illumina 450k CpG methylation data from blood samples of 1128 individuals from the Heinz Nixdorf Recall Study. The methylation arrays encompass six CpGs from the *POMC* intron 2-exon 3 region, one of which is located within the ASM region, although outside of the most variable part, while five CpGs are at the border or just outside of the ASM region. The methylation levels of the latter five CpGs were significantly correlated with nearby SNPs. Three of these SNPs are located in the *EFR3B-POMC* region and known to be eQTLs for *POMC*. One of these SNPs is mildly correlated to BMI in our cohort. In summary, our studies suggest that the variation in DNA methylation at the intron 2-exon 3 border of *POMC* might be affected by genetic variation. Additional CpGs within the ASM region are currently being analysed for association with SNPs.

P-BasEpi-015

Capturing transcriptional dynamics of transposons by single-cell RNA-Seq of 2500 cells in the male mouse germ line

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Activation of transposable elements in the germ line provides a source of genetic variation and, in rare cases, causes mutations that lead to disease. To protect the integrity of the genome passed on to the next generation, transposon activity in the germ line is a highly controlled process with several stages of activation and inactivation of TE families and repression mechanisms. However, most studies performed in the field to date relied on histology or bulk RNA from whole tissue or enriched subpopulations. As these methods rely on the expression of few, if any, markers, they are very limited in terms of resolution.

We performed single-cell 3' RNA-Seq on 2,500 cells from testis tissue of two C57Bl/6 wild type mice, achieving a mean read depth of 150,000 reads per cell (33% saturation). Most cells displayed a relatively low abundance of TE transcripts, with levels ranging from no TE detection at all to approximately 1% of RNAs stemming from transposons. For most expressed elements, transcript levels were high during early stages of spermatogenesis, followed by a drop towards the end of meiosis I. Some elements showed immediate reactivation, while in other cases this was delayed until the round spermatid stage. After histone-to-protamine transition, no TE transcripts could be observed, while simple repeats remained active.

In summary, single-cell RNA-Seq provides a high-resolution map of transposon activity in the germ line. Furthermore, it allows expression dynamics of repressor genes to be studied in relation to TE activity, yielding further insight into the mechanisms of repression in specific cells. Our results demonstrate that methods based on few markers are insufficient to capture the variability inherent in the continuous differentiation of germ line

cells. Single-cell RNA-Seq considering transposon and repeat element expression in addition to protein-coding genes may aid cell classification significantly.

P-BasEpi-016

Identification of a putative KCND3 (SCA19) mutation in an ataxia patient

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To date, at least 35 different loci for autosomal dominant inherited spinocerebellar ataxias (SCAs) have been mapped. Among those, 26 disease-causing genes have been identified. Common SCAs are caused by repeat-expansions either in coding, or noncoding parts of the respective genes. In contrast, rare SCA types are originated by point mutations in genes with different functions in the cell. Among those, protein-kinases (*TTBK2*, SCA11 and *PRKCG*, SCA14), a nuclear encoded mitochondrial protease (*AFG3L2*, SCA28), fatty acid elongase (*ELOVL5*, SCA38), and ion channels (*KCNC3*, SCA13 and *KCND3*, SCA19) are affected (1). Although the SCAs have a genetically heterogeneous background, typical clinical symptoms, such as gait and limb ataxia, abnormal eye movements and dysarthria occur in almost all SCAs making a proper clinical diagnosis difficult.

Among the SCAs associated with impaired channel function, SCA19 is caused by point mutations in the *KCND3* gene encoding a voltage-gated potassium channel (Shal-related subfamily, Kv4.3) (3). Kv4.3 is a multi-pass membrane protein involved in the repolarization phase of the action potential. Interestingly, Brugada syndrome - a severe tachyarrhythmia - is also associated with KCND3 mutations (3).

To date, nine variations associated with SCA19 pathology have been detected in single families of Caucasian, and Asian origin (4, 5). The seven missense mutations, one 3-bp deletion, and a 9-bp duplication are located in the first two transcribed exons coding for the six transmembrane domains of Kv4.3 resulting in impaired channel function.

To investigate the mutation spectrum in SCA19, we screened 51 ataxia patients of German origin for novel variations in *KCND3*. The majority of known SCA-causing genes (SCA1-3, 6-8, 10, 12, 14, 17, 19, 23, 27, and 28) had been excluded previously. The seven coding exons and adjacent exon-intron borders were sequenced. In the first coding exon, a novel A>G transition resulting in a substitution of asparagine acid by glycine in the N-terminal cytoplasmic domain was detected. Impairment of proper channel function was supported by the following points: (I) conservation of the substituted amino acid among species, (II) absence of the variant in 200 healthy control chromosomes, and (III) absence of the variant in SNP browsers such as 1000 Genomes.

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P-BasEpi-017

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. In most cases, tumor development is caused by biallelic inactivation of the tumor suppressor gene *RB1*. Human *RB1* is imprinted, leading to differential expression from the maternal and the paternal allele controlled by differential methylation of CpG85. CpG85 is methylated on the maternal allele only. On the unmethylated paternal allele, CpG85 acts as promoter for the alternative *RB1* transcript, *RB1-E2B*. Experimental evidence suggests that expression of *RB1-E2B* is causative for skewing of regular *RB1* expression in favor of the maternal allele.

In blood, CpG85 showed 50 percent methylation, as expected for a gametic differentially methylated region (gDMR). Analysis of CpG85 in eight tissues of three individuals, in human embryonic and induced pluripotent stem cells and in retinoblastoma cell lines revealed a general gain of methylation, ranging from 60 percent to full methylation. CpG85 is therefore an unstable gDMR and gains DNA methylation during differentiation, ageing or in vitro culture.

To investigate if DNA methylation at CpG85 is caused by transcriptional read-through from the upstream *RB1* promoter, as has been shown for establishment of a few gDMRs in oocytes, we deleted the regular *RB1* promoter in human embryonic stem cells. *RB1* promoter knock-out lines were generated by CRISPR/Cas9 mutagenesis and characterized for genetic background, *RB1* expression and pluripotency. To determine the stability of the CpG85 imprint in presence or absence of the regular *RB1* promoter, the fully methylated CpG85 allele was exchanged by an unmethylated version in *RB1* promoter knock-out and wildtype cells. Again, the CRISPR/Cas9 system was employed using guide RNAs flanking the complete island of CpG85 and a PCR-generated CpG85 template for homology directed exchange. The exchange template contained an EcoRI restriction site at the position of the PAM sequence of the downstream guide RNA. Presence of this EcoRI site in genomic DNA was indicative for successful CpG85 exchange. Only clones with a homozygous CpG85 exchange were analyzed for gain of DNA methylation by next-generation bisulfite sequencing four weeks after transfection. In two clones with the regular *RB1* promoter present, methylation levels of 55% and 93% were observed, respectively. In three clones with the *RB1* promoter absent, methylation levels were measured to be 11%, 95% and 84%. These results so far do not allow a clear conclusion about the role of regular *RB1* transcription on imprint stability of CpG85. We will analyze more cell lines at earlier time points and need to determine if an incomplete exchange of CpG85 occurred.

P-BasEpi-018

Translating the GEKO guideline for the requirements of the qualifications and the contents of genetic counseling into OB/GYN specialists' practice – Results from the GenBIn2 project

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Background: No empirical data are currently available on how obstetricians/gynecologists (OB/GYN) are applying the Commission on Genetic Testing (GEKO) guideline on genetic counseling at point of care. Data are needed to assess the guideline's impact and to advance both, informed discussion and further research.

Methods: As part of the GenBIn2 project, an exploratory research project was conducted applying a stepwise approach: (i) set-up of a multidisciplinary expert group tasked to identify key areas to be addressed for assessing the translation of the guideline into practice; (ii) development of an extensive questionnaire addressing identified key areas; (iii) followed by a pre- study/inquiry to validate key-areas addressed in the questionnaire.

Results: 150 OB/GYN from all over Germany representing different types of practice ranging from private solo practice (51%) to teaching hospitals and university settings (9%) volunteered to participate anonymously in the inquiry in 2017. 80% of the participants possessed the qualification "Fachgebundene genetische Beratung", obtained by passing a knowledge test at a "Landesärztekammer" (97%). On average, per quarter, 303 (mean: 45; mode: 50) patients requiring genetic counseling were seen. 48% reported changes in the management of patients incurred by the guideline, 61% reported more referrals to a genetic specialist for counseling. Genetic counseling provided by OB/GYN themselves mostly include counseling before first trimester screening (76%) and after first trimester screening with normal results (77%), before (64%) prenatal ultrasound for fetal anomalies (DEGUMII/III), before NIPT for Trisomy 21 (57%) and after NIPT (57%) with normal results. Additional work and documentation load, time constraints in busy practice settings, lack of reimbursement and struggling with the complexity of the guidelines were commonly reported. In addition, comments indicated that limited understanding of the guideline's underlying concepts and terminology are conflicting with established approaches to delivering care in daily practice. Positive responses included: increased sensitivity for skills required for genetic counseling and increased referrals to counseling.

Conclusions: The GenBIn2 inquiry provides first hand insights and information on how the GEKO guideline on genetic counseling is translated into daily medical practice by OB/GYN. By identifying numerous challenges the inquiry helps to better understand factors influencing the translation process and provides groundwork for future assessment studies.

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P-BasEpi-019

Simultaneous single cell transcriptome and epigenome analysis in an automated microfluidics system

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In various diseases, epigenetic modifications like methylation of cytosines play a major role in the development of the disorder. As epigenetic modifications have a direct influence on the transcriptional level of a cell, it is the aim of this work to correlate these epigenetic changes directly with transcriptional variation. Therefore, the individual epigenome and the transcriptome of single cells are assessed in a high-throughput and cost and time efficient manner. A sophisticated microfluidics system manages the separation of RNA and DNA of single cells by the use of droplets and specific micro-particles with unique cell- and molecular barcodes. This enables single cell identification while massive parallel sequencing on an NGS platform.

The system is used to characterize murine neurons and microglia of a neurodegenerative disease animal model to investigate the role of epigenetic modifications in the development of neuropathological processes. Side by side, circulating tumor cells of patients with prostate-carcinoma are explored to specify the heterogeneity of the tumor and to gain possible insights into individualized tumor-therapy.

Sequencing-data analysis is incorporated in an homemade analysis pipeline based upon GNU-licensed tools. Downstream analyses like principle component analysis or gene ontology enrichment deliver a better correlation of epigenetic and transcriptional changes with the development of a neurodegenerative disorder.

The application of the herein described method for different kind of cell types illustrates the broad usage of the automated and user-friendly system for the combined processing of both epigenome and transcriptome for high throughput sequencing based multi-parameter-analyses of single cells in many scientific research fields.

P-BasEpi-020

Functional consequences of altered hsa-miR-129-5p expression by HDAC inhibitors Vorinostat and Romidepsin in hepatocellular carcinoma

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Background and aims: Histone acetyltransferases and histone deacetylases (HDACs) regulate chromatin remodeling and the transcriptional activity of specific genomic regions. In hepatocellular carcinoma (HCC) an overexpression of HDACs has become evident causing a global loss of histone acetylation. We, therefore, aimed to identify tumor suppressive miRNAs deregulated by histone deacetylation and to understand their functional effects in HCC tumorigenesis.

Methods: HCC cell lines (HLE, HLF, Huh7, HepG2, Hep3B) and immortalized liver cell lines (THLE-2 and THLE-3) were treated with two HDAC inhibitors Vorinostat and Romidepsin to induce histone acetylation. Apoptosis, proliferation and changes in histone acetylation after HDAC inhibition were determined. To identify epigenetically deregulated miRNAs we performed miRNA-sequencing and validated findings by qRT-PCR. After transfection of miRNA mimics, apoptosis, proliferation and migration of HCC cells were analyzed. Putative target genes were examined by siRNA-mediated knockdown, western blotting, luciferase reporter assays and ribonucleoprotein immunoprecipitation (RIP) with subsequent qRT-PCR.

Results: After HDAC inhibition by Vorinostat and Romidepsin, apoptosis and histone acetylation of HCC cells were significantly upregulated, whereas proliferation was diminished. MiRNA-sequencing revealed 52 differentially expressed miRNAs that were strongly induced after HDAC inhibition: 16 of these miRNAs were re-expressed after Romidepsin treatment, 3 miRNAs after Vorinostat treatment and 33 miRNAs after both Vorinostat and Romidepsin treatment. One of the 33 up-regulated miRNAs is miR-129-5p that holds tumor suppressive potential and displays a reduced expression in different tumor types. Overexpression of miR-129-5p in HCC cells induced apoptosis and inhibited proliferation and migration. 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, and also in HCC, and its expression correlates with a poor prognosis. Incubation of HCC cells with Vorinostat and Romidepsin or transfection with miR-129-5p reduced expression of *HDGF*. *HDGF* knockdown attenuated cell viability and migration of HCC cells and increased apoptosis. Moreover, the expression of the death receptor *Fas*, a potential downstream target of HDGF, is also regulated by miR-129-5p. However, a direct interaction of miR-129-5p and *HDGF* could not be confirmed by luciferase assays and RIP.

Conclusions: Chromatin remodeling results in a reduced expression of the tumor suppressor miR-129-5p which plays a fundamental role in hepatocarcinogenesis by regulating, inter alia, *HDGF* and *Fas*. Inhibition of

histone deacetylation and small molecules against target genes of epigenetically deregulated microRNAs may direct new therapeutic strategies. In the future, some epigenetically deregulated miRNAs may be considered for miRNA replacement therapy.

P-BasEpi-021

Expanding the mutational and phenotypic spectrum of KMT2B

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Methylation of histone 3 on lysine 4 (H3K4) is an important epigenetic mark for the regulation of gene transcription and is performed by at least six KMT2 family members in mammals including the methyltransferase KMT2B. By analyzing whole-exome sequencing data, we now identified novel *de novo* variants in *KMT2B* in five individuals presenting with syndromic forms of primary microcephaly, namely two frameshift (c.5462insC, [p.Leu1822Thrfs*12] and c.5682del, [p.Thr1895Profs*39]), one splice site (c.3789+1G>A) and two missense variants (c.3746G>C, [p.Arg1248Pro] and c.7852G>A, [p.Glu2618Lys]). Mutations in *KMT2B* have already been described in patients with childhood-onset dystonia. However, in the present study, three out of five patients show no signs of any movement disorder, although two of them already reached the ages of 10 and 12 years, making a childhood-onset dystonia manifestation unlikely. Furthermore, one patient has a very severe form of congenital microcephaly (-7 SD) which has not been described in patients with *KMT2B* mutations before. Analyses in patient derived induced pluripotent stem cells (iPS) and zebrafish models are still in focus of ongoing research and will help to finally understand the underlying pathomechanism. In conclusion, our findings expand the mutational and phenotypic KMT2B spectrum and propose *KMT2B* as a gene associated with syndromic forms of primary microcephaly without dystonia manifestation.

P-BasEpi-022

Modeling the genetic and molecular basis of atrial fibrillation with patient-specific iPS cells

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Atrial fibrillation (AF) is the most prevalent cardiac rhythm disturbance worldwide, but the underlying molecular mechanisms are still largely unknown. Several studies have demonstrated that AF has a substantial genetic component, where common single-nucleotide polymorphisms (SNPs) as well as rare mutations in transcription factors and modifiers have been shown to play a role in the development of the disease. In clinical practice, AF often coexists with sinus node dysfunction. The sinoatrial node (SAN) automaticity is essential for maintaining normal cardiac function. It consists of a specialized group of cardiac muscle cells located in the right atrium, which act as the primary pacemaker of the heart. The formation is defined by SAN-specific genetic programs at an early stage of embryonal development.

We were able to show that the homeodomain transcription factor SHOX2 plays an important role in the development of the cardiac conduction system CCS, especially the SAN formation and specification. Mutational analyses of the SHOX2 gene have revealed a significantly associated 3'UTR variant (c.*28T>C) and an unique missense mutations (p.H283Q) in a cohort of patients with early-onset AF, which interfere with the function of SHOX2 as a transcriptional activator.

The aim of the present project is to elucidate the pathogenic mechanisms underlying the identified genetic mutations.

By using human induced pluripotent stem cells (hiPSCs), we are planning to establish a novel in vitro cellular disease model for AF. We therefore reprogrammed hiPSCs out of blood samples derived from AF patients harboring these mutations and characterized the cells regarding their pluripotency potential and karyotype. Patient-specific iPS cells were differentiated into cardiomyocytes via alteration of the Wnt signaling pathway. A major challenge in modeling a cardiac subtype-specific disease such as AF in iPSC-derived cardiomyocytes is the purification of the respective cell type. Thus, we isolated specific cardiac cell subtypes (SAN-like, atrial-like, ventricular-like), to dissect the transcriptional profiles and electrophysiological properties of subtype-specific cardiomyocytes. To purify cardiac sybtypes, we generated lentiviruses that contained fluorescent proteins under the control of lineage-specific promotors. Infection of the cardiomyocytes revealed that cells of all subtypes were present after differentiation. Subtype-specific cardiomyocytes were isolated via fluorescent activated cell sorting (FACS) for detailed expression analysis and electrophysiological studies to finally assess whether the cells recapitulate the disease phenotype seen in the patients. Our strategy for generating these populations can be applied to any other cardiac iPSC-based disease model as it is independent of the underlying disease type, the genetic background of the cells and even the chosen differentiation strategy.

P-BasEpi-023

Increased prevalence of filaggrin deficiency in 51 patients with recessive X-linked ichthyosis presenting for dermatological examination

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X-linked ichthyosis (XLI) is a keratinization disorder caused by deficient activity of steroidsulfatase. In contrast, ichthyosis vulgaris is due to semidominant mutations of the filaggrin gene (FLG). In view of phenotypic variations of these ichthyoses we speculated that XLI may be influenced by additional FLG mutations in a significant number of patients. We characterized a group of 51 patients with XLI and systematically analyzed them for additional FLG mutations (R501X, 2282del4, R2447X, S3247X). The study was complemented by morphological analyses. Full FLG sequencing for rare mutations was performed in special cases. Interestingly, prevalence of FLG mutations was significantly increased compared to a population-based control cohort of 1,377 individuals (17.6% vs. 8.4%, p=0.038). Palmoplantar hyperlinearity was significantly associated with the FLG mutation status. Ichthyosis severity score seemed to be increased in XLI with FLG mutations, but the difference was not significant (p=0.124). To our surprise, percentages of atopic manifestations were highly prevalent in both subgroups, 40% and 33% in XLI without and with filaggrin deficiency, respectively. Of note, reduction of filaggrin staining or keratohyalin could not be explained by FLG mutations in all patients. However, we conclude that FLG mutations represent a significant genetic modifier of XLI. [196 words]

P-BasEpi-024

Array-based DNA methylome analyses of classical Hodgkin lymphoma

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Classical Hodgkin lymphoma (cHL) is an atypical germinal-center derived B-cell lymphoma, in which the tumor cells have lost parts of their B-cell identity and instead up-regulate expression of genes characteristic for other hematopoietic lineages. The nature of the transcription factors initiating and maintaining Hodgkin-Reed-Sternberg-specific gene expression as well as the mechanisms of simultaneous down-regulation of many B-cell-specific genes remain poorly understood. In theory, gene silencing could be achieved by mutations, absence of transcription factors or by epigenetic silencing. Indeed, previous studies suggest that epigenetic modifications could be involved in this cHL-associated B-cell reprogramming. The aim of the present study was therefore to investigate the DNA methylation patterns that are specific for cHL.

As the tumor cell content in primary cHL biopsies is usually very low (~1%), we performed our analyses on five well characterized cHL cell lines (L1236, L-428, L-540, SUP-HD1 and HDLM-2). We investigated the DNA methylation of these cHL cell lines using the Infinium HumanMethylation450 BeadChip Array (Illumina) and compared these findings to 38 non-Hodgkin lymphoma and 6 lymphoblastoid cell lines with the aim to identify differences in DNA methylation that are specific for cHL. For selected cell lines we additionally performed whole genome bisulfite sequencing.

After normalization of the array 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. Hence, 462,452 loci finally entered subsequent analyses. We compared the DNA methylation profiles of cHL cell lines versus non-Hodgkin lymphoma and lymphoblastoid cell lines in order to remove differences in DNA methylation that are cell line and normal B-cell specific. We identified 2617 cHL-specific loci that are differentially methylated (σ/σ_{max}>0.5; q<1e-3), 2271 (86.8%) of which were hypermethylated. A significant enrichment of those hypermethylated loci was observed in enhancer and promoter regions, which might alter gene expression in cHL. Moreover, these hypermethylated loci were significantly enriched in binding sites of the transcription factors BCL11, EBF, ELF1, MEF2, MTA3, NFIC, PU.1, RUNX3 and PML (OR>2) which might also contribute to the aberrant B-cell phenotype in cHL. This is in line with previous analyses showing that also the expression of the transcription factors PU.1 and EBF themselves is drastically reduced in cHL which correlated with the hypermethylation of the respective promoter. Our analyses, thus, further support the notion that epigenetic processes play a major role in B-cell-specific gene silencing in cHL.

P-BasEpi-025

Analysis of DNA damage repair mechanisms in fibroblasts derived from patients with microcephaly and developmental delay and a homozygous TFIP11 missense variant

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Microcephaly is defined as an occipitofrontal head circumference <-2 standard deviations below the mean for sex, age, and ethnicity. Anomalies leading to microcephaly may exclusively affect the cerebral development or are associated with other clinical abnormalities. In this study we report three individuals, two Indian siblings and one Palestinian boy, from consanguineous parents who presented with secondary microcephaly and global developmental delay. The two siblings developed retinopathy, while the other child had nystagmus and poor vision in both eyes with only light perception. Whole-exome sequencing in the two families revealed homozygous missense variants in the *TFIP11* gene. The variant c.1697T>C [p.(Leu566Pro)] was found in brother and sister and the variant c.1784G>C [p.(Trp595Ser)] in the single affected child; parents were heterozygous carriers. Both changes affect highly conserved amino acid residues and represent very rare (no homozygous carriers) or absent variants in the general population (ExAC and gnomAD browsers). To characterize the functional effects of the missense variants in *TFIP11* we used fibroblasts derived from the three patients and healthy individuals. We did not detect any difference in TFIP11 protein amount in patient- and control-derived fibroblasts by western blotting. In addition, immunocytochemistry revealed the same nuclear localization of wild-type and mutant TFIP11 in fibroblast cells. Recent data suggest a role of TFIP11 in DNA double-strand break repair. Therefore, we analysed DNA damage repair mechanisms in patient- and control-derived fibroblasts. After X-radiation of cells with a dose of 6 Gy followed by a recovery time of 24 h immunostaining with an antibody against the phosphorylated histone H2AX, gammaH2AX as a marker for DNA damage, was performed. By counting the number of gammaH2AX-positive foci per cell, we obtained preliminary evidence of higher DNA damage in patient compared to control cells. Furthermore, the amount of the cell cycle regulator p53 and its downstream target p21 seemed to be slightly increased in the patient fibroblasts. Our findings may suggest alterations in DNA damage repair and regulation of p53 and p21 protein level in fibroblasts from patients with a homozygous *TFIP11* missense variant, however, further experiments are ongoing to confirm these initial data.

P-CANCER GENETICS

P-CancG-026

Case Report: HNPCC patient with one heterozygous mutation in each MSH2 and MLH1

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Hereditary non-polyposis colorectal cancer (HNPCC, Lynch syndrome) is the most frequent form of hereditary colon cancer. It is caused by a mutation in one of the four DNA mismatch repair (MMR) genes *MLH1*, *MSH2*, *MSH6* and *PMS2* and is inherited in an autosomal dominant manner. Mutations in these MMR genes lead to defective DNA repair mechanisms that result in microsatellite instability (MSI). The gene products of *MLH1* and *PMS2* as well as *MSH2* and *MSH6* form protein complexes, respectively. In immunohistochemically analysis mutation in a single gene can therefore result in expression loss of the total protein complex. Clinically, many HNPCC patients develop colorectal cancer before the age of 50. Moreover they have an increased lifetime risk for endometrial, ovarian, gastric, small bowel and renal pelvis cancer, bile duct and pancreatic carcinoma, and tumors of the central nervous system and the sebaceous glands (Muir-Torre syndrome).

Here we describe a highly striking consanguineous family with HNPCC-associated carcinomas in at least four generations. In total 18 cases of colorectal, endometrial and gastric carcinomas, acute myeloid leukemia and brain tumors were reported. Our index patient had a mucinous carcinoma in the colon ascendens at the age of 38. Immunohistochemical staining showed a loss of *MSH2* and *MSH6* expression. Additionally, MSI was shown for tumor DNA. We analyzed DNA from peripheral blood using Next Generation Sequencing (NGS) and performed MLPA analysis to detect larger deletions/duplications within the MMR genes. First, we identified the heterozygous deletion of exon 9 of the *MSH2* gene, which is in line with the expression loss of the MSH2/MSH6 complex. Additionally we detected the heterozygous mutation c.1667G>C (p.Ser556Thr) in exon 14 of the *MLH1* gene which we classified as class 4. Concurrent heterozygous mutations in the genes *MSH2* and *MLH1* are rare but have been observed before in five cases. Here we present recent results regarding carrier status of family members.

P-CancG-027

„Identification of BAP1 germline mutations in patients with monosomy 3 in uveal melanoma"

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Uveal melanoma (UM) is the most common primary cancer of the eye in adults. Metastatic progression is strongly associated with loss of one chromosome 3 in the tumour (monosomy 3, M3). Most UM with M3 show mutational inactivation of BAP1, a tumour suppressor gene located at 3p21. In most patients these variant alleles are the result of somatic mutations. However, some rare patients are constitutional heterozygous for mutationally inactivated BAP1 alleles. This genotype results in heritable predisposition to diverse neoplasms including UM, mesothelioma, renal cancer, skin cancer (BAP1 Cancer Syndrome, BAPCaS). Most patients with BAPCaS have been ascertained via families showing aggregation of cancer cases and this mode of ascertainment is expected to result in overestimation of risk figures. To reduce this kind of bias we performed BAP1 mutation analysis in all UM patients treated in the department of ophthalmology between Oct. 2014 and Nov. 2016 who had a tumor with M3 and agreed to participate in the study. Sequencing and gene dosage analysis by MLPA of the all BAP1 coding exons was performed on DNA from tumor samples obtained after enucleation (n=31) or biopsy (n=32). DNA from blood of patients was tested for the presence of the pathogenic variant alleles identified in the tumour DNA.

Hemizygous pathogenic variant alleles of the BAP1 were identified in tumors of 47 of 63 patients (75%). In all but one patient, these variant alleles were found to be the result of somatic mutations. We identified one patient with a 2 bp constitutional BAP1 deletion affecting the first two nucleotides of exon 6 which is predicted to result in a frame shift and consequently in a premature termination codon, a functional type of alteration that is typical for somatic BAP1 mutations in UM. According to our results, we estimate that roughly 2% of UM patients with M3 tumours might have a BAPCaS. The age at diagnosis (60 years) of this patient was the same as the mean age of diagnosis of all patients who had a somatic mutation in the tumor. In addition individual medical and family history of the BAP1 mutation carrier identified here were not apparently distinct from that

of the other patients in the cohort. This suggests that cancer risk of patients with BAPCaS that were not ascertained via familial aggregation is lower compared to previous estimates.

Nonetheless identification of patients and relatives with BAPCaS is a prerequisite for the development of strategies for early detection and treatment. Implementation of such strategies may improve prognosis of individuals diagnosed with BAPCaS.

P-CancG-028

Precise ERBB2 copy number assessment in breast cancer by means of molecular inversion probe array analysis.

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HER2/ERBB2 amplification/overexpression determines the eligibility of breast cancer patients to HER2-targeted therapy. This study evaluates the agreement between ERBB2 copy number assessment by fluorescence in situ hybridization, a standard method recommended by the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP), and newly available DNA extraction-based methods. A series of n=29 formalin-fixed paraffin-embedded breast cancers were subjected to ERBB2 copy number assessment by fluorescence in situ hybridization (FISH, Vysis, Abbott). Following macrodissection of invasive breast cancer tissue and DNA extraction, ERBB2 copy number was also determined by molecular inversion probe array analysis (MIP, OncoScan, Affymetrix) and next generation sequencing combined with normalized amplicon coverage analysis (NGS/NAC, AmpliSeq, Ion Torrent). ERBB2 copy number values obtained by MIP or NGS/NAC were tightly correlated with ERBB2 copy number values obtained by conventional FISH ($rs = 0.940$ and $rs = 0.894$, $P < 0.001$). Using ASCO/CAP guideline-conform thresholds for categorization of breast cancers as HER2-negative, equivocal or positive, nearly perfect concordance was observed for HER2 classification by FISH and MIP (93% concordant classifications, $\kappa = 0.87$). Substantial concordance was observed for FISH and NGS/NAC (83% concordant classifications, $\kappa = 0.62$). In conclusion, MIP facilitates precise ERBB2 copy number detection and should be considered as an ancillary method for clinical HER2 testing.

P-CancG-029

Exome sequencing identified potential causative candidate genes for unexplained hereditary tumor syndromes

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Purpose: The aim of the study was the detection of novel, high penetrant cancer predisposition genes in two unrelated index patients with rare, early-onset tumor entities (*lipomatosis and embryonal tumor types*) and two families with a *Li-Fraumeni-like syndrome (LFLS)*. No germline mutation in established cancer predisposition genes corresponding to the phenotype had been identified beforehand by routine panel diagnostics.

Methods: To identify rare cancer related germline mutations in the families, we performed whole exome sequencing (Illumina HiSeq) of leukocyte DNA in the index patients and their healthy parents and/or in additional affected relatives. Variants were filtered for being rare (MAF: biallelic ≤ 1%, heterozygous ≤ 0.1% according to dbSNP, EVS, and ExAC), truncating (frameshift, nonsense, conserved splice sites), and for rare missense variants (predicted by ≥ 2/3 prediction tools as pathogenic). In the two single index patients variants were filtered for *de novo* mutations. To analyze the datasets and filter the variants, the GATK software and the Varbank Software were used. Subsequently, potentially causative variants were prioritized according to pathway analysis and biological processes. All selected variants were validated by Sanger sequencing and the zygosity of supposed biallelic variants was ascertained by testing of relatives, where applicable.

Results: After stringent filtering, manual exclusion of artifacts and considering a recessive or dominant model of inheritance, respectively, rare variants in 5-10 genes per patient / family remained, the majority of which were heterozygous and missense variants. The most interesting findings are presented in the following. In the lipomatosis patient, we identified a X-chromosomal missense variant in *KDM6A*, which might be associated with cancer and adipogenesis. In the patient with embryonal tumors, a homozygous missense variant in *LRRC16B* was discovered, whereas the parents were heterozygous carriers. Interestingly, this gene

is upregulated in fetal and tumor tissues. In one index patient with LFLS (multiple primary tumors) and healthy parents, we identified a de novo truncating heterozygous variant in *MINK1* as potentially causative. This gene has been linked to lots of cellular processes and is part of the MAP kinase pathway. In the second LFLS family we filtered for variants, which are present in the two affected sisters and absent in a healthy sister. The most interesting heterozygous truncating variant is a *CALML4* mutation, which functions as a regulator of signal transduction. When comparing the two LFLS families, no rare variants in the same genes were found.

Conclusions: The preliminary analysis of exome data identified a few promising, potentially causative genes in patients with a suspected hereditary tumor syndrome. However, to evaluate the clinical relevance, segregation data, additional affected patients and / or functional analyses are needed.

P-CancG-030

Quantitative investigation of BRIP1 and PALB2 expression level in breast cancer patient in compare with normal

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Quantitative investigation of BRIP1 and PALB2 expression level in breast cancer patient in compare with normal

Abstract

Background: Breast Cancer is the most common Cancer in the world and it is the second reason of death between women after the Lung Cancer. Studies show that spreading of this kind of Cancer in Asian Countries population like Iran is increasing. Breast Cancer consists 33% of present Cancers among Women and it is responsible of 19 percent of deaths for those who suffered it. Since the Cancer is influenced by heritage so screening and trace is necessary for the women who is facing with the danger of it, however the patients with personal history or family back ground are the most suspicious people for this kind of Cancer. Studies showed that more than 30% breast cancer caused by known biomarkers which could transfer from parents .Investigation of tumor biomarker expression level in patients who are apt to the breast cancer (BRIP1,PALB2) we could find some indicators as bio markers to investigate during screening of breast cancer samples at different stage of cancer , especially in pre-diagnostic studies.

Methods: In this study, we investigate expression level of two main biomarkers (BRIP1, PALB2) among 50 breast cancer patients in compare with 50 control people. Total RNA from Blood samples were extracted and cDNA is synthesized. The specific primers for detection of markers are designed and expression level of BRIP1, PALB2 in presence of gene GAPDH by using Real Time PCR method was quantitatively studied.

Results: Assessment of quantitative comparison showed that the two genes (BRIP1, PALB2) expression level increased significantly in breast cancer patient in compare with control population.

Conclusion: Based on this study results we could predict the expression level of (PALB2, BRIP1) gens in suffered patients quantitatively which could use as biomarker indicator during screening of breast cancer samples. So could be used in screening of population for early detection to inhibit cancer.

P-CancG-031

Genetic panel sequencing for advanced stage melanoma in the clinic.

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Background:

Genetic profiling of tumor tissue has become a fundamental component for personalized oncology. Targeted sequencing of tumor material enables the identification of genetic alterations in genes and their corresponding biological pathways. The results may lead to the possibility of a patient-tailored therapy. We designed a comprehensive gene panel to interrogate clinically relevant genes across the full spectrum of cancers. **Methods:**

The panel targets the coding region of 673 cancer associated genes, promotor regions and fusion breakpoints using the SureSelectXT technology by Agilent. The tumor material is either fresh frozen, formalin fixed paraffin embedded tissue or circulating cell free DNA. Blood is used as the normal sample. All samples were sequenced on Illumina devices (NextSeq500 / HiSeq2500). Raw data processing is done by our in house bioinformatics pipeline, publicly available on GitHub (https://github.com/imgag/megSAP). Best practice filters are used to narrow down the variant list, e.g. a minimum somatic allele frequency of 5 % is required. Copy number alterations are identified using our own tool CNVHunter and the published tool CNVkit (Talevich,

Shain, Botton, & Bastian, 2016). Once a variant list is generated all alteration are annotated for actionability using the QIAGEN Clinical Insight (QCI™) software and publically available Cancer Genome Interpreter (Tamborero, 2016). The result is further discussed with the treating physician and the interdisciplinary molecular tumor board (MTB) at the Comprehensive Cancer Center (CCC) in Tuebingen-Stuttgart. **Results:** Over the past 18 month 62 advanced melanoma patients were sequenced, primarily those that did not respond to immune checkpoint inhibitor therapy. All pairs were sequenced to a mean depth of 433x. We were able to identify 805 somatic mutations in total resulting in a median number of eigth somatic mutations per patient. This translates to an overall mutational burden of 4 mutations per megabase. The top ten altered genes are well known tumor suppressor and oncogenes affecting druggable signaling cascades like the RAS/RAF/MAPK or PI3K pathway as well as cell cycle control and the DNA damage repair. Since the majority of the cases are non-responders to immune checkpoint inhibitor therapy, additional alterations which might explain the resistance was found in the genes *B2M*, *MYC* and *JAK2*. **Conclusion:** Our results show that genetic screening of the cancer genome with a targeted gene panel is technically feasible in clinical setting of advanced cancer patients. But the search for actionable alterations and resistance mechanism is still challenging. For this reason the interpretatio of genetic findings should be a multistep process involving the use of public databases and group of experts: Which is why at the CCC Tuebingen-Stuttgart all genetic findings are discussed in an interdisciplinary molecular tumor board.

P-CancG-032

Gene Ontology term enrichment analysis following prostate cancer whole exome sequencing study

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The perception of prostate cancer as a complex disease encompasses the search for causal genes with related function beyond the conventional identification of genetic variants. Thus, in a sample of 25 pairs of brothers, who were all affected by prostate cancer and participated in a whole exome sequencing study initiated at Ulm University Medical Center, genetic variation was re-evaluated by means of Gene Ontology (GO) term enrichment analysis.

As a starting point, three scores for the deleteriousness of single variants were taken into consideration which then served to form summary scores to assess genes as a whole: the REVEL score ("an ensemble method for predicting the pathogenicity of rare missense variants"), the CADD score ("combined annotation dependent depletion"), and a further score based on how many of the five methods SIFT, Polyphen2, mutation taster, mutation assessor, and FATHMM judged a variant as deleterious. Applying these scoring methods to the variants observed in the WES-study, the highest scored 10% of genes yielded by each method were united (leading to U10, in total about 1000 genes) or intersected (I10: 2 or more score values among the best 10%, about 300 genes). These sets were contrasted to their complementary sets taken out of more than 18.000 genes. Quoting only GO classes with false discovery rate <= 0.05, at least 2-fold enrichment and a moderate number of GO genes (<= 20), we found amongst others the terms "ATPase activity", "acyl-CoA dehydrogenase activity", "flavin adenine dinucleotide binding", "fatty acid oxidation", "lipid oxidation" and "rho guanyl exchange factor activity" to be over-represented in U10 or I10.

In addition, these three scores were weighted to incorporate information whether (1) the sample frequency of variants was increased in comparison with population frequencies (measured by -log(p-value)), (2) the variants were predominantly shared by the two siblings in a pair (measured by -log(p-value) of a corresponding test), and (3) the gene showed or changed expression in normal and tumor tissue (measured by standardized values taken from the Human Protein Atlas). Essentially, the major part of evidence derived from the raw scores could be retrieved using the modified scores. E. g., a couple of genes covered by the term "acyl-CoA dehydrogenase activity", had already been identified among metabolic quantitative trait loci in samples from a study of prostate cancer in Swedish men.

Finally, omitting assessment of deleteriousness and taking into account only information on frequency, inheritance and gene expression ((1) to (3) above), again leads to "rho guanyl exchange factor activity". Guanyl exchange factors activate GTPases which are critical regulators of cell-cell junctions and could be involved in invasion and metastasis in prostate cancer. This supports that enrichment of specific "common" variants without measurable deleteriousness may contribute to disease development.

P-CancG-033

Early onset gynecological malignancies in Lynch syndrome

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Lynch syndrome (HNPCC) is an autosomal dominant disease caused by germline mutations in the DNA mismatch repair (MMR) genes MLH1, MSH2, MSH6 and PMS2. It is characterized by the clustering of colorectal cancer, gastric carcinoma, small bowel carcinoma, upper urinary tract cancer, as well as additional endometrial and ovarian carcinoma in women. In female MSH2 and MSH6 mutation carriers, the lifetime risk for endometrial cancer is estimated to be up to 44% (average age at diagnosis is 47-49 years) and for ovarian cancer up to 8% (average age at diagnosis is 42 – 48 years). Here, we present extraordinary courses of gynecological disease in two female patients identified in our DNA testing collective.

In family A, a 25 year old woman was diagnosed with adenocarcinoma of the cervix. Two sisters of the healthy mother developed gynecological cancer presumably of the cervix at the age of 35 and 45 years, respectively, a maternal cousin suffered from gynecological carcinoma at the age of 25. The father of the patient is unknown. Molecular pathology in lymph node metastasis, performed due to unusual histology of the cervical cancer, showed high-grade microsatellite instability and protein expression failure in MSH2 and MSH6. In the subsequent DNA analysis, a heterozygous pathogenic c.942+3A>T mutation was detected in the MSH2 gene. Genetic testing of the mother could not identify the c.942+3A>T mutation.

In family B, a 22 year old female patient developed a low grade serous ovarian cancer. Her maternal grandmother was diagnosed with ovarian cancer at age 75, her grandmother's sister died of colon cancer at the age of 40. A brother of the maternal grandfather died of colon cancer at age 44. Suggesting hereditary breast and ovarian cancer syndrome (HBOC) or Lynch syndrome, DNA testing of ten HBOC core genes identified no pathogenic mutation. Subsequent molecular analysis of the four MMR genes revealed a heterozygous pathogenic c.2335dupT, p.(Cys779Leufs*6) mutation in the MSH6 gene. In the 54 year old healthy mother, carrier status for the c.2335dupT mutation was confirmed.

Our case report demonstrate that gynecological malignancies may occur at extraordinary young ages in Lynch syndrome and also may lead to rare carcinomas of the lower uterine segment.

P-CancG-034

Screening for deleterious germline variants in the mismatch repair (MMR) genes using multi-gene panel testing in patients with hereditary breast cancer

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BACKGROUND:

Mutations in the BRCA1 and BRCA2 genes (BRCA1/2) are the most common causes of hereditary breast and ovarian cancer. About 10-12% of BRCA1/2-negative patients have pathogenic mutations in other high or moderate cancer risk genes. Deleterious germline variants in the mismatch repair (MMR) genes MLH1, MSH2, MSH6, and PMS2 predispose to Lynch syndrome and cause an increased risk of malignancies. Patients with MMR mutations can often present with cancers that are not considered part of Lynch syndrome. Finding pathogenic mutations in the MMR genes is important for cancer prevention in patients and/or their families.

METHODS:

To estimate the frequency of germline pathogenic mutations (class 4 and class 5 variants) and large rearrangements in the MMR genes, we analyzed 1700 women with a diagnosis of hereditary breast cancer using next-generation sequencing and digital MLPA. The deleterious germline variants were confirmed by Sanger sequencing or by standard MLPA.

RESULTS:

Six patients (0,4%) had pathogenic or likely pathogenic germline variants in two MMR genes. We identified two previously reported mutations in MSH2 gene and one mutation in PMS2 gene. Three novel large rearrangements in PMS2 gene were detected by digital MLPA: whole gene deletion, duplication of exon 11 and deletion of exon 12-14. No pathogenic mutations were detected in the genes MSH6 and MLH1.

CONCLUSIONS:

These results suggest that individuals with MSH2 and PMS2 mutations may present with a hereditary breast and ovarian cancer phenotype. In addition to breast cancer screening, these patients also need special cancer prevention for colorectal cancer. These data also highlight the need for further investigation of breast and ovarian cancer risks in large cohort with MMR mutations.

P-CancG-035

The GPRC5A frameshift mutation c.183delG is not associated with increased breast cancer risk in BRCA1 mutation carriers

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Introduction: The orphan G-protein coupled receptor GPRC5A has been associated with several cancer related pathways, such as the EGFR and the double-strand break repair pathway. Accordingly, dysregulation of *GPRC5A* expression has been reported in several cancer entities including lung, pancreatic, and breast cancer (BC).

Hypothesis: Within a consanguineous family of Turkish origin, we identified a homozygous frameshift mutation c.183delG (p.Arg61Serfs*51) in the first coding exon of *GPRC5A* in two sisters affected by early-onset BC at the age of 26y and 33y, respectively. The variant was identified via exome sequencing and verified as candidate via subsequent linkage analysis, suggesting an autosomal recessive trait. Interestingly, Sokolenko et al. reported a ten-fold increase in the heterozygous *GPRC5A* c.183delG mutation in carriers of the Russian *BRCA1* founder mutation c.5266dupC (5.1%; 6/117). Additionally, they provided evidence for a potential interaction between *GPRC5A* and *BRCA1* via RNA interference (RNAi) experiments and expression analyses in breast tumor tissue, suggesting that *BRCA1* expression is depending on *GPRC5A* expression. Therefore, we hypothesized that *GPRC5A* could act as a recessive BC risk gene and a modifier of the breast cancer risk in *BRCA1* and *BRCA2* mutation carriers when heterozygously inactivated.

Methods: We genotyped a large sample of *BRCA1*- (n=1,650) and *BRCA2*-positive (n=1,048) BC and BC/OC index patients recruited in the centers of the German Consortium for Hereditary Breast and Ovarian Cancer and geographically matched healthy or non-cancer controls (n=5,625) for the *GPRC5A* frameshift mutation c.183delG. Knockdown of either *GPRC5A* or *BRCA1* was carried out in the BC cell line MDA-MB-231.

Results: We did not observe a significant accumulation of *GPRC5A* c.183delG in BC patients carrying a BRCA1 mutation (0.79%; 13/1,650; OR: 1.136(0.606-2.129); p = 0.6909) compared to control individuals (0.71%; 40/5,625). These findings are further supported by preliminary RNAi data revealing only a slight reduction of *BRCA1* expression upon *GPRC5A* knockdown. However, in BC patients with *BRCA2* mutation we observed a significant accumulation of *GPRC5A* c.183delG (1.53%; 16/1,048; 2.217(1.237-3.975); p= 0.0061).

Summary and Outlook: We propose that the *GPRC5A* frameshift mutation c.183delG is not a modifier of BC risk in *BRCA1* mutation carriers, but may be a modifier in *BRCA2* mutation carriers. Further investigation is mandatory to validate these findings and *GPRC5A* as a recessive BC risk gene. Therefore, we generated a CRISPR-Cas9 induced *GPRC5A* knockout to further elucidate the role of *GPRC5A* deficiency in BC risk and its effect on *BRCA1* and *BRCA2* expression.

P-CancG-036

Incidental variant findings in BRCA genes in a large retrospective Swiss monocentric series

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Pathogenic variation in *BRCA* genes has been estimated to account for 20-30% of all familiar breast cancer cases. If known in a family at high risk, cumulative life time penetrance in *BRCA1/2* mutation carriers of 50%-70% for breast cancer and 15%-55% for ovarian cancer have been demonstrated, respectively. Thus, considering the substantial decrease of disease risk by intensified surveillance and prophlyactic surgery, knowdlege of the *BRCA* mutation status plays a crucial role in oncologic care. Since the advent of the early high-throughput sequencing technologies, organised *BRCA1/2* screening independent of individual and family cancer history has therefore been multiply proposed. As different ethnic and geographic regions have different *BRCA* variant spectra and prevalences, one critical prerequisite of a potentially general *BRCA1/2* testing is the

knowledge of the expected carrier frequency and of possible founder mutations among the population to be screened.

Herein we systematically assessed for the first time the prevalence of incidental *BRCA* variants in a Swiss monocentric cohort consisting of n = 404 individuals evaluated by Whole-Exome Sequencing (WES) (exome captured by Agilent SureSelectXT Kit, 125 bp paired-end sequencing on HiSeq2500 Illumina Platform) for neurodevelopmental disorders (NDD). Among these are n = 184 unaffected parental couples and n = 23 singles as well as n = 13 NDD index patients for which no individual history of cancer has been documented (average age of 43 years, R = 3–77 years). The wide majority of the study cohort is of Caucasian ethnicity (n = 340, 84.2%) and of Northwest European ancestry (n = 205, 50.7%) among which for n = 171 individuals (42.3%) an autochthonous Swiss descent has been assumed.

After filtering for rare protein changing and splice site variants (including 12 intronic baise pairs) with an overall minor allele frequency (MAF) below 2% a total of 33 and 61 different sequence alterations have been encountered for *BRCA1* and *BRCA2*, respectively. Interestingly, in respect to this total sequence variation yield, a percentage of 32.7% (n = 132 individuals) harbors at least one rare *BRCA* variant (9.9% and 24.0% for *BRCA1* and/or *BRCA2*, respectively). If categorized according to the current ACMG guidelines, for *BRCA1* gene a single ENIGMA curated pathogenic mutation and 13 variants of unknown significance (VUS) have been identified (carrier frequencies of 0.24% and 3.2%, respectively). Among the latter, six VUS – four missense and two non-coding variants - are novel and have been reported neither in any locus- specific mutational database (LSMD) nor in any reference population database. For the *BRCA2* gene, two *bona-fide* pathogenic frameshift muations have been found in two female individuals of Italian ancestry (carrier frequency of 0.50%). Furthermore, 19 incidental BRCA2 VUS could be detected in n = 21 individuals (carrier frequency of 5.20%) consisting of 16 missense and three non-coding alterations being novel in about a third of the alterations.

In summary, the presented large cancer-unrelated cohort demonstrates the high prevalence of *BRCA1/2* pathogenic variants in the general population as well as a substantial frequency of variants of unclear clinical significance warranting further investigation.

P-CancG-037

Heterozygous and hemizygous TET2 mutations in myeloid dysplasias

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Introduction: The *TET2* gene encodes a protein that plays a key role in DNA demethylation. It is involved in myelopoiesis and defects in *TET2* have been associated among others to various types of myeloid dysplasias, including acute myeloid leukemia, chronic myelomonocytic leukemia and myelodysplastic syndromes. We herein present investigations of the *TET2* mutation status in 7 patients with myeloid malignancies.

Methods: We analyzed bone marrow samples of 7 patients with a myeloid dysplasia by conventional karyotyping using R-banding (7 cases) and fluorescence in situ hybridization (FISH) with a *TET2* Deletion probe (5 cases) (Metasystems, Altussheim, Deutschland). Next-generation sequencing (NGS) was performed on DNA of 5 cases using a MiSeq benchtop sequencer (Illumina, San Diego, USA). Sanger sequencing of exons 2 to 11 of TET2 isoform A was performed on DNA of the remaining 2 cases.

Results: A normal karyotype was seen in 6 cases. One case presented an abnormal karyotype with an isolated trisomy 14. Deletions in chromosome 4q24, where *TET2* is located, were not seen by R-banding. By FISH, a heterozygous deletion of *TET2* was identified in 2 cases. 1 patient harbored one mutation, in 3 patients 2 mutations in *TET2* were found. The mutations lead to frameshift or nonsense-changes and, thus, resulted in a truncation of the encoded protein. Three patients showed 3 missense mutations with unknown effect (unclassified variants). Interestingly, one of the two cases with a deletion of *TET2* evidenced two *TET2* mutations in exon 3 either simultaneously or in 2 clones (one nonsense mutation and one frameshift mutation).

Conclusions: *TET2* mutations occur both as heterozygous and hemizygous events in patients with myeloid dysplasias. Concomitant chromosomal deletions of *TET2* were detected by FISH in 2 out of 5 cases and were cryptic for conventional cytogenetic analyses. Moreover, in one of these two latter cases, two hemizygous *TET2* mutations affecting the remaining allele were seen. Whether these two mutations are present concomitantly in one clone or separately in different clones is subject of ongoing investigations. Additional studies of a larger group of patients are required to determine incidence of hemizygous *TET2* mutations and their clinical significance.

P-CancG-038

Philadelphia chromosome positive acute lymphoblastic leukemia (PH+ ALL) or chronic myeloid leukemia in lymphoid blast phase (CML-BC-lymph) in childhood?

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Background: Although described as a characteristic chromosomal aberration in chronic myeloid leukemia (CML), the BCR-ABL1 fusion resulting from the reciprocal translocation t(9;22) is also detected in other leukemias including ALL. About 2-3 % of pediatric ALL patients present with a BCR-ABL fusion. The discrimination between patients with CML-BP-lymph and Ph+ ALL at the time of diagnosis can be particularly challenging. While CML is a multilineage disease with a stem cell precursor and, in the terminal stage of blastic phase both, lymphoid and myeloid cells are affected, ALL is generally considered to be lymphoid-restricted. The aim of this study was to define an easy and effective method to discriminate between Ph+ ALL and CML-BP-lymph based on the affected cell types and, therefore, offer the optimal therapy to all patients.

Material und Methods: We performed a retrospective analysis on four pediatric patients that were diagnosed with Ph+ ALL. We used fluorescence in situ hybridization (FISH) for staining BCR-ABL1 fusion in interphase nuclei. Co-staining with DAPI allowed the discrimination between segmented nuclei (granulocytes) and mononuclear cells. Furthermore, we separated granulocytes and mononuclear cells via density gradient centrifugation using Ficoll-PaqueTM Plus solution. Following this, a nested PCR was performed in the different fractions for the detection of BCR-ABL1 transcripts on the cDNA level.

Results: Using nested RT-PCR subsequent to cell separation we detected BCR-ABL1 transcripts in both fractions of all patients analyzed. This led us to the conclusion that even slight contamination with other cell fractions can result in false positive BCR-ABL1 transcript detection using this method. FISH with DAPI co-staining on the other hand allowed the morphological discrimination between granulocytes and mononuclear cells and, therefore, contamination of the fractions is no major concern. In three patients diagnosed with Ph+ ALL we were able to detect BCR-ABL1 fusions in granulocytes indicating a multilineage disease such as CML.

Conclusions: The discrimination between patients with CML-BP-lymph and Ph+ ALL at the time of diagnosis still is a major concern today. Analysis of BCR-ABL1 fusions in granulocytes via FISH offers an easy method in routine diagnostics for discrimination of these two entities. We recommend performing FISH on granulocytes if possible prior to treatment. As the majority of patients do not have sufficient numbers of granulocytes due to predominate lymphoblastic proliferation at the time of diagnosis, cell separation can be used to enhance the proportion of granulocytes thus allowing the analysis via FISH. Alternatively, analysis in granulocytes can be performed shortly after start of cytoreductive treatment, e.g. within the first two weeks of therapy.

P-CancG-039

Next generation sequencing (NGS) based detection of copy number variations (CNV) in hereditary breast and ovarian cancer germline diagnostics

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NGS (Next Generation Sequencing) based CNV (Copy Number Variation) detection is a new possibility to detect large rearrangements in genes. We used the TruRisk® gene panel (Agilent SureSelect) which covers 34 cancer risk genes/candidate genes for NGS analysis. Sequencing was performed on Illumina NextSeq devices. For bioinformatic analyses of sequencing data, we employed the Sophia Genetics DDM platform. All cases met the inclusion criteria of the German Consortium Hereditary Breast and Ovarian cancer (GC-HBOC) for germline testing.

Of the 4,933 datasets included in our CNV study 98% (4,813) were successfully analysed. As expected, most CNVs were detected in the BRCA1, BRCA2 and CHEK2 gene. Yet, CNVs were also observed in further established cancer predisposition genes (*ATM*, *BARD1*, *FANCA*, *MLH1*, *MSH2*, *PALB2*, *PMS2*, *RAD50*, *RAD51C*, *RAD51D*, *TP53*). About 70% of the detected CNVs were confirmed by either MLPA or aCGH at the time of data collection. However, no CNVs were detected in *MSH6*, *CDH1*, *NBN*, *FANCM*, *PTEN*, *STK11*.

In summary, bio-informatic analyses of NGS gene panel data robustly detects CNVs in a large sampleset. However, it remains to be determined whether sensitivity/specificity of *in silico* CNV detection meets diagnostic criteria.

P-CancG-040

*** 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 carcinoma (ACC) is a rare disease. Due to an often late diagnosis and limited treatment options prognosis for patients are poor with a 5 year overall survival rate of 7 to 35%. Although integrated studies identified complex molecular patterns related to outcome over the last years, no reliable molecular prognostic factors, as well as no effective targeted therapy or personalized treatment approach emerged to date. The aim of this study was to establish a reliable method to define a molecular signature of ACCs that could be used for prognostic classification, planning an individualized therapeutic approach and identification of potential targetable molecular events with data gained from formalin-fixed and paraffin-embedded (FFPE) samples that are routinely used in clinics for cancer diagnostics.

DNA from 107 FFPE tumor and matched blood samples was enriched with the Human Comprehensive Cancer Panel (Qiagen), which includes coding sequences from 160 genes associated with different entities of cancer, sequenced on a NextSeq500 (Illumina) and analysed with GensearchNGS (PhenoSystems). Pyrosequencing was performed on a PyroMark Q96 MD Pyrosequencing System (Qiagen) to determine the methylation status of the four tumor suppressor genes GSTP1, PAX5 and PAX6 and PYCARD in ACC and analysed with PyroQ-CpG software (Biotage).

Sequencing data were used to detect somatic single nucleotide variants (SNV), small insertions and deletions (smallInDel), copy number variations (CNV) and methylation status. Sequencing data were then compared to clinical data e.g. tumor stage, Ki67-index and time of PFS to define molecular prognostic factors.

In 77 of 107 patients (72%) one or more somatic SNVs and smallInDels were detected. Mutations were frequently found in TP53 (22%) and CTNNB1 (17%), and other genes that had already been described in ACC (e.g.NF1, ZNRF3, APC, MEN1, ATRX and GNAS). We also found genes that have not been associated with ACC before (e.g. NOTCH1, CIC, KDM6A, BRCA1 and BRCA2). 43% of samples were affected by CN gains in CDK4. 33 tumors (31% of cases) presented a mean methylation pattern >25% which was considered as hypermethylated.

Correlated to clinical outcome, data showed that patients with two or more mutations as well as those with alterations in Wnt/ß-catenin signalling pathway or Wnt/ß-catenin and p53 signalling pathway have a shorter PFS survival. Pyrosequencing data showed that patients with a mean methylation of ≥25% have a shorter PFS compared to patients with lower mean methylation.

Interesting targetable events were CN gains at CDK4 locus as well as mutations in genes of the DNA repair system (ATM, BRCA1, BRCA2).

This study demonstrates the feasibility of targeted DNA-sequencing in FFPE samples. Our findings will help to provide a molecular risk stratification of ACC patients, and to identify new potential targeted therapies for a precise medical approach.

P-CancG-041

Searching the needle in a haystack: regulatory pathomechanisms in DNA mismatch repair genes

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In patients with a clinical suspicion of Lynch Syndrome (LS) predisposing for early-onset hereditary colorectal cancer and associated tumors, an immunohistochemical loss of expression of DNA mismatch repair (MMR) proteins MLH1 and/or PMS2 or MSH2 and/or MSH6 combined with a high microsatellite instability in the tumour hints towards an underlying germline defect in the respective gene.

In approximately 15-20% of these patients suspected of having LS, no causative germline MMR variant is detectable, and the heredity and molecular basis of their tumor predisposition remain unsolved. For these patients, further defects may exist for the MMR genes.

Our aim was to search for regulatory mechanisms in the germline of these four MMR genes.

We defined a cohort of 124 moleculargenetical unsolved patients meeting one of the Bethesda criteria. Prerequisite was an immunohistochemical loss in at least one MMR protein (28 MLH1/PMS2, 19 PMS2 isolated, 51 MSH2/MSH6, 26 MSH6 isolated) in the tumor and an inconspicuous routine germline diagnostics for MMR sequence variants and larger deletions or duplications. 38 patients with MLH1-deficiency showing MLH1 promoter methylation in tumor tissue but not in germline were excluded.

We analysed the promoter region of the respective MMR gene which was absent in the tumor by Sanger sequencing in lymphocyte DNA of our 124 unsolved patients. For 79/124 patients we also investigated germline methylation and copy number variation (CNV) in the MMR promoters by applying MS-MLPA kit Me011 (MRC Holland).

As a result, promoter sequence variants of unknown significance and a low allelic frequency in controls were detected in four patients, two in MSH2 and one each in MLH1 and PMS2.

A 50% germline promoter methylation is known to exist in rare cases for MLH1; we recently described 16 patients, one with a causative promoter variant. Our results here reveal absence of germline promoter methylation for MSH2, MSH6, and PMS2, and we did not discover additional cases for MLH1.

Patients with deletions or duplications of a coding MMR gene region (potentially involving the promoter as well) were sorted out by routine diagnostics. We did not detect additional CNVs limited only to the promoter region of MLH1, MSH2, MSH6, or PMS2 in our 79 unsolved patients investigated by MS-MLPA.

We conclude that MMR promoter changes involving variants, methylation and CNVs are no frequent pathomechanisms in unsolved patients with LS-suspicion, so that regulatory defects based on promoter changes are therefore estimated to play a minor role. For the four MMR promoter variants the functional relevance in colon tissue expression remains to be investigated.

In patients with no family history of cancer, somatic MMR mutations in the tumor may be the reason for the LS phenotype. For patients with familial clustering of tumors, however, further MMR gene defects or germline mutations in other DNA repair genes such as POLD1, POLE might be the underlying cause.

P-CancG-042

Characterization of SUP-T11 as a model cell line for T- Prolymphocytic Leukemia

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T-cell prolymphocytic leukemia (T-PLL) is a rare aggressive post-thymic T-cell malignancy accounting for about 2% of mature lymphocytic leukemias in adults. The median age at diagnosis is 65 years. The chromosomal hallmark is an inv(14)(q11q32/t(14;14)(q11;q32) or less frequently the translocation t(X;14)(q28;q11), juxtaposing the TCRAD locus (14q11) to the oncogenes TCL1 (14q32) or MTCP1 (Xq28), respectively. As a consequence TCL1A or the highly homologous gene MTCP1 are expressed in T-PLL cells. The mechanisms leading to the leukemogenesis of T-PLL are not yet fully understood, although several signaling pathway including the JAK-STAT pathways have been identified to be recurrently altered.

No human tumor cell lines derived from T-PLL exist to model the disease in vitro. Nevertheless, the cell line SUP-T11 is frequently used for studying T-PLL, as it carries the hallmark cytogenetic aberration of T-PLL. But, the SUP-T11 cell line is derived from a patient with T-acute lymphoblastic leukemia. The aim of this study is to characterize the SUP-T11 cell line on cytogenetic and molecular genetic level to ascertain if it is indeed a suitable T-PLL model system.

In this study, we characterized the SUP-T11 cell line by cytogenetics including karyotyping and fluorescence in situ hybridization (FISH) using break apart probes for the TCL1A, TCRAD and MTCP1 genes, immunophenotyping by cytometry and analysis of chromosomal alterations using SNP6.0 arrays. Furthermore, we analyzed the TCL1A expression on RNA-level by quantitative real time PCR (qPCR) and on protein level by Western Blot. Finally, we analyzed the coding region of TCL1A gene for the occurrence of mutations by Sanger sequencing.

In a first step, we analyzed the chromosomal alterations in SUP-T11 cell line which revealed a highly complex karyotype including a t(14;14)(q11;q32). By FISH, we observed signal constellations indicating breaks for both TCRAD and TCL1A , in line with the results of karyotyping. Using SNP-array, we detected a total of 61 copy number alterations including gains in 8q and losses in 5q, 11p 13q, 12p and 17p. Immunophenotyping

showed an expression of T-cell markers such as CD2, CD3 and CD7, while lack of CD1a expression. Remarkably, we could detect TCL1A mRNA by qPCR whereas we could not detect TCL1A protein expression in Western blot analyses in repetitive attempts. To exclude that the protein expression is impaired due to a mutation, we performed Sanger sequencing of all 5 exons of TCL1A by which we could not detect any mutations on genomic level.

Taken together, based on cytogenetic and genetic analysis we can show that SUP-T11 harbors hallmark chromosomal abnormalities as observed in T-PLL. Also, an expression of TCL1A could be detected at RNA level, but not on protein level. Hence, and considering that the pattern of imbalances is quite different from T-PLL and its derivation from a T-ALL, it is questionable if SUP-T11 is indeed a suitable model for T-PLL.

P-CancG-043

Uniparental Disomies (UPD) in acute lymphoblastic leukemia: Integration of SNParray analysis

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Background: Hypodiploid B-cell precursor acute lymphoblastic leukemia (pB-ALL) with <45 chromosomes represents an extremely rare subtype of disease and has been correlated with high risk of treatment failure and disease relapse. Correct diagnosis of hypodiploid pB-ALL in conventional karyotyping is frequently hampered by chromosomal doubling of the founder clone resulting in a so-called masked hypodiploidy with >46 chromosomes. The primary aim of this ongoing project is the identification of masked hypodiploidies by detecting uniparental disomies (UPD) with SNParrays. Secondly, in a prospective approach sporadic UPDs in hyperdiploid pB-ALL samples are recorded to enable correlation with longitudinal disease course data.

Methods: Pediatric pB-ALL patients within the BFM-ALL 2009 trial were analyzed for risk stratification markers including translocations (*BCR-ABL1* and *ETV6-RUNX1*), rearrangements (*KMT2A* and *TCF3*), and hypodiploidy (<45 chromosomes) using cytogenetics and PCR analysis. Samples negative for these markers qualified for genome-wide copy number analysis on high resolution arrayCGH (400k eArray, Agilent). For this project, a subset of samples with chromosome numbers >46 were additionally evaluated for UPDs (copy-neutral losses of heterozygosity) using SNParrays (CytoScan HD or OncoScan, Affymetrix).

Results: Between February and November 2017, a total of n=336 pB-ALL samples were obtained. Within these samples, n=199 had to be run on microarray due to absent risk stratification markers, including n=101 samples with >46 chromosomes based on cytogenetic and molecular cytogenetic observations. From these hyperdiploid samples n=73 were analyzed for UPD. This approach identified two cases of masked hypodiploidy with 56 chromosomes [arr(1-3,5-7,9,11-17,19-20,22,X)x2 hmz, (4,8,10,18,21)x4, (Y)x0] and 68 chromosomes [arr(1,5,6,8,10,11,14,19,21,22,X)x4, (2-4,7,9,12,13,15-18,20)x2 hmz], respectively. Analysis of the remaining hyperdiploid cases identified sporadic UPDs affecting a maximum of three chromosomes per case. UPDs were frequently observed for chromosomes 7, 9 and 12 (5.6%, 11.3% and 5.6% of 71 cases).

Conclusion: Our SNParray analysis identified masked hypodiploidy in two patients with hyperdiploid clones as detected by karyotyping and FISH. These findings are relevant for risk stratification and patients were treated according to the high-risk protocol. The aberrant karyotypes, harboring five and eleven tetrasomies, are a likely result of chromosomal doubling of clones with 28 (near-haploid) and 34 chromosomes (low hypodiploid), respectively. In line with such mechanism, samples showing rare tetrasomies accompanied by numerous trisomies did not show masked hypodiploidy. Sporadic UPDs, primarily of chromosome 9, have been reported previously in hyperdiploid ALL and leukemogenesis might be influenced by loss of heterozygosity. Sequence data analysis (RNA or Exome) to detect potential unmasking of recessive pathogenic alleles will follow as well as comparison of treatment responses between hyperdiploid pB-ALL with and without sporadic UPDs.

P-CancG-044

*** Large B-cell lymphoma with IRF4-rearrangement harbor recurrent IRF4 and CARD11 mutations

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A rare subtype of B-cell lymphoma are large B-cell lymphoma with IRF4-rearrangment (IRF4+LBCL) which is considered as a distinct provisional entity by the WHO classification update in 2016. These lymphomas present either as follicular lymphomas grade 3B or as diffuse large B-cell lymphomas with strong expression of the transcription factor IRF4/MUM1 usually due to an IRF4-translocation by which IRF4 is juxtaposed to the immunoglobulin heavy chain region (IGH) or one of its light chain variants. Little is known about the genetic characteristics of these lymphomas. Hence, the aim of our analysis was to characterize the genetic landscape of these lymphomas.

We recruited 9 LBCLs with proven IRF4-rearrgangement of which 4 were analyzed within the ICGC MMML-Seq project. 8/9 cases were shown by immunohistochemistry to be positive for IRF4/MUM1 protein expression. We performed whole exome sequencing (WES, N=5) and whole genome sequencing (WGS, N=4) to investigate the mutational landscape. To study copy number alterations, we performed OncoScanTM CNV FFPE arrays (N=5) of those cases with WES data, whereas on cases with WGS data we applied the ACE-seq algorithm (Kleinheinz et al, submitted). Additionally, 8 described IRF4+LBCL (Salaverria et al., Blood 2011) were screened for IRF4 mutations in exon 2.

In a first step we mined the sequencing data for the breakpoints of the IG-IRF4 translocation. In 4 cases, we could detect the breakpoints in IRF4 and IGH or IGK and could validate 3 of them by Sanger sequencing. Interestingly, 3 of the breakpoints were located downstream from IRF4 whereas in one case the breakpoint was located within the IRF4 gene. Analysis of the sequencing data revealed recurrent mutations in IRF4 (7/9) and CARD11 (4/9), which both play a role in the B-cell receptor signaling pathway. Interestingly, a median of 2 coding IRF4 mutations per case was identified. All coding mutations mapped within the 2nd exon all located on the same allele. 4 of the 8 additional IRF4+LBCL cases harbored IRF4 mutations. In addition to the coding IRF4 mutations, we detected in 7/16 cases (44%) a mutation at the same position within the Kozak translation initiation sequence which might influence the translation of the IRF4 protein. This mutation did not occur in any other B-cell lymphoma of the ICGC MMML-Seq cohort. Next, we screened the cohort for recurrent copy number alterations. We could identify chromosomal alterations affecting the CD79B and GRB2 genes known to participate in the B-cell receptor signaling pathway.

Taken together, we could show that the IRF4+LBCL harbor a specific mutational landscape with recurrent IRF4 and CARD11 mutations. In addition to the mutations, recurrent copy number alterations affecting the B-cell receptor signaling pathway have been identified. Thus, the described genetic alterations indicate a crucial role of B-cell receptor signaling for the lymphomagenesis of the IRF4+LBCL which might be an applicable target for a specific therapy.

P-CancG-045

*** *ALK*-fusion gene expressing large B-cell lymphoma harbor a different genetic and epigenetic landscape than other large cell lymphoma variants

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Anaplastic lymphoma kinase (ALK)–positive (+) large B-Cell Lymphoma (LBCL) is a rare subtype of diffuse LBCL (DLBCL). It affects mainly male patients and one third of cases occur in the pediatric age group. ALK+LBCL show an aggressive behavior with poor response to standard therapies. The hallmark of ALK+LBCL is a chromosomal translocation leading to an activation of the *ALK* gene. In vitro and in vivo experiments have shown that treatment of this tumor with an ALK-inhibitor efficiently leads to cell death. Besides this translocation not much is known about the genetic and epigenetic landscape and drivers of ALK+LBCL lymphomagenesis. Hence, the current aim was to examine the genetic and epigenetic changes in ALK+LBCL.

We recruited 10 ALK+LBCL for our present study. We performed OncoScan™ FFPE CNV array in 7 cases to analyze the chromosomal imbalances as well as whole exome sequencing (WES) in 5 cases to identify recurrently altered genes. In addition, we analyzed the DNA methylation profile of 6 ALK+LBCL by HumanMethylation450 BeadChip array. We contrasted the findings to those in mature B-cell lymphomas (BCL) including 19 DLBCL, 25 Burkitt lymphomas (BL) and 26 follicular lymphomas (FL) which were analyzed in the framework of the ICGC-MMML Seq as well as to 24 multiple myelomas (MM) previously published (Agirre et al., Genome Research, 2015). As controls we included publicly available DNA methylation data of 93 B-cell populations of different developmental stages. In addition, we included an ALK+LBCL cell line (CL) model in all analyses and performed whole genome bisulfite sequencing in the CL.

Analysis of the copy number alterations did not reveal any highly recurrent alterations. Nevertheless, we identified alterations recurrent in mature BCL like gains in *BCL6* or losses in *TP53* in 2/7 and 4/7 cases, respectively. By WES we identified 10 genes to be mutated in 2/5 cases. Among those was the gene *NSD3* encoding a histone methyltransferase. Interestingly, genes recurrently mutated in DLBCL like *CREBBP* or *EP300* were not mutated in any ALK+LBCL. Analysis of the DNA methylation profile revealed ALK+LBCL to show a massive loss of global methylation similar to the pattern observed in MM. Unsupervised DNA methylation analysis of all lymphoma subtypes revealed that ALK+LBCL form a cluster with MM but apart from other BCL (σ/σ_{max}=0.8, 2348 CpG). In line with this, a phylogenetic analysis indicates a similar maturation stage of the ALK+LBCL and MM.

In conclusion, due to the lack of recurrent mutations and no highly recurrent copy number alterations, we assume the *ALK*-translocation to be the main driver of lymphomagenesis. Furthermore, based on the epigenetic profiling a cell of origin similar to that of MM and different from that of DLBCL can be assumed. Thus, the genetic and epigenetic characterization improves the understanding of the biological and clinical differences compared to other DLBCL variants.

P-CancG-046

Hereditary TP53 mutations identified and classified by NGS gene panel sequencing

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Background *TP53* (MIM #191170) is one of the five most significantly mutated genes in the most common human cancers, including breast- and ovarian cancer. Recent molecular profiling studies have identified four major subtypes of breast cancer: luminal A, luminal B, basal like, and *HER2*. The frequency of *TP53* alteration

in these subtypes ranges from 12% in luminal A to more than 80% in basal like. In colon cancer, mutations occur less frequently in tumors with deficiencies in mismatch repair genes associated with high-microsatellite instability. The *TP53* tumor suppressor protein is activated in response to a variety of stress signals and suppresses cellular transformation by triggering cell-cycle arrest, DNA repair, and apoptosis. In addition, a role for *TP53* in processes such as metabolism, fertility, angiogenesis, immune responses, and stem cell maintenance has been shown.

Patients & Methods We applied NGS gene panel sequencing searching for hereditary, disease-causing mutations in HNPCC-, breast- and ovarian cancer- and phenotypically diagnosed Li Fraumeni syndrome-cases. Both, the HNPCC and the breast- an ovarian cancer panel included the *TP53* gene. Approx. 1.900 samples were analyzed, 120 samples on HNPCC panel and 1.680 samples on the breast- and ovarian cancer panel, respectively.

Results In total, we identified likely pathogenic (class 4) or pathogenic (class 5) *TP53* mutations in nine cases. One intronic splice-site mutation hasn´t been classified before. We applied RNA sequencing by Sanger and by NGS in parallel to characterize this mutation in more detail, i.e. its effect on splicing. Our study shows, that NGS RNAseq is much more suitable for characterizing this kind of mutation than Sanger sequencing, circumventing pitfalls like allele drop outs.

P-CancG-047

Investigating the Molecular Basis of Glioblastoma using Transcriptome and Pathway Analysis

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Glioblastoma (GBM) is the most common and aggressive primary central nervous system malignancy, with median survival of 6-12 months. Although the etiology of GBM has not been fully elucidated, viruses like human cytomegalovirus, ionizing radiation and occupational exposure to rubber and petrochemical materials are associated with increased risk. To gain deeper insights into the molecular processes involved we studied transcriptome wide expression profiles using RNAseq of normal/tumor tissue pairs of 12 GBM patients and systematically analyzed the differentially expressed genes.

Fresh paired normal and tumor tissue samples were obtained from 12 patients with GBM undergoing surgery in Neurosurgery Department at Istanbul University Medical Faculty. Immunostaining was performed at Pathology Department validated the nature of both tissue groups. After RNA extraction and reverse transcription, we prepared libraries with the Ion Ampliseq Transcriptome Human Gene Expression Kit. Templated spheres were achieved by emulsion PCR using the Ion One Touch system and were sequenced with an Ion S5 semiconductor sequencer. Subsequently we performed an extensive pathway analysis using Ingenuity Pathway Analysis.

Three pairs were excluded as a result of the principal component analysis (PCA), as normal tissue from these patients showed tumor-like characteristics. We found 3,062 differentially expressed genes ($p<0.001$) which were subsequently subjected to Ingenuity Pathway Analysis.

In order to elucidate perturbations in gene regulatory networks leading to glioblastoma formation, we screened for potential upstream regulator genes. As major signaling pathways, such as cAMP, CREB, and MAPK signaling were dysregulated in the tumor samples, upstream analysis was perfomed to prevent incorrect attribution of secondary effects. At a more relaxed threshold of $p<0.05$, 120 common genes identified as upstream regulators and differentially expressed ($p<0.05$) were evaluated according to gene expression in the individual samples, and selected according to significance and biological roles in glioblastoma. This list of genes contained well-known factors involved in glioblastoma formation, such as *ERBB2*, *TP53*, *TP73*. Other candidates such as *CPXM1* and *KDM3A* were not previously associated with this type of tumor. Tumor tissues displayed an upregulation of proliferation and invasion pathways and downregulation of neuronal and glial differentiation. Validation and replication in further 23 tissue pairs by qPCR experiments are in progress. The results will help uncovering novel regulatory networks in glioblastoma formation.

P-CancG-048

European Reference Network for Genetic Tumor Risk Syndromes (GENTURIS) – the future of patient-care in Europe

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Rare or low-prevalence complex diseases affect around 30 million citizens of the European Union (EU). For many of these diseases standardized concepts for treatment are still lacking, and especially for poorer countries it is difficult to provide high-quality care for these people. In March 2017, the European Commission approved 24 European Reference Networks (ERN), many involving German centers. ERNs are virtual networks of health-care providers and centers of expertise across Europe, which aim at improving access to diagnosis and treatment as well as providing high-quality care for patients with rare diseases all across Europe.

The ERN for Genetic Tumor Risk Syndromes (GENTURIS) is concerned with families affected by hereditary tumor predisposition syndromes. GENTURIS patients are at very high risk of developing common cancers, which are often located in multiple organ systems. Affected individuals and closely related relatives need different treatment and more intense surveillance programs as compared to patients with non-hereditary cancers. According to estimates, about 70-80 % of families are still not diagnosed meaning that neither the index patients nor their relatives receive appropriate care to prevent cancer.

The main objectives for GENTURIS are to improve the identification of patients with genetic tumor risk syndromes, to develop evidence based guidelines and educational materials on these syndromes, and to make them accessible to all physicians and patients in Europe. Concomitantly, research projects to find new genetic causes for GENTURIS diseases in yet unexplained families are being designed. To deal with these complex tasks, four thematic and six working groups have been established. Patient representatives have been involved in the GENTURIS governance and in the different working groups from the beginning to make sure that the measures meet the requirements oft the people they are meant for.

Three expert centers from Germany meeting the high requirements of the European Commission are involved in GENTURIS: the Center for Hereditary Tumor Syndromes at the University Hospital of Bonn, the Institute for Human Genetics at the University of Dresden, and the Medical Genetics Center in Munich. These centers coordinate the interdisciplinary collaboration at their location and act as contact persons for other health-care provider caring for patients with genetic tumor risk syndromes in Germany.

The ERN GENTURIS is meant to improve both health-care and research for families with genetic tumor risk syndromes nationally and across Europe and to promote communication and collaboration of European experts. Further information is provided on the GENTURIS homepage (www.genturis.eu).

P-CancG-049

Next Generation Sequencing and Pediatric Brain Tumors: Detection of Hereditary Cancer Predisposition in CNS Tumor Patients and their Families

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The international study ‘Molecular Neuropathology 2.0’ (MNP2.0) is a comprehensive approach offering histological and molecular genetic diagnostics to a large cohort of pediatric patients with primary central nervous system (CNS) tumors, also aiming to identify molecular targets for potential therapeutic options. By assessing tumor and germline DNA variations of all study subjects, this study bridges the gap between scientific genetic analysis and medical care. The study’s workflow takes the conditions of a multicenter study, legal stipulations, as well as the needs for close interdisciplinary cooperation into consideration. To date, gene panel analysis was performed on tumor tissue- and blood derived DNA of 458 CNS tumor patients. Here we present a workflow illustrated by case reports of patients diagnosed with different cancer predisposition syndromes (CPS). The diagnosis of a CPS and subsequent family analysis are of substantial importance for

all presented patients and their families. Germline analysis within the ongoing MNP 2.0 study provides additional information about the prevalence and distribution of potential underlying germline mutations in a large population-based cohort of pediatric neuro-oncology patients. In addition, the results of this study have the potential to identify CNS tumor entities- or molecular subgroups with a high prevalence of underlying CPS.

P-CancG-050

Long-term survival and second malignancies in patients with retinoblastoma: a report from the German referral center from 1940-2008

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Retinoblastoma is the most common eye tumor in childhood. The 5-year overall survival for children with retinoblastoma is excellent. However, those 50 % of patients who have heritable predisposition to retinoblastoma have an increased risk to develop second primary malignancies (SPM) later in life and mortality due to SPM is high. Here we present a study on long-term survival and the incidence of SPM in German retinoblastoma patients. At the German referral center, 1194 national patients were treated for retinoblastoma between 1940 and 2008. Overall survival and the influences of constitutional *RB1* mutation, tumor extent, decade of diagnosis and treatment were analyzed. The 5-year overall survival rate was 95.4 % after diagnosis of retinoblastoma between 1940-2008 in Germany. Despite a 5-year overall survival rate of 97.4 % (95 % CI: 96.0- 98.8%) for survivors of heritable retinoblastoma with tumors restricted to the eye (IRSS stage 0 or I), the 50-year overall survival rate was significantly decreased compared to patients with non-heritable disease. The most common cause of death were SPMs. The cumulative incidence ratio (per 1000 person years) of second cancers in survivors of heritable retinoblastoma was 8.6 (95 % CI 7.0; 10.4). The type of eye-preserving treatment of retinoblastoma and constitutional *RB1* mutation influenced the incidence of second cancers and overall survival. The results of this follow-up study emphasize that long-term side effects of eye-preserving treatment need to be balanced carefully and alternative eye-preserving treatments are urgently required. For all survivors of heritable retinoblastoma, life-long regular oncological follow-up is crucial.

P-CancG-051

Combined BRCA1/2 mutation and copy number analysis in native ovarian cancer tumor samples

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BRCA1/2 status of tumor cells is of great relevance in the treatment of ovarian cancer with PARP inhibitors. Genetic studies in tumors are usually restricted to sequence analyses. We have devised a cost-effective massive parallel Sequencing (MPSeq) approach which enables detection of both *BRCA1/2* mutations as well as copy number changes in tumor tissues in a routine diagnostic setting. 46 shock-frozen ovarian cancer tumor samples were obtained from 43 patients.

Target-genes were enriched by using hybridization-based Nextera® Rapid Capture (TruSight™ Cancer Sequencing Panel, Illumina) and subsequently sequenced on a MiSeq platform (Illumina). Sequence data analysis was carried out with the SeqNext Software (JSI). All variants in over 5% of the reads were classified according to the consensus recommendations of the American College of Medical Genetics (Richards et al. 2015) as (likely) pathogenic (denoted mutations), unclassified, or (likely) irrelevant variants. To distinguish germline mutations from somatic mutations, the presence of mutations was assessed in DNA from matched blood-lymphocytes. Quantitative analysis was established by comparing data from 180 blood-lymphocyte samples analyzed with methods recently evaluated by us (Povysil et al. 2017) and validated for tumor samples.

Loss of heterozygosity (LOH) – deletion-based or copy-number-neutral – was determined taking into consideration the frequency of non-pathogenic variants. Evidence for generalized genomic instability could be obtained by quantitive analyses of a large number of genes. *BRCA1/2* germline and somatic mutations were detected in 13% and 5% of the analyzed tumor samples, respectively. *BRCA1/2* loss was seen in approx. 20% of the samples and was significantly associated with LOH. Interestingly, up to 40% of the samples showed *BRCA1/2* copy number changes and/or LOH without *BRCA1/2* mutations.

Taken together, our results show that routine MPSeq of native ovarian cancer tumor samples allows reliable detection of *BRCA1/2* mutations and copy number variations. In addition, we provide evidence that LOH in *BRCA1/2* is due to deletion of the wildtype-allele in most cases. Detection of copy number changes

and/or LOH without (point-)mutation in *BRCA1/2* may indicate for other *BRCA1/2* inactivating mechanisms or genomic instability which can also be identified using our approach.

P-CancG-052

Exome sequencing identifies potential new causative genes for hereditary renal cell carcinoma

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Background

Renal cell carcinoma (RCC) can be associated with several established hereditary tumor syndromes. In addition, a genetic cause is suggested in patients with early-onset disease or familial clustering of RCC, however, in the majority of those cases the underlying etiology remains unknown so far.

Methods

To uncover potentially causative germline mutations, the exomes of five unrelated index patients with early onset RCC were sequenced (Illumina HiSeq) using leukocyte DNA. From one patient the healthy parents and from another patient two family members with multiple benign tumors or an early-onset glioma and the healthy father were available for exome sequencing as well. The germline variants were filtered for rare (MAF: ≤ 1% for biallelic and ≤ 0.1% for heterozygous variants according to dbSNP and ExAC), truncating, and missense variants (predicted to be pathogenic by ≥ 2/3 prediction tools; for the recessive model at least one of the variants had to fulfill this criterion). The Varbank NGS Software was used for data analysis and filtering.

Results

After stringent filtering and manual exclusion of artifacts, we primarily selected genes with heterozygous variants in at least two index patients. Based on this dominant model, 24 variants in 11 genes including three truncating variants (two frameshift in CNTLN and one nonsense in PCNT) were found. After extensive data mining regarding gene function and expression, three genes (IPO8, SUPT5H, PCNT) remained as potential pathogenic. The most interesting gene was PCNT, which is involved in the cell cycle. Furthermore, in one patient a de novo heterozygous missense variant in BTNL8 was detected. Assuming a recessive mode of inheritance, ten homozygous missense variants (one variant in one patient, two variants in two patients and five variants in one patient) in different genes were found; another patient had potentially compound-heterozygous missense variants in three genes. The most interesting candidates with assumed biallelic variants are EFHD1, which has been described as down-regulated in RCC, and TRIM66, which is presumably a transcription repressor. In the family with several affected members, 41 heterozygous variants (four inframe indels and 37 missense variants) and four homozygous variants (one nonsense, one frameshift indel and two missense variants) were shared between 2-3 affected family members. After data mining, five candidate genes remained, the most interesting finding was a heterozygous missense variant in the tumor suppressor PTPRG. All variants of the 11 most interesting genes were validated by Sanger sequencing.

Conclusion

Based on exome sequencing of five index patients including one case with a suspicious family history, variants in 11 candidate genes were found, which are potentially causative for RCC considering the pathogenicity of variants and the gene function. To evaluate the clinical relevance, additional affected patients and / or functional analyses are needed.

P-CLINICAL GENETICS, GENETIC COUNSELLING AND PRENATAL DIAGNOSIS

P-ClinG-053

De novo MYH7 mutation in a patient with facioscapulohumeral muscular dystrophy (FSHD) phenotype

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The standard diagnostic procedure for patients with clinical presentation of limb-girdle muscular dystrophy (LGMD) phenotype with involvement of facial muscles consists firstly of the exclusion of facioscapulohumeral muscular dystrophy 1 (FSHD1) and 2 (FSHD2). If there are no pathological findings the next step most

probably will be a gene panel analysis including genes for LGMD1 and 2. However, this strategy of genetic testing does not always unravel the genetic defect of patients with this common and yet specific phenotype.

Here we report a 31-year-old male patient with LGMD phenotype and additional facial muscle involvement in which the aforementioned standard diagnostic procedure was without pathological findings. Further gene panel analysis of overall 204 genes associated with a wide range of different myopathy types revealed a mutation in the MYH7 gene, which to our knowledge has not been described previously. Segregation analysis showed that this MYH7 mutation was de novo. The MYH7 gene encodes one of the two beta heavy chains of myosin (MHC-beta) and is primarily expressed in cardiac muscle, but also in slow twitch (type I fiber) skeletal muscle. Although mutations in the MYH7 gene are known to predominantly cause autosomal dominantly inherited hypertrophic cardiomyopathy (HCM), a few cases have been described in which MYH7 mutations are causative for other forms of myopathies, i. e. distal myopathy and scapuloperoneal myopathy with facial muscle weakness in some. Since the here identified de novo MYH7 mutation is most likely the genetic cause of the clinical symptoms seen in our patient, he represents one of these rare cases in which MYH7 mutations lead to a phenotype resembling LGMD rather than cardiomyopathy.

Taken together, this case report on the one hand demonstrates the broad phenotypic spectrum of MYH7 gene mutations and on the other hand emphasizes the wide genetic heterogeneity present in patients with LGMD phenotype and facial muscle involvement. Hence, we conclude that MYH7 should also be considered as a putative disease-causing gene in such patients.

P-ClinG-054

Molecular Analysis of twenty-seven Iranian Patients with Usher Syndrome

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Usher syndrome (USH) is an autosomal recessive genetic disease that demonstrates combined deafness and blindness. It is characterized by sensorineural hearing loss (SNHL), retinitis pigmentosa (RP) and manifests with or without vestibular dysfunction. The prevalence of USH has been estimated to be between 1/6000 and 1/25,000. Depending on the age of onset, the severity of the retinal and hearing phenotypes, as well as the presence or absence of vestibular dysfunction, USH is classified into three major sub-types (USH1, USH2, and USH3). There are presently six, three and two genes recognized to be associated with USH1, USH2 and USH3, respectively. A fourth clinical subtype has been recently proposed, which regroups atypical forms of USH such as those resulting from mutations in three genes.

USH1 accounts for 35% of USH cases and is the most severe form. It is characterized by congenital severe to profound deafness with severe vestibular ataxia. USH1 patients develop RP within the first decade of life. USH2 patients have moderate to severe hearing loss, normal balance and usually develop RP in adolescence. USH3 patients have normal or near-normal balance, progressive hearing loss and RP that develops usually during adolescence.

Due to extensive clinical and genetic heterogeneities, which make it difficult for clinicians to name a proper clinical diagnosis due to subclinical or atypical manifestations in some patients, we applied whole exome sequencing and bioinformatics analysis to characterize the mutation spectrum in 27 unrelated Iranian patients. The identified pathogenic variants were validated using Sanger sequencing and subjected to segregation analysis. Copy number variation analysis using whole exome data ensued. As the majority of patients had parental consanguinity, we employed homozygosity mapping in patients in whom no pathogenic variants were identified. Using this approach, we were able to uncover likely pathogenic variants in the majority of our probands. We present a molecular and clinical summary from our collective cohort and disclose a strategy for comprehensive genetic diagnosis of USH.

P-ClinG-055

De novo missense mutation in a conserved DNA-binding and dimerization domain of NFIX causes Sotos syndrome 2 or Malan syndrome

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NFIX mutations have been associated with Sotos syndrome 2 (MIM # 614753) characterized by tall stature, macrocephaly, distinct facial appearance, and intellectual disability (ID). Furthermore, NFIX mutations located

within exons 6 to 10 have been shown to cause Marshall-Smith syndrome (MIM # 602535) a more severe clinical presentation with developmental delay and respiratory difficulties often leading to neonatal death.

We report on a 2-year-old Turkish girl born to non-consanguineous parents after ICSI treatment. Pregnancy was unremarkable besides an increased nuchal translucency documented at the 13th week of pregnancy. The girl had postnatal macrocephaly (+3,3 SDS), tall stature (88 cm at the age of 18 months, P95), craniofacial abnormalities (prominent forehead, long face with deeply set eyes and a short nose), vision impairments (intermittent nystagmus left eye, strabism divergens intermittens alternans, hyperopia), hypotonia, mild ID, and motor retardation. She is able to stand up and walk with help. Behavioural abnormalities were not present. Brain MRI showed a deficit of the white matter, periventricular gliosis, delayed myelinisation and hypoplasia of the optic nerve (DD: atrophy) on both sides.

We used diagnostic exome sequencing to detect a heterozygous de novo missense mutation in NFIX in exon 2 (NM_002501.3: c.A337G; p.Lys113Glu). The mutation is located in the DNA binding/dimerization domain, affecting an evolutionary conserved aminoacid. Other changes in this domain have been previously associated with a Sotos-like phenotype (Sotos syndrome 2) also known as Malan syndrome (PMID:26200704).

P-ClinG-056

The Two Faces Of RAB39B

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In the 1990ies several non-specific mental retardation families had been mapped to distal Xq28, among these the Sardinian family MRX72 (Russo et al.,2000). In the following, a mutation in the Small GTPase Gene *RAB39B* was identified in affected members of MRX72 as the cause of X-linked Intellectual disability, accompanied by autism, epilepsy and macrocephaly. The two-exon gene *RAB39B* is a member of the RAS oncogene family with known expression in neurons and neuronal precursors, particularly in the hippocampus (Giannandrea et al.,2010). In 2014, genetic analysis of three males displaying clinical features of early-onset parkinsonism and intellectual disability identified a deletion resulting in the complete loss of *RAB39B* (Wilson et al., 2014). Since then functional studies have repeatedly supported the role of RAB GTPases as the key players in the molecular pathway of Parkinson's Disease with *RAB39B* in particular being involved in autophagy regulation, intracellular trafficking events and the modulation of α-synuclein (Shi et al., 2017).

X-linked intellectual disability and early-onset parkinsonism, also referred to as Waisman Syndrome (WMSN, OMIM 311510), has been described in only a few families so far, but it can be expected that exome sequencing will continue to add new patients and families.

Here, we describe one adolescent with severe intellectual disability and epilepsy in whom we identified a new predicted pathogenic *RAB39B* variant c.535dup (p.(Glu179Glyfs~49)) as well as an adult male with early-onset parkinsonism and mild intellectual disability who carries the mutation c.215+1G>A (p.?) originally found in the Sardinian family MRX72.

Together with a look at the patient's families and a review of the patients described so far in the literature we elucidate the expanding phenotypic spectrum of *RAB39B* mutations and their role in X-linked intellectual disability as well as early-onset Parkinson`s Disease.

P-ClinG-057

Expanding the phenotype of LRP6-associated non-syndromic oligodontia to ectodermal dysplasia.

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Heterozygous *LRP6* [MIM 603507] mutations were recently reported as a cause of selective tooth agenesis-7 [STHAG7; MIM 616724] which is characterized by oligodontia concerning the permanent dentition (4 to 20 teeth missing except wisdom teeth) and taurodontia, ankyloses, enamel defects, and tooth shape anomalies in some patients. Altogether, 12 cases/families are reported in the literature so far. Except for oligodontia, apparently none of them showed other ectodermal symptoms.

Here we report on three further cases with a heterozygous nonsense or splice site *LRP6* mutation. In all cases mutational analysis of *EDA, EDAR, EDARADD, WNT10A* (all including MLPA also), *MSX1*, and *PAX9* genes yielded normal findings.

Case 1 is an 11 years old boy who presents with fine and sparse hair, laterally thinned eyebrows, thin and brittle fingernails, dry skin, and rough skin on the elbows. Orthopantomogram revealed three missing

permanent teeth and oral inspection showed an underdeveloped lateral upper incisor on the right and a broad diasthema in addition. The boy lives in a foster family and nothing is known about the dental status of the biological parents. In the *LRP6* gene, the heterozygous mutation c.4591_4592ins A, p.(Pro1531Hisfs*8) was found.

Case 2 is a 21 years old woman with sparse hair who reported to have had a complete deciduous dentition but agenesis of nine permanent teeth. In the *LRP6* gene, the heterozygous mutation c.1067T>G, p.(Leu356*8) was found.

Case 3 is a 12 years old girl who presents with fine and sparse hair, five missing permanent teeth according orthopantomogram, broad diasthema, and slowly growing toe nails. Teeth of the first dentition were complete and normal. Her mother has similar symptoms such as sparse hair, dysplastic nails on the fifth toes, and three permanent teeth missing. In the *LRP6* gene, the heterozygous mutation c.1545+1G>T, p.(?) was found in both, mother and daughter.

The phenotype in patients with LRP6 mutation mimics tooth findings of *WNT10A* [MIM 606268]-associated types of ectodermal dysplasia where monoallelic mutations may lead to selective tooth agenesis-4 [MIM 150400] with hypodontia (up to 6 teeth missing) and biallelic mutations leading to odonto-onycho-dermal-dysplasia [MIM 257980] with severe oligodontia with or without other ectodermal symptoms. However, in contrast to *WNT10A*, monoallelic mutations in the *LRP6* gene appear to cause both mild and severe phenotypes regarding tooth agenesis as known from the literature also. Since LRP6 functions as a co-receptor in the canonical Wnt/β-catenin signaling cascade, further similarities could have been expected, such as hair, skin, and nail anomalies, which were not reported so far. However, the cases reported here show that the phenotypic spectrum caused by *LRP6* mutations is indeed much broader and, in addition to the teeth, may include other ectodermal structures also.

P-ClinG-058

The Role of WNT3 and WNT7A in Tetra-Amelia and Fuhrmann Syndromes. Novel Mutations and Review of the Literature.

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Mutations in the Wingless homologous genes WNT3 and WNT7A have been associated with different syndromes mainly characterized by limb malformations. One nonsense mutation in WNT3 was reported as the cause for tetra-amelia in a single family in 2004, and in 2015 a WNT3 missense mutation was associated with bladder exstrophy. Apart from these two, no other WNT3 mutations have been reported. Mutations in WNT7A have been published in 2006 to cause Fuhrmann and Al-Awadi-Raas-Rothschild syndromes. Since then, only five other mutations have been reported in patients affected by those syndromes. Here we report a novel mutation in WNT3 in tetra-amelia syndrome and a novel WNT7A mutation causing Fuhrmann syndrome, and give a review of the role of WNT3 and WNT7A in the pathogenesis of these limb malformation syndromes.

P-ClinG-059

Isodisomic paternal uniparental disomy of chromosome 15 due to maternal Robertsonian translocation (14;15) by monosomy rescue

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Angelman syndrome (AS) is a neurodevelopmental disorder caused by deletion of the maternally inherited 15q11q13 region, paternal uniparental disomy 15 [upd(15)pat], an imprinting defect of the maternal chromosome region 15q11q13, or a pathogenic mutation of the maternal UBE3A allele. Predisposing factors for upd(15)pat, such as non-homologous Robertsonian translocations involving chromosome 15, have been discussed, but no evidence for this predisposition has been published. Chromosomal analysis was performed in the index individual, both parents and the maternal grandparents. Methylation-specific multiplex ligation dependent probe amplification (MS-MLPA) analysis was performed on DNA of the index individual, and microsatellite analysis was performed on DNA of the index individual and his parents. Cytogenetic analysis had shown that the mother and maternal grandfather are carriers of a Robertsonian translocation (14;15). The index individual has a numerically normal karyotype, but MS-MLPA and microsatellite analyses confirmed the clinical diagnosis of AS and revealed a pattern highly suggestive of isodisomic upd(15)pat. This is the first report of an AS-affected individual with isodisomic upd(15)pat and a numerically normal karyotype that most

likely results from a Robertsonian translocation (14;15) associated meiotic error in the maternal germline followed by monosomy 15 rescue in the early embryo.

P-ClinG-060

A 820kb microdeletion in 11p14.1-p13 leads to isolated aniridia without neurodevelopmental disorder

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Aniridia is an autosomal dominant inherited disorder characterized by partial or total hypoplasia of the iris as well as other ocular anomalies. Isolated aniridia is usually caused by mutations in the *PAX6* gene. Aniridia also occurs as part of the Wilms tumor-aniridia-genital anomalies-retardation (WAGR) syndrome, a contiguous gene syndrome due to deletion of the *PAX6* and the *WT1* genes. Downstream of *PAX6* there are several regulatory elements, including an ultraconserved enhancer in intron 9 of the *ELP4* gene.

Microdeletions of *ELP4* have been suspected to be associated with neurodevelopmental disorders as intellectual disability, language impairment or autism spectrum disorders. However, since these deletions have been frequently inherited from healthy parents, reduced penetrance must be assumed.

Here we present a father and his daughter with isolated aniridia, but sequencing of *PAX6* revealed no mutation. However, MLPA detected a deletion of the *ELP4* and the *DCDC1* genes downstream of *PAX6*. Array-CGH confirmed an 820kb deletion in 11p14.1-p13 encompassing five genes (*DCDC5*, *DCDC1*, *DNAJC24*, *IMMP1L*, *ELP4*) including the *PAX6* enhancer in the intron 9 of the *ELP4* gene.

We present the clinical and molecular data of the patients and support the assumption that deletions including *ELP4* can lead to isolated aniridia without any hint of neurodevelopmental dysfunction.

P-ClinG-061

Autosomal Dominant Robinow Syndrome Associated With a Novel DVL3 Splice Mutation

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Robinow syndrome is a clinically and genetically heterogeneous disorder characterized by mesomelic limb shortening, distinctive facial features, and variable oral, cardiac, vertebral and urogenital malformations. We identified the novel *de novo* splice acceptor mutation c.1715-2A>C in *DVL3* in a 15-year-old female patient with typical features of Robinow syndrome. By studying *DVL3* transcripts in this patient, we confirmed expression of both wild-type and mutant alleles. Mutant *DVL3* mRNAs were found to harbor a deletion of four nucleotides at the beginning of exon 15 and encode a protein product with a distinct -1 reading-frame C-terminus. The data demonstrate that altered DVL3 proteins associated with Robinow syndrome show truncation of the C-terminus and share 83 novel amino acid residues before the stop codon confirming highly specific *DVL3* alterations to be associated with this syndrome. The phenotype of the Robinow syndrome-affected female reported here is typical as she shows mesomelia and mild hand anomalies as well as characteristic facial anomalies. She also exhibited a supraumbilical midline abdominal raphe which has not been observed in other patients with Robinow syndrome. In contrast to the clinical data of four previously reported individuals with *DVL3*-related Robinow syndrome, short stature was not present in this individual. These findings expand the clinical spectrum of Robinow syndrome associated with *DVL3* mutations. To date, comparison of clinical data of *DVL3* mutation-positive individuals with those of patients with genetically different forms did not allow delineation of gene-specific phenotypes.

P-ClinG-062

Prenatal diagnosis of a de novo TUBA1A associated brain malformation

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We report a case of a prenatally diagnosed TUBA1A associated brain malformation. During the second, and initially unremarkable pregnancy of a healthy 26-year-old woman the following ultrasound abnormalities were detected at 31 gestational weeks: microcephaly, severe cerebellar hypoplasia, lissencephaly, suspicion of agenesis of the corpus callosum, ventricular dilatation, external ear malformation. A fetal MRI was performed and revealed microlissencephaly, agenesis of the corpus callosum, as well as hypoplasia and dysplasia of the cerebellum, the cerebellar vermis, the basal ganglia and the brainstem. Array-CGH analysis showed no aberrations. A de novo heterozygous TUBA1A mutation (c.791G>A, p.Arg264His) was detected by rapid NGS panel diagnostic within one week including 188 genes involved in abnormal brain development. The mutation has already been reported in a patient with tubulinopathy (Alby (2016) Birth Defects Res A Clin Mol Teratol). The TUBA1A associated lissencephaly is a congenital developmental disorder with abnormal neuronal migration. The clinical spectrum includes children with epilepsy and severe intellectual and motor developmental delay, depending on the severity of associated brain malformations. In the presented case a very poor neurological outcome could be predicted. The couple was extensively counselled and opted for a late termination of pregnancy in the 33rd week.

With rapid NGS gene panel diagnostics the cause of complex fetal brain malformations can be detected which enables accurate counselling and decision making for the parents.

P-ClinG-063

Comparison of whole exome sequencing to targeted next-generation sequencing in patients with developmental and neuromuscular disorders

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In disorders with common clinical features but a heterogeneous genetic background, NGS has opened up new possibilities in the search for disease-causing mutations. The identification of the underlying genetic defect provides a clear diagnosis for patients influencing their management. Last year we reported on our three years diagnostic experience with a NGS gene panel. Gene panels are increasingly replaced by whole exome sequencing (WES) because of a supposed higher mutation detection rate and cost efficiency. We compared the use and efficiency of WES to our NGS gene panel in routine clinical diagnostics of patients with syndromic or non-syndromic developmental delay or neuromuscular disorders.

54 patients (age 1-75 years; mean 20.8) with either syndromic (S: 26) or non-syndromic (NS: 10) developmental delay or with neuromuscular symptoms (NM: 18) were analyzed with WES. For enrichment the SureSelect Human All Exome V6 kit from Agilent was used. In 27 patients (50%) chromosomal rearrangements and copy number variations had been excluded by high resolution SNP array analysis. Sequencing was carried out on an Illumina NextSeq500 system (2x126bp PE). Raw reads where mapped to the reference genome using BWA-MEM and variant discovery was performed using GATKs best practice guidelines. The resulting VCF files were annotated with the ENSEMBL VEP. All detected variants where checked against dbSNP150, ClinVar, the 1000 Genomes Project, the gnomAD population database, the OMIM catalog, the professional version of the HGMD, and other sources in a self-implemented user interface. For exclusion of technical artifacts and segregation testing of all likely disease-causing variants, PCR and Sanger sequencing were performed according to standard protocols.

Class 5 mutations were identified in 8/54 patients (NS: 1; S: 3, NM: 4) and 8 different genes. In further 13 patients (NS: 3; S: 6; NM: 4) likely disease causing but previously unreported variants could be detected (class 4). 7 variants (NS: 2, S: 2; NM: 3) were classified as class 3; segregation analyses 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 (overall detection rate: ~38%, S: 34%, NS: 40%, NM: 44%) support the clinical utility of WES in identifying monogenic causes in a genetically heterogeneous cohort of patients. Compared to the detection rate of our targeted gene panel (overall: 26%, S: 35, NS: 20%, NM: 25%) we found an increase in the overall detection rate of 12% (ΔS: -1%, ΔNS: +20%, ΔNM: +19%). This increase of solved cases might be due to the greater number of genes included in the enrichment and better bioinformatic algorithms, but also to growing

public databases with genotype-phenotype information. The results show the usefulness of WES to identify the underlying cause of disease, which can often take the burden of guilt from the family.

P-ClinG-064

Is a partial deletion of only the WHSC1 gene in two affected consecutive fetuses with renal abnormalities, anhydramnios and IUGR a Wolf-Hirschhorn syndrome?

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Wolf-Hirschhorn syndrome (WHS) is normally a contiguous gene deletion syndrome with mental retardation, intrauterine and postnatal growth retardation, seizures, specific craniofacial appearance ("Greek warrior helmet facies"), club feet, urogenital malformations and high mortality in the first two years of life. Usually, large deletions from the short arm of chromosome 4 are cytogenetically visible spanning several Mb or detectable with FISH probes. In this region, haploinsufficiency of the genes WHSC1 and LETM1 is thought to be a major contributor to the pathogenesis of WHS (Anderson et al. 2014). WHSC1 has a complex expression pattern in early embryonal development and it is expressed preferentially in rapidly growing embryonic tissues. Furthermore, it is localizing to site of DNA damage and replication stress and is required to inhibit the DNA damage (Hajdu et al. 2011).

Here we report about two consecutive fetuses of a 31-year-old woman with bilateral multicystic kidney abnormalities, anhydramnios and intrauterine growth retardation (IUGR) in 24 weeks of gestation of fetus 1. Fetus 2 showed bilateral dysplasia of kidney, oligohydramnios and mild IUGR in 17 weeks of gestation.

Intensive molecular investigation of 11 genes for multicystic kidney disease in fetus 1 and 44 additionally genes in fetus 2 using NGS showed no pathological findings.

Karyotyping of both parents using all subtelomere probes revealed a cryptic terminal 8p deletion in the phenotypic unremarkable mother.

Array CGH on fetal DNA disclosed a de novo 68kb partial deletion of only WHSC1 in both fetuses and in addition in fetus 1 the maternal inherited 8p deletion.

To our knowledge, this is the first report about isolated partial WHSC1 deletion with prenatal severe abnormal ultrasound of kidney and IUGR. Based on minimal diagnostic criteria (Zollino et al. 2003), these fetuses do not meet a WHS diagnosis. But it is also difficult to describe the typical face in WHS patients prenatally. So can we go along with the opinion from Zollino et al., dividing WHS into a classical and a mild form, depending on clinical features in correlation with size of deletion?

P-ClinG-065

Identification of a patient with CAPOS syndrome and auditory neuropathy

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Hearing loss (HL) belongs to one of the most common sensory disorders in humans and is characterized by its extremely heterogeneous appearance. About 1 in 1000 newborns is affected at birth. There are presently over 400 forms of syndromic HL recognized. In stark contrast to non-syndromic deafness, the genetic landscape of syndromic HL is poorly characterized. The ongoing investigation of genes and mutations involved in syndromic HL enables improved genetic counseling and future treatment opportunities.

The aim of the current project was to identify likely pathogenic mutations in known and novel HL-associated genes in selected Iranian, Pakistani and German families that was achieved through whole exome sequencing. Bioinformatics analysis was performed using GensearchNGS and an in-house exome analysis pipeline. In addition to the discovery of mutations in genes already associated with HL, we were able to identify a heterozygous missense mutation in the autosomal-dominant *ATP1A3*-gene (OMIM: 182350) in a German boy.

The identified missense mutation c.2452G>A (NM_152296.4, p.Glu818Lys) in *ATP1A3*, encoding the neuron-specific alpha subunit of the Na+/K+-ATPase α3, is known to be responsible for CAPOS syndrome. "CAPOS" is an acronym for cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural HL and represents a rare neurological disorder first described in 1996. We performed segregation analysis with the unaffected mother, father and brother of the patient and concluded that this mutation occurred de novo. The patient suffers from a slight cerebellar ataxia in the form of an unsteady gait, episodic migraine and stable

moderate low-frequency HL with auditory neuropathy since the age of 6. His speech recognition scores are worse than what would be expected from his pure-tone audiogram thresholds. At the time of his last clinical examination, he has not shown signs of areflexia, pes cavus or optic atrophy in addition to no febrile episodic decline.

To date, only 22 patients with CAPOS syndrome have been described, underscoring its rarity. We present the youngest reported patient and argue that initial clinical onset may be variable and can be mild as was the case in our patient. Our patient has been integrated in a study that included detailed audiological description in 18 patients diagnosed with the c.2452G>A mutation (Tranebjærg, Strenzke, Bitner-Glindzicz et al.). This study also provides evidence of an auditory neuropathy in association with CAPOS syndrome which has potentially important implications for hearing rehabilitation.

P-ClinG-066

Identification of a novel nonsense mutation in SLC39A13, causative for spondylocheiro dysplastic Ehlers Danlos syndrome (SCD-EDS), in a patient presented for myopathy

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Both hereditary myopathies and connective tissue disorders comprise a wide clinical and genetic spectrum, with some subtypes showing considerable overlap between these two entities. Therefore, establishing the correct diagnosis has proven difficult in some cases, especially before the era of next-generation sequencing (NGS).

The 14-year-old German patient presented here was initially diagnosed with myopathy based on his clinical, radiological and muscle biopsy findings. Targeted analyses did not reveal the underlying genetic cause. We therefore performed exome sequencing using the Ion Torrent™ technology (Life Technologies) with data analysis based on the NextGENe V2.4.2 software. With this approach we identified a novel homozygous nonsense mutation (c.830G>A, p.W277*) in the *SLC39A13* gene, causative for spondylocheiro dysplastic Ehlers Danlos syndrome (SCD-EDS), thus establishing the diagnosis of a very rare connective tissue disorder in our patient. There was no evidence for (additional) pathogenic mutations in myopathy genes.

To date, only nine affected individuals from four families (including our patient) have been described for SCD-EDS. *SLC39A13* encodes the ZIP13 protein, representing a zinc transporter which participates in maintaining cellular zinc homoeostasis; however, the exact pathogenetic mechanisms are still unknown. Typical symptoms include growth retardation, sensitive and transparent skin, hand and joint abnormalities, bone deformities with radiological pathologies, protuberant, grey-bluish sclerae and an abnormal urinary lysyl pyridinoline/hydroxylysyl pyridinoline ratio. Although most of these features were present in our patient, the myopathy rather than the skin features had been the clinically leading symptom. Interestingly, the previously reported patients did not show obvious evidence of myopathy, suggesting a broader clinical spectrum for SCD-EDS than originally suspected.

Our report therefore extends the current knowledge on the mutational and clinical spectrum of SCD-EDS, a very rare connective tissue disorder, and further illustrates the potential of exome sequencing in clinically ambiguous cases.

P-ClinG-067

Congenital myasthenic syndrome caused by novel COL13A1 mutations

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Collagen XIII is a nonfibrillar transmembrane collagen which has been long recognised for its critical role in synaptic maturation of the neuromuscular junction. More recently, bi-allelic COL13A1 loss-of-function mutations were identified in three patients with congenital myasthenic syndrome (CMS), a rare inherited condition with defective neuromuscular transmission, causing abnormal fatiguability and fluctuating muscle weakness and often successfully treated with acetylcholinesterase inhibitors. Here we report three additional CMS families in whom affected children carried homozygous loss-of-function mutations in the COL13A1 gene.

The clinical presentation of our cases was similar to the previously reported patients including prominent eyelid ptosis, respiratory difficulties, and negative response to acetylcholinesterase inhibitor treatment. Our data further support the causality of COL13A1 variants for CMS and suggest that this type of CMS might be clinically homogeneous and requires alternative pharmacological therapy.

P-ClinG-068

First International Consensus Guideline for Beckwith-Wiedemann syndrome

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The imprinting disorder Beckwith-Wiedemann syndrome (BWS) is mainly characterized by overgrowth, but the variable phenotype also includes macroglossia, anterior abdominal wall defects (including exomphalos), neonatal hypoglycemia, hemihypertrophy and predisposition to embryonal tumors (particularly nephroblastomas). Its incidence is at least 1 in 10,500. The genetics of BWS is complex, but most cases have a molecular abnormality that alters the expression/function of imprinted gene products (in particular IGF2 and CDKN1C) in the chromosome 11p15.5 imprinted gene clusters. The delineation of the molecular defect in an individual case has major impact on the prediction of the risk (and type) of an embryonal tumor and of familial recurrence risks. Despite recent advances in knowledge there is marked heterogeneity in clinical diagnostic criteria and care. To enhance the diagnosis, investigation and management of BWS an international consensus group was assembled and agreed on 72 recommendations for the guidelines for clinical and molecular diagnosis and treatment. The consensus includes a definition of BWS spectrum disorder (BWSp) to cover classical BWS without a molecular diagnosis and BWS-related phenotypes (including isolated lateralised overgrowth) with an 11p15.5 molecular anomaly. The consensus recommendations comprise a clinical scoring system, comprehensive protocols for the molecular investigation, care and treatment of BWS from prenatal diagnosis to adulthood. A consensus was agreed to recommend a tumor surveillance program targeted by molecular subgroups as favored in some European centers. However, it is recognized that screening might differ according to the local health care system (e.g. in the United States) and that it is important that the results of targeted and universal screening are evaluated prospectively. Given that BWSp is a rare disorder, international collaboration, including prospective audit of the results of implementing the consensus recommendations, is required in order to expand the evidence base for the design of optimum care pathways.

P-ClinG-069

Paternal mosaicism of STXBP1 mutation causes epileptic encephalopathy in half-siblings

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Epileptic encephalopathies are characterized by an early onset, severe electroencephalographic abnormalities and refractory seizures causing developmental delay or rather regression and intellectual disability. In this context, STXBP1 mutations should be considered as one of the most common cause for epileptic encephalopathies with its broad phenotypic spectrum, including infantile spasms and severe mental retardation as the main consistent features. Although, the majority of STXBP1 mutations occurred de novo.

Here, we report a 13-year-old male patient with epileptic encephalopathy. Lack of hand grasping, and eye contact and frequent vomiting were noticed in infancy. The first seizures (focal seizures) occurred at 21 months, which evolved into a secondarily generalized and treatment-resistant epilepsy. Additionally, early development was delayed, as he was able to sit independently with 11 years, but never walked without assistance and depends on a wheelchair. Last, he exhibited stereotypic and dyskinetic movements of the head, arms and body. He possibly understands simple prompts. The MRI shows a cerebral atrophy. Interestingly, the father has a daughter resulting from a new partnership, with similar clinical symptoms like his son. This half-sister was delivered in 34 weeks of gestation. An epilepsy occurred at 3 months. Just like her brother, she developed psychomotor retardation including lack of eye contact.

Towards an Array CGH analysis of the index patient, a multi-gene panel testing via sequencing by synthesis was conducted, including candidate genes contributing to epileptic encephalopathy.

We revealed the missense mutation p.Arg190Trp (c.568C>T) in the STXBP1 gene. The mutation is well known and described as disease causing for an early infantile epileptic encephalopathy. Interestingly, the half-sister also inherited the STXBP1 mutation. And in addition, the father did not carry the mutation in peripheral blood leukocytes. Certainly, no other tissue from him, e.g. saliva, buccal cells or skin cells, was available to exclude tissue-specific differences of the STXBP1 mutation expression. But the recently described STXBP1

mosaic mutation was detectable in the blood as well as in other tissue, suggesting a somatic mosaicism of a parent. Hence, there is a strong evidence for a gonadal paternal mutation in our described family. In another pregnancy of the second partnership, the STXBP1 mutation was excluded.

To our knowledge, this is the first report of a gonadal mutation causing a familiar STXBP1-related epileptic encephalopathy. Therefore, a parental mosaic mutation carrier should be considered when determining recurrence risk in families with a child with epileptic encephalopathy. Accordingly, prenatal diagnostics should be offered in further pregnancies examining the familial mutation.

P-ClinG-070

Pathway analysis of miRNA revealing immunologic alteration in lymphocyte immune response and T cell differentiation in Fontan patients with Protein Loosing Enteropathy (PLE)

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The development of protein loosing enteropathy (PLE) in Fontan patients represents a severe complication of surgically repaired congenital heart defects with single ventricle morphology. Approximately 3-15% of all patients develop PLE associated with increased morbidity and mortality up to 50% in five years. PLE has been clinically characterized in Fontan patients by mild to severe diarrhea with elevated fecal alpha-1 Antitrypsin, hypoproteinemia, hypoalbuminemia, and dysregulation of salt and water homeostasis, including the development of third space volume retention and lymphangiectasia. Immunologic changes, including lymphopenia, CD4+ T-cell deficiency have been described earlier in Fontan patients with PLE. Growing evidence suggests that miRNAs regulate the intestinal immune system via the intestinal epithelial tight junction permeability, intestinal interleukine and T cell expression, cell differentiation, and inflammatory cell trafficking. We therefore hypothesized that miRNAs may contribute to the pathogenesis of PLE.

We now performed comprehensive miRNA expression profiling in whole blood using a commercial microarray in a group of 40 Fontan patients (including 10 patients with PLE) compared to control group of patients with biventricular corrected transposition of the great arteries. 481 miRNAs were significantly ($p < 0.05$) downregulated in PLE, while 303 miRNAs were upregulated. GO-term enrichment analysis performed on target genes of the differentially regulated miRNAs revealed a large number of pathways affected (31 for downregulated miRNAs; 1734 for upregulated miRNAs). Ontological comparisons of the regulated pathways showed a pronounced enrichment in a large cluster of 38 interconnected immunological gene signatures associated with, among other terms, T-cell apoptosis and survival. Other clusters included nucleotide- and cytoskeleton catabolic processes (34 terms) and reactive oxygen species metabolism (27 terms).

Indeed cell sorting of whole blood unveiled a severe T cell deficiency including dramatically decreased CD4+ lymphocyte counts, resulting in a remarkably altered CD4+/CD8+ ratio. For the first time we observed severe alterations in CD4+ and CD8+ T cell differentiation in Failing Fontan patients with a loss of naïve T cells and increased Treg frequency. The observed changes in CD4+ and CD8+ cell differentiation suggest an ongoing immune reaction in Failing Fontan patients, where naïve T cells rapidly differentiate to memory cells after antigen recognition.

Our results on the blood miRNA profile reflect the pathological processes of T cell differentiation. In further prospective analyses of miRNAs found differentially expressed in our study we will investigate their potential role as prognostic biomarkers.

P-ClinG-071

Structural variants in patients with Silver-Russell syndrome features – Curation of a disease database

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Silver-Russell syndrome (SRS) is a clinically and molecularly heterogeneous growth disorder in which the clinical diagnosis can be confirmed by molecular analysis in about 60 % (loss of methylation of the H19/IGF2:IG-DMR on 11p15.5 (50%); maternal uniparental disomy of chromosome 7 (5-10%). In addition to these two major molecular alterations, there are numerous publications on structural chromosomal variants identified in patients with SRS and SRS-like features, but an overview and comparison of these cases, even those with seemingly similar alterations, was not available because the chromosomal breakpoints were defined with different methods at different times, and a standardized and generally accepted clinical scoring system was not available. These problems can now be overcome by standardized molecular nomenclature and a consented clinical scoring system (Netchine-Harbisons clinical score). We evaluated all genetic and clinical reports on patients with structural variants diagnosed as SRS, and generated for the first time a respective database. Data of 73 patients with structural variants were submitted to the publicly web accessible LOVD database (https://databases.lovd.nl/shared/diseases/00232) using the LOVD software version 3.0. This study provides a framework for both research and diagnostic purposes through facilitating a standardized comparison of (epi)genotypes with phenotypes of patients with structural/sequence variants. Furthermore, it may help to propose additional clinical definitions in line with those recently adopted for SRS (Tümer et al., Human Mutation, under revision).

P-ClinG-072

Clinical course and distinguishing features in two adult patients with Schaaf-Yang syndrome displaying a Prader Willi syndrome-like phenotype

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Schaaf-Yang syndrome (OMIM #615547) is a recently characterised disease entity that results from truncating mutations in MAGEL2, a maternally imprinted, paternally expressed gene located in the Prader-Willi syndrome critical region on the long arm of chromosome 15 (15q11q13). Patients with Schaaf-Yang syndrome usually satisfy the major diagnostic criteria of Prader-Willi syndrome but are distinguished by additional features, mainly symptoms from the autism disorder spectrum and hand joints abnormalities. Here, we describe the development of the clinical features and course of the syndrome in two adult patients over time. At 34 and 28 years of age these are to our knowledge the oldest reported cases of Schaaf-Yang syndrome. In addition, we draw comparisons with the limited number of reported cases of Schaaf-Yang syndrome in the literature and offer conclusions on the necessity of considering a differential diagnosis of Schaaf-Yang syndrome in patients with a PWS-like phenotype where a genetic confirmation of the diagnosis is lacking.

P-ClinG-073

Whole exome sequencing reveals novel compound heterozygous mutations in PIBF1 as a cause of Joubert syndrome

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Background: Joubert syndrome (JBTS) is a genetic heterogeneous disorder characterized by a congenital malformation of the brainstem and cerebellar vermis leading to a specific MRI finding called "molar tooth sign" (MTS). In 2015, the gene PIBF1 has been identified as causative for JBTS by a siRNA-based functional genomics screen for the identification of ciliopathy genes. The affected individuals showed developmental delay, ataxia, oculomotor apraxia, and a brain phenotype consistent with Joubert syndrome. None of the patients had other features commonly associated with JBTS, including retinal dystrophy, nephronophthisis and liver involvement.

Clinical report and genetic findings: The patient is the first child of non-consanguineous healthy parents from Germany. She was born at term with a birth weight of 2620 g (-2 SDS), length of 48 cm (-1,7 SDS) and head circumference (OFC) of 34 cm (20th centile). Soon after birth a spastic tetraparesis and hypotonia of the trunk was noticed. She had feeding difficulties and a failure to thrive. At the age of six months atypical eye movements became apparent. At the same age elevated liver enzymes were noted and persisted during childhood. Psychomotor development was delayed from the beginning. Brain MRI at age 24 months showed polymicrogyria, hypoplasia of cerebellar vermis, and a mild MTS. At age 4;2 years she presented with severe global developmental delay, no speech, spastic tetraparesis, a submucosal cleft palate and poor growth (weight 11,47 kg (-3,1 SDS), length 85 cm (-5 SDS), OFC 49.5 cm (-1,4 SDS)). Conventional chromosome analysis and molecular karyotyping were without pathological findings. Testing of by then known genes causative for Joubert syndrome (NPHP1, TMEM216, CC2D2A, MKS3, RPGRIP1L, OFD1 and MKS4) was performed with normal results. Whole exome sequencing showed the novel compound heterozygous PIBF1 variants c.1453C>T; p.(Q485*) and c.1508A>G; p.(Y503C), which, according to ACMG-guidelines, were classified as pathogenic and likely pathogenic, respectively. The PIBF1 mutations have not been described before and were associated with impaired ciliogenesis in Xenopus derived multiciliar cells.

Discussion: Since the discovery of PIBF1 as a gene for Joubert syndrome in 2015 no further patients with mutations in this gene have been described in the literature. To our knowledge, we describe the first individual with PIBF1 related JBTS with liver involvement, severe growth retardation and cleft palate, therefore expanding the phenotypic spectrum. Additional investigations on PIBF1 function in Xenopus cells are in progress.

P-ClinG-074

Rare somatic KIT mutation in a patient with congenital diffuse cutaneous mastocytosis

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Diffuse cutaneous mastocytosis (DCM) is an extremely rare disease associated with abnormal proliferation of mast cells and their accumulation in the entire skin. DCM mainly affects newborns and infants.

We report on a 15 year-old girl, who had prominent bullous and erosive skin lesions shortly after birth, initially confused with impetigo. Systemic mastocytosis with diffuse cutaneous manifestation was diagnosed after bone marrow biopsy showing mast cell infiltrates in the first year of life. Over the years, the clinical manifestations evolved to generalized erythrodermia, thickening of the skin, submammary blistering, variable pruritus and headache.

The mother, two sisters, her brother and several further family members are not affected by DCM, but suffer from intense pruritus after physical exercise with vibratory stimulus.

In our patient, genetic analysis of the 21 coding exons of KIT including 10 bp of flanking introns revealed a heterozygous mutation c.1504_1509dupGCCTAT, p.Ala502_Tyr503dup in the blood sample, but not in DNA isolated from hair follicles and nail keratinocytes. In buccal swabs, the mutation was detectable to a very low grade, probably caused by mast cells present in the saliva. The mutation could also be excluded in the mother.

The present tandem duplication at exon 9 is known to act as a gain-of function-mutation for the tyrosin kinase of KIT and is a rare cause of sporadic gastrointestinal stromal tumors (GIST). So far, this mutation has only been reported twice in mastocytosis, once in a patient with DCM and once in urticaria pigmentosa.

Imatinib is a selective tyrosine kinase inhibitor and is clinically used for treatment of GISTs and others neoplastic diseases. The inhibitory effect of imatinib on phosphorylation of KIT-Dup-Ala502Tyr503 has already

been demonstrated in transfection experiments. We therefore assume that imatinib might also be an effective treatment in our patient, although the family has yet to decide on its use in our patient.

Taken together, we report the third patient with mastocytosis associated with the imatinib-sensitive mutation KIT-Dup-Ala502Tyr503.

P-ClinG-075

Short stature, optic nerve atrophy and Pelger-Huët anomaly (SOPH) syndrome is a differential diagnosis for autosomal recessive cutis laxa type IIIA and IIIB.

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The autosomal recessive conditions associated with cutis laxa (ARCL) are heterogeneous multisystem disorders. Affected individuals show lax, thin and/or translucent skin with visible veins, intrauterine and/or postnatal growth restriction and a typical facial gestalt leading to a progeroid appearance.

Gene panel analysis of nine affected individuals fulfilling the major diagnostic criteria of autosomal recessive cutis laxa type IIIA and IIIB was performed. In 7/9 we identified disease causing mutations in *ALDH18A1* and *PYCR1*, encoding enzymes involved in mitochondrial proline biosynthesis. However, in two patients no convincing mutations were detectable. Using exome and subsequent Sanger sequencing we identified in both patients compound heterozygous mutations in *NBAS* (Neuroblastoma-amplified sequence), coding for a protein involved in Golgi-to-ER transport. Additional features like hepatomegaly, optic nerve atrophy, and Pelger-Huët anomaly supported the molecular genetic diagnosis of SOHP (short stature, optic nerve atrophy and Pelger-Huët anomaly) syndrome. A comparison with all patients suffering from SOPH syndrome so far described showed that atrophy of the subcutaneous fat, lax, thin and translucent skin with visible veins, increased bone age and a typical facial gestalt were common features, especially in early infancy. Interestingly, these features overlap with ARCL caused by *PYCR1* or *ALDH18A1* mutations.

In summary, using our established workflow for the molecular genetic analysis of cutis laxa related conditions we identified two cases with SOPH syndrome. This suggests that SOPH syndrome is a differential diagnosis for ARCL type IIIA and IIIB and that the *ALDH18A1*, *PYCR1* and *NBAS* gene defects trigger overlapping pathomechanisms in spite of their different function.

P-ClinG-076

Looking for the hidden mutation: a long journey to a mosaic 10q23 microdeletion involving *PTEN* and *BMPR1A*

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Background:

Germline heterozygous pathogenic variants in the tumor suppressor gene *PTEN* are associated with the *PTEN* hamartoma tumor syndrome (PHTS).

Case reports:

Here, we present two patients, a 16-year-old female patient and a 15-year old male patient, with juvenile polyposis (JP), macrocephaly, intellectual disability, and pigmented macules, who were clinically suspected for Bannayan-Riley-Ruvalcaba syndrome (BRRS).

Results and discussion:

Array-CGH analysis identified an interstitial 10q23.1q23.3 deletion (arr[GRh37] 10q23.1q23.3(86048561_92004208)x1) in a blood sample of the 16-year-old patient that encompassed 46 genes including *PTEN*, *BMPR1A*, and *KLLN*. In contrast, no pathogenic variant was identified in *PTEN* and *BMPR1A* in a blood sample of the 15-year-old patient. Array-CGH analysis of a blood sample did not reveal any relevant copy number variation. However, in a surgical specimen of the thyroid gland, which was resected due to multinodular goiter, high-level mosaicism for a 10q23.2q23.3 deletion (arr[GRCh37] 10q23.2q23.3(87991551_92402440)x1) involving 45 genes was observed (log_2-ratio -0.77). In addition, a pathogenic frame shift variant ENST00000371953:c.956_959delCTTT p.(Thr319LysfsTer24) in *PTEN* was detected in the thyroid tissue. The patient's phenotype was highly suspicious of a germline variant, encouraging us to search for a mosaic status. The frame shift variant was neither detected in the patient's

blood sample nor his buccal mucosa sample. Low-level mosaicism for the microdeletion was identified in a buccal swap sample (log_2-ratio -0.20), while re-analysis of the blood sample suggested marginal-level mosaicism for the 10q23.2q23.3 deletion (< 10%). The 10q23.2q23.3-deletion mosaicism was also identified in a subsequently resected colonic polyp (log_2-ratio -0.54). In both cases, the diagnosis of a 10q23-microdeletion syndrome that clinically presented as a BRRS-JP overlap syndrome was established.

Conclusion:

In summary, the study expands the BRRS-JP spectrum and highlights the relevance to consider mosaicism in BRRS and other *PTEN* hamartoma tumor syndromes. We conclude that in all patients with a clear clinical suspicion of PHTS, in which genetic analyses of DNA from blood and buccal swap samples fail to identify causative genetic variants, it is important to thoroughly investigate additional tissues, e.g. colonic polyps.

Joint first authors: Golas, MM. and Auber, B.

P-ClinG-077

CMTRID as a rare cause of neuropathy with HSP-like phenotype – Report of a novel splice-site mutation in the COX6A1-gene

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Charcot-Marie-Tooth (CMT) disease is the most common inherited sensomotoric neuropathy. Over 50 associated genes have been described so far. Recently a homozygous 5-bp deletion in the COX6A1-gene has been described in three families with early-onset, intermediate CMT.[1,2] COX6A1-gene encodes the cytochrome-c oxidase subunit VIa polypeptide 1, a component of mitochondrial respiratory complex IV.

We like to present the phenotype of a 44 year old man of German descent, who showed high grade paraparesis of the legs, atrophy of the intrinsic hand muscles and impaired fine motor skills. In addition electrophysiological examination revealed high grade sensomotoric polyneuropathy. Onset of disease was in childhood with progressive bilateral foot flexor paresis as first symptom. Family history was negative for neurological diseases. To the knowledge of the index patient his parents were non-consanguine.

The patient was transferred for genetic counseling with suspected diagnosis of hereditary spastic paraplegia. Analysis of HSP-associated genes (e.g. BSCL2) revealed no causative mutations. Because of concomitant sensomotoric polyneuropathy genetic testing for CMT was initiated. A PMP22-duplication could not be detected. Further sequencing of CMT-associated genes revealed a homozygous donor splice-site mutation c.103+1G>T in intron 1 of COX6A1-gene (hg19:NM_004373.3). According to prediction tools this donor splice-site mutation results in loss of exon 1 and is therefore most likely pathogenic.

To the best of our knowledge this is the first report of CMTRID (recessive, intermediate CMT Type D) in Germany with a novel splice-site mutation in COX6A1-gene.

[1] Tamiya G. et al., Am J Hum Genet. 2014 Sep 4;95(3):294-300.

[2] Laššuthová P. et al., Clin Genet. 2015 Aug 25.

P-ClinG-078

Effective identification of putative disease-causing mutations in patients with ataxia or paraplegia using disease specific gene panels

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Neurodegenerative diseases with ataxia or paraplegia are highly heterogeneous (>200 genes) and show autosomal dominant, recessive and X-linked forms of inheritance. Work package five of the EU funded NeurOmics project aimed to identify putative disease-causing mutations in cohorts of 100 patients per disease group using two custom-made disease specific gene panels.

In order to achieve this objective we established further developed disease specific gene panels (HaloPlex, Agilent) which contained 201 target genes for ataxia and 192 genes for paraplegia in the last versions. The target region of 679kb (ataxia)/ 630kb (paraplegia) was mainly sequenced on a MiSeq (Illumina) with 2x 151bp paired-end runs. The read sequences were analyzed by an in-house bioinformatics pipeline.

Currently, index patients from 175 families (ataxia: 107/ paraplegia: 68) have been sequenced. HaloPlex disease panel approach achieved a high efficiency: median target region coverage ≥20 reads: >96,9% (ataxia)/ >98,9% (paraplegia); median depth: 219 (ataxia)/ 404 (paraplegia).

Boosting regions which constantly appeared with a low average coverage (<20x) by adding more region specific probes to the panel design increased the average depth per million reads at these regions by about 14x although being sequence with similar amounts of total reads per sample.

Mapping the reads to the human genome (hg19) followed by annotation to different databases resulted in large lists of variants per sample (ataxia: 204-490/ paraplegia: 133–435) which were further filtered for rareness (in-house database, ExAC, Kaviar) and for functional relevance (ns, indel). This led to a reduced number of 1-51 (ataxia)/ 0-18 (paraplegia) variants per patient. Several rare, disease-causing and potentially causative mutations were found in different genes (ataxia: SPG7, SACS, CACNA1A, AFG3L2 …/ paraplegia: CYP7B1, SPG11, SPG7, KIF1C, IFIH1 …). Panel sequencing elucidated the molecular basis in 20 ataxia (19%) and 25 paraplegia families (37%) respectively. For both disease groups, potentially disease causing variants of yet uncertain significance could be identified in another 22%/ 28% of cases.

For highly heterogeneous disorders with ataxia or paraplegia genetic testing by gene panel NGS is clearly the method of choice in terms of the overall processing time of the diagnostic target regions in patients DNA, the increased diagnostic yield which raised up to 35% and the costs which are less than one fiftieth in contrast to stepwise analyses with standard approaches like Sanger sequencing.

P-ClinG-079

Autosomal recessive Noonan syndrome caused by a homozygous LZTR1 mutation

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A boy with features suggestive of a RASopathy was born to unaffected parents who are first cousins once removed originating from Syria. When first seen at our outpatient clinic, the two-year-old boy presented with developmental delay, a complex heart defect (atrial septum defect, hypoplastic pulmonal artery with pulmonary stenosis and right ventricular hypertrophy), short stature, facial dysmorphic features (widely spaced eyes, down-slanted palpebral fissures, low-set ears and increased posteriorly angulated ears) and curly hair. He had unilateral cryptorchidism and a right-sided inguinal hernia. At the age of 3 years 4 months his height was 88 cm (<3rd centile), weight 13.8 kg (10-25th centile) and head circumference 51 cm (50-75th centile). A clinical diagnosis of Noonan syndrome (NS) was considered. He had an unaffected twin sister and two unaffected older sisters. His parents reported three prior miscarriages, two of which were twins and one triplets of unknown affection status and zygosity. We performed multigene panel sequencing using Illumina Nextera® Rapid Capture Custom Enrichment Kit covering the entire coding sequence of the established RASopathy genes (PTPN11, SOS1, SOS2, RAF1, RIT1, KRAS, NRAS, RRAS, HRAS, SHOC2, LZTR1, A2ML1, BRAF, MAP2K1, MAP2K2, NF1, SPRED1, and RASA2). With this method, no heterozygous mutation in any of these 18 genes was found. However, we detected two apparently homozygous variants in LZTR1: c.508C>T; p.(Arg170Trp) (minor allele frequency (MAF) 3/245,898 in gnomAD) and c.614T>C; p.(Ile205Thr) (MAF 2/245,670 in gnomAD). The unaffected parents were heterozygous for both variants, and the three unaffected siblings were not homozygous for any of the two variants. Thus, parental consanguinity and the identification of homozygous cosegregating variants were in line with an autosomal recessive form of NS. Notably, all molecularly confirmed forms of NS have been inherited in an autosomal dominant pattern to date, and heterozygous mutations of LZTR1 are a rare cause of autosomal dominant NS and familial schwannomatosis. Yet, no history of schwannomas was reported in the family. A mutation similar to p.(Arg170Trp) identified here, p.(Arg170Gln), has been reported in heterozygous state previously as the likely cause of schwannomatosis, so we suggest that p.(Arg170Trp) is more likely to be causative than p.(Ile205Thr).

In a large international collaborative study (to which our report contributed), biallelic LZTR1 mutations have been identified in a total of 21 patients from 12 families with NS compatible with an autosomal recessive mode of inheritance (Johnston et al., Genetics in Medicine, accepted for publication). These data confirm the existence of an autosomal recessive form of NS, a finding with important implications for the molecular diagnosis of NS, the evaluation of recurrence risks for families with LZTR1 variants, and the pathogenesis of familial schwannomatosis.

P-ClinG-080

Preimplantation genetic diagnosis for monogenic disorders in Regensburg

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In Germany preimplantation genetic diagnosis (PGD) requires a positive vote from the local PGD ethics committee. In Bavaria the first applications were ruled on in December 2015. We here report on our results of the first 36 consecutive PGD cycles for monogenic disorders and compare them to the previous treatment results at our center after PGD obtained by polar body diagnosis (PBD).

Methods: preimplantation genetic diagnosis for 144 blastocysts from 25 families with mutations in 17 genes associated with monogenic inherited disorders using in house genetic assays. Parallel genetic testing for aneuploidies was performed for 12 of these blastocysts using VeriseqTM PGS (Illumina).

Results: 546 oocytes were obtained in 40 treatment cycles. 5 of these oocyte retrieval cycles were collection cycles only without genetic testing (12,5%), in order to increase the number of blastocysts for PGD. Blastocysts were obtained for 144 oocytes (26,4%) and biopsied on day 5 or 6, with trophectoderm samples collected and subsequent blastocyst freezing. PGD was performed with family specific PGD assays incl. 3-7 linked markers for segregation analysis and additional direct mutation detection for 18 families. Genetic testing of 133 trophectoderm biopsies (TEB) resulted in a genetic diagnosis for 124 (93,2%). For 62 of these transfer was recommended based on the assumption, that the embryo was not affected.

Transfer of 26 embryos in 17 subsequent transfer cycles (1,5 embryos/transfer cycle) for 15 families resulted in 10 clinical pregnancies, delivery of 3 children and 7 ongoing pregnancies. For the remaining families 36 frozen blastocysts are still available for transfer. In comparison to PBD at our center the clinical pregnancy rate per embryo transfer increased from 23,6% after PBD (116 families, 237 cycles to PGD, 61 clinical pregnancies with delivery in 46 = 39,6% of the families) to 58,8 % after PGD on trophectoderm samples. Additional testing for aneuploidies was performed for 12 blastocysts unaffected for the family specific disorder, with genetic diagnosis for 10 blastocysts from 3 families. Transfer of 4 embryos without indication for aneuploidies in 3 transfer cycles resulted in 3 pregnancies (100%), compared to 7 pregnancies out of 22 embryos transferred in 14 cycles (50%) without aneuploidy screening.

Discussion: We confirm an increased pregnancy rate after PGD when compared to the results after PBD at our center without a substantial increase of pure oocyte collection cycles without PGD. First results at our center confirm feasibility of simultaneous testing for monogenic disorders and aneuploidies, which in the presence of a sufficient amount of blastocysts may further increase the pregnancy rate per embryo transfer and reduce the time to pregnancy.

P-ClinG-081

Diagnostic exome sequencing identifies an AUTS2 nonsense mutation in a 26-year-old patient with developmental delay and phenotypic features resembling Marfan syndrome

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The 26-year-old male patient is the only child of healthy parents. Family history is unremarkable concerning the occurrence of genetic diseases. During pregnancy polyhydramnion had been observed. Fetal chromosomal analysis showed a normal male karyotype. After birth (caesarean section at 41 weeks of gestation) poor sucking and muscular hypotonia were noticed. The child showed delayed motor and speech development. Microdeletion 22q11.2 was ruled out as were neurometabolic diseases by biochemical screening. At the age of 17 years the patient's height was 192 cm [>P97] (father 183 cm, mother 176 cm), head circumference was 54.5 cm [P3-P10]. Clinical features included nasal speech, narrow hands and feet with long fingers and toes, palmar crease at left hand, camptodactyly of 4th fingers of both hands, myopia (-5dpt), and microretrognathia. Despite extensive support, hand writing and reading could not be accomplished, but manual skills in general were described as quite remarkable by the parents. Differential diagnoses included Lujan-Fryns syndrome, however, genetic analyses were not performed at this stage due to the parental feeling of a lack of consequences. At the age of 25 years, the family asked for re-evaluation of the patient's features and further diagnostic work-up. SNP microarray analysis and exome sequencing were carried out leading to the identification of a de novo nonsense mutation in the AUTS2 gene (c.1165C>T, p.Gln389*).

A syndromic form of intellectual disability caused by exonic deletions in the AUTS2 gene was first described in 2013; about 70 patients are known to the literature. AUTS2 syndrome is characterized by borderline to severe developmental delay, microcephaly, feeding difficulties, and features of autism. A friendly

personality was described in most patients. Congenital malformations are not typically associated with this syndrome. The mutational spectrum comprises mainly intragenic deletions leading to haploinsufficiency, nonsense mutations are rarely described. Tall, marfanoid stature (as in our patient) is not a typical feature of this syndrome as most patients' height is reported to be in the lower percentile range. Therefore, the genotypic (nonsense mutation) and phenotypic (tall stature, no microcephaly) spectrum of the AUTS2 syndrome is further expanded by this patient's characteristics.

P-ClinG-082

*** Cerebral MR imaging based genetic assessment of brain malformations

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Introduction: Structural brain malformations are an important cause of early psychomotor retardation, intellectual disability and seizures. Identification of the underlying mutations allows not only more precise genetic counseling of the affected families on the clinical spectrum and long-term course as well as the recurrence risk for further offspring, but for some patients may also provide additional information directly relevant for further diagnostic workup or therapeutic decisions. We here report the results for 140 patients with brain malformations consecutively tested by individual cMR imaging based genetic testing incl. next generation multigene panel sequencing.

Methods: Initial evaluation of available cMR images (37 patients), clinical data and individual genetic testing by either Sanger sequencing/MLPA (8 patients) or multi-gene panel sequencing (132 patients; on average analysis of 7,1 genes was reported per patient) after Nextera Enrichment (Illumina) and bioinformatic assessment of called variants with our in house pipeline including SeqNext (JSI medical systems) and evaluation for copy number variations using an in house JAVA-based skript.

Results and discussion: For 7 patients with typical cMRI pattern of hydrocephalus with aqueduct stenosis or classic lissencephaly a conventional single gene analysis was employed allowing to identify the causal L1CAM or LIS1/PAFAH1B1 mutations, respectively. Overall causal mutations or likely pathogenic variants (class 5 or 4) were identified for 51 of the 140 tested patients (36,4%), with large differences in the mutation detection rate for the specific brain malformations: hydrocephalus 4/20 = 20%; Walker-Warburg syndrome/Muscle eye brain disease 6/7 = 86%; Periventricular nodular heterotopia 1/6 = 16,7%; Lissencephaly/Double cortex 13/18 = 72%; Polymicrogyria 7/25 = 28%; Cerebellar hypoplasia 8/11 = 73%; Holoprosencephaly/Septooptic dysplasia 7/28 = 25% and Microcephaly 5/25 = 20%.

As expected, NGS panel sequencing substantially increased the number of identified variants of unknown significance (class 3), which were observed in 32% of the overall patient cohort. E.g. rare heterozygous class 3 variants (8x) or class 4/5 variants (2x) were observed in 10/25 patients with unexplained microcephaly. Three holoprosencephaly patients with identified heterozygous mutation in one of the core genes were heterozygous for an additional class 3 variant, suggestive for digenic inheritance.

Our data emphasize the importance of the individual clinical and imaging data for the individual testing strategy as well as for the final clinical classification of identified sequence variants. Furthermore, this combined clinical NGS approach may also allow extending the previously known gene-specific phenotypic spectrum and to offer new insights into signaling pathways and the impact of genetic modifiers.

P-ClinG-083

Prenatal diagnosis of Matthew-Wood syndrome in a fetus with multiple structural anomalies

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We present a case of prenatal diagnosis of Matthew-Wood syndrome (MWS) in a fetus with multiple structural anomalies caused by homozygous mutation in the STRA6 gene. To our knowledge there are currently only 44 reported cases of MWS syndrome worldwide.

The presenting couple is consanguineous and two previous pregnancies ended with spontaneous abortion or termination of pregnancy because of severe structural anomalies of the fetus. In addition, one early miscarriage occurred. The couple has a healthy daughter.

The woman was referred to us in 20th week of her fifth pregnancy because of an external diagnosis of bilateral hydronephrosis, oligohydramnios and intrauterine growth restriction. NIPT (Harmony-Test) in 12th week had been unremarkable. Ultrasound examination showed multiple structural anomalies: microcephaly,

anophthalmia, heart defect, hypoplasia of thorax and lungs, hydronephrotic kidneys and short long bones. Amniocentesis was performed and karyotyping showed no chromosomal aberrations. Pregnancy was terminated in 22th week and dysmorphology examination of the fetus showed fused eyelids, thick eyebrows, low-set ears, small nose with flat nasal bridge, long philtrum and camptodactyly of the left hand. Beyond that, pathological examination confirmed anophthalmia, nuchal oedema, ventricular septal defect, small lungs and hydronephrosis. Searching the Winter-Baraitser Dysmorphology Database revealed - besides numerous other possible syndromes with anophthalmia as key feature - the STRA6-associated Matthew-Wood syndrome (syn. PDAC syndrome) as a best match for the presenting features of the fetus. We performed NGS based clinical exome analysis and detected a homozygous mutation c.50_52delACTinsCC, p.Asp17Alafs*55 in the STRA6 gene. Blood samples of the consanguineous parents were not available so far but it is highly likely that both are heterozygous carriers of the mutation. The detected mutation has been reported twice in the literature (Golzio et al 2007, Sadowski et al 2017) as causative for MWS.

In summary the molecular genetic detection of the homozygous mutation in the STRA6 gene confirms with high probability the diagnosis of a Matthew-Wood syndrome for the fetus and it appears very likely for the fetuses of the former pregnancies with unfavorable outcome.

In subsequent pregnancies prenatal diagnosis can be offered to the couple. Also preimplantation diagnosis (PGD) could be an option. This case exemplifies that in recurrent pregnancies with fetal anomalies molecular genetic diagnostics represents an invaluable tool for appropriate counseling of concerned couples in regard to future pregnancies.

P-ClinG-084

First report of somatic mosaicism and parent to child transmission of DeSanto-Shinawi syndrome (DESSH)

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DeSanto-Shinawi syndrome (OMIM # 616708) is a recently described neurodevelopmental disorder caused by loss-of-function mutations in *WAC* located in 10p12.1. This autosomal dominant condition is characterized by developmental delay, hypotonia, behavioural problems, eye abnormalities, feeding problems, constipation and characteristic facial features including a prominent forehead, full cheeks, posteriorly rotated ears, hypertelorism, synophrys, a flat nasal bridge and a bulbous nasal tip.

To date only a small number of individuals with DeSanto-Shinawi syndrome have been described. The *WAC*-mutations identified were de novo heterozygous mutations including missense and nonsense mutations, small deletions and insertions. Microdeletions of chromosome 10p12-p11 including *WAC* have been reported in some individuals with developmental delay, intellectual disability or autism spectrum disorders.

We herein report a further case of DeSanto-Shinawi syndrome caused by a novel splice site mutation in *WAC* (c.497+1G>A, NM_016628.4). The patient, a 5 year-old female, was born at 34 weeks of gestation with postnatal hypotonia, global development delay, intellectual disability and dysmorphic features (brachycephaly, prominent forehead, full cheeks, posteriorly rotated ears). Tall stature and macrocephaly were also apparent, probably inherited by her father, who we presume to have an overgrowth syndrome, which still needs to be validated. Her 25 year-old mother, who carries the same splice site mutation as a somatic mosaic, had developmental delay, mild learning disability and facial dysmorphic features including midface retrusion and full cheeks.

To our knowledge, this is the first case of somatic mosaicism and moreover the first report of a familial transmission of DeSanto-Shinawi syndrome (DESSH). Our data expand the clinical and mutational spectrum of the *WAC* associated phenotype.

P-ClinG-085

Maternal transmission of a mild Coffin-Siris syndrome phenotype due to a SOX11 missense mutation

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In 2014 mutations in *SOX11* have been described to cause a mild Coffin-Siris syndrome (CSS) phenotype. Classical CSS is characterized by aplasia or hypoplasia of the distal phalanx or nail of the fifth and additional digits and/or toes, developmental delay, characteristic facial features, hypertrichosis and sparse scalp hair.

SOX11 is a transcription factor of the PAX6-BAF complex, which is proposed to play a role in brain development.

We report on two daughters of non-consanguineous parents with 14 and 10 years of age. The older girl was born at term. The pregnancy was uneventful. She had learning disability and muscular hypotonia. The body measurements were in normal range. Mild facial features consisted in low-set ears. A brain MRI was normal. The younger sister was born preterm at 31 weeks of gestation via cesarean section due to growth restriction. She also presented with low-set ears. Her developmental delay was more severe. Body measurements were in normal range. Cogan ocular motor apraxia was found in both sisters. Mother and both daughters showed hypoplastic nails of the fifth toes as sign of mild CSS. There was no history of seizures. The mother had a history of learning difficulties. She had a coloboma of the iris on the right side.

Karyotyping and array-CGH gave normal results. NGS CSS panel showed a missense variant in *SOX11* (c.139G>A; p.(Gly47Ser)) in both sisters and their mother. Four *in silico*-Tools (PROVEAN, SIFT, Polyphen-2 and MutationTaster) predicted the mutation as probably pathogenic.

Here we report for the first time of a maternal transmission of CSS due to a *SOX11* missense mutation. A review of the literature showed that until now only five patients were described with mutations in *SOX11*. In addition, seven patients having a deletion involving *SOX11* were reported. All of them showed developmental delay, especially concerning acquired language. Most of them had feeding difficulties. Half of them had hypoplastic nails of the fifth toes. It is of note that some of these patients show Cogan ocular motor apraxia. The facial dysmorphic features seem not to be specific. We suppose that the combination of Cogan ocular motor apraxia, developmental delay and hypoplastic nails of fifth toes are important diagnostic criteria for recognizing patients for mutations in *SOX11*.

P-ClinG-086

Familial locus heterogeneity in non-syndromic hearing loss: evidence from whole exome sequencing in Iranian families

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The clinical characteristics of non-syndromic hearing loss (NSHL) are diverse and vary according to type (conductive or sensorineural), onset (prelingual or postlingual), severity (mild to profound), affected frequencies (low, mid, or high), progressivity, and laterality. 50% of all cases are due to a genetic etiology, in which one of over 100 presently identified genes could be involved. Depending on the gene and in several cases on the allelic variant, the inheritance follows recessive (75-80%), dominant (20%) or X-linked (2-5%) patterns. The high rate of consanguineous marriages in Iran increases the overall prevalence of recessive NSHL. One-third of inherited hearing loss involves additional symptoms that may be challenging to recognize based on the delayed manifestation of multiple organ system involvement, as well as potential pleiotropic outcomes when pathogenic variants in so-called NSHL genes also cause syndromic features. These phenotypic and genotypic diversities characterize NSHL as a heterogeneous disorder. One layer of this enormous heterogeneity can be seen in the form of familial locus heterogeneity.

In our study, we whole/clinical exome sequenced over 140 Iranian probands who were largely pre-screened for pathogenic variants in *GJB2* and *STRC*. Libraries were prepared using the TruSight One and Nextera Rapid Capture Exome enrichment protocols and were sequenced using the MiSeq and NextSeq 500 desktop sequencers (Illumina). GensearchNGS and an in-house exome analysis pipeline guided variant analysis. When likely pathogenic variants were identified, family members were recruited for segregation analysis. Interestingly, in five consanguineous families, the likely pathogenic variant segregated in the nuclear family, but not in all affected members of the family. These probands were further analyzed by exome sequencing or by testing carrier variants of the index. Homozygous *SLC26A4* and *CIB2* pathogenic variants could be detected in one family, whereas another family disclosed homozygous pathogenic variants in *CABP2* and *CDH23*, respectively. In these two cases, the additional likely pathogenic variant could be identified, but in other cases that included intra-sibship heterogeneity, the disease causing variant is still unknown. This suggests the involvement of novel genes. Additionally, two branches of a consanguineous pedigree segregated two different *MYO7A* alleles that were compound heterozygous in the index. These examples underscore the importance of segregation analysis. In conclusion, these cases highlight familial locus heterogeneity and illustrate a challenge for the identification of causal variants in the face of phenotypic and genetic heterogeneity.

P-ClinG-087

A novel KDM5C nonsense mutation in two siblings with developmental delay

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An increasing number of neurodevelopmental diseases have been associated with disruption of chromatin remodeling in eukaryotes. The KDM5C gene encodes a specific H3K4me3 and H3K4me2 demethylase, and acts as a transcriptional repressor through the RE-1-silencing transcription factor (REST) complex and is essential for neuronal survival and dendritic growth. Several studies pointto a KDM5C pathogenic mutational frequency of up to 4 % among males with X-linked intellectual disability (XLID), both syndromic and non-syndromic. The phenotypical spectrum ranging from mild to severe ID, often accompanied by symptoms such as behavioral disturbances, hyperreflexia, short stature and epilepsy.

Here we report on two siblings with developmental delay. The boy was born at term with a birth weight of 2880 g, length 50 cm and head circumference 34 cm. Surgery was performed due to a synostosis of the sagittal suture. Developmental delay was noticed during the first year of life. Sitting age was 2 years. At age 2 years and 6 months he presented with profound speech impairment, short stature, dystrophy and microcephaly. He showed a happy disposition, scaphocephaly, a short neck, ptosis and spastic tetraparesis. At age 4 years grand mal epilepsy occurred. MRI studies revealed myelination defects.

His sister was born at 38 weeks of gestation with a birth weight of 2465 g, length 50 cm and head circumference 35 cm. Sitting age was 14 months. At age 15 months she presented with muscular hypertonia, short stature, dystrophy and plagiocephaly.

Exome sequencing revealed in both siblings and their healthy mother a novel nonsense mutation in KDM5C [c.1277G>A, p.(Trp426*)]. For evaluating X-inactivation status, the androgen receptor (CAG)n variable repeat region was used and showed non-random X-inactivation in the girl (94%) and her mother (100%). Few authors have described KDM5C mutations in females with intellectual disability and until now only five affected females were thoroughly described in the literature. This case supports the growing evidence of phenotypic manifestation of formerly recessive XL genes in females.

Both affected children showed verbal deficiency, short stature and spasticity and the boy presented additionally with epilepsy and microcephaly as previously reported in male cases. Nevertheless, myelination defects in MRI were not described and may represent a rare finding.

P-ClinG-088

Xq26.3 duplication with position effect on SOX3 in a family with hypopituitarism

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Mutations in *SOX3* (Xq27.1) are associated with growth hormone deficiency and variable additional anomalies (OMIM 312000 and 300123). We report on a family in which three male patients (two cousins and a maternal uncle) had short stature, growth hormone deficiency, cryptorchidism and hypoplasia of the pituitary gland. Array CGH analyses revealed a 3.3 Mb duplication in Xq26.3-q27.1 that was located 86 kb downstream of *SOX3* in all three patients. Female carriers of the duplication were healthy. We hypothesize that this duplication exerts a position effect on *SOX3* transcription.

P-ClinG-089

*** Targeted Sequencing with Expanded Gene Panel Enables High Diagnostic Yield in non-5q Spinal Muscular Atrophies

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Introduction: Spinal muscular atrophies (SMA) without deletions or point mutations in the SMN1 gene (non-5q-SMA) are a clinically and genetically heterogeneous group of disorders characterized by muscular atrophy, weakness and hypotonia due to the pathology in lower motor neurons. We aimed to test the clinical utility of targeted sequencing in the diagnosis of non-5q-SMAs by using two different gene panels with various sizes.

Methods: We first tested 30 unrelated individuals, for whom extensive clinical data had been collected and 5q-SMA had been excluded, on an LMND gene panel including 65 genes associated with SMA, hereditary motor neuropathy (HMN) and amyotrophic lateral sclerosis (ALS) using IonTorrent-AmpliSeq target enrichment. For 72 patients referred to us with a diagnosis of a SMA/LMND, we continued with a broader gene panel (NMD panel) including up to 479 genes with Agilent-SureSelect target enrichment method. Additional genes for neuromuscular disorders were included e.g. hereditary spastic paraplegias (HSP), ataxias, myopathies, myasthenic syndromes, muscular dystrophies, and muscle channelopathies. We updated the NMD panel four times by adding new genes and optimizing low-covered regions.

Results: In total, we have performed targeted sequencing of 93 unrelated affected individuals in both panels. 70% of the affected individuals had the disease onset at paediatric age (<18 years). Of all 93 cases 50 had an autosomal-recessive, 13 an autosomal-dominant, one an X-linked inheritance, while 25 were sporadic cases. Inheritance pattern in 4 families was not certain. Parental consanguinity was present in 37% of families. Thirty individuals were tested by the small LMND panel of which 4 (13%) were diagnosed. Nine unsolved cases from the LMND panel and additional 63 individuals were tested by large NMD panel, which yielded a definite diagnose of 31 individuals (43%). Among the 31 disease causing mutations identified by using the NMD panel, we found only 5 mutations in 3 genes that were implicated in non-5q-SMA (IGHMBP2, GARS, HSPB1). The remaining cases with a suspected non-5q-SMA were diagnosed with mutations in 3 genes involved in ALS, 6 genes in HMN, 3 genes in ataxia, 2 genes in HSP, 2 genes in distinct polyneuropathies (FXN, GAN), and one gene for arthrogryposis (LGI4). Of note, we found causative mutations in 3 muscle/extracellular matrix related genes (COL6A1, COL6A2, COL12A1) in 4 patients with a suspected non-5q-SMA. Finally, the diagnostic rate in the paediatric age group was with 42% significantly higher than in adult group reaching only 28%.

Conclusion: Our experience supports the use of gene panels covering a broad disease spectrum for NMD diseases that are highly heterogeneous and clinically difficult to differentiate

P-ClinG-090

De novo loss-of-function variant in HIVEP2 in a 27-year-old woman with mild intellectual disability

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Heterozygous pathogenic variants in *HIVEP2* (Human immunodeficiency virus type I enhancer binding protein 2) have recently been reported as a cause of neurodevelopmental abnormalities. To date, only eight patients with nonsense or frameshift mutations and one patient with a missense mutation in *HIVEP2* have been published. They all showed developmental delay and/or mild to moderate intellectual disability. We report on a 27-year-old female patient in whom exome sequencing revealed a heterozygous stop mutation (c.[6609_6616delTGAGGGTC];[=], p.[Glu2204*];[=]) in the last exon (exon 10) of *HIVEP2*. In line with a *de novo* occurrence of the change, Sanger sequencing confirmed the variant in patient's DNA but not in blood-derived parental DNA samples. Besides mild developmental delay and intellectual disability (IQ 58), the patient

didn't show any medical issues, dysmorphic features, malformations, seizures, neurological abnormalities or behavioural disorders. This is the oldest reported patient with a *HIVEP2* mutation so far, and we present detailed clinical information from birth to adulthood. Furthermore, we review the literature and compare the phenotype of the other reported patients with *HIVEP2* mutations.

P-ClinG-091

A recessive type of congenital contractural arachnodactyly is caused by biallelic mutations in FBN2

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Fibrillin-2, encoded by FBN2, plays an important role in the early process of elastic fiber assembly. To date, heterozygous mutations in FBN2 have been shown to cause congenital contractural arachnodactyly (CCA; Beals-Hecht syndrome) inherited as an autosomal-dominant trait. CCA is characterized by a Marfan-like appearance with tall stature, long, slender fingers and toes, ear deformities, joint contractures at birth, club feet, muscular hypoplasia often accompanied by multiple cardiovascular or gastrointestinal anomalies. CCA-associated FBN2 mutations cluster in the so called neonatal region (exons 24-36) and comprise missense, splice and in-frame deletions/duplications, with the exception of a single nonsense mutation, suggesting a possible gain-of-function effect.

Here, we report on a 15-year-old girl with classical CCA and biallelic mutations in FBN2. The girl inherited the novel missense mutation c.3563G>T/p.(Gly1188Val) in exon 27 from her unaffected father and the novel nonsense mutation c.6831C>A/p.(Cys2277*) in exon 54 from her healthy mother. By qualitative cDNA analysis we could detect only a small amount of FBN2 transcripts harbouring the nonsense mutation in leukocyte-derived mRNA from the affected girl and her mother suggesting nonsense-mediated mRNA decay of the majority of c.6831A-bearing transcripts. As the father does not show any clinical signs of CCA we hypothesize the missense mutation c.3563G>T to be a hypomorphic allele.

Taken together, our data suggests that a classical CCA can also be inherited in an autosomal-recessive manner by a combination of a hypomorphic allele together with a null allele of the FBN2 gene, likely giving rise to monoallelic expression of transcripts encoding a mutant FBN2 protein.

P-ClinG-092

Novel homozygous PDE10A mutation in two sisters affected by an early-onset hyperkinetic movement disorder

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Both dominant and recessive mutations in *PDE10A* (Phosphodiesterase 10A; OMIM *610652) have recently been described to cause a childhood onset of chorea (Diggle et al. 2016, Mencacci et al. 2016). In comparison with patients with dominant *PDE10A* mutations, reported patients with homozygous *PDE10A* mutations (6 individuals of a consanguineous family of Pakistani origin and two brothers of a consanguineous family from northern Finland) showed a more severe clinical presentation with axial hypotonia and a generalized hyperkinetic movement disorder characterized by limb and orofacial dyskinesia within the first six months of life. Dyskinesia was accompanied by dysarthria in all affected individuals. While all affected individuals of the Pakistani family were able to walk without support and had normal intellectual function, the two finish brothers had a markedly developmental delay of speech (expression of a few words at the age of seven years) and motor function (require a wheelchair). Furthermore the younger brother of the finish family developed focal epilepsy at the age of 3.5 years. In contrast to patients with dominant *PDE10A* mutations who showed characteristic bilateral striatal T2 hyperintensities on cerebral MRI, cerebral MRI of five affected individuals with biallelic *PDE10A* mutations was normal.

Here we report on two sisters of consanguineous healthy parents from Syria affected by marked ongoing motor restlessness, hyperkinetic and choreatic movements and dystrophy. Whole exome sequencing revealed a novel potentially deleterious homozygous missense mutation NM_001130690.2:c.1994T>C; p.(Val665Ala) in the *PDE10A* gene which was not present in dbSNP or the Exome Aggregation Consortium database. In addition to the above mentioned features, the seven-year-old sister showed microcephaly (-47.5 cm; -3.67 z), severe mental retardation and generalized epilepsy. She is weelchair-bound. Her younger one-year-old sister showed marked developmental delay and severe muscular hypotonia. EEG and cerebral MRI of the younger sister were normal.

The family broadens the mutational and clinical spectrum associated with mutations in *PDE10A*.

P-ClinG-093

Microphthalmia is not a mandatory finding in X-linked recessive Lenz microphthalmia syndrome

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Mutations in BCOR cause an X-linked form of syndromic microphthalmia (MCOPS2). Truncating variants in BCOR follow an X-linked dominant pattern and underlie Oculofaciocardiodental syndrome (OFCD) in females. This phenotype includes variable microphthalmia with congenital cataracts, facial dysmorphism, cleft palate, cardiac and dental anomalies. Intelligence is normal. A recurrent missense variant p.(Pro85Leu) has been identified in three families with X-linked recessive Lenz microphthalmia. All reported, affected males have ocular anomalies such as microphthalmia, anophthalmia, microcornea, glaucoma and coloboma. Other common clinical aspects include microcephaly, ear anomalies, hearing loss, dental findings, skeletal anomalies such as syndactyly or kyphoscoliosis and genitourinary anomalies. More than half of the patients have mild to severe intellectual disability or developmental delay.

We saw two brothers referred to our clinics due to mild developmental delay and variable additional anomalies. The older boy was born with severe microphthalmia, hypoplastic optical nerves, facial and neck asymmetry, and renal reflux requiring surgery. Walking age was 2 years, and age of first words was one year. At age 12 years he attended school with a teaching assistant. Under treatment with growth hormones since age 6 years his height was 10th to 25th centile, and his weight and head circumference were below the third centile. He has a relatively wide mouth with large teeth, prominent finger joints, broad distal phalanges and sandal gaps. Syndromic microphthalmia had been suspected, but previous arrayCGH and testing of SOX2, PAX2, SIX1 and SIX6 had been normal.

The younger brother had no major ophthalmologic anomalies apart from myopia and astigmatism. He presented with feeding difficulties after birth and developmental delay (walking age 23 months, delayed speech development). He had progressive scoliosis, bilateral radioulnar synostosis and cryptorchidism. At age 6 years his body measurements were below the 3rd centile. He had mildly dysplastic ears, a long philtrum and sandal gaps.

Due to the phenotypic differences we simultaneously performed exome sequencing in both. Unexpectedly, this revealed the recurrent BCOR mutation p.(Pro85Leu) in both boys and in their healthy mother. While the older brother's phenotype completely fits the described phenotypic spectrum, the younger brother only shows developmental delay, microcephaly and skeletal anomalies, but not the key feature microphthalmia. In contrast to the previously published families, our findings demonstrate incomplete penetrance for p.(Pro85Leu)-associated microphthalmia in males.

Our findings demonstrate the large variability of BCOR-associated, syndromic microphthalmia and expand the spectrum to developmental delay with skeletal anomalies but without ocular malformations.

P-ClinG-094

Monozygotic twins with POMK-associated Walker-Warburg Syndrome and occipital meningocele

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Walker-Warburg syndrome (WWS) represents the most severe end of a phenotypic spectrum of similar disorders caused by defective glycosylation of alpha-dystroglycan, collectively known as dystroglycanopathies. Alpha-dystroglycanopathies are genetically heterogeneous autosomal recessive disorders; mutations in 16 underlying genes have been identified so far.

We report on monozygotic male twins (monochorionic-diamniotic), born to healthy consanguineous parents. Six siblings are healthy, one died postnatally due to complications of a congenital hydrocephalus.

Prenatal ultrasound investigations showed a strikingly similar clinical manifestation of WWS with hydrocephalus, occipital meningocele and hypoplastic cerebellum in both fetuses. The boys were born by C-

section at 35^{+2} weeks of gestation with normal measurements. Postnatal examination confirmed the meningocele and revealed eye anomalies in both twins. CK was increased above 1000 U/l. Cranial MRI scans in both twins showed an occipital meningocele with dorsally enlarged fourth ventricle, hypo-/aplasia of the cerebellar vermis, cortical malformation with generalized polymicrogyria-like cobblestone malformation, temporo-occipital subcortical band heterotopia, and eye malformations (microphthalmia with coloboma and caudal cyst in twin 1; persistent hyperplastic primary vitreous body and posterior staphyloma in twin 2).

Based on the clinical signs, the elevated CK and the radiological findings with brain and eye malformations, the tentative diagnosis of WWS was established. Molecular panel analysis revealed a homozygous nonsense mutation in *POMK* (protein O-mannosyl kinase, OMIM *615247) that was confirmed in both twins by Sanger sequencing. These molecular findings confirmed the diagnosis of a *POMK* associated WWS in both twins (OMIM #615247). Both parents were confirmed to be heterozygous mutation carriers.

So far, with only five different *POMK* mutations published in three families (Jae et al., 2013, von Rennesse et al. 2014, Di Constanzo et al., 2014), *POMK* mutations represent a very rare cause of alpha-dystroglycanopathies. Meningo/encephaloceles have been reported so far as a rare finding in WWS including one patient with *POMK*-related WWS (Jae et al., 2013). The observation of occipital meningoceles at identical positions in both twins appears interesting and might point to a more important role of *POMK* in the pathogenesis of neural tube defects.

P-ClinG-095

Novel SBF2 mutations and clinical spectrum in patients with autosomal recessive Charcot Marie Tooth disease type 4B2

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Biallelic mutations in the SBF2 gene cause Charcot-Marie-Tooth disease type 4B2 (CMT4B2), a demyelinating neuropathy with autosomal recessive inheritance and association to early-onset glaucoma. Since discovery of the causal gene in 2003, only a limited number of additional CMT4B2 patients and families have been identified. Here we report seven new CMT4B2 families and nine different mutations in the SBF2 gene. Revisiting genetic and clinical data from our cohort and the literature, SBF2 variants were generally private mutations, including multi-exon deletion and de novo variants. The neuropathy, which is always demyelinating, starts in the 1st decade after normal early motor development, is predominantly motor and progresses slowly. Increased intraocular pressure and glaucoma were seen in about half of the cases and were not always congenital. Comparing loss-of-function and missense mutations, there were no genotype-phenotype correlations with regard to occurrence of glaucoma or onset and progression of neurological symptoms. We conclude that genetic testing for demyelinating CMT should include analysis of the SBF2 gene, irrespective of the presence of early-onset glaucoma. Despite autosomal recessive inheritance, de novo variants should be considered to ensure accurate reporting of recurrence risk.

P-ClinG-096

Diagnostic exome in neurometabolic disease: a single center experience

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Molecular diagnosis of neurometabolic diseases is challenging due to the large number of established and candidate disease genes associated with an extreme heterogeneity of clinical presentations at variable ages. Especially in infancy-onset of inborn errors of metabolism an early diagnosis is crucial to guide downstream clinical management and treatment decisions. Since 07/2016 next generation sequencing (NGS)-based genetic testing is accepted in the German health system in principle. However, its application as a first line diagnostic tool is hindered by bureaucratic hurdles. Towards this end, concerted efforts of researchers, clinicians, and managers are needed to promote and realize the potential benefit of approaches such as whole exome / genome sequencing (WES/WGS).

We here report on the results of exome-based diagnostics of a cohort of 46 index cases with clinically suspected neurometabolic disease, presumably resulting from impaired mitochondrial energy metabolism.

Coding genomic regions were enriched with a SureSelect Human All Exon Kit V6 (Agilent technologies) for subsequent sequencing as 2x125 bp/2x100 bp paired-end reads on a Illumina HiSeq2500/NovaSeq6000 system. Generated sequences were analyzed using the megSAP pipeline (https://github.com/imgag/megSAP). Clinical variant prioritization included different filtering steps (e.g. MAF < 0.1 % in gnomAD, in-house database) and was conducted in parallel by at least two analysist according to an in-house standard operating procedure.

In 21 (46%) cases, we identified likely pathogenic or pathogenic variants in genes that have been associated with mitochondrial diseases (14), other neurometabolic diseases (3) or severe encephalopathies (3). In 16 cases (35%) we identified variants of unknown significance were follow up studies for functional analyses are pending (mitochondrial diseases: 11, neurometabolic diseases: 2, encephalopathies: 2, ataxia: 1). Moreover we newly identified 3 candidate genes affecting the mitochondrial calcium entry (1), mitochondrial morphology (1) or selenoprotein metabolism (1). However 9 cases (20%) remain unsolved. Further ongoing investigations of the latter in a research setting include the additional analysis of parental DNAs (trio analysis) as well as full genome and transcriptome sequencing. Although presenting with a wide phenotypic spectrum WES facilitated a definite diagnosis in 46% and a possible diagnosis pending follow up in 35% of the cases.

P-ClinG-097

A novel reciprocal translocation t(9;11)(p24.3;p15.4) as a cause of transgenerational Beckwith-Wiedemann syndrome

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Identifying the molecular underpinnings of Beckwith-Wiedemann syndrome (BWS) can be challenging due to its etiological heterogeneity. The vast majority of BWS cases are associated with altered methylation of chromosome 11p15.5. However, these epigenetic abnormalities may be induced by various molecular mechanisms like epimutations, uniparental disomy, copy number variations, and structural rearrangements. In addition, loss-of-function mutations in *CDKN1C* (OMIM: 600856) need to be considered, especially in familial forms of BWS. A patient`s family history as well as a thorough clinical examination can favour specific diagnostic approaches. Despite a good comprehension of the disease and its pathogenesis, the molecular background remains unknown in 30% of characteristic BWS patients.

We describe an Iranian family with a transgenerational form of BWS, including two affected females. After the determination of an abnormal methylation pattern of the BWS critical region on chromosome 11p15.5 by bisulfite pyrosequencing (hypermethylation of the imprinting control region 1 and hypomethylation of the imprinting control region 2), subsequent quantitative real-time PCR revealed a heterozygous copy number gain of 11p15.5. The extent of this duplication was further assessed through array-comparative genomic hybridization. The co-occurrence of a heterozygous copy number loss of the distal part of chromosome 9p in both patients led to the assumption of a paternally inherited unbalanced segregation product derived from a balanced reciprocal translocation in the respective fathers. Fluorescence *in situ* hybridization analyses confirmed a reciprocal translocation between chromosomes 9p and 11p in the fathers of both patients and a copy number gain of chromosome 11p translocated to chromosome 9p in both affected individuals.

To our knowledge, we describe the first family with a reciprocal translocation between chromosomes 9p and 11p resulting in BWS through paternal inheritance.

P-ClinG-098

Mutations in PIH1D3 cause X-linked primary ciliary dyskinesia with outer and inner dynein arm defects

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Primary ciliary dyskinesia (PCD) is a genetically heterogeneous disorder characterized by chronic airway disease, male infertility and randomization of left-right body asymmetry due to defects of motile cilia frequently

resulting in permanent lung damaged. PCD is mostly caused by defects involving components of dynein arm complexes, responsible for cilia beat regulation and generation. Despite the fact that many components of cilia/flagella are well characterized, even though in the model organism *Chlamydomonas*, the mechanisms underlying cilia formation and function, and in particular the cytoplasmic pre-assembly of dynein arm complexes that power ciliary motility, are only poorly understood. Here we report a novel gene located on the X-chromosome, *PIH1D3*, involved in the preassembly of both outer (ODA) and inner dynein arms (IDA) of cilia and sperm flagella. We identified inherited as well as de novo hemizygous loss-of-function mutations in 4 male PCD individuals from a Dutch and a German family. We show that loss-of-function mutations in *PIH1D3* result in absence of ODAs and IDAs from ciliary axonemes, causing ciliary and flagellar immotility. Further, we show that PIH1D3 interacts and co-precipitates with the previously described cytoplasmic ODA/IDA assembly factors DNAAF2 and DNAAF4. This is the first report of an X-linked mode of inheritence in individuals with a classic PCD phenotype without cosegregation of other syndromes such as intellectual disability or retinitis pigmentosa. We demonstrate that mutations can be inherited by the mother but also can occur as de novo events. The identification of a novel PCD gene located on the X-chromosome is of major clinical importance to genetically unsolved male cases with ODA and IDA defects as well as for genetic counseling.

P-ClinG-099

More or less than 25 kb: a question of costs or expertise? Critical evaluation of applications for extended NGS analysis at statutory health insurance in Germany

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Next-generation sequencing (NGS) analysis has been proven as a powerful tool for analysis of heterogeneous genetic diseases. High diagnostic yield has prompted its clinical application as first line approach to allow for early and cost-effective molecular diagnosis in children with rare and unspecific conditions. In Germany, coverage of ambulant medical services in patients with statutory health insurance is regulated by the EBM catalogue (Einheitlicher Bewertungsmaßstab der Kassenärztlichen Bundesvereinigung). Recent adaptation of the EBM allowed using NGS for analysis of up to 25 kb of DNA sequence. Reimbursement of costs for analysis of more than 25 kb (EBM 11514) needs to be case-based approved. Application for approval requires a research-based justification including clinical information, a precise description of the extent of the needed analysis, and a critical evaluation regarding therapeutic and prognostic consequences. Statutory health insurance may evaluate applications based on the recommendations given by a consultant of the MDK (Medizinischer Dienst der Krankenkassen). We established a standardized system to apply for reimbursement of >25 kb NGS analysis in patients with intellectual disability and/or epilepsy referred for genetic analysis to our molecular lab. Up to now, we sent out 68 applications that are supported by a research-based expert opinion fulfilling the EBM requirements. We received a final statement in 33 cases. Application was approved in 9 cases (27%) and rejected in 24 cases (73%). Of the 24 rejections, reasoning of the MDK consultants (specialties: e.g. gastroenterology, oral and maxillofacial surgery, ENT) were available in 11 cases. The consultant reasoned rejections based on variable arguments. These included formal mistakes (8/11, 73%), insufficient argumentation (8/11, 73%), low benefit for the patient (4/11, 36%), missing therapeutic relevance (6/11, 55%), insufficient pre-genetic investigations (5/11, 45%), insufficient previous genetic workup (6/11, 55%), non-approved method (4/11, 36%), and genetic epilepsy not considered as a rare disease (2/11, 18%). Apart from these formal points, there was no research-based argumentation. Our results reveal a major gap between evidence-based clinical and technical progress and its utilization in clinical practice. This forces health care-providers to perform multiple clinical and genetic investigations instead of straight forward application of NGS analysis as first line approach to cut diagnostic odysseys and costs. Health care regulations and implementation of NGS analysis in genetic diagnostics for patients with heterogeneous rare diseases should be made evidence-based by experts and should be free from lobby influence. We therefore argue against restriction of genetic analysis in routine diagnostics based on non-scientific advisory recommendation by non-experts in the field of Human Genetics.

P-ClinG-100

Homozygous Nonsense Mutation in TECRL in a Patient with Overlapping Clinical Symptoms of both LQTS and CPVT

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Inherited arrhythmogenic cardiac disorders are associated with significant mortality and are a known risk factor for sudden cardiac death. Here, we present a case report of a patient from a consanguineous Caucasian

family with overlapping clinical symptoms of both LQTS (long QT syndrome) and CPVT (catecholaminergic polymorphic ventricular tachycardia) caused by a homozygous nonsense mutation in TECRL. The patient, a 15-year-old male who collapsed during sport and lost consciousness, was hospitalized in the Department of Pediatric Cardiology at Essen University Hospital due to recurrent ventricular fibrillations. Stable sinus rhythm was achieved after several defibrillations plus cardiopulmonary resuscitation and the administration of Amiodarone together with Suprarenin, an L-form of adrenaline. Shortly after Suprarenin injection, the rhythm resulted in several polymorphous ventricular extra systoles and short-term ventricular tachycardia, which then returned to ventricular fibrillation. The initial ECG showed a left precordial disturbance of repolarization as well as a prolonged frequency corrected QT time (QTc) of 460-500 ms. An ICD was implanted and the patient is currently being treated with 3 x 40 mg Propranolol. To date, he has had no further cardiac events.

In order to clarify the genetic cause of this cardiac event, DNA samples from the patient were submitted for NGS panel diagnostics consisting of 18 LQTS and CPVT related genes (*AKAP9, ANK2, CACNA1C, CALM1, CALM2, CASQ2, CAV3, KCNE1, KCNE2, KCNE3, KCNH2, KCNJ2, KCNJ5, KCNQ1, RYR2, SCN4B, SCN5A* and *SNTA1*). Since pathogenic mutations in TECRL have been described in three independent families with overlapping features of both CPVT and LQTS, we included this gene in our extended research panel in order to report it as part of the Multiple Integration of Data Annotation Study (MIDAS) for phenotype/genotype correlation. Here, we were able to identify the homozygous nonsense mutation NM_001010874.4:c.415C>T, p.(Gln139*) leading to a premature termination of translation in exon 4 and thus to the loss of the 3-oxo-5-alpha steroid 4-dehydrogenase domain and three transmembrane segments. No other pathogenic, likely pathogenic or variants of uncertain significance were found in the remaining LQTS and CPVT genes. In the non-affected parents, the mutation was detected in the heterozygous state, whereas it was not found in the non affected sibling. The evolutionarily conserved TECRL gene is primarily expressed in the endoplasmic reticulum of myocardial cells and seems to plays an important role in the intracellular calcium balance. Thus, it might have a crucial function in the heart.

P-ClinG-101

Two novel LRP5-mutations causing Osteoporosis-Pseudoglioma Syndrome

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We report on a 10 month-old son of non-consanguineous healthy parents presenting with a combination of ocular and skeletal anomalies.

The pregnancy was uneventful and the child was delivered spontaneously at term. Birth weight (3370g), head circumference (34 cm) and length (50 cm) were in the normal range.

At the age of six weeks microphthalmia, leukocoria, persistent hyperplastic primary vitreous (PHPV) and ablatio retinae with blindness on both sides were diagnosed, so that Norrie syndrome was suspected. No additional abnormalities were detected. Nine months later the boy had two spontaneous fractures without any adequate trauma. Furthermore the X-ray revealed abnormalities of the bone structure. In this context the mother reported, that she herself experienced multiple fractures during childhood attributed to osteoporosis and an osteogenesis imperfecta (OI) was suspected.

Because of the combination of severe abnormalities of the eyes and the bones osteoporosis-pseudoglioma syndrome (OPPG) was taken into account as a differential diagnosis after NDP-mutations (Norrie syndrome) were excluded. OPPG is a very rare disease characterized by early-onset osteoporosis along with bone fragility and eye abnormalities (i.a. vitreoretinal). Further symptoms e.g. short stature, microcephaly, cardiac defects, hyperextensible joints may occur. The intelligence is normal in most cases, mild mental retardation is possible.

OPPG is caused by homozygous or compound-heterozygous mutations in the gene LRP5. The protein encoded by LRP5 (low density lipoprotein receptor-related protein 5) impairs bone accrual during growth through Wnt signaling pathway. Furthermore LPR5 plays an important role in the establishment of peak bone mass (Wan et al. 2013, Gong et al. 2001) and i.a. in the regression of fetal hyaloid vessels (Kheir et al. 2016).

Sequence analysis of LRP5 showed the heterozygous mutations c.1480C>T (p.R494W) and c.2588G>A (p.W863*). Both mutations have not been described before. In silico analyses for the missense mutation c.1480C>T predict a pathogenic effect. The mutation c.2588G>A induces a premature stop-codon resulting in nonsense mediated mRNA decay or if translated most probably leads to a truncated non-functional protein. Segregation analyses confirmed compound heterozygosity in the boy as the mutation c.1480C>T was inherited by the father and the mutation c.2588G>A was inherited by the mother.

The detection of two novel biallelic mutations in LRP5 confirmed the diagnosis of OPPG in the boy. The mother´s repeated fractures most probably result from a reduced bone mass which is reported in heterozygous LRP5 mutation carries (Maltese et al. 2017, Kheir et al. 2016). Genetic diagnosis allows preventive treatment in the boy and his parents.

P-ClinG-102

Exome Sequencing reveals a novel missense mutation in PIGA associated Multiple Congenital Anomalies-Hypotonia-Seizures Syndrome 2

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PIGA (Phosphatidylinositol N-acetylglucosaminyltransferase subunit A) codes for a subunit of an enzyme involved in the first step of biosynthesis of the GPI (glycophosphatidylinositol) anchor. The GPI-anchor, a glycolipid, plays an important role in attaching glycoproteins to the outer membrane of eukaryotic cells. Germline PIGA-mutations are associated with the X-linked Multiple congenital anomalies-hypotonia-seizures syndrome 2 (MCAHS2, OMIM: 300868), characterized by distinct dysmorphic features, neonatal hypotonia, epilepsy and variable congenital anomalies. We present a case of MCAHS2 in a 9-year-old male patient, with a previously undescribed hemizygous PIGA-mutation.

Our patient presented with severe epileptic encephalopathy refractory to treatment, developmental regression after initially normal development until the age of 8 months, lack of speech development, cortical blindness as well as dysmorphic features including gingival hyperplasia and widely spaced teeth.

Through Trio-WES we detected a novel hemizygous mutation in exon 2 of the PIGA-gene (c.154C>T/p.His52Tyr) in our patient. Segregation analysis showed heterozygosity of the mother, while the father did not carry the mutation. Analysis of protein levels of GPI-anchored proteins showed a reduced expression of CD16, CD14 and CD24 in patient blood cells compared to the healthy parents, consistent with reduced GPI-biosynthesis. The patient's mother also showed slight reduction of CD24 expression in comparison to the father. The phenotype of the patient compares well to clinical findings in previously described cases, including the dental anomaly and the arrest and regression of psychomotor development, and therefore further supports the diagnosis of PIGA-associated MSAHS2.

P-ClinG-103

A novel mutation in WDR45 in a girl with developmental delay, loss off speech and motor skills

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Mutations in WDR45 have been shown to cause an X-linked dominant form of neurodegeneration with brain iron accumulation (NBIA) designated as the "beta-propeller protein associated neurodegeneration". More recently, WDR45 mutations were also identified as the cause of "static encephalopathy of childhood with neurodegeneration in adulthood (SENDA)". Here we present a girl, who was born after an mostly uneventful pregnancy at 34 weeks. The initial motor and speech development was mildly delayed, but in the second year of live she rapidly lost most of her motor and language skills. Testing for Angelman, Rett and Pitt-Hopkins syndromes were normal, as were karyotype and array CGH. After the development of febrile seizures at 2 years of age, SCN1A and PCDH19 were also tested with normal results. Due to the severe phenotype and the wish of the family to have more children, we decided to perform NGS testing by means of the TruSight One Panel (Illumina) and evaluate the data for mutations in genes related to the phenotype. We found a novel intronic variant predicted to result in disturbed splicing. Sanger sequencing in the child and her parents confirmed the variant and showed the de novo occurrence. Although initial MRI investigations only showed delayed myelinisation, a new investigation at 7 years confirmed iron accumulation in the globus pallidus and the substantia nigra. We present the case and a review of the literature.

P-ClinG-104

Extreme phenotypes of TOR1A mutation-associated arthrogryposis multiplex congenita

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Torsion dystonia-1 is a long-recognised low-penetrance autosomal dominant disorder typically caused by a single amino acid deletion in the torsinA protein, which is encoded by the TOR1A gene. Recently, bi-allelic TOR1A mutations have been linked to a congenital contracture syndrome complicated by global developmental delay. Here we report the extended clinical spectrum of this new entity, ranging from infants with massive contractures, profound developmental delays and a fatal course due to respiratory failure to an 8-year-old patient with only mild contractures, motor delay, spastic paraparesis and cognitive deficits. We found that arthrogryposis-related TOR1A variants lead to decreased expression or abnormal subcellular localization of mutant torsinA. Our data also suggest that the levels of remaining torsinA protein are inversely related to the severity of the phenotype. In conclusion, TOR1A mutations have a previously unanticipated broad range of consequences depending on inheritance pattern and the type of mutation and should be considered in patients with variable forms of arthrogryposis.

P-ClinG-105

Factors impacting approval of GOP11514 – experiences from 397 applications

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Clinical and genetic heterogeneity of rare diseases together with the tardiness of traditional single gene testing approaches pose a major hurdle towards a genotype-differentiated treatment and informed decision making by families and physicians. Technological advances now allow for the sequencing and analysis of full genomes within a day with a diagnostic yield of up to 57 % and the potential to augment or modify medical management.

Since the change in the Einheitliche Bewertungsmaßstab (EBM) in 07/2016 the choice of method for genetic testing is free allowing for next generation sequencing-based analyses in principle. However, only 25 kb (about 10 average-sized genes) of coding sequences can be interpreted without prior approval by the insurance within the Gebührenordnungsposition (GOP) 11513. Any interpretation of additional sequences above 25 kb requires the approval of this analysis (GOP 11514) by the responsible insurance after a formalized application (GOP 11304). This application is usually forwarded to the Medizinische Dienst (MDK) which provides a recommendation for decline or approval of the test.

We here report on our experiences from 397 applications for extended genetic testing. Of the 281 cases with a final decision only 38 (9.9 %) have been approved while the majority has been declined. Amongst others, reasons for a negative votum included formal shortcomings of the application, general denial of the efficacy of NGS-based diagnostics, lack of evidence of direct therapeutic consequences, and reference of ongoing research activities at the Institute. There was no clear difference between acceptance rates between different insurers. Processing time of the applications by the insurance/MDK was highly variable with about one third not receiving a decision within 5 weeks. Tracking of the applications status is challenging due to the complex communication between insurances, patients / guardians, clinicians, and genetic laboratory.

It's undebatable that an indication for genome-wide NGS-based genetic testing needs to be placed with caution. However, the current situation hinders genetic diagnostics and management of rare disease patient at international standards, promotes inefficient usage of financial resources, and might even put patient's health at stake. We therefore welcome an initiative by the KV (TRANLATE-NAMSE, Innovationsfond) aiming to coordinate and improve not only NGS-diagnostics but also overall clinical management of patients with rare diseases.

P-ClinG-106

Identification of compound-heterozygosity for two novel *YY1AP1* splice mutations in a family with Grange syndrome by whole-exome sequencing

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We here report on three siblings with a severe steno-occlusive arterial disorder. The 25-year-old female index case presented with chronic hypertension, occlusion of the left internal carotid artery (ICA), stenosis of the contralateral ICA, mesenteric arterial and renal artery stenosis. In addition, an intracranial steno-occlusive disease with a transient ischemic attack (TIA) at the age of 16 had been reported. The younger sister and brother did not only present with multiple vascular stenotic lesions but also with cutaneous syndactyly of their hands and feet.

By whole-exome sequencing for both affected sisters, two intronic variants in the *YY1AP1* gene (OMIM *607860) could be identified which were predicted to interfere with normal splicing. *YY1AP1* encodes the transcription coactivator yin yang 1-associated protein 1. Loss-of-function variants in this gene have only very recently been identified as cause of Grange syndrome (OMIM # 602531) which is a rare autosomal-recessive condition characterized by severe steno-occlusive arterial lesions of multiple vascular systems (Guo et al., Am J Hum Genet. 2017, 100:21-30). Furthermore, bone fragility, heart defects, syndactyly, brachy¬dactyly and learning disabilities have been reported for some of the originally described patients.

The substitution NM_001198903.1:c.826-1G>A identified in this family is located in intron 5 of *YY1AP1* and listed in gnomAD with only three heterozygous carriers. The alteration of the acceptor splice site is predicted to lead to skipping of exon 6. *In silico* splice predictions for the second point mutation c.997+23T>G which had not been previously described consistently indicated the creation of a novel donor splice site in intron 6 whose use was predicted to result in a frameshift due to the exonisation of 22 intronic nucleotides. Sanger sequencing confirmed compound-heterozygosity for the two variants in all affected siblings while their parents and the unaffected sister were shown to be heterozygous carriers. RT-PCR on RNA from blood lymphocytes confirmed both splice defects.

The family presented here adds clinical and genetic information to the still limited number of patients with Grange syndrome described in literature so far. A minigene assay for the c.997+23T>G variant is ongoing to test the splice correction efficiencies of antisense oligonucleotides.

P-ClinG-107

Familial periodontal Ehlers-Danlos syndrome caused by the novel missense variant c.926G>A; p.Cys309Tyr in the C1R gene

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Ehlers-Danlos syndrome (EDS) is a connective tissue disorder characterized by skin hyperextensibility, joint hypermobility, tissue fragility and abnormal wound healing. The predominant clinical features of periodontal EDS (pEDS; EDS type VIII) are early severe periodontitis and gingival recession. Recently, Kapferer-Seebacher et al. showed that heterozygous mutations in the complement 1 subunit genes *C1R* and *C1S* cause pEDS. Besides this publication, no other patients with pEDS caused by pathogenic variants in *C1R* have been described so far.

Here we describe three affected females with familial pEDS. The index patient (aged 29), her mother (60), as well as the maternal aunt (54) presented with gingival recession and premature tooth loss. Further clinical findings include thin, translucent skin with easy bruising and bleeding (primarily of lower legs), abnormal wound healing, internal bleeding after minor traumas, thrombosis, diverticular disease with ruptures, progeroid appearance, and myopia. All three have recurrent infections (e.g. bronchitis, upper airways, urinary tract). Additionally, the index patient was diagnosed with fibrotic pericarditis in adolescence. The aunt had stomach cancer and suffers from progressive rheumatoid arthritis. The mother has signs of a demyelinating disease.

The index patient was initially diagnosed with vascular EDS or progeroid EDS. In order to identify the underlying genetic defect, we performed panel based next generation sequencing (NGS) for 44 relevant genes (diagnostic panel “connective tissue disorders”, Praxis für Humangenetik Tübingen) in 2016. As we identified no potentially causative variants, we performed a whole exome based analysis for the three affected family members in September 2016 (Praxis für Humangenetik Tübingen). We filtered for shared variants and excluded variants in genes with no known association with connective tissue disorders. Again, no potentially causative variant was identified. Based on the accordance of the familial phenotype with pEDS, the exome

data were reevaluated in 2017, focusing on the genes *C1R* and *C1S*. We identified a heterozygous missense variant (c.926G>A; p.Cys309Tyr) in the *C1R* gene in all affected family members, which had been excluded in the previous analysis, as *C1R* mutations were only associated with autosomal recessive Complement C1r deficiency then. The substitution affects a highly conserved amino acid residue in a functionally relevant domain of the protein, and a known pathogenic missense variant affects the same codon (p.Cys309Trp). Thus this variant is considered to be most likely pathogenic and to be causative for the familial pEDS.

In conclusion, we present three affected individuals with familial pEDS, caused by a novel missense variant in the *C1R* gene. The provided clinical data broaden the spectrum of manifestations of this rare disorder. Furthermore, this case illustrates the importance of reevaluations of genetic data in certain time frames.

P-ClinG-108

De novo KAT6A mutation in a 5-year-old girl and delineation of the phenotype

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We report on a 5-year-old girl with severe global developmental delay, secondary microcephaly and absent speech. She was born at 40 weeks of gestation with normal measurements. Feeding difficulties and muscular hypotonia were present after birth. She had unilateral renal agenesis, an atrial septal defect and a patent ductus arteriosus (PDA). At the age of five, she was unable to walk independently. She showed facial dysmorphism with hypertelorism, tented upper lip and broad nasal tip. Stereotypic hand movements with restoration of hand function and hyperventilation episodes triggered by happiness were noticed.

Chromosomal analysis, CGH array and an eight gene panel for Angelman-like syndrome (*ARX*, *FOXG1*, *MECP2*, *MEF2C*, *SLC9A6*, *TCF4*, *UBE3A*, *ZEB2*) revealed no clearly pathogenic results. An extended NGS analysis revealed a heterozygous, truncating variant in *KAT6A* (c.3385C>T; p.Arg1129*), which was described previously. Segregation analyses in both parents confirmed de novo origin.

De novo *KAT6A* mutations have previously been reported in 20 patients (including a pair of monozygotic twins) to cause a syndromic form of intellectual disability (ID) characterized by severe developmental delay, absent speech, microcephaly, facial dysmorphism and congenital heart defects (CHD). Facial dysmorphism include subtle features consisting of bitemporal narrowing, broad nasal tip, thin and tented upper lip and microretrognathia.

KAT6A encodes for a histone lysine (K) acetyltransferase, which is part of a histone 3 acetylation complex and thus plays an important role in transcriptional activity and gene expression.

As *KAT6A* mutations were detected in up to 1% of ID patients in larger cohorts, it may be one of the more common causes of syndromic developmental delay and should be considered as a potential differential diagnosis in patients with syndromic ID including microcephaly, subtle facial dysmorphisms and CHD.

P-ClinG-109

PCYT1A-associated spondylometaphyseal dysplasia in two patients without cone-rod dystrophy revealed by gene panel analysis

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Spondylometaphyseal dysplasia is a genetically heterogenous group of bone dysplasia. One rare form is spondylometaphyseal dysplasia with cone-rod dystrophy (SMDCRD). This autosomal recessive disorder is characterized by profound short stature, rhizomelia with bowing of the lower extremities and early-onset progressive visual impairment associated with a pigmentary maculopathy and electroretinographic evidence of cone-rod dysfunction. SMDCRD results from mutations in the PCYT1A gene encoding the phosphate cytidylyltransferase 1, cholin, alpha isoform on chromosome 3q29. Here, we report a 10-year-old female patient with disproportional short stature with short limbs, bilateral clinodactyly V, broad feet, genu varum and lumbar lordosis. Earlier ophtalmologic examination revealed mild myopia and astigmatism. Using gene panel analysis, we identified the homozygous mutation c.968dupG in the PCYT1A gene. The mutation was confirmed by Sanger sequencing. Also the 7-year-old sister of our patient, presenting with the same phenotype, carries this homozygous mutation, while the unaffected parents are heterozygous carriers respectively. By now, mutations in the PCYT1A gene were described in patients with spondylometaphyseal dysplasia with cone-rod dystrophy (SMDCRD) or patients with retinal dystrophy without skeletal malformations. In both patients presented here, opthalmologic examination showed no evidence for changes of the macula or the retina up to now. In our patient cohort, we investigated another family with three affected children with suspected

autosomal recessive spondylometaphyseal dysplasia in 2014. The three girls (4, 12 and 13 years old) carried the above mentioned mutation in the PCYT1A gene compound-heterozygous with a missense mutation and presented with macula and retinal abnormalites in opthalmologic examination repsectively. Due to the missing eye involvement single gene testing of PCYT1A-gene was not considered in the patients with signs of spondylometaphyseal dysplasia presented here. Therefore, gene panel analysis is an appropriate method to detect causative mutations in case of a disease with genetic heterogeneity or an ambiguous clinical phenotype.

P-ClinG-110

A potential splice site mutation of NDS1 gene in a girl with Sotos syndrome type 1

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Sotos syndrome type 1 is a rare, autosomal dominant genetic disease, which appears to be a fully penetrant condition. The typical symptoms are excessive growth, especially during childhood, distinct craniofacial characteristics and a variable degree of mental impairment. These three cardinal features occur in ≥90% of affected individuals [1]. Different types of mutations in the NSD1 gene have been described in Sotos syndrome. The NSD1 gene encodes a histone methyltransferase with functional domains regulating chromatin structure and function [2]. Specifically, the catalytic domain which is subdivided into pre-SET, SET and post-SET domains are involved in histone methylation.

Here, we report a 6 4/12 year old girl with the clinical diagnosis of Sotos syndrome type 1 presenting with characteristical craniofacial features such as a long and narrow face, pointed chin, broad and prominent forehead, hypertelorism, strabism and rising eyelid axis. In addition, the girl manifested overgrowth (1,96 SDS), macrocephaly (3,74 HC SDS) and a delay in speech development. A first febrile seizure occurred at 18 months, absence epilepsy was diagnosed at 48 months of age.

We performed multi-gene panel testing via sequencing by synthesis, which includes the core genes associated with Sotos syndrome. We identified a novel intronic sequence variation c.6152-14G>A in intron 20 of the NSD1 gene in the index patient that was absent in the clinically unaffected parents. Although a few NSD1 splice site mutations have been described in the literature, splice site mutations beyond the 5` or 3` splice site-associated GT and AG dinucleotides have not been published. Further bioinformatics analysis indicates, that the identified intronic variation c.6152-14G>A causes a shift of the splice acceptor site in intron 20 to position -14. To investigate the consequence of this variant on splicing, we sequenced NSD1 mRNA in the index patient. This analysis revealed an in-frame addition of 12 base pairs in the open reading frame at 5`site of exon 21 resulting in 4 additional aminoacids located in the catalytic domain of the NSD1 protein. This findings indicate that NSD1 protein function may be impaired and suggest that the identified NSD1 splice variant is a disease-causing mutation.

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[2] Nader et al. (Biomol NMR Assign. 2016;10(2):315-20.

P-ClinG-111

Interdisciplinary case based learning in within the Competency oriented Duesseldorf Curriculum of Medicine – a concept to teach interdisciplinary counselling in hereditary cancer

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Interdisciplinary teamwork is essential for an effective and safe patient care in a highly diversified health system. To be able to take over the role of a member of an interdisciplinary team is one of the seven superordinate roles which have been defined in the National Competence Based Catalogue of Learning Objectives for Undergraduate Medical Education in Germany (NKLM). In the NKLM, learning objectives (LO) have been described, which have to be addressed to achieve this competency. To address these LO in the Duesseldorf Curriculum of Medicine (DCM) the educational format "Patient complaint guided, simulation patient supported interdisciplinary case handling" (PSICH) was created. LO for team development as well as for communication, collaboration and reflection in the team were developed. Cases with a chronic disease and

additional health problems were selected. Simulation patients (SP) were trained to the respective multimorbid clinical picture. A group of six students took the history of the SP, did the clinical examination and evaluated their findings according to the underlying diseases. They built teams of medical specialists of different expertise to cope with the diverse health problems of the patients. They took over the roles of the respective medical specialists, collected the diagnostic and therapeutic information by self-directed learning, and drafted an interdisciplinary therapy plan. To communicate the plan to the medical colleagues, the students wrote an interdisciplinary medical report. The students discussed the therapy plan with the SP according to the concept of shared decision making in an interdisciplinary counselling setting. The counselling session was evaluated by direct feedback of the SP to the students, and by peer feedback among the students. The overall performance of the team with respect to the correctness of the therapy plan was evaluated by a senior physician. The process of the team work was critically reflected by peer feedback of the group. Each group was guided through the entire process by a senior student. The educational format was evaluated by the students using an online tool. Since start in 2015 a total of about 900 medical students successfully completed the format. An overall satisfaction with the format ranging from 2.0 (range 1-6, very satisfied – very dissatisfied) in 2015 to 1.9 in 2017 in the online evaluation indicated, that the format was generally well accepted. PSICH is a multidimensional educational format covering examination and communication skills, patient centered thinking and acting, self-directed learning capabilities as well as team work competency. Genetic counselling in hereditary cancer is an interdisciplinary task involving human geneticists, clinical oncologists, pathologists and psychooncologists.. The PSICH format covers all aspects of interdisciplinary counselling in cancer families and may therefore be used to educate students in this task.

P-ClinG-112

First report on Chudley-McCullough syndrome in a patient of German origin

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We report on a rare case of Chudley-McCullough syndrome in a patient of German origin. Chudley-McCullough syndrome is a rare autosomal recessive neurologic disorder characterized by early-onset sensorineural deafness and specific brain anomalies on MRI, including hypoplasia of the corpus callosum, enlarged cisterna magna with mild focal cerebellar dysplasia, and nodular heterotopia. Some patients also present with hydrocephalus. Psychomotor development is normal (summarized by Alrashdi et al., 2011). We report on a 2 8/12 years old girl with shunted hydrocephalus due to obstruction caused by an interhemispheric cyst, partial agenesis of corpus callosum, frontal polymicrogyria and dysplastic cerebellar hemispheres. Her development was characterized by dystrophy and developmental delay without expressive language. There was no suspicion of deafness in the newborn screening, but at age of 2 8/12 years bilateral deafness was diagnosed. These specific brain anomalies as well as the deafness suggested Chudley-McCullough syndrome, which was confirmed by the finding of compound heterozygous mutations in the GPSM2-gene, a nonsense mutation in exon 13 of maternal origin and a deletion of exons 11-15 of paternal origin. This is also the first report on a deletion of several exons in GPSM2-gene.

P-ClinG-113

Panel diagnostics of inherited platelet disorders via high-throughput sequencing

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Inherited platelet disorders (IPD) once considered as rare, are today recognized with increasing frequency. With an estimated prevalence of 10-100/10000 individuals they are in the range of common bleeding disorders like the von Willebrand Disease. The clinical spectrum of IPD is heterogeneous and ranges from mild conditions that may remain unaffected throughout life to severe haemorrhage recognized shortly after birth. Thereby, the observed IPD might be caused by receptor/cytoskeleton defects, secretion disorders or signal transduction defects of the platelets. Due to this enormous variety many specialized tests are usually required to reach a putative diagnosis. Comprehensive diagnostics including molecular genetic characterization are important to better understand IPDs and to support an efficient therapy. To this end, we have established a targeted high-throughput sequencing (HTS) panel that comprises 87 genes covering IPDs including thrombocytopenia and platelet function defects as well as genes associated with acquired thrombocytopenia, e.g. immune thrombocytopenia for routine diagnostics. Validation of our HTS IPD panel was initially performed

on 18 patients with already confirmed IPD. Sequencing was carried out on an Illumina MiSeq system covering 96% of target regions with >100 reads. In 8 patients of our cohort we could thereby reconfirm the variants of previous Sanger sequencing. The rest of the cohort was studied single-blinded and matched with their clinical diagnostics after interpretation of sequencing results. Overall, we were able to identify all missense mutations (12/18), small insertions/deletions (5/18) as well as splicing variants (1/18) that cause inherited thrombocytopenia (8), hypofibrinogenemia (2), Hermansky-Pudlak syndrome (3), Bernard-Soulier syndrome (2), von Willebrand disease (1), CAMT (1) and Glanzmann thrombasthenia (1) in our cohort. Some of the identified mutations were already described as pathogenic in the Human Gene Mutation Database (HGMD), whereas other variants have not been published so far. Those new variants were rated by several bioinformatics programmes for their pathogenicity and classified according to the guidelines published by the American College of Medical Genetics and Genomics.

Following validation the HTS IPD panel has been implemented in routine diagnostics at Labor Berlin, revealing a relatively high diagnostic yield thus far. Hence, we believe that application of our HTS panel will help to simplify provision of a definitive diagnosis for patients with IPD.

P-ClinG-114

Novel KMT2A (MLL) mutations and atypical clinical presentation in Wiedemann-Steiner syndrome

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Wiedemann-Steiner syndrome (WSS) is characterized by hypertrichosis cubiti associated with short stature, mild to moderate intellectual disability, behavioral difficulties, hypertrichosis on the back, and distinctive facial features with long eyelashes, thick or arched eyebrows with a lateral flare, and downslanting and vertically narrow palpebral fissures. The causative gene was identified by whole-exome sequencing in patients with WSS, where truncating mutations in the KMT2A (MLL) gene were found in five of six patients (Jones et al. 2012). To date 27 KMT2A mutations have been described in HGMD as causative for WSS, whereby the majority are either nonsense mutations, frameshift mutations or larger deletions, most likely resulting in haploinsufficiency for MLL. Only four missense mutations have been described so far, of these three reside in the CXXC-type Zinc finger domain. We present six further patients with de novo novel KMT2A mutations, five truncating mutations and one missense mutation (p.R1154W) that affects a highly conserved amino acid within the CXXC-type Zinc finger domain and results in a slightly aberrant phenotype.

P-ClinG-115

Extended genetic analyses in patients with non-obstructive azoospermia

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Male infertility is a clinically and genetically highly heterogeneous disease, mostly caused by spermatogenetic failure, clinically noted as oligo- or azoospermia. The most common form of the latter phenotype is non-obstructive azoospermia (NOA), which can be caused by various genetic defects such as chromosomal aberrations, microdeletions or monogenic defects. In the majority of NOA cases, a genetic origin is suspected but current genetic testing, comprising cytogenetic analysis and AZF deletion screening, only discovers the cause in about 17%.

In 2017, we expanded our analyses of clinically well-characterized men with NOA that attended the Centre of Reproductive Medicine and Andrology (CeRA). First, the routine chromosomal and AZF analyses were performed. In a second step, sequence analysis of three genes *NR5A1*, *DMRT*1 and *TEX11* was carried out in men with apparently normal karyotypes and without AZF deletions.

Chromosomal analyses were performed in 323 patients. Altogether 46 patients (14.2%) were identified with numerical (47,XXY; 47,XYY) or structural aberrations (46,XX; aberrant Y chromosomes; translocations; inversions). AZF deletions were found in 1.9% (6 of 310) of the NOA patients.

The coding sequence of *TEX11*, *NR5A1* and *DMRT1* was analysed in 80 patients. Potentially pathogenic variants were identified in 4 patients (5.0%), one mutation in the *DMRT1* and *TEX11* gene, respectively, and two different mutations in *NR5A1*. The mutation in *TEX11* (c.450C>T) and one of the *NR5A1* mutations

(c.712G>A) were already published, whereas one novel mutation was detected each in *NR5A1* (c.1079C>T) and *DMRT1* (c.308A>G).

In conclusion, the basic genetic analyses in men with NOA by conventional cytogenetic analysis and AZF screening revealed the expected number of abnormalities. Through sequencing of three genes, which have been confirmed as responsible for spermatogenetic failure, we were able to identify an additional 5% of men carrying possibly pathogenic variants.

This work was carried out within the frame of the DFG Clinical Research Unit ‚Male Germ Cells: from Genes to Function' (CRU 326).

P-ClinG-116

New patient with Marden-Walker syndrome phenotype: central nervous system abnormalities characterization and overview

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Blepharophimosis, mask-like face, camptodactyly/arthrogryposis and severe mental delay combination compose a distinct pattern of rare Marden-Walker syndrome (MWS, OMIM#248700, autosomal recessive inheritance, approximately 40 reported patients). Central nervous system (CNS) pathology, cleft palate, hearth, renal and genital defects registering with variable frequency form a complete phenotypic spectrum.

Genetic defect is not identified. PIEZO2 gene heterozygous mutation was detected in one patient with MWS phenotype.

We present a new patient with MWS spectrum features focusing on brain abnormalities comparing them to the published data.

Non-consanguineous parents and 2 sibs were healthy. G6 (proposita) was complicated by severe polyhydramnion. Fetus malformations (corpus callosum agenesia, ventriculomegaly, pyeloectasy, aberrant subclavia artery) were detected by US screening at 20 weeks. Karyotype (GTG, amniocytes): 46,XX.

Newborn girl born at term (BW=3270g, BL=50cm, OFC=34cm) showed reduced reflexes, blepharophimosis, hypertelorism, microgenia, foramen ovale (FO), patent ductus arteriosus, dolichomegacolon, lobulated spleen, hydronephrosis II-III, clitoris and small labia hyperplasia, hymen abnormality, arthrogryposis. At age 9 months the proposita presented growth delay (W=6850g, L=67cm), motor milestones and mental development retardation, lower paraparesis, expressionless face, abnormal pupil shape, iris dystrophy, optic nerves disks partial atrophy, dysgenesia of retina, glaucoma, partial ptosis (left), FO, hepatomegaly, umbilical hernia, camptodactyly, dislocation of the left hip and knee, right knee flexion contracture, clubfoot. CNS abnormalities included microcephaly (OFC=41cm), a characteristic signs of corpus callosum agenesia (colpocephaly, septum pellucidum agenesia, dilated distance of anterior horns, large posterior horns of lateral ventriculis, 3-d ventricle dilatation), changes of brain gyries architectonics.

Reported informative for brain data cases (US, CT, MRI studies) demonstrate a wide spectrum of CNS abnormalities: microcephaly, hydrocephalus/ventricular dilatation, cerebral atrophy, hypoplasia of cerebellar hemispheres/vermis and brainstem, corpus callosum agenesia/hypoplasia/dysgenesia, Dandy-Walker malformation. Microscopic changes - diffuse proliferation of astrocytes with swollen nuclei, atrophic oligodendrocytes, slight astrocytic proliferation in caudate nucleus, thalamus, hypothalamus, basal ganglia were identified by postmortem examination.

Conclusion. Clinical findings of our patient, including multiply brain lesions, strongly correlate with reported MWS phenotypic spectrum. CNS pathology (especially, cerebellar and corpus callosum abnormalities) can be helpful in distinguishing between MWS and other distal arthrogryposis syndromes.

P-ClinG-117

Novel MED13L mutation in a patient with intellectual disability, sensorineural hearing loss, preauricular ear tag and auricular fistula

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We report on a five-year-old boy, the first child of healthy, non-consanguineous German parents, with intellectual disability (ID), sensorineural hearing loss, preauricular ear tag, auricular fistula, hyperopia, strabismus, hypotonia and distinctive facial features (including upslanting palpebral fissures, flat nasal bridge and low-set posteriorly rotated ears). Genetic testing including conventional cytogenetic analysis, Microarray-CGH and molecular analysis of the genes FMR1 and ATRX was unremarkable.

Subsequent trio whole-exome sequencing identified a de novo heterozygous mutation in MED13L (c.4011_4024del / p.Ile1338Hisfs*22) leading to a premature stop codon and most probably resulting in a truncated and functionally impaired protein.

Mutations in the mediator complex subunit 13 like gene (MED13L) on 12q24.21 have been identified to cause mental retardation and distinctive facial features with or without cardiac defects (MRFACD; OMIM 616789). The patients with MRFACD described so far all showed developmental delay (DD) and/or intellectual disability (ID) and a spectrum of facial anomalies. Initially MED13L was reported in patients with congenital heart defects, in particular in isolated dextro-looped transposition of the great arteries (TGA; OMIM 608808). To date, congenital heart defects seem to be a variable feature with incomplete penetrance of the broad clinical spectrum of this condition (Asadollahi et al., 2017). Hearing loss has been described in three patients with MED13L haploinsufficiency so far (Adegbola et al., 2015; Caro-Llopis et al., 2016)). Low set ears were seen in 11 out of 21 patients (Asadollahi et al., 2017). Since our patient also shows a preauricular ear tag and an auricular fistula, similar to other previously reported patients (Adegbola et al., 2015; Cafiero et al., 2015), we suggest that MED13L haploinsufficiency seem to affect the complex development of the ear and dysmorphic features concerning the external ears might be a consistent feature of the MED13L haploinsufficiency syndrome.

In conclusion, the presented case adds up to the yet small number of reported cases of patients with a heterozygous mutation in the MED13L gene and thereby contributes to the clinical spectrum of MRFACD.

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P-ClinG-118

Genetic counselling services in Germany 1996 to 2016, still rising steeply

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Background: Over many years demand for genetic counselling has been constant, in Germany. From 1996 to 2004 on average about 47,000 cases per year with minor fluctuations were reimbursed by the German statutory sickness funds (Pabst and Schmidtke, Gendiagnostik in Deutschland, BBAW, p. 195-203, 2007). Reimbursement data collected for the “GenBIn2”- project from the ZI_KBV database for the years 2009 to 2014 showed a steep rise in reimbursed genetic counselling cases: 41,243 (2009), 45,525 (2010), 46,691 (2011), 51,316 (2012), 54,739 (2013) 61,308 (2014), (Schmidtke et al., medizinische genetik 29, 152-153, 2017). Based upon these data we originally assumed the marked increase in demand for genetic counselling services not to be coincidental but to be attributable to the enactment of the German Act on Genetic Testing (GenDG, February 1, 2010). Because data referring to the period 2005 to 2008 were no longer kept by the ZI-KBV database, additional data were needed to substantiate this assumption.

Methods: To control whether the increase in reimbursed genetic counselling cases started already earlier than 2009 and continued to rise after 2014: (i) 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) were collected for 2015 and 2016 from the ZI-KBV database , (ii) missing data for the 2005 to 2008 period were obtained from a number of “Kassenärztliche Vereinigungen” of several German federal states.

Results: From these new data it becomes evident (i) that the rise of genetic counselling services already started in the years 2005/2006 and is thus not directly related to the enactment of the GenDG and (ii) that demand for counselling services continues to rise steadily (62,981 cases/2015; 67,672 cases/2016).

Discussion: We suggest that the observed rise in demand for genetic counselling services has multifactorial causes including a general increase of awareness of the importance of genetic counselling, fuelled by novel technologies and the public and professional debate surrounding the GenDG. Our data relate to services delivered by specialists in human genetics only (195 full time equivalents in statutory health insurance in 2016). We estimate that, on average, a specialist in human genetics in ambulatory health care is currently devoting about 40% of his or her working hours directly to genetic counselling. It can be expected

that the demand for genetic counselling will continue to rise and that the counselling workload will continue to increase for human genetics specialists.

P-ClinG-119

The ethics of prenatal programming: Design of a survey on attitudes towards the potential role and detection of fetal epigenetic changes

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Recent research reveals how prenatal programming can influence the development of predispositions and diseases e.g. by changing epigenetic marks in the fetus. In the focus of interest are maternal influences during pregnancy and their possible consequences for the child. Epigenetic programming is blamed to contribute to maladaptation in the womb and metabolic disorders such as diabetes and obesity, as well as an increased risk of cancer later in life. In case of known susceptibility, conceivably epigenetic prenatal diagnosis could lead to earlier treatment. The discussion, however, is sometimes controversial: the pathogenicity of many epigenetic modifications is not proven or completely unknown. Altogether, epigenetic approaches to assess maternal imprinting would open up a new dimension of genetic testing and raise questions about the underlying ethical principles. Remarkably, hardly any studies on the ethics of epigenetic testing yet exist. In contrast, technically, it is already possible to localize epigenetic changes non-invasively on the fetal genome (e. g., differential methylation between mother and fetus to diagnose trisomy 21).

To address the ethical issues related to prenatal programming and non-invasive detection of fetal epigenetic patterns and changes we have initiated a research project aiming at determining the attitudes in the affected population.

The first step in our project is a comprehensive survey of the knowledge on (epi)genetics and of personal basic attitudes to (epi)genetic testing with a focus on prenatal expositions and diagnostics. For this purpose, we are developing questionnaires to be handed out to people in theoretical decision-making situations, i. e. to persons potentially faced with epigenetic testing which is not yet available but possibly in the near future. Primarily in gynecological, neonatological or pediatric institutions, we intend to invite pregnant women and mothers of newborn children, as well as their partners, to participate. In addition, we are planning to recruit a control group with similar distribution of gender and age, composed of people not directly involved in such circumstances, e. g. from other hospital units or recruited via public announcements. A statistical power analysis has yielded 200 couples and 200 control persons to be envisaged for study participation.

In a second step, after the survey, we plan to have experts comprehensively inform small groups of interested participants in the above survey (20 couples and 20 control persons) about the actual possibilities and perspectives, relevance, consequences and limits of genetic and especially epigenetic diagnostics and prediction. With these, we will partly repeat the above survey about 3 months after the education. The effect of education on potential changes of ethical positions will then be evaluated. We plan to invite further institutions to participate and estimate a duration of two years for the entire project.

P-ClinG-120

Clinical heterogeneity in hereditary transthyretin amyloidosis

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The most common type of hereditary amyloidosis is transthyretin amyloidosis (hATTR amyloidosis), which is endemic in Portugal, Sweden, and Japan. The prevalence in the German population is unknown. Deposition of an abnormal transthyretin protein can occur in multiple organs of the body leading to the allelic diseases familial amyloid polyneuropathy (FAP), cardiac amyloidosis, and leptomeningeal amyloidosis. In addition, an age-dependent accumulation of wild-type transthyretin protein has also been described and can result in senile systemic amyloidosis (SSA). If untreated, death usually occurs 5 to 15 years after onset of symptoms. As therapy of hATTR amyloidosis is different from SSA, early diagnosis and precise knowledge of disease causes is mandatory for patient management. To further elucidate the variability of the familial TTR-related amyloidosis, we present two specific cases identified in our DNA testing collective.

In family G, a hypertrophic cardiomyopathy with considerable reduced diastolic dysfunction was diagnosed in an 84 year old female patient. DNA testing revealed a likely pathogenic c.88T>C, p.Cys30Arg mutation in the TTR gene. At age 85, with onset of polyneuropathy she is on treatment with Tafamidis, the only approved

drug therapy for this condition. Two of her six children (a 53 years old son and a 63 years old daughter) were tested positive for the familial TTR gene mutation. Both are asymptomatic and under clinical control.

In family H, a 59 year old male suffered from polyneuropathy (loss of walking ability, urinary and bowel incontinence), gastro-intestinal involvement (gastritis, diarrhea) and hypertrophic cardiomyopathy. Genetic testing revealed a pathogenic c.148G>A, p.Val50Met mutation in the TTR gene. The patient was listed for liver transplantation, but deceased at the age of 60 years.

Our cases show the clinical variability of hereditary ATTR amyloidosis and emphasize the importance of early diagnosis. Different treatment options include drug therapy, liver transplantation and potentially targeted therapy in the future.

P-ClinG-121

Autosomal recessive cutis laxa type 1: clinical and molecular analysis of 17 families, comprehensive literature review and delineation of the phenotype.

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Autosomal recessive cutis laxa type 1 (ARCL1) presents a heterogeneous disease spectrum, characterized by generalized wrinkled, redundant, inelastic, and sagging skin. Systemic manifestations, like pulmonary emphysema, vascular complications, and gastrointestinal and genitourinary involvement, lead to early mortality. The ARCL1 subtypes described so far are caused by mutations in the large genes FBLN5 (ARCL1A), EFEMP2 (ARCL1B), and LTBP4 (ARCL1C), encoding for components of the extracellular matrix. Due to considerable clinical overlap, genetic testing results are important to support the clinical diagnosis. In the current study we analysed 17 unrelated individuals diagnosed with ARCL1 using a Next Generation Sequencing (NGS) based approach. We identified four novel homozygous FBLN5 mutations in 4/17 probands, one known and four novel homozygous EFEMP2 mutations in 5/17 probands, and eleven novel biallelic LTBP4 mutations in 8/17 probands. A comprehensive review of 79 previously reported individuals with molecular confirmed ARCL1 revealed characteristic facial similarities. Most common features included lax and wrinkled skin, sagging cheeks, long philtrum, large ears, hypertelorism, inguinal and diaphragmic hernias, and pulmonary emphysema. Arterial tortuosity was exclusively present in patients carrying EFEMP2 mutations, while cutis laxa was less frequently observed in this subgroup. Our findings expand the mutational and clinical spectrum and delineate the phenotypic differences of ARCL1 disorders. Furthermore, we show that NGS offers an accurate and efficient procedure to establish a molecular diagnosis in patients with ARCL1.

P-ClinG-122

Complex multiorgan disorder due to a de novo muation in the ACTG1 gene

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We report on a girl, who presented first at the age of 9 months, with a complex multiorgan disorder including congenital diaphragmatic hernia with enterothorax, hypoplasia of the right lung, recurrent bronchial infection and respiratory distress, intestinal malrotation, cardiac dysfunction, PFO and PDA, colobomata of iris and retina, failure to thrive, unilateral renal malformation, craniofacial dysmorphism (hypertelorism, ptosis, low set ears), and profound developmental delay.

She is the second child to a healthy non-consanguineous couple of Serbian origin. The elder sister is completely healthy. The girl was born spontaneously in the 41st week and due to respiratory maladaptation (APGAR 4) was transferred to a neonatal intensive care unit where the diaphragmatic hypoplasia was diagnosed. She received ECMO treatment and reconstructive surgery of the diaphragm and the abdominal wall. Due to a severe failure to thrive nutrition via percutaneous jejunostoma was necessary.

Conventional chromosome analysis of lymphocytes and fibroblasts (Pallister-Kilian syndrome?), and CHD7 mutation search (CHARGE-syndrome?) were normal. Array-CGH showed a de novo deletion of 15.5kb in the chromosomal region 17q25.1 comprising the ACOX1 gene. Biallelic ACOX1 alterations result in a peroxisomal Acyl CoA oxidase deficiency (MIM 609751) which does not clinically fit with the presentation of the child. Biochemical testing excluded an ACOX1-deficiency.

In March 2017, the mother was pregnant again. Given the severity of the girl´s handicap and the uncertain recurrence risk we offered a trio NGS-analysis. In the ACTG1 gene the mutation c.767G>A (p.Arg256Gln) was detected in heterozygosity in the girl but not in her parents. In silico prediction suggested pathogenicity. Arg256 is evolutionary highly conserved and the mutation p.Arg256Trp has already been published as pathogenic (Rivière et al 2012, De Donato et al 2016).

Pathogenic mutations in the ACTG1 gene are known to cause Baraitser-Winter-syndrome (BRWS), a rare disorder characterized by intellectual disability, distinctive facial features with hypertelorism, bilateral ptosis and metopic ridging, ocular colobomata, congenital cardiac disease, genitourinary malformations and cortical malformations (e.g. pachygyria). The majority of the congenital malformations and the facial gestalt at the age of 1 ¾ years in our patient fit quite well with the syndrome.

The couple asked for prenatal diagnosis and the mutation was not found in fetal DNA.

To our knowledge, diaphragmatic hypoplasia has not been described in BRWS yet. Thus, we cannot exclude, that the diaphragmatic defect has another etiology. Therefore, repeated ultrasound examinations are performed and up to now, no fetal abnormalities were seen.

In conclusion, our case illustrates that “fast track” NGS-analysis may allow the identification of the molecular basis of the disease even in an ongoing pregnancy enabling the parents to have an early invasive diagnosis.

P-ClinG-123

Ankylosis of the thumb metocarpophalangeal joints as diagnostic clue in a boy with Renpenning syndrome

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Renpenning syndrome is a rare X-linked disorder caused by hemizygous mutations in PQBP1. Major symptoms are intellectual disability of varying degree, short stature, microcephaly, small testes, and subtle dysmorphism. As the facial phenotype in Renpenning syndrome is often non-specific, clinical diagnosis mainly relies on more specific features which may only be detected by thorough clinical examination.

Here, we describe how particular skeletal findings led us to the diagnosis of Renpenning syndrome in a 16-year old boy with mild cognitive impairment (IQ 69), short stature (157.5 cm, - 2.9 standard deviation) and pronounced microcephaly (50.0 cm, - 4.4 standard deviation). His early motor development was normal, but he had speech delay. Recently he finished regular school (Polytechnical School) and is now looking for an apprenticeship. He has a slender build, asymmetry of the axial skeleton with thoracolumbar scoliosis and subtle dysmorphic signs (full hair, triangular face, long eyelashes, short philtrum, protruding ears, prominent nasal root). On clinical examination he was noted to be unable to flex the thumb metacarpophalangeal joints and to have limited forearm pronation and supination, two findings which have been described as characteristic for Renpenning syndrome. This diagnosis was confirmed by sequence analysis which revealed a recurrent hemizygous frameshift mutation in PQBP1 [c.459_462delAGAG (p.Arg153SerfsTer41)]. The boy´s clinically unaffected mother was found to carry the mutation in a heterozygous state.

The present case emphasizes the importance of a thorough clinical examination. Little details, in particular the ankylosis of the thumb metocarpophalangeal joints and the restricted forearm supination, were crucial for making the diagnosis in this otherwise mildly affected boy.

P-ClinG-124

*** A novel syndrome characterized by severe primary microcephaly, progressive myoclonus epilepsy, and hypotonia is caused by a specific homozygous splice-site mutation in GOSR2 leading to defects glycosylation

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Progressive myoclonus epilepsy (PME) is a very heterogeneous group of disorders and also phenotypic expression is highly variable. Recently, a founder homozygous missense mutation in the SNARE domain of golgi SNAP receptor complex member 2 (*GOSR2*) c.337-12T>A (p.Gly144Trp) was associated with PME.

GOSR2-associated PME is characterized by onset of ataxia in the first years of life, followed by action myoclonus and seizures later in childhood, and loss of independent ambulation in the second decade. The cognition is usually normal, however mild memory problems may appear in the third decade. Worldwide, only seventeen GOSR2-associated PME patients were diagnosed. All of them share the same founder mutation and showed remarkably similar clinical presentation.

Here, we report identification of a novel mutation in *GOSR2* gene in 12-year old girl of consanguineous Turkish family presenting with a novel syndromic form of PME characterized by severe primary microcephaly, intellectual disability, mild facial dysmorphism with coarse face, early onset myoclonus epilepsy, hypotonia, and hypertrichosis. Taking advantage of whole exome sequencing, we identified the novel homozygous c.337-12T>A splice-site mutation in *GOSR2* affecting the splicing of exon 5. RNA analysis demonstrated aberrant transcripts leading to in-frame deletion of 47 amino acids (p.Asp113_Lys159del) in the SNARE domain of GOSR2 protein. GOSR2 also named membrin is a part of SNARE complex required for subcellular trafficking within Golgi apparatus. Deletion of the major part of SNARE domain leads to complete loss of function of membrin and Golgi dysfunction. We hypothesized that this might lead to glycosylation defects in the patient and indeed, we could show abnormalities in glycosylation patterns. Moreover, functional analysis of Golgi-function is currently done in patients fibroblasts.

Our data demonstrate that a novel mutational mechanism in *GOSR2* leads to aberrant transcripts, partial loss of function of membrin and subsequently to glycosylation defects in a patient with a novel PME-related syndrome.

P-ClinG-125

Routine diagnostics in patients with heritable pediatric liver disease

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Background: The liver plays an essential role in many bodily functions including immunity, protein synthesis, metabolism, and storage. The spectrum of liver diseases covers a wide range of phenotypes. Heritable pediatric liver diseases although rare, often have serious comorbidities, and an early and precise diagnosis is of vital importance to enable an effective therapy. Molecular genetic analyses offer further diagnostic avenues, in addition to conventional imaging and laboratory investigations. In contrast to classical Sanger sequencing, next-generation sequencing (NGS) allows the parallel sequencing of all known genes involved in liver disease.

Method: A routine liver panel has been established containing 123 genes which are subdivided in 8 subpanels according to distinct clinical criteria. For a further patient group, we performed trio whole exome sequencing, which includes the parallel analysis of patient and parents. This method offers i.a. the possibility of novel associations between variants in candidate genes and distinct liver phenotypes.

NGS was performed using the HiSeq 4000 platform (Illumina), followed by bioinformatics analysis.

Results: For panel diagnostics, pathogenic variants most likely explaining the disease were identified 22% of the cases, while the diagnosis of 12% remained uncertain.

Within the trio exome analysis group we detected pathogenic variants in 36% of the cases, all identified variants except one were in genes which were included in the routine liver panel, and within two analyses putative novel candidate genes for liver diseases were identified.

Conclusion: Our data indicate panel diagnostics are a highly reliable and very cost-efficient tool enabling the diagnosis of patients with clinically heterogeneous and very rare liver diseases within a short time-frame as four weeks. Furthermore, trio exome analysis contributes to a better understanding of genotype-phenotype correlations, and allows for the identification of novel liver disease causing candidate genes.

P-ClinG-126

A case of Chorea-acanthocytosis presenting as autosomal recessive epilepsy

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Chorea-acanthocytosis (ChAc) is a rare adult-onset disease characterized by neurodegeneration and acanthocytes in the blood. It presents as a complex movement disorder with a variable symptomatology and age of onset (20-50 years). Most patients develop orofacial dyskinesia with dysarthria and dysphagia. Other symptoms include choreatic movements, tics, epilepsy, dystonia, Parkinsonism, muscle and brain atrophy,

cognitive decline, and behaviour and personality changes. The disease is progressive, incurable and inevitably leads to premature death. ChAc is caused by mutation of the VPS13A gene and is transmitted in an autosomal recessive style.

We report on a consanguineous Turkish family with three affected siblings, aged 37, 33 and 31 years. All of them developed seizures at age 25-30 years diagnosed as grand mal, tonic-clonic seizures or temporal lobe epilepsy respectively. Intelligence was regular, dysmorphisms or malformations excluded. None of the parents or other relatives had a history of seizures. As no other symptoms of ChAc were reported to us we suspected an autosomal recessive form of epilepsy. Exome sequencing detected the homozygous novel truncating mutation c.4326 T>A (p.Tyr1442*) in VPS13A in the three siblings. The parents were confirmed as heterozygous carriers. By this mutation detection in VPS13A the seizures were identified as the first symptoms of ChAc.

This case highlights the importance of exome sequencing to establish a correct diagnosis in familial cases of neurological disorders with unspecific symptoms.

P-ClinG-127

A girl with a rare combination of Down's Syndrome and Prader-Willi-Syndrome with complications after cardiac surgery

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Down's Syndrome is a common genetic disorder (prevalence ca. 1:2000) and in most cases caused by a trisomy of chromosome 21. It is characterized by a typical facial gestalt, intellectual disability, muscular hypotonia and a high risk for heart defects, duodenal atresia, hypothyreoidism, leukemia and early onset Alzheimer disease.

Prader-Willi-Syndrome (PWS, prevalence ca. 1:25.000) is caused by a lack of expression of the paternally derived PWS region of chromosome15q11.2-q13 by one of several genetic mechanisms (Deletion on one chromosome 15, maternal uniparental disomy (UPD) 15, methylation defect). It is characterized by a severe hypotonia in infancy followed in later infancy by excessive eating and a development of morbid obesity. All individuals have a intellectual disability and a hypogonadism.

We report on a girl that was prenatal diagnosed with Down's Syndrome, Prader-Willi-Syndrome and a large ventricular and atrial septal defect (VSD, ASD). A Non-invasive-prenatal testing was made because of an increased maternal age which indicated existence of trisomy 21. Chromosome analysis was made adjacent to an amniotic fluid puncture that confirmed trisomy 21 and revealed an additional marker chromosome in 7 of 30 metaphases. Fluorescence in vitro Hybridization verified the marker chromosome as chromosome 15. A methylation analysis revealed a maternal UPD 15 in the unborn girl which confirmed Prader-Willi-Syndrome.

Due to a pathological placentary perfusion and intrauterine growth retardation a caesarean section was performed at the gestation age of 34 + 2 weeks with a birth weight of 1220g. Prenatal cardiac diagnosis was confirmed by echocardiography. Neonatal care focused on congestive heart failure with respiratory distress. After successfully gaining of weight to 3kg the child was transferred to our tertiary center and underwent corrective cardiac surgery with pericardial patch closure of the VSD and ASD and mitral valve repair with eight weeks of life. After relief of pulmonary hypertension a delayed sternal closure was performed three days later. The postoperative course was protracted by persistence of intractable chyli-form effusions, respiratory distress, recurrent systemic infections and intermittent high-grade atrioventricular blocking that made long-term intensive care inevitable. After another eight weeks it was decided to implant an epicardial single chamber pacemaker to prevent from symptomatic bradycardias and referred the child to a secondary center. The later course was uncomplicated so far.

Considering the moderate complexity of the congenital heart defect we conclude that the perioperative course was complicate above the ordinary. It could be attributed to the prematurity and the small for gestational age state on the one hand but also to the extremely rare combination of PWS und Down's Syndrome.

P-ClinG-128

Gómez-López-Hernández Syndrome - A clinically distinct phenotype but an unsolved genetically puzzle -

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Gómez-López-Hernández Syndrome (GLHS) is an easily recognizable syndrome. The disorder is characterized by the triad of rhombencephalosynapsis, parietal alopecia, and trigeminal anesthesia. Furthermore, patients infrequently present a broad range of distinctive congenital malformations and neuropsychiatric disorders comprising hydrocephalus, brachyturricephaly, corneal opacity/ anesthesia, intellectual disability, autism, major depression, schizophrenia, ataxia and spasms. To the best of our knowledge, to date only sporadic cases with an equal gender distribution are reported. It is hypothesized that the mode of inheritance is autosomal-recessive. The genetic factors, which are involved in the origin of GLHS, are unknown.

Here, we report the first affected siblings, both exhibiting the typical symptoms of GLHS. The 1-year old girl and her 4-year old brother were born with brachyturricephaly and parietal alopecia. MR revealed triventricular hydrocephalus and rhombencephalosynapsis. The psychomotoric develeopment is markedly delayed in the siblings. The boy moreover displayed corneal anesthesia and ataxia.

We performed trio-based WES of blood samples of the family as wells as RNA and genome sequencing of fibroblasts. Furthermore, we performed whole genome sequencing and RNA sequencing of blood samples of two additional sporadic patients with GLHS. However, we did not identify any pathogenic mutation. The genetic causes of GLHS remain elusive. An in-depth understanding of the underlying genetic causes and involved pathways is still missing. This reflects the complexity of the underlying molecular signatures and the demand for new scientific strategies. One likely explanation – besides others - for missing mutational profile are mosaic conditions with probably varying tissue specific mutational level. Unravelling the molecular causes and the biology of GLHS might be the key towards an improved understanding of this characteristic disorder.

P-ClinG-129

Implementation of whole exome sequencing in the molecular diagnostics of primary immunodeficiencies

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Introduction: With more than 300 genetically defined diseases and extreme heterogeneous phenotypes, the diagnostic of primary immunodeficiencies (PIDs) is challenging and time-consuming. The accurate identification of causative variants using high-throughput methods like whole exome sequencing (WES) is crucial. This provides the opportunity for timely treatment to prevent life-threatening infections, as well as information regarding prognosis and recurrence risk.

Objective: We sought to investigate the ability of whole exome screening to detect disease-causing variants in patients with PIDs.

Patients and Methods: We obtained the clinical findings including flow cytometry of patients from 21 families with probably autosomal recessive PID since more than one sibling was affected and/or the parents were related. We performed whole exome sequencing of the index patients and initially focused on 327 known or candidate PID genes.

Results: We identified five previously reported variants in IL2RG, XIAP, PRF1, CD40LG, and NFKBIA. We identified nine novel variants in the previously described genes; ARPC1B, BLNK, G6PC3, IL21R, RAG1 (two families), RFXANK (two families), and STAT3. Interestingly, in BLNK, IL21R and ARPC1B, which have so far few reported pathogenic variants, our identified likely pathogenic variants were associated with an extended immunophenotype. In two patients with agammaglobulinemia 4 (MIM:613502) characterized by non-detectable serum antibodies and absent B cells, the identified variant in BLNK may extend the phenotypic spectrum of BLNK deficiency to include almost absent natural (NK) killer cells. Also, compared to the clinical phenotype of IL21R immunodeficiency (MIM:615207) reported by OMIM, the identified variant in IL21R caused more severe phenotype characterized by inverted CD4+/CD8+ T cells ratio, almost absent NK cells and reduced serum levels of IgG, IgA, and IgM. Lastly, the identified variant in ARPC1B validated it as a PIDs gene

after it was reported only once early this year in three patients with platelet abnormalities, cutaneous vasculitis, eosinophilia and predisposition to inflammatory diseases (MIM:617718). Strikingly, in one consanguineous family of Jordan descent in which we have identified no candidate variants in PIDs associated genes; one homozygous in silico pathogenic missense variant was identified in CISH encoded cytokine-inducible SH2-containing protein. Although, this result suggest CISH as a potential candidate for PIDs, more genetic and functional studies must be performed to confirm this correlation.

Discussion: Genetic testing for PIDs associated genes using whole exome sequencing provides rapid molecular diagnoses and improves the diagnostic yield by enabling the detection of disease-related variants in unexpected genes. Still, a third of the cases are not solved; new candidate genes are to be detected.

P-ClinG-130

Sequential analysis of KIF24 in patients with Müllerian anomalies

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Müllerian anomalies include fusion anomalies of the Müllerian ducts (didelphic, bicornuate, unicornuate, septate uterus and vaginal septa) and the Mayer-Rokitansky-Küster-Hauser syndrome (MRKHS). MRKHS type 1 is characterized by congenital absence of uterus and vagina (CAUV) whereas in MRKHS type 2 CAUV is associated with renal or skeletal malformations, hearing defects and less frequently cardiac anomalies. The incidence of fusion anomalies is about one in 250 and the incidence of MRKHS is about one in 4.500 women.

To date, the etiology of malformations of the Müllerian ducts is not completely enlightened. However, array-CGH analyses in patients with MRKHS and in patients with fusion anomalies show recurrent microdeletions and –duplications. Furthermore, mutations in *TBX6*, *WNT9B*, *LHX1* and in a subgroup of patients with MRKH and hyperandrogenism mutations in *WNT4* have been described as being causative.

Recently, a deletion in 9p13.3 was found in a patient with a fusion anomaly of the Müllerian ducts. In this deletion-interval the gene *KIF24* (OMIM *613747) is located, which encodes a kinesin-motor-protein. This protein acts as a negative regulator of ciliogenesis. Disturbance of ciliogenesis cause ciliopathies like the Bardet-Biedl-, McKusick-Kaufman- or the Meckel-Gruber-syndrome, which in some cases are associated with genital malformations. Therefore, it has been supposed, that a subgroup of MRKHS might be addressed as ciliopathies.

Thus, sequential analysis of *KIF24* has been performed in a cohort of 230 patients with Müllerian anomalies (108 patients with MRKHS and 122 patients with fusion anomalies of the Müllerian ducts). Three different missense variants in *KIF24* have been found in three patients (3/230, 1.3 %) and one silent variant have been found in 7 patients (7/230, 3.0 %). The three missense variants are due to their in silico score classified as pathogenic such as the silent variant which is predicted to affect splicing.

In summary, we found four possibly pathogenic variants in 2.3% (10/230) patients with malformations of the Müllerian ducts. Therefore, we conclude that variants in *KIF24* are associated with Müllerian anomalies.

P-ClinG-131

Design and establishment of a new NGS gl for microphthalmia and anophthalmiaene pane

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Microphthalmia and anophthalmia are rare ocular malformations describing the presence of a small eye or the complete absence of the globe. Microphthalmia is defined by the shortening of the axial length of the eye by at least two standard deviations in comparison to the age-matched controls. Both, microphthalmia and anophthalmia, can be present unilateral or bilateral. The prevalence is up to 1 per 33.000 live births. Sporadic cases have been described as well as different inherited forms. In approximately 30% of the cases microphthalmia appears as part of a complex syndrome, frequently in combination with other eye abnormalities, like coloboma, and additional facial or skeletal malformations. Until now several genetic causes of isolated and syndromic forms of microphthalmia/anophthalmia have been described including several eye specific transcription factors, like RAX, SOX2 and MITF. Due to a high level of heterogeneity the genetic diagnosis of microphthalmia/anophthalmia is very difficult.

Only a few diagnostic centers provide a panel specifically for analyzing the genetic basis of microphthalmia/anophthalmia at the moment. Therefore, we have developed a next-generation-sequencing (NGS) gene panel encompassing 53 genes that are associated with microphthalmia/anophthalmia. In addition to genes known to cause isolated forms of microphthalmia/anophthalmia we also included genes for different syndromic forms, like CHARGE-, Lenz-, BOSMA and Meckel syndrome, ensuring a broad coverage of the most common causes of microphthalmia.

The panel design was established using SureDesign (Agilent Technologies) on the basis of the Clinical Research Exome V2 and encompasses 7585 probes with a total probe size of 247.093 kb. Enrichment was performed using the SureSelectQXT (Agilent Technologies) and sequencing was done on the MiSeq platform (Illumina). The generated sequencing data was analysed with the software SeqPilot using the NGS-data analysis module SeqNext (JSI medical systems GmbH). The performance of the gene panel and the quality of the generated data was validated by analysing DNA of five control patients with known mutations in genes included in this panel. We were able to detect all of these mutations correctly. Coverage of target regions was significantly higher compared to different whole-exome sequencing approaches. All coding regions of included genes and regulatory elements, in which mutations have already been described, were fully covered with a minimum coverage of at least 50 reads.

In summary, we established a targeted NGS panel as a new diagnostic tool for the gene identification in patients with syndromic as well as non-syndromic forms of microphthalmia/anophthalmia which provides a fast and cost effective tool for diagnosis.

P-ClinG-132

Description of a novel case with small patella syndrome caused by *TBX4* mutation

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The small patella syndrome (SPS) is a rare autosomal dominant developmental disorder of the lower limb characterized by absent or hypoplastic patella and by anomalies of the pelvis and feet. Childhood-onset pulmonary arterial hypertension (PAH) is a facultative concomitant disease of SPS. Although delineation from nail-patella syndrome or isolated familial patella aplasia-hypoplasia syndrome can be achieved by radiographic and clinical features, genetic analysis of the SPS causative gene *TBX4* is important to ensure the diagnosis. So far, a few deleterious heterozygous *TBX4* sequence variants (n=6) have been described in patients with clinical suspicion of SPS. Furthermore, *TBX4* mutations (n=3) or *TBX4*-containing deletions (n=3) were detected in 6 out of 20 children with PAH who appeared to have previously unrecognized SPS. Here, we report on the first de novo intragenic *TBX4* deletion causing SPS in a 25 year old female patient with long lasting history of gonarthrosis. We describe the clinical, radiograpic and genotypic findings in detail and discuss the patient management from an interdisciplinary point of view.

P-ClinG-133

Novel PPM1D mutation in a patient with IDDGIP syndrome (intellectual disability with gastrointestinal difficulties and high pain threshold)

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Truncating mutations in PPM1D have recently been reported as a cause of autosomal dominant intellectual disability with gastrointestinal difficulties and high pain threshold (IDDGIP, OMIM 617450) in 14 patients. PPM1D was shown to be expressed in the fetal brain which suggests a role in fetal brain development. It is hypothesized that the ID-associated mutations in PPM1D lead to a reduced cell proliferation by decreased G1-to-S phase progression. All mutations described yet were located in the last or penultimate exon.

We report on a 5-year-old male patient with mild intellectual disability, strabismus, short stature and brachymesophalangy. He also had cleft lip/palate, simian crease and aberrant right subclavian artery which had hitherto not been reported in this disorder. Next-generation sequencing analysis revealed a novel heterozygous de novo mutation in the last exon of PPM1D: c.1529delA, p.(Asn512fs).

The patient reported here broadens the clinical spectrum of IDDGIP syndrome. Reports of additional patients with PPM1D mutations will be needed for a more detailed clinical delineation of this novel and probably underdiagnosed disorder.

P-ClinG-134

Early onset dystonia: a new genetic disorder or a new aspect of valproate fetopathy ?

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Background: Hereditary dystonia often starts in childhood, adolescence or in adult, while manifestation in the neonatal period is rare. Here we report a Syrian girl suffering from dystonia from birth onwards.

Case: The girl is the second child of consanguineous Syrian parents (second degree cousins). Because of epilepsy the mother was on valproate treatment throughout pregnancy. The girl was delivered per Caesarean section in the 42. week of gestation because of refractory epilepsy of the mother. Soon after birth, the patient was admitted to the neonatal intensive care unit because of respiratory distress, ophisthotonus and hyperirritability. Newborn screening for inborn metabolic diseases was normal. CMV-DNA in urine was detected by PCR at 30 days of age. The EEG revealed no evidence of epilepsy. The cranial MRI was normal. The patient shows characteristic craniofacial features of fetal valproate syndrome with a high forehead, low nasal bridge with short nose, midface hypoplasia and long philtrum with a thin vermilion border, and micrognathia. Based on the clinical-neurological findings, early onset dystonia was suspected. Treatment of dystonia with L-dopa/Carbidopa did not alleviate symptoms, but the combination therapy with L-dopa/Carbidopa and Trazodon slightly reduced dystonic movements.

Family history: The mother suffers from epilepsy since childhood without intellectual disabilities. The 7 year old brother has a mild mental developmental delay with characteristic craniofacial features of fetal valproate syndrome but no dystonia.

Genetic analysis: Chromosome analysis and array CGH revealed no abnormalities. We analyzed the exomes of the girl and her parents. Common SNPs with a minor allele frequency of < 0.01, intronic and synonymous variants, as well as ncRNA variants were excluded.

Results: Based on a suspected autosomal recessive inheritance and consanguinity of the parents, we searched for homozygous variants in our patient, which are heterozygous in her parent and her brother. In our patient, we identified in total 22 such variants in 18 different genes. No variants in known dystonia associated genes were identified. Among the 22 variants, 21 were non-synonymous and 1 was one base pair insertion. Among these 18 genes, 7 genes were not registered in the OMIM database, 5 genes don't have phenotype OMIM number.

Discussion and Conclusion: We could not identify any disease causing mutation in one of the known 26 dystonia genes. Although we cannot exclude that the CMV infection or the valproate exposure may contribute to the phenotype, we rather suspect a rare autosomal recessive dystonic movement disorder.

P-ClinG-135

Two novel mutations of Tenascin-X cause an autosomal-recessive subtype of Ehlers-Danlos-Syndrome

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Ehlers-Danlos-syndrome (EDS) is a genetically heterogeneous group of connective tissue disorders. Most subtypes are due to defects affecting the structure of collagene1, but also defects in other extracellular matrix proteins (EMP) and even intracellular pathways may cause EDS 2. It has been shown that defects of the EMP tenascin-x can also be responsible for EDS 3 causing an autosomal-recessive subtype of EDS 4. Tenascin-x is encoded by the *TNXB-gene*, which contains 39 exons.

In this case-report we describe a 38-year-old woman whose features are strongly suggestive for an EDS. She shows skin hyperextensibility with multiples hematomes, joint hypermobility especially concerning the fingers, striae distensae and myopia, but no vascular features of EDS. The patient's brother, the mother and a maternal uncle show also features of EDS highlighting hypermobility.

By multi-gene panel analysis with next generation sequencing, we identified two novel heterozygous mutations in *TNXB*. To make sure both variants concern the *TNXB-gene* and not the non-functional pseudogene we performed a long-range-PCR. The mutations were confirmed by Sanger sequencing. The first variant (c.9616C>T, p.Gln3206*) is a novel pathogenic mutation in exon 28. The second novel variant

(c.11381-10A>G) was found in intron 34 of the *TNXB-gene* causing probably an alternative splicing-site, which is predicted by five different splicing prediction programs (SSF, MaxEnt, NNSPLICE, GeneSplicer, HSF). We are currently conducting a RNA-analysis to confirm the predicted alternative splicing-site.

We also conducted a segregation analysis, involving the brother and the patient's parents. Compound heterozygosity for these mutations was confirmed by analyzing the parents. The analysis showed that the splice site mutation lays on the maternal allele; the nonsense mutation lays on the paternal allele. The patient's brother, who also shows features of EDS, is also a carrier of both mutations.

TNXB mutations are a rare cause of EDS. Our case supports the clinical utility of additional multi-gene panel testing for EDS-patients who do not have a pathogenic mutation in the classical genes.

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P-ClinG-136

TEX15-mutations as a possible cause for male infertility

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Background: Approximately 7% of the total male population worldwide are affected by male infertility. About 10% of these cases are due to non-obstructive-azoospermia (NOA), which often has a genetic background. Less than 15% of cases of NOA are caused by chromosomal abnormalities. Single gene defects are another reason for NOA. One of these genes is the *testis-expressed 15* (*TEX15*) gene. It has previously been shown that truncating mutations in this gene may cause NOA. However, also a decrease of sperm concentrations over time has been described in patients with a nonsense mutation of *TEX15*[1].

Experiments with mice show that males with homozygous deletions of *TEX15* have a significantly reduced testis volume and a meiotic arrest, while female mice with a deletion of *TEX15* are fertile.

TEX15 is required for meiotic recombination and seems to play a crucial role in the repair of double-strand breaks (DSB) by leading DNA repair proteins to the sites of DSB. The expression of *TEX15* is testis-specific and dynamic throughout spermatogenesis.

Patients & Methods: Here, we report the cases of two infertile, but otherwise unremarkable patients attending the Centre of Reproductive Medicine and Andrology (CeRA). After exclusion of chromosomal abnormities and Y-chromosomal-microdeletions, we performed whole-exome-sequencing. Patient 1, who is an offspring of consanguineous parents, had a testis biopsy demonstrating a Sertoli-cell-only syndrome. Patient 2 had oligozoospermia.

Results: In Patient 1, we identified a homozygous in frame deletion in TEX15 (c.3070_3072delGAA; rs759164949). In patient 2, we detected two frameshift mutations (c.1425_1428delAACA, p.Thr476IlefsTer16, rs565708396 and c.5725_5728delTCAC, p.Ser1909LysfsTer11, rs754041709), giving the possibility of a compound-heterozygous case of *TEX15* deficiency. We are currently trying to perform segregation analysis involving the patient's parents to demonstrate whether the two mutations map on different alleles. All three variants have been described previously, but at very low frequency.

Conclusions: Regarding our results, we provide further evidence that homozygous or compound-heterozygous mutations of *TEX15* are a possible causes for male infertility, indicating a recessive pattern of inheritance.

This work was carried out within the frame of the DFG Clinical Research Unit ‚Male Germ Cells: from Genes to Function' (CRU 326).

1. Okutman O, Muller J, Baert Y, et al. Exome sequencing reveals a nonsense mutation in TEX15 causing spermatogenic failure in a Turkish family. Hum Mol Genet. 2015;24(19):5581-5588. doi:10.1093/hmg/ddv290.

P-ClinG-137

Splice mutation in TMEM5 expands the mutation and phenotypical spectrum of alpha-dystroglycanopathy

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Dystroglycan 1 is a large precursor protein processed to alpha- and beta-dystroglycan, together they link the extracellular matrix to the sarcolemma. Dystroglycanopathies are a diverse group of disease, caused by an increasing number of genes. *TMEM5* is one of the latest genes connected to alpha-dystroglycanopathies,

it encodes for a protein involved in the O-glycosylation of alpha-dystroglycan. Mutations in *TMEM5* are associated with autosomal recessive congenital muscular dystrophy-dystroglycanopathy with brain and eye anomalies type A 10 (OMIM: # 615041). Patients display characteristic cobblestone lissencephaly, visceral malformations, neural tube defects and gonadal dysplasia.

The index patient analysed is a 15 year old boy suffering from a congenital muscle dystrophy with proximal muscle weakness. He presented with elevated serum creatine kinase (up to 2000 IU/l) and almost complete absence of alpha-dystroglycan in immunohistochemistry of muscle tissue, while the rest of the sarcolemmal proteins were normally expressed. Although his motor and cognitive developments were delayed, his clinical course was milder than reported cases and did not include brain or eye defects. The patient's parents are first-degree cousins; however no cases of muscle disease were reported in the family.

For molecular diagnosis, next generation sequencing (NGS) was applied to analyse 65 myopathy related genes. Genomic DNA of the patient was amplified using Nextera Rapid Capture (Illumina) and sequenced on a MiSeq desktop sequencer (Illumina). We identified a homozygous variant (NM_014254.1: c.914+6T>G) in *TMEM5* (OMIM: * 605862). The variant is close to the donor splice site of exon 5 and *in-silico* prediction tools (SpliceSiteFinder-like, MaxEntScan, NNSPLICE, and Human Splicing Finder) calculated a slight reduction in the efficiency of the 5' splice site. To validate the variant's effect on the splice site, we isolated RNA from whole blood (PreAnalytiX GmbH) and retranscriped it into cDNA (Thermo Fisher Scientific). Sequencing data of exon 4 to 6 confirmed the expression of a shortened transcript of *TMEM5* lacking exon 5, which leads to an *in-frame* deletion in a possible exostosin domain of the protein. However a small amount of wild type transcript remains, most likely due to an only partly inactive splice site. Segregation analysis and the immunohistochemistry supported the homozygous variant.

In summary, we present the case of a boy suffering from muscle dystrophy due to a mutation in *TMEM5*. The mutation affects the donor splice site of exon 5 and leads to an *in-frame* deletion of exon 5 in most transcripts. The remaining small amount of wild type transcript could explain the milder phenotype of the patient compared to previously reported cases. Patients with mild phenotypes have been reported for mutations in other O-glycosylation genes as well.

P-ClinG-138

Familial Carvajal syndrome caused by DSP gene mutations

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Carvajal syndrome (CS, AR, OMIM#605676; AD, OMIM#615821) is a rare disorder caused by Desmoplakin gene mutations (DSP, mapped on 6p24). Protein product is a component of desmosomes, which play main functional role for intercellular junctions, including myocites adhesion. Desmosomal proteins defect affects the tissues most exposed to mechanical stress: skin, heart. Disorder manifests by distinct cardiocutaneous features: wooly hair, palmoplantar keratoderma, dilated mainly left-sided cardiomyopathy. CS dominant form additionally displays teeth abnormalities. Patients with intractable heart failure, severe biventricular involvement may need cardiac transplantation.

We present familial CS clinical and molecular data focusing on cardiologic pathology.

Non-consanguineous healthy parents. Both affected sibs showed normal pre/postnatal growth, motor, speech, mental development. Wooly hair, transient mucocutaneous blisters (resemblance to epidermolysis bullosa simplex) manifested at birth. Palmo-plantar keratosis, appearing at age 1-2 years showed progressive course: at 1 year old – hyperkeratotic skin on soles, mild on palms; at 3 years – dry skin, diffuse hyperkeratosis of palms and soles. Dystrophic lesions of nails. Teeth were normal. Proposita (a boy) was referred for genetic counseling due to dilated cardiomyopathy symptoms started at 8 years-old. Follow up displayed severe cardiac failure. Cardiac data at 10 years old: fatigue, weakness, palpitation, systolic murmur. ECG: ventricular tachycardia, extrasystoles, right-axis deviation, low voltage, T-wave inversion in leads V3 to V6. X-ray: cardiomegalia. Echocardiography: all chambers dilatation (predominantly left), global hypokinesia, biventricular systolic dysfunction, mitral and tricuspid valves regurgitation. Ejection fraction LV 17%. Successful heart transplantation was performed at 11 years old. His 3-year-old sister showed normal results of complex cardiologic examination.

DSP gene testing revealed compound heterozygous mutations in exon 24: known nucleotide substitution c.7123G>C(p.Gly2375Arg) and substitution c.6986T>C(p.Leu2329Pro) has not been published before to our knowledge.

Major differential diagnosis: Naxos syndrome (NS, AR, OMIM #601214), Plakoglobin gene (JUP, 17q21.2), characterized by similar skin, hair, nail abnormalities, arrhythmogenic right ventricle cardiomyopathy, predominantly developing in adulthood.

Conclusion. Our observation demonstrates AR inheritance, early severe hearth pathology, strong necessity of cardiologic monitoring for patients with wooly hair and palmoplantar keratoderma. Life expectancy

may be increased in case of early treatment of heart failure including implantable cardioverter defibrilator or heart transplantation. Cardiologic status of patients with DSP gene mutations will be discussed

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

P-Compl-139

Shared genetic etiology of body-mass-index and body fat distribution with Barrett esophagus and esophageal adenocarcinoma: Insights from large genome-wide association studies

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Barrett esophagus (BE) is a precancerous condition of esophageal adenocarcinoma (EA). It is characterized by the replacement of normal stratified squamous epithelium by metaplastic columnar epithelium in the distal esophagus. Both BE and EA are multifactorial disorders that share polygenic effects contributing to the risk of one or both diseases. Besides genetic components and gastroesophageal reflux (GER), obesity is one of the major risk factors. Hypothesized mechanisms explaining these associations are (i) increased intra-abdominal pressure that promotes GER and (ii) systemic inflammatory processes within the adipose tissue. Notably, the association between obesity and EA seems to be stronger than for any other types of obesity-related cancers.

The aim of the present study was to elucidate the overall shared genetic etiology between BE/EA and obesity. For this, we applied cross-trait linkage disequilibrium score regression (LDSR), a method that estimates genetic correlation on a genome-wide level. We used the summary level results of (i) our recently published genome-wide association study (GWAS) meta-analysis for BE/EA and (ii) the GIANT consortium (Genetic Investigation of ANthropometric Traits). For the GIANT data, we extracted genetic data for (i) the body mass index (BMI), which assesses general obesity, and (ii) the waist-hip-ratio (WHR), a measure that is closely related to abdominal obesity. LDSR analyses were performed both sex-specific and sex-combined. In addition, we performed analyses on single marker level and compared risk alleles for all genome-wide significant SNPs in the BMI and WHR studies with those reported in BE/EA GWAS meta-analysis.

In the sex-combined analysis, the LDSR revealed a genetic correlation (r_g) of 0.13 (standard error, se=0.04; $p=2\times10^{-4}$) between BE/EA and BMI and 0.12 (se=0.05, p=0.01) between BE/EA and WHR. The sex-specific analysis revealed a stronger correlation with BMI in women, whereas the correlation with WHR was stronger in men. Analysis on single risk marker level revealed a statistical significant enrichment of BMI/WHR associated variants in BE/EA GWAS data (p=0.0086).

Our results provide evidence that the WHR in men and the BMI in women are genetically correlated with BE/EA. This is in line with epidemiological findings and might at least partially explain the associations. In women, systemic inflammatory processes within the adipose tissue - associated with general obesity - might be the predominant mechanism driving the risk for BE/EA. In men, the accumulation of visceral fat - associated with abdominal obesity - might increase intragastric pressure which in turn promotes GER. Further research into elucidation of the mechanisms is required. This is the first systematic study analyzing the shared genetic etiology between BE/EA and the risk factor obesity on a genome-wide level. Our data point towards sex-specific mechanisms by which obesity mediates the risk for developing BE/EA.

P-Compl-140

Preliminary results of a genome-wide association study on athletic performance: flexibility and coordination

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Introduction. Athletic performance is connected with a set of physical traits and is significantly determined by a complex interaction of genetic factors. Investigation of these factors can be useful for sport testing. Particularly, flexibility and coordination are important athletic characteristics that are assumed to be genetically-conditioned. Here we describe the preliminary results of a genome-wide association study on flexibility and coordination.

Materials and method. The study included 94 participants from Russia: 29 women and 65 men, both professional and amateur athletes. Median age: 29 years, interquartile range: 24 – 34 years. The types of sport activities included: speed skating, cycle sport, powerlifting, skiing, skeleton and swimming. Median time of training: 10 years, interquartile range: 6 – 15 years. According to Russian Unified Sports Classification System, 60 achieved the title of Candidate for Master of Sport and higher. The participants reported their level of flexibility and coordination using a 4-grade scale, where 1 was bad estimate and 4 was excellent one. All the participants were genotyped using DNA chip InfiniumOmniExpressExome-8 v 1.4 with around 1,000,000 markers. Data processing was conducted using R v3.4.2, association testing – using Plink v1.90, and variant annotation – using Ensemble VEP v90.

Results. We conducted two investigations with different designs. The first one included two phenotypes – flexibility and coordination – in ordinal scale (from 1 to 4) and used regression analysis to estimate the association between the phenotype measures and detected variants. The second one included two phenotype as binary values (derived from the most pronounced values of the both phenotypes) and used chi-square test. At each step there were around 30,000 variants with p-value lower than 0.05 but after multiple testing correction (Bonferroni, Holm methods and false-discovery rate) none of the variants appeared to be significant. However, a set of resulting odds ratios from the second step was significantly higher than the one on the first step (calculated as the exponent of the regression coefficient): Welch's t-test p-value < 10^{-16}. Significant differences were also obtained comparing only specific genes (e.g. the collagen family). Although the second step did not produce any significant variants, it revealed a trend which might be confirmed with larger samples.

Conclusion. These preliminary results show a trend of potential association between genetic markers and both flexibility and coordination as important athletic characteristics. Increasing of sample size may help to confirm the findings and increase statistical significance of the results.

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P-Compl-141

The role of Neanderthal alleles in risk for age-related macular degeneration

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Purpose: Age-related macular degeneration (AMD) is the leading cause of vision loss in Western societies. A large portion of disease risk can be attributed to genetic risk factors affecting the function of the innate immune system. Recent advances facilitate the identification of Neanderthal derived alleles in the human genome. Here, we investigated the role of Neanderthal alleles in shaping the AMD disease risk profile.

Methods: First, we extracted 5,562 variant alleles known to tag haplotypes of Neanderthal origin outside of known AMD associated loci from imputed genotypes. Next, we computed a genetic score by calculating the sum of Neanderthal alleles for each individual. Consequently, a higher genetic score indicates individuals with more Neanderthal alleles than average. We then computed the association with late-stage AMD using a dataset provided by the International AMD Genomics consortium (IAMDGC) including 16,144 late-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 of Neanderthal origin.

Results: On average, AMD patients had 469.78 (SD: 24.06) and controls had 470.59 (SD: 23.66) alleles of Neanderthal origin (P < 0.001). Adjusting the analysis for 52 known AMD associated variants resulted in a strong increase in association strength (P = 6.56x10-05). Further adjustment for additional covariates like sex, age and the first ten principle components did not significantly influence the observed association. None of the 5,562 tag variants individually was significantly associated with late-stage AMD after adjustment for multiple testing.

Conclusion: Our findings demonstrate a contribution of Neanderthal alleles to risk for late-stage AMD independent of known genetic AMD risk factors.

P-Compl-142

Analysis of rare CNVs in patients with generalized pustular psoriasis

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Generalized pustular psoriasis (GPP) is a rare, potentially life-threatening disease, which is characterized by infiltration of neutrophils into the skin and a multi-systemic inflammation. In contrast to the more common forms of psoriasis, it is considered to be an oligogenic disease. Mutations in the gene IL36RN encoding the IL-36 receptor antagonist have been identified to be disease-causing, while genetic risk factors in two further genes contribute to disease susceptibility. Known genetic factors explain the genetics of ~25% of GPP patients. Besides SNPs, copy number variations (CNVs) have been shown to contribute to and cause genetic diseases as well. We used data of whole exomes of 18 GPP patients and analyzed them with two algorithms, Conifer and XHMM, to reveal potentially disease-associated CNVs. 1,042 further exomes of individuals without GPP served as healthy controls.

We considered rare CNVs in the control group as candidate variants (MAF: <0.24%). By using Conifer, we identified 86 different CNVs, with XHMM 67 CNVs. Among these latter 67 CNVs, 25 CNVs (37.3%) were also found by using Conifer. We identified three GPP patients carrying an overlapping duplication (carrier frequency of 0.6% in controls and of 16.7% in GPP patients (p (Fisher's exact test) = 3.2E-04). Overall 2 genes (PRKA, MIR58N) were affected by the CNV in the minimally overlapping region of ~1,8Kb on chromosome 2.

Based on these results, we screened these two candidate genes for (additional) rare variants in exomes of the same patients as well as in additional exomes of 24 further patients. We but did not identify a single rare candidate variant. Interestingly, one candidate gene coded a ubiquitously expressed protein kinase that mediated the effects of interferon in response to viral infection. Furthermore, the second gene was a so far poorly characterized microRNA expressed in CD4+ T-cells, epithelial cells and CD14+ cells in adipose tissue. This gene has been described to downregulate host antiviral response. The expression profile and the gene functions rendered these genes interesting candidates for GPP. Therefore we are currently analyzing this CNV and affected genes in further genetic and functional studies which we will present at the meeting.

P-Compl-143

Serotonin type 3 receptor variants in Irritable Bowel Syndrome - a multi-centre replication study

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Irritable Bowel Syndrome (IBS) is a common gastrointestinal (GI) disorder of multifactorial origin affecting 15% of the population worldwide. Evidence of disturbed serotonergic function in IBS accumulated for the 5-HT3 receptor family. 5-HT3Rs are encoded by HTR3 genes and control GI function, in particular, peristalsis and secretion and 5-HT3R antagonists are beneficial in the treatment of IBS-D. Furthermore, they are relevant in emotional processing, mood and visceral perception and have been associated to anxiety and depression that represent comorbid phenotypes in IBS. We previously reported SNPs in *HTR3A* c.-42C>T (rs1062613), *HTR3C* p.N163K (rs6766410) and *HTR3E* c.*76G>A (rs62625044) to be associated with IBS-D. The *HTR3A* and *HTR3E* SNPs represent cis-regulatory variants. The c.-42C>T SNP locates within the 5'UTR of *HTR3A* and c.*76G>A within the 3'UTR of *HTR3E* in a microRNA miR-510 binding site, respectively. Both SNPs seem to impair expression regulation and cause up-regulation of receptor expression. Lately, they have been replicated with IBS-D in three Chinese studies and a recent GWAS showed a trend for association for *HTR3A* and *HTR3E* to be nominally associated with IBS. The p.Y129S in *HTR3B* which we previously have found to be associated with anorexia and depression was also reported in the context of IBS in a Japanese sample, in particular with an increased anxiety score and alexithymia. Some additional evidence for the role of *HTR3* variants arose from genetic imaging studies.

Our hypothesis is that the predisposing SNPs in *HTR3A, HTR3B, HTR3C* and *HTR3E* lead to changed expression levels that encode 5-HT3 receptors of changed structure and function, thereby making people prone to develop IBS. Joining efforts of the German IBS research network and the COST Action BM1106 GENIEUR (The Genes in Irritable Bowel Syndrome Research Network Europe; www.GENIEUR.eu) enabled us to perform a European multicentre study to validate previous results and provide further evidence for the relevance of these SNPs to the aetiology of IBS. To confirm this, we aimed to perform comparative expression analysis in three different GI regions of 67 IBS patients and 51 controls. In addition, we genotyped the respective polymorphisms *HTR3A* c.-42C>T (rs1062613), *HTR3B* p.129YS (rs1176744), *HTR3C* p.N163K (rs6766410) and *HTR3E* c.*76G>A (rs62625044) in DNAs isolated from GI tissue (jejunum, ileum and colon), compared expression levels of patients and controls and subsequently correlated the expression level with the

genotype status. Last but not least, we genotyped the SNPs in samples of more than 3000 IBS patients and 10.000 controls from 12 cohorts from Chile, Germany (2), Greece, Ireland, Spain, Sweden (2), the UK (2) and the USA (2) to validate the association findings with IBS. Final data analysis (meta-analysis) is currently ongoing and we will report on replication and expression data.

P-Compl-144

*** Is EBF1 a negative regulator of WNT10A in the development of androgenetic alopecia?

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GWAS have identified >100 risk loci for androgenetic alopecia (AGA) but little is known about the underlying molecular mechanisms at most of these loci. An exception is the WNT10A-locus (2q35), where we have previously reported significantly lower expression of WNT10A in hair follicles (HF) of risk allele vs. non-risk allele carriers for the most strongly associated variant (Heilmann et al. 2013). This variant is in nearly perfect LD (r2=0.96, D'=0.99) with another AGA associated variant, located within a binding site for the transcription factor (TF) EBF1. Interestingly, the gene encoding EBF1 is located at a second risk locus at 5q33.3. We therefore hypothesized that EBF1 is a regulator of WNT10A in AGA and that allele-specific differences in binding affinity of EBF1 to its target site at 2q35 explain the differences in WNT10A expression. To test this hypothesis, we performed luciferase reporter assays in HEK cells by co-transfecting (i) luciferase vectors that contained the predicted WNT10A promoter sequence and the 2q35-EBF1 binding site harboring either the AGA risk or the alternate allele and (ii) an EBF1 expression vector. Our experiments showed that EBF1 activates the WNT10A promoter and that the WNT10A/EBF1 interaction was increased with the risk allele, suggesting that EBF1 acts as a negative regulator of WNT10A expression in AGA. As dermal papilla cells (DPCs) have been reported as the most relevant cell-population and hormonal regulation is one of the major etiological components in AGA, we decided to further follow up the detected WNT10A/EBF1 interaction in Dihydrotestosterone (DHT) stimulated (w/) and unstimulated (w/o) DPCs from balding and non-balding human scalp. RT-PCR revealed expression of EBF1 in both balding and non-balding DPCs w/ and w/o DHT, while WNT10A was not detectable. These findings suggest that the observed WNT10A/EBF1 interaction is likely to evolve its effect on AGA pathobiology in HF cell populations outside the dermal papilla. To identify the relevant HF cell populations, we analyzed for a co-expression of EBF1 and WNT10A in a published single-cell RNA-Seq data set from mouse HF and performed immunofluorescence co-staining in human HF and skin. Here the strongest co-expression was observed in HF keratinocytes. Therefore, the initial luciferase reporter assays are currently repeated in human keratinocytes (HaCaTs). Additionally, we are using real-time qPCR to confirm the hypothesis that EBF1 is a negative regulator of WNT10A expression. The results of these experiments will be presented at the meeting. Taken together, our data provide the first evidence of a functional interaction between two AGA-associated loci and suggest an underlying molecular mechanism where the risk allele at 2q35 leads to an altered binding affinity of the TF EBF1 (5q33.3) to its target site at 2q35 and a subsequent downregulation of WNT10A expression. This mechanism may contribute to hair cycle changes and impaired hair growth in AGA.

P-Compl-145

*** Large-scale re-sequencing study of nsCL/P candidate genes in 1061 nsCL/P cases and 1591 controls

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Nonsyndromic cleft lip with or without cleft palate (nsCL/P) is one of the most common congenital malformations with a prevalence of around 1 in 1,000 in the European population. The etiology of nsCL/P is thought to be multifactorial and the heritability has been estimated to be more than 90%. To date, 40 common risk loci have been identified for nsCL/P in different populations, explaining about 25% of the heritability. We hypothesize that some of the remaining genetic etiology is explained by rare dominant *de novo* mutations. This is supported by the frequent observation of sporadic nsCL/P cases, and a higher nsCL/P recurrence risk in offspring compared to parents. Based on recent results from our whole exome sequencing study (WES), we defined candidate genes and re-sequenced them in a large-scale next-generation sequencing-based study.

Previously, we performed WES in 50 sporadic nsCL/P patients of Central European ancestry and their unaffected parents. We could validate 35 *de novo* events excluding synonymous variants, of which 33 have a MAF ≤ 1% in publicly available databases (1000 Genomes, ExAC/gnomAD, GoNL) and our genotyped in-house population-matched control sample (n=956). All variants were located in different genes. Among these 33 genes, which were defined as “candidate genes”, are *TRMO* and *CDH1*, which have been previously reported as nsCL/P candidate genes.

These candidate genes were subjected to a multiplex re-sequencing study using single molecule molecular inversion probes (smMIPs) in a multiethnic case/control sample of Arabian, Mexican and Central European ancestry (n_{cases}=1,061, $n_{controls}$=1,591).

The smMIPs assay was designed using the standard MIPgen pipeline and was successful for 32 genes. After rebalancing on a MiSeq to improve performance, libraries were sequenced in high-throughput on an Illumina HiSeq2500 2x125bp using Illumina v4 paired-end chemistry. Raw reads were aligned with BWA and variants were called with UnifiedGenotyper. Downstream analysis included filtering for CADD ≥ 15 and MAF ≤ 0.1% followed by a validation step and segregation analysis.

At the time of writing 989 cases and 1,468 controls have been sequenced and are now being forwarded to downstream analyses. Results of our re-sequencing study will be presented at the conference.

Identification of additional rare variants in the nsCL/P candidate genes in affected individuals would support these genes as true causative genes, and strengthen our hypothesis of dominant *de novo* events adding to the genetic etiology of nsCL/P.

P-Compl-146

Identification of highly penetrant variants in the etiology of Major Depressive Disorder via Next Generation Sequencing in ten associated genes in 1500 cases and controls

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Major Depressive Disorder (MDD) is a complex disorder with a lifetime prevalence of around 14% and a moderate heritability of 40%. It is characterized by recurrent episodes of a variable symptom complex including loss of interest or pleasure, depressed mood, feelings of worthlessness or guilt and poor concentration. The most recent MDD GWAS meta-analysis by the Psychiatric Genomics Consortium (PGC) comprised 130,664 patients and 330,470 controls and identified 44 independent genome-wide significant loci. As the cumulative impact of common alleles with small effect may only explain around 20-25% of the phenotypic variance for MDD, rare variants of high penetrance have been suggested to contribute to the development of MDD.

The aim of the present study was to identify rare sequence variants (MAF<1%) in promising candidate genes located at the 44 MDD-associated loci using Next Generation Sequencing. For the prioritization of candidate genes we further investigated the 44 loci using publicly available databases including the UCSC Genome Browser. We identified eight loci harbouring only one protein-coding gene within a 200,000 bp window up- and downstream. The eight implicated genes (*NEGR1, VRK2, TENM2, ASTN2, SORCS3, SOX5, LRFN5* and *RBFOX1*) were selected for resequencing. eQTL effects of associated SNPs were identified for *NEGR1* and *LRFN5* using data generated by the Genotype-Tissue Expression (GTEx) project, providing further evidence for their possible involvement in MDD susceptibility. Interestingly, the LRFN5 gene is involved in synapse formation and presynaptic differentiation.

In addition, we included the two genes *OLFM4* and *TCF4* in the resequencing analyses as both genes reached a Bonferroni adjusted p-value < 10^{-10} in gene-based analyses of the PGC MDD GWAS.

The ten selected MDD candidate genes were investigated in 1500 MDD patients and 1500 controls of German descent. For resequencing we use the single molecule molecular inversion probes (smMIPs) technology which enables multiplex targeted resequencing in large cohorts. The smMIPs sequences were designed with the empirically trained algorithm MIPgen (Boyle et al., 2014) and sequencing is currently performed on the Illumina HiSeq2500 platform. In addition to single variant tests, we will also perform gene burden tests to evaluate cumulative effects of rare variants within one gene. Results will be presented at the upcoming conference.

P-Compl-147

Re-sequencing of schizophrenia candidate genes in a large case-control cohort

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Schizophrenia (SCZ) is a multifactorial disorder with a complex genetic architecture. Established genetic risk factors include common single nucleotide polymorphisms and rare copy number variants. The availability of next-generation sequencing technologies has shifted the scientific focus towards the identification of very rare DNA-sequence variants. Particularly, *de novo* mutations are valuable for unraveling the pathogenesis of SCZ: (i) it is well recorded that the incidence of the disorder remains fairly stable over time despite the reduced fecundity rate in patients, and (ii) increased paternal age at the time of conception is a known risk factor for SCZ. There are at least two likely explanations for the previously mentioned observations: (i) mutations that are selected out of the gene pool are replaced by *de novo* mutations, and (ii) the number of *de novo* mutations arising in the male germ line increases with advancing age. Therefore, genes hit by a *de novo* mutation are interesting candidate genes for SCZ.

So far, twelve studies analyzing *de novo* mutations in about 1,400 patients with SCZ were published. We combined these data with our own in-house, unpublished data of 40 SCZ proband-parent-trios and filtered for the most promising candidate genes. For the follow-up analysis, we included all genes that (i) were hit by at least two *de novo* mutations that were *in silico* predicted to be pathogenic and (ii) were *in silico* predicted to be intolerant of mutations. The 20 genes fulfilling these criteria (including *SETD1A*) are currently re-sequenced using single molecule molecular inversion probes on an Illumina HiSeq 2500 in 2,500 independent SCZ patients and 2,500 healthy controls.

This study will provide further genetic evidence for promising candidate genes and new insights into the genetic architecture of SCZ. Analyses are still in progress and results will be presented at the upcoming conference.

P-Compl-148

Association analyses of functional NCF1 variants in psoriatic arthritis and psoriasis vulgaris

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Psoriatic arthritis (PsA) and psoriasis vulgaris (PsV) are common chronic inflammatory disorders of complex etiology. In a mouse-model for psoriasis, skin and joint symptoms were aggravated in a NADPH oxidase deficient strain, indicating regulation by reactive oxygen species (ROS). A subunit of the human NADPH oxidase is encoded by *NCF1*, a gene located in a structurally complex genomic region on chromosome 7q11.23 including two homologous pseudogene copies. The considerable homology of the whole genomic region comprising **NCF1**, its pseudogenes and further genes predisposes to genomic rearrangements resulting in variability of copy number. A reduced copy number of *NCF1* and a functional missense variant

(c.269G>A/p.Arg90His, rs201802880) in *NCF1*, causing a reduced ROS production, have recently been identified as strong genetic risk factors in other autoimmune diseases. Those association findings in combination with the findings in the mouse model prompted us to analyze both variants in large cohorts of 1,248 PsA and 1,157 PsV patients and 932 healthy blood donors.

NCF1 and pseudogene copy numbers were determined by qPCR in comparison to copy number of the house-keeping gene albumin using the comparative Ct ($2^{-\Delta\Delta CT}$) method. The coding variant rs201802880 was genotyped with a nested PCR strategy followed by a TaqMan based genotyping assay. Frequencies of carriers of one *NCF1* copy in PsV and PsA patients (0.54% and 0.42%, respectively) were comparable to the control group (0.57%). Analysis of genotypes of rs201802880 in carriers of the most frequent copy number ratio of 4:2 (pseudogene / *NCF1* gene copies) did not indicate association with neither PsA nor PsV despite 97% power to detect nominally significant association with PsA or PsV. Due to the low frequency of carriers of one *NCF1* copy, the power of our study to detect association of PsA or PsV with reduced NCF1 gene copy number was lower (29%). Our negative association findings in PsA and PsV do not indicate a role of these functional *NCF1* variants, associated to reduced ROS production. As the molecular mechanism of oxidative burst seems to play a role in autoimmune disorders, other subunits of the NADPH oxidase complex or other pathways resulting in deviant ROS production might play a role in the pathogenesis of PsA or PsV.

P-Compl-149

Whole exome sequencing of multiplex 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 around 1% of the general population. The disease is characterized by recurrent episodes of mania and depression and has a high heritability of around 70%.

Since the cumulative impact of common alleles with small effect may only explain around 25-38% of the phenotypic variance for BD, rare variants of high penetrance were suggested to contribute to BD risk.

In the present study, we investigated 226 individuals of 68 large multiply affected families of European origin using whole exome sequencing (WES). For sequencing, we selected two to five affected individuals with BD or recurrent major depression from each family. WES was executed on the Illumina HiSeq2500 platform and for data analysis we used the Varbank pipeline of the Cologne Center for Genomics. All variants shared within each family were filtered for a minor allele frequency <0.1% and potentially damaging impact on protein structure predicted by at least four of five different bioinformatics tools.

In total, WES identified 1214 rare, segregating and functional variants implicating 1122 different genes, of which 903 were expressed in the human brain. Subsequently, we applied the Residual Variation Intolerance Scores (RVIS, Petrovski et al., 2013) and performed a gene enrichment analysis with the genes that were ranked among the 20% most intolerant genes in the genome. A significant enrichment for a total of 18 pathways ($p<0.001$) including neuron projection, post-synapse and cell adhesion was observed.

For follow up analyses, we prioritized genes that were either implicated 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 2000 independent BD cases and 2000 controls with European ancestry. Among the multiply implicated candidate genes is *SYNE1*, which was also identified as a susceptibility locus for schizophrenia and autism.

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-150

COMPARATIVE EXPRESSION PROFILING IN RECTAL BIOPSIES OF GIARDIA-INDUCED POST-INFECTIOUS IRRITABLE BOWEL SYNDROME – A PILOT STUDY

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Irritable bowel syndrome (IBS) is a common gastrointestinal disorder, by affecting over 15 % of the population worldwide; it is the most frequent cause for referral to a gastroenterologist. IBS represents a multifactorial disorder in which genetics, bacteria in the digestive system, lifestyle, stress and other psychological factors, infections of the gut, environmental factors, and food intake contribute to the manifestation of the disease To date, evidence accumulated on differential microRNA (miRNA) mediated gene expression regulation in IBS. Complementing analysis of miRNA target genes revealed differential expression of genes relevant for immune and mast cell signalling as well as intestinal barrier function. In order to further unravel the role of differential miRNA and target gene expression in IBS, we performed comparative expression profiling of miRNAs in microdissected gut biopsies from Giardia lamblia post-infectious IBS patients (PI-IBS) versus healthy controls (HC) in an extraordinary entity of patients from Bergen in Norway.

miRNA profiling on rectal biopsy samples from 12 healthy controls and 12 PI-IBS cases revealed miRNA expression changes in the epithelial layer of PI-IBS samples. Biological functions related to differentially expressed miRNAs included gastrointestinal disease, inflammatory response and immunological disease. Validation was performed using a customized nCounter miRGE CodeSet panel profiling the top 5 candidate miRNAs and 100 potential mRNA targets in both, the epithelium layer and lamina propria of 16 healthy controls and 17 PI-IBS cases. De-regulation of the selected miRNAs could not be verified in a larger sample set; however, target genes involved in immunology (*Tryptase, TGFB1, ZEB2, NR3C1*), barrier function (*HNF4A, CLDN3, OCLN, MUC1-2*) and epigenetic modulation (*HDAC1, DNMT1-3A*) were differentially expressed in both, the epithelial layer and lamina propria. Complementing immunostaining for selected candidates on 16 healthy controls and 17 PI-IBS cases confirmed the upregulation of tryptase as well as a decreased and different subcellular expression of OCLN at the protein level.

In conclusion, genes relevant to immune and barrier function as well as stress response and epigenetic modulation are differentially expressed in PI-IBS and presumably contribute to the manifestation of the disease.

P-Compl-151

A fifty-year follow-up of familial Hirschsprung disease

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This report describes familial Hirschsprung disease with histologically proven congenital intestinal agangionosis (OMIM 142623) in the the proposita, her two brothers, her two affected daughters, and in the extended family three first cousins (two males and one female). This family was originally described in 1967 as part of a systematic study of the genetics of Hirschsprung disease (Family 21 in E. Passarge, New Eng J Med 1967; 276:138-143). It presumably involves a family with the highest number of affected indiduals

documented. In nearly all the non-syndromic long segment disease (HSCR type 2) is present. The proposita (IV-31 in the original report) underwent surgery two days after birth for meconium ileus, megacolon, and abdominal distension. Her aganglionic colon was completely removed. At the age of 8 months she was admitted to Cincinnati Children´s Hospital for ileostomy in part under the care of one of the authors (EP), then a resident in pediatrics. During the following 15 years she required additional 41 surgical procedures of her GI tract or the rectum. She is well adjusted to her disorder. This family was re-investigated in November 2017, including two daughters of the proposita who also are affected with Hirschsprung disease. In one daughter a 40-cm aganglionic segment of the colon has been removed, in the other a 26-cm long segment. Both daughters also have required multiple additional surgical procedures. A mutation S32L has been detected in the RET gene (OMIM 164761) in the mother and the two affected daughters. However, in the light of the complex genetics of Hirschsprung disease this is unlikely to be the only cause. Further molecular studies are under way.

Hirschsprung disease is a genetically heterogeneous and clinically variable disorder. In populations of European origin it occurs in 1 in 5000 newborns with a male-to-female ratio of 5:1. It results from the absence or malfunction of intestinal ganglion cells leading to megacolon, abdominal distension and other GI complications. It always involves the distal colon. Short segment (type 1) extends from the distal colon to the upper sigmoid. It occurs in about 60-85% of patients. Long segment (type 2) reaches beyond the splenic flexure into the transverse colon. It occurs in about 15-25%. In about 3-5% the aganglionic part also involves the small intestines.

At least three signal effector pathways are required for normal migration and development of intramural intestinal ganglion cells, (i) the RET tyrosine-kinase receptor and its ligand GDNF (glial-cell-derived neurotrophic factor, OMIM 600837), (ii) endothelin type B receptor (EDNRB, OMIM 600837) and its ligand EDN3 (endothelin 3, OMIM 131244), and (iii) SOX10 transcription factor (OMIM 602229). Pathogenic changes in the RET gene, including three non-coding sequences, and in about 12 additional genes contribute to the causes in 30-60% of patients. In general one mutation alone is neither necessary nor sufficient in this low-penetrance complex disorder.

P-Compl-152

Analysis of microRNAs in Lithium Response in Bipolar Disorder

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Bipolar disorder (BD) is a common, highly heritable neuropsychiatric disease characterized by recurrent episodes of mania and depression. Lithium represents the best-established long-term treatment for BD, even though individual response is highly variable. Evidence suggests that some of this variability has a genetic basis. This is supported by the largest genome-wide association study (GWAS) of lithium response to date conducted by the International Consortium on Lithium Genetics (ConLiGen) (Hou et al., 2016). A genome-wide analysis of the involvement of miRNAs in the development of BD identified nine miRNAs (Forstner et al., 2015). It is unknown, however, whether these miRNAs are also associated with lithium response in BD.

We aimed to determine whether common variants at any of the nine BD-associated miRNAs contribute to the variance in lithium response. Furthermore, we systematically analyzed whether any other miRNA is implicated in the response to lithium. We performed gene-based tests on the ConLiGen GWAS data (n=2,563

patients) where the Alda scale was applied to create a dichotomous and a continuous measure for the evaluation of long-term treatment response.

For the gene-based tests, a set-based testing approach adapted from the versatile gene-based test for GWAS (VEGAS2) (Mishra and Macgregor, 2015) with a minor correction for the top-0.1-test option (Hecker et al., 2017) was used. The 10% most significant SNPs for each miRNA and their ± 20kb flanking sequence were summarized, miRNA-based P-values calculated and corrected for multiple testing.

Exclusively miR-499a revealed a nominally significant association with lithium response in the candidate approach hence providing some evidence of involvement in the development and treatment of BD. Intriguingly, an upregulation of miR-499a in the prefrontal cortex of patients with depression (Smalheiser et al., 2014) and differential expression in the postmortem brains of BD patients compared with controls has been demonstrated (Banigan et al., 2013). Altogether, the results of the candidate approach suggested that the nine BD-associated miRNAs together do not significantly influence responses to lithium treatment.

In the genome-wide miRNA analysis, 71 miRNAs showed nominally significant associations with the dichotomous and 106 with the continuous treatment response. The intergenic miR-633 demonstrated the strongest association with the continuous measure (p=9.80E-04). Interestingly, miR-633 differentiated relapsing-remitting from secondary progressive multiple sclerosis courses suggesting this miRNA as a potential biomarker for disease course in multiple sclerosis (Haghikia et al., 2012).

No association between miRNAs and treatment response to lithium in BD in either of the tested conditions withstood multiple testing correction. Given the limited power of our study, the investigation of miRNAs in larger GWAS samples of BD and lithium response is warranted.

P-Compl-153

Analyzing allele-specific transcription factor binding events in a cellular model for orofacial clefting.

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Non-syndromic cleft lip with or without cleft palate (nsCL/P) is a multifactorial malformation with a prevalence of about 1 in 1,000 livebirths. Despite recent success in the identification of genetic risk factors underlying nsCL/P, their functional effects are only poorly understood. Furthermore, a substantial fraction of the heritability is not yet identified, some of which might be attributed to common genetic variants with low effect sizes. In the present study we hypothesize that transcription factor (TF) binding events, which occur in relevant cell types and act in an allele-specific manner at genetic risk variants, contribute to a further understanding of the biology of nsCL/P. To investigate this, we use human embryonic palatal mesenchyme (HEPM) cells, a cell line obtained from the facial region of a ten-week old female fetus. This time point falls into the critical period for palatal development and therefore the cells are considered an appropriate in vitro model to gain insights into molecular processes of this craniofacial defect.

We first identified TFs that are expressed in the HEPM cells by performing 3'RNA-Seq (Lexogen) on three replicates and analysed the data using an in-house pipeline. Of the several TFs which were highly expressed in HEPM, we decided to focus our molecular work on TFAP2A, for several reasons: (i) In vivo knockout models provided evidence for it being linked to craniofacial malformations, (ii) TFAP2A has been shown to preferentially bind a well-established risk variant at an IRF6 locus ,linked to orofacial clefting, and (iii) the gene TFAP2A itself is located at the previously identified nsCL/P risk locus on chr. 6p24.

Currently we are establishing a ChIP-seq pipeline in the HEPM cells, with promising outcomes obtained at certain benchmark steps of the protocol (including several replicates of positive/negative controls, gel electrophoresis, qPCR with melting curve, and PCR). Upon successful establishing, this ChIP-seq pipeline will be performed using a monoclonal validated antibody against TFAP2A and libraries will be sequenced on a MiSeq. Genomic regions identified as TFAP2A-binding sites will be analysed for (i) overlap with genetic regions associated with nsCL/P using our in-house GWAS-data and (ii) preferential allele-specific reads at heterozygous positions. Validations of selected regions will be performed using qPCR.

Our strategy will shed light on molecular events that are triggered by associated risk variants and help to identify regulatory networks in the developing palate. Furthermore, our study might contribute to an identification of novel risk loci associated with nsCL/P. Finally, the experimental design we develop could be easily applied to different TFs and also be performed for other complex traits.

P-Compl-154

Barrett's esophagus and esophageal adenocarcinoma: systematic integration of eQTL data with associated genetic variants and their replication

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Barrett's esophagus (BE) is a disorder of the lower esophagus characterized by a metaplastic transformation of the squamous epithelial tissue into columnar epithelium. It is a precancerous condition for the development of esophageal adenocarcinoma (EA), though only a small minority of patients with BE progress to EA. However, clinical surveillance to reduce incidence and mortality of EA is difficult and prevalence is rising. The etiology of both BE and EA is multifactorial. Genome-wide association studies (GWAS) have recently led to the identification of 14 susceptibility loci for BE/EA. Nevertheless, the vast majority of genetic risk factors still await elucidation.

In the present study, we used data from our recent genome-wide meta-analysis which represents the largest genetic association study on BE and EA to date. The aim of our study was (i) to link the genetic data with gene expression levels through expression quantitative trait loci (eQTLs) and (ii) to replicate eQTL-SNPs in an independent cohort.

First, we performed a systematic integrative analysis with our GWAS data and data from the GTEx consortium using esophageal tissue from gastroesophageal junction and mucosa. To identify potentially interesting eQTLs, we filtered our GWAS meta-analysis results for SNPs that (i) revealed a p-value of $5\times10^{-5} > p > 5\times10^{-8}$ and (ii) were an eQTL in at least one of the esophageal GTEx tissues. Here, we identified 32 independent eQTLs that show association to BE/EA ($r^2<0.4$ and/or 500 kilobases (kb) distance). Amongst others, identified eQTLs regulate genes involved in DNA break repair (upregulation of *C7orf49* on chromosome 7) and genes that have been previously implicated in gastric cancer (downregulation of both *PTPRCAP* on chromosome 11 and *BTN3A2* on chromosome 6).

In order to confirm the association of the identified eQTLs and to increase the statistical power, we are currently performing a replication study in an independent cohort consisting of 1,406 BE/EA cases and 992 controls. SNP genotyping is already finished and data analysis is currently ongoing. Our data will provide new insights into the etiology of BE/EA on the genome and transcriptome level.

P-CYTOGENETICS AND CNVS

P-CytoG-155

Jumping translocations – short telomeres or pathogenic TP53 variants as underlying mechanism in AML and MDS?

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Background: Chromosomal rearrangements, which involve one donor chromosome and two or more recipient chromosomes are called jumping translocations (JT). JT recurrently occur in hematologic malignancies. To date only few cases of acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) with JT have been described. The underlying mechanisms for JT have not yet been entirely understood.

Material and Methods: In our study we analyzed 11 AML patients and 5 MDS patients with JT (13 patients showed a complex karyotype). The patients were analyzed by karyotyping, FISH analyses, measurement of telomere length via T/C-FISH and/or qPCR and next generation sequencing with a panel for MDS/AML associated genes (TruSightTM Myeloid Sequencing Panel, Illumina).

Results: 6 patients showed recipient chromosomes with a breakpoint in the telomeric region and 7 patients showed recipient chromosomes with a breakpoint in the centromeric region. Patients with JT showed significantly ($p<0.01$) shorter telomeres in comparison to healthy age-matched controls. Furthermore, neo-telomeres were found in two patients. The telomere length did not significantly differ between patients with recipient chromosomes with a breakpoint in the telomeric or centromeric region. All patients with a centromeric breakpoint in the recipient chromosome had a pathogenic variant in the TP53 gene (n=6) and/or a loss of TP53 (n=5). In contrast, no pathogenic variant or loss of TP53 was identified in a patient with a telomeric breakpoint.

Conclusions: Our results divide the cohort of AML and MDS patients with jumping translocations into two groups: the first group with a telomeric breakpoint of the recipient chromosome is characterized by short telomeres and a possibly telomere-based mechanism of chromosomal instability (CIN) formation. The second group with a centromeric breakpoint of the recipient chromosome is defined by mutation and/or loss of TP53. Therefore, we assume that both critically short telomeres as well as pathogenic variants of TP53 have an influence on JT formation.

P-CytoG-156

Inherited derivative chromosome 9 [der(9)t(7;9)(q32;p24.3)] causing 46,XY sex reversal in one of two Syrian sisters with global developmental delay.

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We report two sisters - 11 and 7 years old - both with severe intellectual disability, muscular hypotonia, microcephaly, absent speech and facial dysmorphic signs, as well as epilepsy in the younger sister.

In order to clarify the cause of the severe developmental delay, chromosomal analysis from cultured peripheral lymphocytes was performed and an identical derivative chromosome 9 was discovered in both sisters, leading to a partial trisomy 7q and partial monosomy 9p. The chromosome analysis showed XX constellation in the older sister and, surprisingly, XY constellation in the younger sister. Testing the parental origin of the chromosomal imbalance we detected a balanced translocation t(7;9)(q32;p24.3) in the father, which was confirmed by fluorescence *in situ* hybridization (FISH). Subsequent array CGH analysis (Cytochip Oligo 4x180K OGT, Oxford, UK) of the younger sister showed a 29 Mb terminal duplication 7q32.2q36.3, a 1.4 Mb terminal deletion 9p24.3 and also a de novo 328 kb duplication 15q11.2, which could not be detected by quantitative PCR in either parent.

Distal 9p deletions that include the three major candidate genes for gonadal dysgenesis (*DMRT1, 3* and *2*) are associated with sex reversal in 46,XY individuals but not in 46,XX individuals. However, it is not clear which genetic mechanisms are involved in the downstream pathways of sex determination. Both patients present symptoms typical of trisomy 7q32-qter. However, the terminal deletion 9p24.3 is the cause of 46,XY-sex reversal (female phenotype) only in the younger sister with the 46,XY karyotype and has no influence on the sex development of the older sister with the XX constellation. In the literature, the duplication 15q11.2 seen in the younger sister (containing the protein coding genes: *NIPA1*, *NIPA2, CYFIP1* and *TUBGCP5*) is described in connection with developmental neurological abnormalities and as a cause of epilepsy.

This case illustrates that a combination of different (conventional and molecular) cytogenetic methods is helpful to elucidate complex chromosome rearrangements and consequently to explain the symptoms.

P-CytoG-157

Radiation-induced loss of Y chromosome in human fibroblast clones

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Radiation-induced genomic instability (RIGI) is defined as the occurrence of *de novo* DNA damage including cytogenetically changes, gene mutations and amplifications, formation of micronuclei and delayed cell death expressed in the progeny of cells surviving the radiation exposure. Chromosomal instability is the most intensive studied endpoint of RIGI and plays an important role in radiation-induced carcinogenesis. Besides structural chromosome aberrations, many cancer genomes possess numerical chromosome changes.

To study aneuploidy in the progeny of irradiated cells, normal human diploid fibroblast cells from two different male donors were clonally expanded after 2 Gray X-ray exposures. Karyotyping analysis was performed after 20 Population Doublings using conventional chromosome banding. Non-irradiated cells were cloned as controls. We were able to detect homogenous loss of whole Y chromosome in three independent clones. In contrast, autosomal monosomies were not found.

The Y chromosome is the smallest human chromosome and its mosaic loss in blood cells is known to be frequent among aging men. Besides aging, smoking and various disorders could be linked to mosaic loss of Y chromosome (mLOY) in the last years. Researchers showed that the Y chromosome is one of the most commonly deleted chromosomes in human cancer cells leading to our hypothesis that LOY occurs at a high frequency in the progeny of irradiated cells displaying a risk factor for radiation-induced cancer. Therefore, interphase fluorescence *in situ* hybridization with chromosome-specific DNA probes was used to determine the frequency of Y and X chromosome loss in additional 68 irradiated and 32 non-irradiated clones.

Y chromosome loss was confirmed by interphase FISH in all cases detected by GTG-banding. In addition, mLOY was observed in 10 of 68 (15%) irradiated clones. The frequency of Y chromosome loss in irradiated clones ranged from 10.7% to 100%. Using a 10% threshold for mosaicism, none of the analyzed control clones was classified as mosaic. Neither irradiated nor control clones showed loss of the X chromosome. This is consistent with the idea that mLOY is a radiation-specific effect. The mechanism underlying radiation induced mLOY is still unknown. Whole exome sequencing of clones for the identification of radiation-induced genetic variants causing mLOY is underway.

P-CytoG-158

Case report: Partial double trisomy 3 and 9 due to 3:1 segregation of a balanced maternal translocation

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We report on a male patient referred at the age of 4 months for evaluation due to mild psychomotor retardation, hypertelorism, bilateral epicanthus, discrete hirsutism, large fontanelle, auricular deformity, bilateral undescended testicles and suspected strabismus divergens. Furthermore, the boy presented a hyperinsulinism with recurrent hypoglycemia as well as a tumor in the liver suspected as hemangioma. First a syndromic cause such as Rubinstein-Taybi-syndrome was suspected. Molecular karyotyping using microarray analysis revealed a duplication of the chromosomal region 9p24.3q21.13 (approximately 74 Mb) as well as a duplication out of the chromosomal region 3p26.3p25.1 at the terminal short arm of chromosome 3 (approximately 14 Mb). Chromosome analysis was performed for both parents. Karyotype of the father was normal but the mother bears an apparently balanced translocation with breakpoints in the short arm of chromosome 3 and the long arm of chromosome 9, respectively, (46,XX,t(3;9)(p25.3;q21.13)).Thus the structural chromosomal anomaly in the boy resulted from a 3:1 segregation of a maternal reciprocal translocation involving the distal long arm of chromosome 9 and the terminal end of the short arm of chromosome 3.

P-CytoG-159

Alterations of a normal variant locus including the gene STRC in two patients with hearing loss

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The most prevalent sensory perception deficit in humans is syndromic and nonsyndromic hearing loss. It is genetically heterogeneous and 1 in 500 newborns is affected. Inherited nonsyndromic bilateral sensorineural hearing loss (NBSNHL, MIM #603720) is transmitted in an autosomal recessive manner in 80 percent of cases. Up to now, many causal genes have been identified. Aberrations of the gene *GJB2* encoding the protein connexin 26 are the main cause of NBSNHL. Recently, a commonly reported deletion copy number variant (CNV) on chromosome 15q15.3 could be identified in a homozygous state as another genetic cause for NBSNHL. This genetic region contains the gene *STRC*, which is coding for stereocilin (MIM *606440) and is expressed in the sensory areas of the inner ear.

We present two additional cases with deletions on chromosome 15q15.3 in patients with isolated hearing loss.

Array CGH analysis (Oxford Gene Technology CytoSure Constitutional v3 4x180K) of the first patient with deafness revealed a 61 kb homozygous deletion in 15q15.3 including *STRC*. The second patient carries a compound heterozygous mutation of *STRC*. Array CGH analysis showed as well a 61 kb but heterozygous deletion of the corresponding locus. The following sequencing analysis revealed an additional point mutation c.3217C>T in the *STRC* gene on the trans allele leading to a translational stop codon (p.(Arg1037*)). In both patients, deletions could be confirmed by multiplex ligation-dependent probe amplification analysis.

Our data reinforce the role of the 15q15.3 locus in patients with hearing loss and illustrates array CGH analysis to be a useful approach for elucidating hereditary hearing loss.

P-CytoG-160

MSX2 nucleotide variant and chromosomal rearrangements in 5q are associated with Craniosynostosis

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Craniosynostosis is one of the most common congenital craniofacial disorders affecting approximately one in 2500 newborns. It is defined as the premature ossification of one or more calvarial sutures. Compensatory growth of the skull leads to a dysmorphic cranial vault and facial asymmetry. Craniosynostosis can occur either as an isolated condition or as part of a syndrome. There is a high intra- and interfamilial variability and clinical overlap which often hampers straight forward diagnostics. Genetic causes have been identified mainly for syndromic craniosynostoses, i.e. mutations in *FGFR2*, *FGFR3*, *TWIST1*, and *EFNB1*. However, chromosomal rearrangements like i.e. partial deletion of 7p, 9p, and 11p as well as partial duplication of 5q, 13q, and 15q, have been reported in 15% of the syndromic craniosynostosis patients. Thus, our diagnostic approach for identification of the genetic cause in craniosynostosis patients is to analyze the genomic DNA by microarray-based comparative genomic hybridization (array CGH) to detect CNVs genome-wide and subsequently by our next generation sequencing (NGS) panel. Our panel comprises 68 genes including known and candidate craniosynostosis genes as well as genes associated with bone development. Both mutations and chromosomal duplications of the gene *MSX2* (MIM #123101) are known to be associated with premature fusion of the sutures. MSX2 is a homeodomain protein involved in craniofacial development. Up to now two point mutations of *MSX2* have been reported to cause craniosynostosis 2 (MIM #604757). Craniosynostosis 2 is associated with variable expression of craniosynostosis, normal intelligence and less frequent with some digital anomalies. We identified a novel pathogenic variant in *MSX2* (c.442C>G, p.P148A) by our NGS gene panel. The substitution affects a highly conserved residue in the homeodomain of MSX2. The three affected family members show craniosynostosis, visual impairments, and shortened phalanges of the thumbs. Furthermore, we present a patient with clover-leaf skull, hydrocephalus, Chiari II malformation, midface hyplasia, clitoromegaly, and craniofacial dysmorphisms. We identified a chromosomal rearrangement comprising partial 5q duplication, including *MSX2*, and partial 8p deletion. To the best of our knowledge this patient is the first reported craniosynostosis case with this specific chromosomal rearrangement. Our data support the assumption that human craniofacial development is dependent on correct MSX2 dosage and function.

P-CytoG-161

Detection of a complex chromosomal rearrangement in a six year old girl

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Constitutional Complex Chromosomal Rearrangements (CCRs) are rare balanced or unbalanced structural rearrangements involving three or more chromosomes with more than two breakpoints. The clinical effects of CCRs range from phenotypically normal to mental retardation, infertility and/ or congenital abnormalities. The phenotypic anomalies are attributed to gene disruption, cryptic imbalances in the genome, and/ or position effects.

We report on a six year old girl with restricted physical capacity including muscle weakness, mild generalized muscle hypotrophy (CK 5.34 µmol/l) and a mild tremor of the hands. G-banding, fluorescent in situ hybridization and array cgh (Oxford Gene Technology CytoSure Constitutional v3 4x180K) were performed in order to clarify this complex rearrangement.

Karyotype analysis showed a CCR in an apparent balanced state with participation of chromosomes 3, 6 and 18. Analysis of parental samples showed that it had arisen de novo. Gains or losses within the breakpoints could be excluded as far as the resolution limits of array cgh analysis allow. Owing to the fact that panel diagnostic for myopathy revealed no pathological findings the cause of the clinical features of the girl remains still unclear. Additional genetic imbalances within the CCR breakpoints or also elsewhere in the genome could be causative for the patients clinical features.

The detection of a complex chromosomal rearrangement might have far-reaching consequences in regard to family planning when the girl reaches adulthood. The frequency for spontaneous abortion or children with phenotypic abnormalities is high in carriers of complex chromosomal rearrangements so that in adulthood there should be offered a genetic counselling regarding possibilities of prenatal diagnostics.

P-CytoG-162

Array-CGH and Mutation Analysis in Patients with Müllerian Fusion Anomalies

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Fusion anomalies of the Müllerian ducts are associated with an increased risk for miscarriage and premature labor. In most cases polygenic-multifactorial inheritance can be assumed but autosomal-dominant inheritance with reduced penetrance and variable manifestation should be considered. So far, the etiology and pathogenesis of anomalies of the Müllerian ducts is very poorly understood. Further analyses are needed to identify genetic causes of Müllerian fusion anomalies.

The study group encompasses 103 patients with Müllerian fusion anomalies. Among these patients, 72 patients have non-syndromic (isolated) and 31 patients have syndromic Müllerian fusion anomalies.

Array-CGH analyses and sequential analyses of *LHX1* and *HNF1B* were performed in 103 patients with Müllerian fusion anomalies. The detected microdeletion in *TRIM32* in two patients was verified by RT-qPCR.

In eight patients we detected microdeletions and microduplications in chromosomal regions 17q12, 22q11.21, 9q33.1, 3q26.11 and 7q31.1. The rearrangement in 17q12 including *LHX1* and *HNF1β* as well as in 22q11.21 have already been observed in MRKHS (Mayer-Rokitansky-Küster-Hauser syndrome).

Sequential analysis of *LHX1* provided one presumably pathogenic missense mutation (c.416C>A) and two predicted splice-site-mutations (c.171-5del, c.1162G>T) in one patient, respectively. In *HNF1ß* three putative splice site mutations (c.345-105A>T, c.1005C>T, c.1045+12T>C) were found, each in one patient.

In summary we i) detected causative micro-rearrangements in patients with Müllerian fusion anomalies, ii) show that Müllerian fusion anomalies and MRKHS may have a common etiology, and iii) identified new candidate genes for Müllerian fusion anomalies. Furthermore, we identified new causative mutations in *LHX1* and *HNF1B*.

P-CytoG-163

12q21.33 deletion in a patient with posterior amorphous corneal dystrophy

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Posterior amorphous corneal dystrophy (PACD) (OMIM 612868) is a rare autosomal dominant disorder characterized by partial or complete posterior lamellar corneal opacification, decreased corneal thickness and flattening of the corneal curvature. Onset of the disease is in the first years of life. PACD is associated with heterozygous deletions in chromosome band 12q21.33-q22 harbouring the genes DCN (Decorin, OMIM 125255), KERA (Keratocan, OMIM 603288), LUM (Lumican, OMIM 600616) and EPYC (Epiphycan, OMIM 601657) which encode small leucine-rich proteoglycans. Only four families with deletions of this region have been published to date. We report on a 7-year-old male patient with PACD in whom an interstitial deletion in 12q21.33 was detected by array CGH. Subsequent FISH analyses in the parents revealed a balanced insertional translocation of this 12q21.33 segment into the long arm of one chromosome 13 in the mother. This family corroborates the association of 12q21.33 deletions with PACD and constitutes the first example of the involvement of a balanced chromosome aberration that predisposes to this rare disorder.

P-CytoG-164

Pure trisomy 20p – due to a familial whole arm translocation

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A trisomy of the short arm of chromosome 20 is a very rare cytogenetic finding. A majority of cases described in the literature are partial trisomies 20p arising from reciprocal translocation or less often from inversions which are associated with additional imbalances. The clinical phenotype of partial trisomy 20p seems to be very variable depending on the trisomic region and compromises motoric and speech developmental delay with characteristically craniofacial features. In some cases, skeletal, heart and kidney abnormalities are also observed.

Here we present a 2-year old boy with global developmental delay and muscular hypotonia. He showed coarse facial features as well as thorax abnormalities, sandal gaps and curved second toes. Family anamnesis revealed two miscarriages in the mother and six miscarriages in the maternal grandmother.

Chromosomal analysis (GTG-banding, resolution 550 bands) of the patient revealed a derivative chromosome 21, 21p+ . Array-CGH of the patient showed a heterozygous duplication of chromosome 20p13p11.1 (at least 26 Mb) which represents the whole short arm of chromosome 20. Chromosomal analysis of the patient's parents was performed. The father had a normal karyotype, 46,XY whereas the mother had a balanced reciprocal translocation of chromosome 20p and 21p (karyotype: 46,XX,t(20;21)(q10;q10) with breakpoints very close to the centromeres. Hence, our patient presented with a maternal inherited unbalanced translocation resulting in a pure trisomy 20p (karyotype: 46,XY,der(21),t(20;21)(q10;q10)mat.

Whole-arm translocations result from centric fission of 2 chromosomes (usually nonhomologous), followed by a reciprocal exchange. This boy is one of the rare patients with pure trisomy 20p. Little is known about the clinical course of patients with this abnormality and therefore further investigations are necessary. Most likely, the miscarriages in the family might also be associated with the inherited whole-arm translocation.

P-CytoG-165

Familial case of a complex chromosomal rearrangement involving chromosomes 1, 9 and 13 in an infertile male patient with cryptozoospermia

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Complex chromosomal rearrangements (CCRs) are rare structural abnormalities involving at least three chromosomal breakpoints. Carriers of familial CCRs have a higher risk for miscarriage due to either malsegregation of the derivative chromosomes or generation of recombinant chromosomes. It is assumed that men having CCRs are more often infertile than women since spermatogenesis is more susceptible to chromosomal aberrations than female gametogenesis. Here we report on a male patient who was referred to the Centre of Reproductive Medicine and Andrology (CeRA) because of primary infertility. The patient had cryptozoospermia in several semen analyses, i.e. only very few spermatozoa could be identified after centrifugation. Hormone analysis repeatedly showed normal LH and FSH levels but slightly reduced testosterone serum values. Cytogenetic analysis including FISH was performed and demonstrated a complex rearrangement involving four breakpoints on chromosome 1p22.1, 9p22 (two breakpoints) and 13q14.3. Chromosomal material of 9p22->9pter was found distal to 1p22.1 and the material of 13q14.3->13qter at 9p22. The derivative chromosome 13 showed distal to 13q14.3 a small insertion of material from 9p22 followed by chromosomal material of 1p22.1->1pter. The patient has two breakpoints in region 9p22 with a distal breakpoint for the translocation to 1p22.1 and a more proximal breakpoint for the translocation to 13q14.3. The patient´s mother showed the same complex aberrant karyotype. After genetic counselling the couple decided for in vitro fertilization including intracytoplasmic sperm injection. Thus far, two attempts were made without successful pregnancy. In conclusion, we report on a rare maternally inherited CCR found in an infertile male patient. The meiotic problems in the formation of the putative hexavalent might well be the underlying cause for the severely reduced sperm count. This work was carried out within the frame of the DFG Clinical Research Unit ‚Male Germ Cells: from Genes to Function' (CRU 326).

P-CytoG-166

A boy with 8p23 microdeletion syndrome and duplication 17q25 due to an unbalanced familial translocation

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We report on a 6 month old boy with 8p23 microdeletion syndrome and duplication 17q25 due to an unbalanced familial translocation. He is the third child of non-consanguineous parents. The maternal age at birth was 34 and the paternal age was 30 years. The boy was born as the second twin of a dichorial-diamniotic pregnancy at 39 weeks of gestation. He had a birth weight of 2910 g (-0.95 SD), a length of 46 cm (-2.35 SD) and a head circumference of 34 cm (-0.9 SD). Apart of a single umbilical artery, there was no prenatal remarkability. Postnatally, he showed ventricular septal defect, iris coloboma, retrognathia, muscular hypotonia, neonatal hypoglycemia, inspiratory stridor, and apnea. He has distinctive facial features including low set ears, broad nasal bridge and tip, retrognathia, thin lips and smooth philtrum.

Conventional chromosome analysis and FISH analysis revealed an unbalanced translocation between the long arm of chromosome 17 and the short arm of chromosome 8 resulting in a karyotype of

46,XY,der(8)t(8;17)(p23.1;q25.1). Parental karyotyping showed that the mother has a balanced translocation 46,XX,t(8;17)(p23.1;q25.1). SNP-Array specified the size and exact breakpoints of the aberrations. The deletion is 10.4 Mb on chromosome 8 p23.3 to p23.1 (164.984-10.535.377) and contains 121 coding genes, of these 34 are OMIM genes. The duplication is 9.1 Mb long and is on chromosome 17q25.1 to q25.3 (71.916.715-81.060.040) and contains 238 coding genes, of these 140 are OMIM genes.

So far there is only one patient in the Decipher-database with an isolated and comparable duplication of 17q25.1 to q25.3. The girl is described with feeding difficulties, hypertelorism, short stature, intellectual disability and delayed speech development. In contrast, 8p23-microdeletion-syndrome is well known and several reports of patients in the literature suggest that the most common clinical findings are developmental delay or intellectual disability, microcephaly, congenital heart defects, and diaphragmatic hernia. Haploinsufficiency of *GATA4* is thought to play a critical role in the development of congenital heart defects and diaphragmatic hernia. As *GATA4* is not part of the deletion, our results suggest that haploinsufficiency of other genes, in addition to *GATA4*, may play a role in these birth defects associated with 8p23.1 deletions.

Overall, the complex phenotype of our patient is caused by the unbalanced, not yet reported, translocation and is consistent with the reported clinical findings of the 8p23-microdeletion-syndrom in combination with the duplication of 17q25. Apnea and neonatal hypoglycemia are not reported in other patients with 8p23 deletion. Thus, we assume it is rather correlated to the duplication 17q25, for which barely comparable cases exist.

P-MONOGENIC DISEASE – FROM GENE IDENTIFICATION TO MOLECULAR MECHANISM

P-MonoG-167

Specific changes in hippocampal-cortical projections underlie intellectual disability in patients with Tuberous Sclerosis

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Tuberous sclerosis (TS) is an autosomal dominant genetic disease caused by mutations in either the TSC1 or the TSC2 gene. Mutations are thought to result in an increase in mTOR (mechanistic target of rapamycin) activity and an upregulation of downstream signalling. The mTOR kinase is the most important regulator of local dendritic and presynaptic protein translation in the brain and controls the synthesis of several proteins involved in synaptic function and plasticity.

Together with autism, epilepsy and benign tumours in several organic systems, intellectual disability (ID) is a characteristic feature of TS. About half of the patients with TS show a significant predisposition for ID with an IQ less than 70. However, not much is known about the neurophysiological mechanisms underlying ID in TS patients. To investigate the development and mechanisms of ID, we are using a heterozygous Tsc2 knockout (KO) mouse model that resembles the heterozygosity of Tsc2 mutations in TS patients.

To confirm the suggestion of previous studies, that memory deficits and ID in TS patients are due to aberrant hippocampal function – Morris water maze (MWM) was shown to be impaired in Tsc2KO mice – we employed a cognitive test battery consisting of MWM and a 24 hours novel object recognition test (NORT), both testing for hippocampal function. Interestingly, performance of both, three to four months and eight months old animals was unaffected in both tests. This led us to the conclusion that ID in TS patients does not occur due to hippocampus damage.

Using a 7 days NORT and a test battery for episodic memory however, while three months old animals were unaffected, a clear decline in eight months old animals in both tests was observed. The 7 days NORT measures memory consolidation, a brain function that relies on projections between the hippocampus, the entorhinal and the prefrontal cortex. Similarly, episodic memory is a domain of brain function that depends on hippocampal-entorhinal projections.

Together our data show that ID in TS (i) develops over time and (ii) is caused by a decline of projections between the hippocampus and cortical regions.

In order to get a deeper insight into the biochemical mechanisms underlying this decline we are currently analyzing the early immediate activity-dependent genes Arc, c-fos and Zif268 by RT-qPCR in different brain regions to further characterize the brain regions involved. With our data in mind, we furthermore plan to conduct specific behaviour testing and functional magnetic resonance imaging in TS patients to specifically analyse memory consolidation and hippocampal-cortical processing.

P-MonoG-168

De novo FBXO11 mutations are associated with intellectual disability, microcephaly and behavioural anomalies

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Intellectual disability (ID) has an estimated prevalence of 1.5-2% and in most affected persons its genetic basis remains unclear. Whole exome sequencing (WES) has proven to be a valuable tool to identify the causative gene defects in research and diagnostic settings. WES studies have shown that a large proportion of sporadic ID cases results from de novo point mutations and small insertions or deletions (indels).

Here, we present two unrelated patients with common clinical features and deleterious de novo variants in *FBXO11* (Homo sapiens F-box protein 11) detected by WES. The *FBXO11* variant in patient 1 was identified in a study to elucidate new candidate ID genes by trio WES in a cohort of 311 patients with unexplained ID and their unaffected parents. The four-year-old boy has mild ID (IQ 53) as well as mild microcephaly, corrected cleft lip and alveolus, hyperkinetic disorder, mild brain atrophy and mild facial dysmorphism. WES verified by Sanger sequencing detected a heterozygous de novo 1bp insertion in intron 3 immediately upstream from the exon/intron border and the donor splice site of exon 4 (chr2(GRCh37):g.48066557dup; NM_001190274.1:c.442+1dup). The variant is predicted unequivocally to result in aberrant splicing. The *FBXO11* variant in patient 2 was identified by diagnostic WES. The three-year-old boy showed ID and pre- and postnatal growth retardation, postnatal mild microcephaly, hyperkinetic and restless behavior, as well as mild dysmorphism. WES followed by Sanger sequencing detected a heterozygous de novo nonsense mutation (chr2:g.48035302_48035303del; NM_001190274.1:c.2738_2739delAT).

FBXO11 encodes a member of the F-box protein family which is characterized by the F-box amino acid motif and constitutes one of the four subunits of ubiquitin protein ligase complex called SCFs (SKP1-cullin-F-box). SCFs play a role in phosphorylation-dependent ubiquitination. FBXO11 can function as an arginine methyltransferase and acts as an adaptor to mediate the neddylation and thus suppression of p53. To our knowledge, only one patient with ID and a de novo deleterious *FBXO11* mutation has been reported so far as part of a larger study on syndromic ID (Martínez et al., JMG 2017;54:87-92). Interestingly, this patient carries the identical mutation as our patient 2 and also displays ID, IUGR, microcephaly, behavioral anomalies (autistic / stereotypic behavior), and dysmorphisms.

Given the genotype-phenotype correlation in three patients, we propose deleterious de novo mutations in *FBXO11* as a novel cause of ID, microcephaly and behavioral anomalies.

P-MonoG-169

*** Investigating the role of tcf12 and twist1 in craniosynostosis with a zebrafish in-vivo model

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Craniosynostosis is a skeletal birth defect with a prevalence of ~1 in 2,200 newborn. It is clinically defined as a premature fusion of the cranial sutures in the skull. The patency of the sutures during childhood is essential to enable the growth of the skull in compliance to the developing brain. An early fusion of the cranial sutures leads to an abnormal skull shape and to neurological damage. Heterozygous mutations in *TWIST1* and *TCF12* have been identified in patients with Saethre-Chotzen syndrome, which is associated with coronal synostosis and in non-syndromic coronal synostosis. It is assumed that TCF12 and TWIST1 form a heterodimeric protein complex and that the dosage of TCF12/TWIST1 heterodimers is critical for normal coronal suture development.

To investigate the specific function and the expression pattern of *tcf12* during suture development and its interaction with twist1, we established several zebrafish (*Danio rerio*) disease models. To detect the dynamic expression patterns of *tcf12* in developing cranial sutures in vivo, we generated a transgenic zebrafish, in which the green fluorescent protein (GFP) is expressed under the control of the *tcf12* promoter. Furthermore, by use of CRISPR/Cas9-mediated genome editing we established a number of loss-of-function mutations in conserved regions of zebrafish *tcf12* and *twist1* to fathom the potential to induce and visualize craniosynostosis. Note that compared to human and mouse suture development cranial sutures in zebrafish stay patent throughout the complete life of the fish.

With the transgenic line we could identify *tcf12* promoter driven gfp expressing cells at the edges of the frontal and parietal bones during zebrafish development, a region where sutures actively form. In addition, *tcf12* expression in adult fish is detected inside all cranial sutures, especially in the coronal sutures. Our loss-of-function experiments reveal that unlike in mice, individuals with a heterozygous mutation in *tcf12* can exhibit partial fusions of the coronal sutures. This observation resembles the situation in human patients properly but is contrary to mouse experiments, where loss of *Tcf12* and *Twist1* are required for suture fusion.

Our future studies aim to examine the effects of *tcf12* and *twist1* double knockouts in zebrafish and to explore the functional consequences of *TCF12* mutations on TCF12 transcriptional activity in humans and zebrafish via Luciferase Assay.

P-MonoG-170

*** RIN1 is the main effector of active HRAS in human immortalized keratinocytes (HaCaT cells)

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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 (e.g. the most common patient mutation HRAS p.G12S or the oncogenic mutant HRAS p.G12V) 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, we generated permanent human keratinocyte cells (HaCaT) stably expressing HA-HRAS^{WT}, HA-HRAS^{G12S} and HA-HRAS^{G12V}, and screened for keratinocyte-specific HRAS binding partners by affinity purification and quantitative mass spectrometry. We identified RIN1 (Ras and Rab interactor 1) as interaction partner of constitutively active variants HRASG12S and HRASG12V but not of HRASWT. We verified the specific interaction of endogenous RIN1 with activated HRAS by immunoprecipitation experiments. The RAS effector RIN1 competes with RAF1 for RAS binding. Immunoblotting analysis demonstrated pronounced RIN1 but only very weak RAF1 expression in HaCaT cells. In line with this, we detected only minimal HRAS-induced modulation of RAF/MEK/ERK signaling in HaCaT cells. These data suggest that RIN1 might be the key effector of HRAS in keratinocytes. As a guanine nucleotide exchange factor (GEF) for RAB5A, RIN1 facilitates receptor endocytosis and is involved in cytoskeletal remodeling. Thus, our findings introduce the possibility, that dysregulation of receptor trafficking and/or cytoskeleton remodeling are relevant in the pathogenesis of Costello syndrome.

P-MonoG-171

*** Diagnostic whole exome sequencing identifies new causative variants in pediatric neurodevelopmental disorders

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We performed diagnostic whole exome sequencing (WES) in 50 cases of pediatric neurodevelopmental disorders in whom a neurogenetic cause seemed highly likely (excluding patients with lesional patterns on MRI, a history of preterm birth or perinatal asphyxia). 64% of the affected individuals presented with predominant intellectual disability, 12% with predominant movement disorders and 24% presented with intellectual disability and movement disorders.

WES analysis revealed causative variants in 13 genes previously indicated in neurological disorders (*ADNP*, *ALDH7A1*, *CASK*, *FOXP1*, *KCND3*, *KIF5A*, *MECP2*, *PPOX*, *STXBP1*, *TBR1*, *UBE3A*, *VAC14* and *WDR26*). While *TBR1* haploinsufficiency was only reported in individuals with autism to date, the individual with a de novo truncating *TBR1* variant reported in this study presented with global developmental delay and bifrontal cortical polymicrogyria without autistic features. This finding broadens the phenotypic spectrum of individuals with pathogenic *TBR1* variants. Bi-allelic *VAC14* mutations have been described in 3 families with childhood-onset striatonigral degeneration which is also seen in the individual examined in this study. *WDR26* haploinsufficiency has only very recently been described in individuals with intellectual disability, seizures and abnormal gait, features which were also seen in the patients examined in this study.

Segregation analysis was performed to determine de novo status for dominant disorders or bi-allelic inheritance in case of recessive disorders. For 2 patients we performed an extended trio analysis resulting in the prioritization of de novo missense and near-splice variants in *CASK* and *STXBP1*, respectively. These results indicate that trio whole exome analysis is a feasible way to identify causative variants in individuals with sporadic neurological disorders.

CNV analysis using the WES coverage data additionally identified a deletion of 6 exons of *GATAD2B* which was also previously indicated in intellectual disability.

For another 17% of cases we identified variants of unknown significance, segregation analyses for these variants are still pending.

Taken these results together we could reveal causative variants in about 30% of cases and identify potentially causative variants in another 17% of cases in a study comprising 50 individuals with pediatric neurological disorders.

P-MonoG-172

MIDAS Case Report: Trio Whole Exome Sequencing (WES) of a patient with developmental delay (DD) identified a pathogenic variant in PUF60 causing rare Verheji syndrome

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Introduction: Clinical and genetic heterogeneity of developmental disorders requires broad genetic testing approaches as well as appropriate software tools for fast and precise data analysis. Here, we present one of over 50 patients with DD sequenced as patient-parent trio as part of the Multiple Integration and Data Annotation Study (MIDAS). The aim of MIDAS is the development and implementation of a central software system for data integration in diagnostics.

Phenotype: We present a 4-year-old boy with developmental delay, brain atrophy, postaxial polydactyly (removed after birth) as well as borderline short stature and decreased body weight. Additionally, he had following symptoms: feeding difficulties, stereotypic behaviors, hyper-extensible skin, joint hypermobility, muscular hypotonia, delayed skeletal maturation, thoracic scoliosis, pectus excavatum, pes valgus, strabismus, conductive hearing impairment, and recurrent upper respiratory tract infections. Standard clinical genetic testing (karyotyping, microarray, fragile X analysis, single gene analysis and gene panel testing) was inconclusive.

Methods: As part of the MIDAS cohort, we sequenced the patient and his healthy, unrelated parents on a NextSeq500 platform (Illumina, San Diego, CA, USA). An enzymatic fragmentation approach was used for library preparation. Exome capture was performed using the SureSelect Human All Exon V6 kit (Agilent, Santa Clara, CA, USA), targeting coding exons and conserved splicing sites of >20.000 genes. The library of the patient was sequenced to 172x mean coverage as 151-bp paired-end reads. 95% of the target region were covered 20x or higher. For data analysis and variant evaluation, the CLC Genomic Workbench 9.5 (Qiagen, Hilden, Germany) as well as annotations from commercial and public databases were used.

Results: By WES trio analysis, we identified the pathogenic *de novo* heterozygous variant consisting of a deletion of two nucleotides and an insertion of 19 nucleotides resulting in a frameshift in the gene *PUF60* (NM_078480.2: c.1072_1073delinsTTGACCCTGGCCCAGCCCC, NP_510965.1: p.(Leu364Argfs*26)). The gene *PUF60* (Poly-U Binding Splicing Factor 60 kDa) encodes a nucleic acid-binding protein, which regulates pre-RNA splicing by facilitating 3-prime splice site recognition at early stages of spliceosome assembly. Microdeletions on chromosome 8q24.3 including the gene PUF60 as well as heterozygous de novo variants in *PUF60* are known to cause the Verheij syndrome (VRJS). VRJS is characterized by growth retardation, developmental delay, dysmorphic facial features and skeletal, mainly vertebral, anomalies. Coloboma, renal defects, and cardiac defects were reported as additional variable features.

Discussion: This case report strongly supports the value of WES for the diagnostics of developmental disorders in children. However, the optimization of analysis pipelines, which is subject of the MIDAS project, is required in order to establish WES in clinical routine.

P-MonoG-173

An unusual deletion in the SMN1 gene: characterization by Nanopore sequencing

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In a boy suspected to have Spinal Muscular Atrophy (SMA) type 2, we detected an unusual deletion in one of the SMN genes in addition to the common deletion of exons 7 and 8 of SMN1 using MLPA. The combined probes for SMN1 and SMN2 repeatedly showed a hybridization pattern consistent with a heterozygous deletion spanning at least exons 1 and 4 of one of the genes. Segregation analysis in the family revealed that the father was heterozygous carrier of the SMN1 exons 7 and 8 deletion, while the mother had a similar, unusual MLPA pattern indicating that she was heterozygous carrier of the unusual deletion. However, using MLPA it was neither possible to distinguish between a deletion in SMN1 or SMN2 nor to delineate its exact size and breakpoints. RNA analysis not only supported the presence of the deletion spanning exons 1 to 4, but also revealed that indeed SMN1 was affected. To further characterize the deletion, we used nanopore sequencing, a 3rd generation long-read sequencing technology. Nanopore sequencing allows reading of several kb of DNA in a row and thus also discriminating the highly homologous sequences of the SMN1 and SMN2 genes. Results will be presented and a possible mechanism will be delineated. This case and similar ones demonstrate that the complexity of the two SMN genes can require additional genetic work-up in affected families, including long-range next-generation sequencing.

P-MonoG-174

Investigating the pathomechanisms of Borjeson-Forssman-Lehmann syndrome in females

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Borjeson-Forssman-Lehmann syndrome (BFLS) is a syndromic form of X-linked intellectual disability, caused by mutations in *PHF6*. Initially, it was reported to follow an X-linked recessive inheritance pattern in families with affected males and unaffected or only mildly symptomatic carrier females. Recently, we identified *de novo* mutations in *PHF6* in several female individuals with intellectual disability and a very distinct, recognizable appearance, only partly overlapping with BFLS in males. Typical phenotypic aspects included variable intellectual disability, a distinct facial appearance, oligodontia, linear skin pigmentation, and finger and toe deformities with hypoplastic nails and/or distal phalanges.

PHF6 contains two plant-homeodomain-like (PHD) domains known from chromatin-interacting proteins, localizes to the nucleus and interacts with the PAF1 transcription initiation complex and with the NuRD complex, a multifunctional epigenetic regulator. Apart from that, little is known about its function and its role in nervous system development so far.

To further delineate the mutational and clinical spectrum of BFLS in females, we have collected two additional cases of female individuals with BFLS and *de novo* variants in *PHF6*. In order to investigate possible genotype-phenotype correlations and to elucidate the pathomechanism of BFLS, we established wildtype and mutant constructs of *PHF6* and transfected HEK293 and SK-N-BE cells. Wildtype PHF6 was found to be ubiquitously located in the nucleus and particularly in the nucleolus. In contrast, mutant PHF6 was also located within the nucleus but forming aggregates. This observation might indicate an impaired chromatin binding capacity of mutant PHF6. For confirmation, we are currently establishing a fractionation assay.

To investigate the possible role of PHF6 and chromatin in transcriptional regulation and to identify its target genes and pathways, we utilized CRISPR/Cas9 to create two different knockout lines, one lacking one of the N-terminal nuclear localization sequences and one lacking the second plant homeodomain. We are currently preparing transcriptome and ChIP-Seq analyses with these cell lines.

Our study will further delineate the mutational and clinical spectrum of PHF6-related phenotypes and will gain better insight into the role of PHF6 in nervous system development and function and into the molecular mechanisms underlying BFLS.

P-MonoG-175

When neurodevelopment meets neurodegeneration

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Vacuolar H+-ATPase-dependent functions are critical for neural proteostasis and are involved in neurodegeneration and brain tumorigenesis. We studied a patient with fulminant neurodegeneration of the developing brain carrying a de novo ATP6AP2 variant encoding an accessory protein of the V-ATPase. Functional studies of induced pluripotent stem cell (iPSC) derived neurons from this patient revealed reduced spontaneous activity and severe deficiency in lysosomal acidification and protein degradation leading to neuronal cell death. Conditional full deletion of this gene in the developing mouse brain impaired V-ATPase dependent functions resulting in degeneration of nearly the entire cortex. We conclude that this gene is key mediator of V-ATPase dependent signaling and protein degradation in the developing human central nervous system.

P-MonoG-176

*** The mutational and clinical spectrum of TUBA1A Tubulinopathy

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In three unrelated individuals with developmental delay we identified 3 heterozygous de novo missense variants in TUBA1A by exome sequencing, two previously described, c.518C>T, p.(Pro173Leu) and c.641G>A, p.(Arg214His), and one novel variant, c.1307G>A, p.(Gly436Asp).

The TUBA1A gene is a member of the large tubulin gene family. The tubulin proteins are essential for the formation of microtubules, cytoskeletal structures involved in various cellular processes including chromosomal segregation, intracellular transport and neuronal migration. During embryonal brain development, the tubulin alpha1 protein encoded by the TUBA1A gene is the major alpha tubulin component in the microtubule architecture. Disease associated variants in TUBA1A and other members of the tubulin gene family have been reported to cause a heterogeneous group of disorders with cortical and subcortical malformations and a variety of clinical features, collectively referred to as “Tubulinopathies”.

We now extensively reviewed the current literature and variant databases to provide a standardized synopsis of neuro-radiological findings and clinical features of TUBA1A Tubulinopathy based on the Human Phenotype Ontology (HPO). The disease associated variants of TUBA1A tubulinopathy were standardized to the Human Genome Variation Society (HGVS) nomenclature.

Thus far, a total of 51 distinct heterozygous missense variants in 82 individuals with TUBA1A Tubulinopathy and 20 fetal cases have been reported. The most common brain malformation, anomalies of the corpus callosum, is present in 94 % of the non-fetal and 100 % of the fetal cases. Lissencephaly is also more common in the fetal cases (85 % vs. 62 %). While the reported percentages of brain anomalies are mostly comparable, fetal cases in general show a more severe manifestation. Common clinical features in mature individuals included microcephaly (73 %), developmental delay (62 %) and seizures (39 %). Most of these common features were shared by the three cases with TUBA1A mutations described here. In addition, they presented with rarely observed clinical features of muscle hypotonia and facial anomalies.

Finally, we analyzed spatial clustering of all variants in the linear gene model and also used publicly available tertiary protein structure data of TUBA1A to provide an insight into the variant distribution. The amino residue Arg402 with two different substitutions (Arg402Cys, Arg402His) is by far the most common (22%) affected position in TUBA1A patients. Variants at this and the second recurrent position (Arg264Cys) are associated with a specific pattern of brain malformations, indicating a genotype-phenotype correlation. Further adoption and enforcement of standardized ontologies and nomenclatures is needed to make the computational analysis of genotype-phenotype relations feasible, thus improving the understanding of rare diseases like tubulinopathies.

P-MonoG-177

A TUBB6 mutation is associated with a new autosomal dominant phenotype comprising non-progressive congenital facial palsy, bilateral ptosis and velopharyngeal dysfunction.

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Introduction: Congenital cranial dysinnervation disorders (CCDD) are a genetically and clinically heterogeneous group of disorders affecting the innervation of specific occulo-cranio-facial muscles. Non-progressive isolated CCDD are in some cases allelic to complex disorders of brain development as illustrated by the example of *TUBB3* where specific heterozygous mutations may cause isolated congenital fibrosis of extraocular muscles (CFEOM) type 3A or cortical dysplasia, complex, with other brain malformations (CDCBM) type 1.

Methods and Results: Using linkage analysis and DNA sequencing, we studied a 5-generation pedigree with autosomal-dominant inheritance of a new phenotype consisting of facial paresis, ptosis and velopharyngeal dysfunction with rhinophonia. A heterozygous *TUBB6* variant strictly co-seqregated with the phenotype and affected an amino residue that is highly conserved in evolution. Exclusion of pathogenic variants in other genes through whole exome sequencing, expression data for TUBB6, 3D molecular modelling, and functional experiments in yeast all supported the interpretation that the *TUBB6* variant c.1181T>C is indeed the causative mutation. *TUBB6* mutation analysis of additional patients with clinically suspected CCDD did not reveal any more mutations.

Conclusions: This is the 1st report implicating *TUBB6* in human disease. A definitive proof that the above described phenotype is a CCDD in the sense of a neurodevelopmental disorder is still lacking. Our study adds to the genetic heterogeneity of tubulinopathies. Further imaging and functional work is planned to understand the mechanisms of TUBB6 action.

P-MonoG-178

A homozygous intragenic deletion in the gene SCAPER causes syndromic intellectual disability

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The S-Phase Cyclin A Associated Protein In The ER (SCAPER) gene is a ubiquitously expressed gene with unknown function in the brain. In dividing non-neuronal cells in vitro SCAPER localizes primarily to the ER and regulates cell cycle progression. Recently, biallelic SCAPER mutations were found in four patients from three families with retinitis pigmentosa and intellectual disability. Here, we expand the spectrum of disease causing mutations in SCAPER and report for the first time an intragenic homozygous deletion in the SCAPER gene in two patients of a consangious family with intellectual disability, retinitis pigmentosa and additional features. The observed deletion spans 10 kb, and includes SCAPER coding exons 16 and 17 causing a frameshift and introducing a premature termination codon. In-silico analyses of SCAPER expression using published RNA-seq data from human embryonic and adult brain as well as RT-qPCR and in situ hybridization in mouse brain revealed an upregulation of SCAPER expression during embryonic development of the cerebral cortex and a higher expression of SCAPER in neurons compared to neural progenitor cells. In the adult brain SCAPER was expressed in several brain regions including the cerebral cortex where it showed a layer-specific expression and was upregulated in lower layer glutamatergic neurons. Our study further supports the role of SCAPER mutations in the etiology of intellectual disability and retinitis pigmentosa, expands the mutational spectrum and highlights the need for functional studies concerning the role of SCAPER during brain development and function.

P-MonoG-179

Copy number variations in CNGB3-linked autosomal recessive achromatopsia account for missing second alleles

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Achromatopsia (ACHM) is a rare autosomal recessive retinal disorder characterized by color vision defects, photophobia, nystagmus, and severely reduced visual acuity. The disease is caused by mutations in genes encoding crucial components of the cone phototransduction cascade (CNGA3, CNGB3, GNAT2, PDE6C, and PDE6H) or in ATF6, involved in the unfolded protein response. CNGB3 encodes the beta subunit of the cyclic nucleotide-gated ion channel in cone photoreceptors and is the major ACHM gene in Europe and the US. Yet a considerable number of cases remain genetically unsolved, in some cases because only a single heterozygous mutation was identified in one of the known ACHM genes. We show here that copy number variations (CNVs) contribute to these missing alleles in CNGB3-lilnked ACHM.

We searched for CNVs by quantitative realtime PCR (qPCR) applying TaqMan Copy Number Assays (Thermo Fisher Scientific, Waltham, MA) and the QuantiTect SYBR Green PCR Kit (QIAGEN, Hilden, Germany);for all coding exons of CNGB3 in 43 single heterozygotes, followed by microarray-based high-resolution comparative genomic hybridization (CGH) using the CytoSure Eye Disease Research Array (Oxford Gene Technology, Begbroke, UK) or the arrEYE (van Cauwenbergh et al. 2017) to confirm and confine the CNVs, and defined the extent of the CNVs by breakpoint mapping.

The qPCR data implied 6 subjects with deletions and 10 with duplications spanning 1-10 consecutive exons of CNGB3. In addition, a homozygous deletion spanning 15 consecutive CNGB3 exons was discovered by diagnostic genetic testing. All CNVs were successfully validated by CGH arrays. While the deletions were unique to each patient or family, a duplication encompassing exons 4−7 was observed in 3 independent families and a duplication of exon 7 in 6 independent families. Precise breakpoint mapping was achieved for all intragenic CNVs. The sizes of the duplications/insertions ranged from 10,599 - 98,770 bp, while the sizes of the mapped deletions ranged from 5,026 - 63,252 bp. All duplicative events were consistently found to be in tandem.

In about 10% of ACHM patients only a single heterozygous CNGB3 mutations had been identified after standard genetic analysis. In this study we identified 9 different heterozygous CNVs in 16 unrelated patients accounting for the missing second CNGB3-allele in these patients. Moreover, one patient with a homozygous CNGB3 deletion encompassing exons 4−18 was identified. These findings highlight the importance of CNV analysis for this gene, and contribute this mutation type to the CNGB3 mutation spectrum.

P-MonoG-180

Compound heterozygous MYH7 carrier with one LoF allele develops early onset cardiomyopathy

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Introduction: Mutations in the MYH7 gene, which codes for the beta (β)-myosin heavy chain in the sarcomere, are a common cause of familial hypertrophic cardiomyopathy, accounting for up to 35 percent of all cases. Most of the reported pathogenic mutations are missense mutations that affect important domains of the protein, e.g. binding sites for actin or ATP, and lead to diminished force transduction properties of myosin (gain/change of function). Heterozygous truncating mutations in the MYH7 gene are not expected to lead to clinical symptoms, as haploinsufficiency of the MYH7 gene is not a known disease mechanism for this gene. The clinical relevance of heterozygous loss-of-function mutations remains unclear, still truncating mutations are uncommon in both the general population and the cardiomyopathy patients.

Results: In this study we present a patient with a loss-of-function mutation in compound-heterozygosity with a missense mutation. The index patient presented at the age of 15 with a severe phenotype of early onset hypertrophic non-obstructive cardiomyopathy, non-compacted apex and severe ventricular arrhythmias. After surviving a sudden cardiac arrest at the age of 18 he got an internal cardioverter defibrillator implanted. The genetic analysis showed a known pathogenic missense mutation (c.1207C>T;p.Arg403Trp) and a splice site mutation (c.1000-1G>A) in compound heterozygous state. The splice site mutation leads most probably to a frameshift deletion, i.e. a loss-of-function allele. The mother was a heterozygous carrier of the missense mutation in the MYH7 gene and showed a mild septal hypertrophy in echocardiography, while the father was asymptomatic carrier of the splice mutation.

Conclusion: Taken together, these findings suggest that loss-of-function mutations in the MYH7 gene are probably not pathogenic in the heterozygous state, as opposed to most cardiomyopathy genes. However, in compound heterozygous state with a pathogenic mutation they lead to a more severe early onset phenotype.

P-MonoG-181

Characterization of Lifr-/- and Lifr+/- mice and patients with heterozygous LIFR mutations to identify the molecular mechanism causing urinary tract malformations in Lifr deficiency and haploinsufficiency

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Congenital anomalies of the kidneys and urinary tract (CAKUT) comprising various structural malformations of the excretory system, including renal agenesis, renal hypodysplasia, and hydronephrosis, are the most common cause of end-stage renal disease in children and young adults. To date, more than 40 genes have been identified to cause CAKUT when mutated. Nevertheless, because CAKUT is genetically highly heterogeneous, the genetic basis remains unsolved in most patients. Recently, we reported a *de novo LIFR* frameshift variant in a patient with severe bilateral CAKUT as well as a form of cryptorchidism, and rare deleterious *LIFR* missense variants in three further CAKUT patients. *LIFR* encodes a transmembrane receptor utilized by IL-6 family cytokines, mainly by the leukemia inhibitory factor (LIF) to stimulate JAK/STAT, MAPK and PI3K signaling. We also demonstrated CAKUT phenotypes including hydronephrosis and reduced ureteral lumen in *Lifr*$^{-/-}$ mice, along with a form of cryptorchidism. As the CAKUT patients carried heterozygous *LIFR* variants, we, here, examined *Lifr*$^{+/-}$ mouse embryos and found the same phenotypes, i.e. hydronephrosis, diminished ureteric lumen, and cryptorchidism, but less frequently or less pronounced than in *Lifr*$^{-/-}$ embryos. We show that the ureteral lumen in *Lifr*$^{-/-}$ and *Lifr*$^{+/-}$ embryos is reduced because of muscular hypertrophy and a thickened urothelium. Furthermore, our work reveals a cellular urothelial anomaly probably associated with CAKUT, which has not previously been described, in the lumen of the ureter and bladder of *Lifr*$^{-/-}$ and *Lifr*$^{+/-}$ embryos, i.e. desquamated *Upk* positive cells. These cells are likely to represent superficial (S-) cells, large hexagonal cells forming the innermost urothelium layer. Normally, S-cells differentiate gradually from intermediate cells located in the next urothelium layer, with a slow turnover rate. However, in *Lifr*$^{-/-}$ and *Lifr*$^{+/-}$ embryos, it seems that S-cell turnover is elevated, and that S-cells are shed from the urothelium without exogenous injury possibly mimicking an inflammatory response. To provide further insights into the role of *Lifr* in urothelial maintenance and differentiation, and in the molecular mechanisms involved in CAKUT caused by *Lifr* deficiency or haploinsufficiency, mutant ureters and bladders will be analyzed on a histological and a molecular level. Additionally, to explore whether S-cell desquamation also occurs in patients with *LIFR* variants, urine sediments of these patients will be analyzed in a reverse phenotyping effort.

P-MonoG-182

De novo IGF2 indel frameshift variant on the paternal allele in a patient with growth restriction

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Both insulin-like growth factors 1 and 2 (IGF1 and IGF2) signal through IGF1R leading to growth and metabolic effects via the downstream PI3K/Akt pathway. Pathogenic variants in *IGF1* and *IGF1R* lead to intrauterine growth restriction and affect postnatal body growth. However, to date there are only three reports of pathogenic *IGF2* variants in individuals with severe pre- and postnatal growth restriction. We identified a *de novo IGF2* indel frameshift variant (NM_000612.5: c.195delC, p.(Ile66Serfs*93)) in a 3.4 years old child with intrauterine growth restriction, postnatal severe growth retardation (height 5.41 SDS, weight 3.51 SDS) in combination with dystrophy, retarded bone age, mild microcephaly (2.36 SDS), brachydactyly with clinodactyly, and facial dimorphism (long philtrum, high forehead, retrognathia, and a Pierre-Robin sequence). Cloning and sequencing of a long-range PCR product harboring the frameshift variant and the SNP informative site chr11:2153634 (rs680, NC_000011.9:g.2153634T>C) demonstrated that the *de novo* variant resided on

the paternal allele. This finding is consistent with a known maternal imprinting of *IGF2*. Structural modelling of the variant suggests that posttranslational IGF2 processing is affected, leading to an enlarged mutant protein and a disturbed IGF2/IGF1R interaction. Our study provides further evidence for the importance of *IGF2* in growth disorders and argues for its inclusion in gene panels designed for routine diagnostics of short stature.

P-MonoG-183

Nicolaides-Baraitser syndrome due to mosaicism for an in-frame deletion of SMARCA2 exons 20 to 27

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Nicolaides-Baraitser syndrome (NCBRS, OMIM #601358) is characterised by severe intellectual disability, seizures and multiple congenital abnormalities, and is caused by heterozygosity for pathogenic missense mutations in SMARCA2 on human 9p24.3 encoding the ATPase subunit hBRM of the SWI/SNF chromatin remodeling complex BAF. We here describe for the first time a female patient with the clinical diagnosis of severe NCBRS and a mosaic SMARCA2 in-frame deletion of exons 20-27.

Sanger sequence analysis of SMARCA2 in the patient's DNA from leukocytes could not disclose any variant. Subsequent CytoScanHD SNP array analysis found no pathogenic copy number variation but revealed a large region (arr[hg38] 9p24.3p13.2(192,128-36,694,479)x2 hmz) with a copy number neutral loss of heterozygosity. Inspection of informative SNPs in the patient and her parents revealed an incomplete (85%) paternal isodisomy with 15% residual maternal alleles. The hmz region contains more than 200 genes including SMARCA2.

Whole genome sequence analyses in the trio confirmed the presence of approx. 85% paternal isodisomy and 15% of the maternal allele, and additionally disclosed an interstitial deletion of 60,692 bp spanning SMARCA2 exons 20-27. This grade of mosaicism is below the detection threshold of the microarray analysis. A deletion-spanning PCR and sequence analysis confirmed the deletion chr9:g.2,092,697_2,153,388del [GRCh38/hg38]. No maternal SNP alleles were retained throughout the entire deletion.

By transcript analysis using total RNA from blood, we verified wildtype and mutant transcripts in the index. In the mutant transcript, exon 19 is spliced to exon 28, leading to an in-frame deletion of exons 20-27, best described as c.2884_3981del (NM_003070.4), and resulting in the amino acids deletion p.Val962_Arg1327del (NP_003061.3). Thus, the mutant protein is missing the entire C-terminal helicase domain (aa1054-1216).

No mutant SMARCA2 gene or transcript could be identified in the parental samples. This makes a de-novo deletion in the index most likely. Although our analyses cannot clarify whether the deletion had occurred in the maternal germline, giving rise to a heterozygous offspring or whether it had occurred post-zygotically, we propose that the observed mosaicism in combination with a high grade of paternal isodisomy is the result of an incomplete rescue of an interstitial SMARCA2 deletion by mitotic recombination events. Acquired uniparental isodisomy of this particular 9p-region is a frequent stem cell defect in polycythemia vera, but acquired UPDs on other chromosomes have also been described in other conditions.

Unfortunately, at the time of writing we had been unable to obtain other tissues from the patient to examine the somatic distribution of the mutant allele. Nevertheless, we suggest to collect not only blood but also additional samples like buccal swabs to enable molecular diagnosis also in those patients who are mutation negative at first glance.

P-MonoG-184

Serum lipid alterations in GBA-associated Parkinson's disease

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Introduction: Mutations in the GBA gene, encoding for the lysosomal enzyme glucocerebrosidase, are associated with Gaucher disease. Alterations in plasma sphingolipids have been reported in Gaucher, and similarly in brain extracts in Lewy body disease. As GBA mutations are prevalent risk factors for Parkinson's disease and overlap of molecular pathways are presumable, here we assessed the lipid profiles in Parkinson's patients with and without GBA mutations.

Methods: We sequenced all GBA exons in 415 Parkinson's patients, previously genotyped for LRRK2. 64 patients (29 GBA positive vs. 35 non-GBA-carriers including 18 LRRK2 positive and 17 non-mutated) were analyzed for chitotriosidase activity and for the concentration of 40 lipid classes using HPLC-MS.

Results: 29/415 patients (6.9%) carried 8 different GBA mutations associated with Gaucher or Parkinson's, including one novel mutation. Chitotriosidase activity was similar across the genetic groups, while the levels of key lipids were altered in GBA mutation carriers: Monohexosylceramide, Ceramide and Sphingomyelin were elevated; while Phosphatidic acid (PA), Phosphatidylethanolamine (PE), Plasmalogen phosphatidylethanolamine (PEp) and Acyl Phosphatidylglycerol (AcylPG) were decreased.

Conclusion: The results suggest an important role for these lipids in GBA mediated Parkinson's disease and assist in the identification of common pathways between Gaucher and Parkinson's. Ultimately, our findings may lead to the identification of novel biomarkers for individuals at increased risk of developing Parkinson's disease.

P-MonoG-185

*** Functional characterization of iPSC-derived RPE cells from patients with autosomal recessive bestrophinopathy (ARB)

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Purpose: Bestrophin 1 (BEST1) encodes an integral membrane protein localized to the basolateral membrane of the retinal pigment epithelium (RPE). Functional BEST1 is a Ca^{2+}-regulated volume-sensitive chloride channel, composed of five homomeric BEST1 subunits. Pathogenic mutations in the BEST1 gene have been associated with distinct retinopathies, including the autosomal dominant Best disease (BD) and the autosomal recessive bestrophinopathy (ARB).

Methods: To understand the functional mechanisms underlying the dominant and recessive forms of BEST1-associated pathology, we generated RPE cells differentiated from induced pluripotent stem (iPSC-RPE) cells from a BD patient (heterozygous for Q238R), an ARB patient compound heterozygous for N99K and R141H and one healthy parent (heterozygous for R141H). We analyzed BEST1 RNA/protein expression and chloride transport function. We furthermore used several homogeneous transfected MDCK II cell lines constitutively expressing normal BEST1 and BEST1 mutations to investigate BEST1 half-life time and degradation pathways.

Results: RNA expression was similar in all iPSC-RPE lines tested. Chloride conductance in the ARB RPE was absent consistent with an absence of BEST1 protein. In contrast, BEST1 protein was reduced to about 20 % in BD and heterozygous R141H iPSC-RPE cells. An impaired channel function was observed only in BD iPSC-RPE cells. MDCK II cells, stably expressing a selected number of BEST1 mutations, showed that normal BEST1 protein remains stable up to 24 hours, whereas the half-life time of dominant and recessive mutant proteins is strongly reduced. Furthermore, the dominant BEST1 mutation is prone to degradation via the endo-lysosomal degradation pathway whereas the recessive BEST1 mutations are degraded by the proteasome.

Conclusion: In iPSC-RPE cells 20 % of residual normal BEST1 protein appears sufficient for proper chloride transport in heterozygous recessive ARB while heterozygous dominant mutations in BD reveal a dominant-negative effect with an abolished chloride conductance. Experiments with MDCK II cell lines

expressing dominant and recessive BEST1 mutations indicate that this is due to different protein stabilities and different degradation pathways.

P-MonoG-186

Bernard Soulier family with novel compound heterozygous variants in GP1BB

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Bernard Soulier syndrome (BSS) is a rare bleeding disorder. The inheritance pattern is usually autosomal recessive with severe deficiency in homozygotes and compound heterozygotes and partial deficiency in heterozygotes. A few cases of an autosomal dominantly transmitted form have been described. The disease is characterized by impaired or absent platelet aggregation by ristocetin, prolonged bleeding time, low platelet counts and abnormally large (giant) platelets whose membrane lacks glycoprotein complex GP Ib/IX/V.

We here report a case of two sisters suspected with Bernard Soulier syndrome. The abnormal laboratory tests included prolonged bleeding time, absence of ristocetin induced agglutination, mild to moderate thrombocytopenia as well as enlarged and very few giant platelets.

Mutation analyses of the genes *GP1BA*, *GP1BB* and *GP9* identified d two sequence changes, c.311_314dupTGGC; p.(Glu109Profs*201) and c.212C>T; p.(Pro71Leu) in *GP1BB*, both not listed in the international mutation and population databases so far. The patients' parents were heterozygous for one of each. The 4-bp-duplication c.311_314dupTGGC theoretically leads to a change in the amino acid sequence from codon 109 onwards and an extended 3'-end of the protein since the original stop codon is not in frame anymore. The mutation affects the extracellular domain of GPIbß and can be classified as likely pathogenic. This is supported by the fact that the patients' mother who is reported to have thrombocytopenia is carrier of this small duplication.

The second alteration identified in both affected sisters is an amino acid change at position 71 of *GPIBB*. While bioinformatic evaluation of this missense mutation supports the assumption of possible functional relevance, the variant remains a VUS according to the ACMG recommendations. Both the patients' unaffected father as well as an unaffected brother are also carriers of this alteration. In the next step, we want to assess whether the clinical phenotype of the mother is comparable to that of the affected patients since this would implicate autosomal dominant inheritance of BSS in our family.

P-MonoG-187

Barth Syndrome: Tafazzin dysfunction triggers a preferential depletion of polyunsaturated cardiolipins

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Barth Syndrome is an X-linked inherited disorder resulting in cardioskeletal myopathy associated with mitochondrial dysfunction and neutropenia. The affected TAZ gene codes for the protein tafazzin, which is a CoA-independent phospholipid-lysophospholipid transacylase that regulates the fatty acyl side chain composition of mitochondrial cardiolipins using other phospholipids as substrates. As part of the cardiolipin remodelling machinery, mutations cause an overall loss of cardiolipin content together with increased levels of monolyso-cardiolipins, altered mitochondrial ultrastructure, and the inhibition of mitophagy and apoptosis. Cardiolipins are phospholipids that are found exclusively in mitochondrial membranes and are functionally involved in maintaining oxidative phosphorylation by stabilisation of respiratory chain complexes, buffering fluctuations of the mitochondrial proton gradient, protecting mtDNA, and mediating oxidative damage-related apoptotic signalling events. Structurally, cardiolipins have a unique dimeric phosphatidyl backbone carrying four fatty acid side chains making them a structurally highly diverse phospholipid class. The side chain composition of cardiolipins is tissue-specific and is regulated by a post-biosynthetic remodeling process, in which tafazzin plays a major role.

We have recently developed a novel reversed phase liquid chromatography – tandem mass spectrometry method, enabling the identification and absolute quantification of each cardiolipin species, including their respective monolyso- as well as oxidised counterparts, resulting in a complete cardiolipidome. Furthermore, by mathematical modelling of MS/MS spectra generated by data-dependent fragmentation, it was possible to

deconvolute the specific fatty acyl composition of each individual cardiolipin species. This allows us to study the molecular structural diversity of cardiolipins in great detail.

When analysing Barth Syndrome patient derived fibroblasts using this methodology, we were able to detect the preferred depletion of (poly-)unsaturated fatty acyl containing cardiolipins in these tafazzin deficient cell lines. The same effect was also observed in TAZ knock-out cell lines generated in a CRISPR/Cas9 gene editing approach. Cardiolipins are predominantly located in the inner mitochondrial membrane facing the matrix side, if however a side chain becomes oxidised it undergoes a structural interconversion leading to translocation to the intermembrane facing side, where tafazzin is located. Upon cleavage of this oxidised side chain a monolyso-cardiolipin is formed, which is then re-acetylated by tafazzin and thus repaired to form a functional cardiolipin. Due to the proximity of cardiolipins to the electron leaking respiratory chain complexes and the susceptibility of unsaturated fatty acyls to oxidative damage, our data indicates that tafazzin does not only play an important functional role in cardiolipin maturation, but also for repairing oxidative damage.

P-MonoG-188

Whole exome sequencing simplifies the identification of mutations associated with retinal dystrophies.

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Monogenic retinal dystrophies (RDs) are clinically and genetically heterogeneous disorders with mutations in about 250 disease-associated genes. Ranging from mild to severe forms, RDs result in mild vision impairment or complete vision loss. More than 2 million people worldwide are affected by different types of RDs.

Whole exome sequencing (WES) allows a simultaneous analysis of many genes. We established a next generation sequencing pipeline to rapidly identify disease-associated mutations in patients and tested the pipeline on selected RD cases.

We present five unrelated patients with different clinical manifestations of RDs. The first patient shows congenital nystagmus (CN) and retinal dysfunction, including night blindness and myopia. The second patient presents with a phenotype of retinitis pigmentosa (RP) with high tone impairment, and the third patient was affected by choroideremia (CHM). The fourth patient has a cone-rod dystrophy (CORD), and the fifth patient shows a typical form of RP.

WES identified eight genetic variants in four known genes associated with RDs. The CN-patient has a hemizygous splice-donor variant (NM_001256789.1: c.2576+1G>A) in the calcium channel voltage-dependent alpha-1f subunit (CACNA1F) gene. Genetic variants in the Usherin (USH2A) gene were found in both, the RP/deafness-patient (NM_206933.2: c.2299del, p. Glu767Serfs*21, and c.4714C>T, p.Leu1572Phe), and the CHM-patient (NM_206933.2: c.2299del, p. Glu767Serfs*21, and c.11864G>A, p.Trp3955*). The CORD-patient presents with two heterozygous variants (NM_001029883.2: c.1709-1728del, p.Gly570Glufs*3, and c.2655del, p.Ser885Serfs*2) in the chromosome 2 open reading frame 71 (C2orf71) gene. In the RP-patient compound heterozygous variants (NM_000329.2: c.1338+1G>A, and c.1207_1210dup, p. Glu404Alafs*4) were identified in the gene encoding the retinal pigment epithelium-specific protein (RPE65). Sanger sequencing analyses confirmed co-segregation of the phenotype and the above mentioned genetic variants in the families.

In the era of genetic therapies, it is important to establish efficient screening strategies to identify mutation in clinically variable diseases, such as RDs.

P-MonoG-189

A novel homozygous ARL13B variant in patients with Joubert syndrome impairs its guanine nucleotide-exchange factor activity

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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.223G>A (p.(Gly75Arg) in the ARL13B gene, which was 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. Both ARL13B and ARL13B-c.223G>A (p.(Gly75Arg) 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-c.223G>A (p.(Gly75Arg) displayed a marked loss of ARL3 guanine nucleotide-exchange factor activity, with 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-190

Homozygous MITF mutation causes severe Waardenburg syndrome

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Waardenburg syndrome (WS) is a genetically and clinically heterogeneous auditory-pigmentary condition triggered by migration and/ or survival defects of neural crest derived cells, the precursors of for example melanocytes. WS can be classified in four major types (WS1 – 4) with several further subtypes. The worldwide prevalence of WS is estimated at around 1:40.000 and accounts for 2 to 5 percent of all cases of congenital hearing loss. Although inherited in an autosomal-dominant manner the penetrance of symptoms of the distinct syndrome types and subtypes vary considerably among affected individuals and even among people in the same family, which makes clinical diagnosis still challenging.

We examined an Argentinean patient (index, male infant, *06/2012) presenting with extensive hypopigmentation of the skin and total absence of hair pigmentation (hair, eyebrows, eyelashes) as well as congenital hearing loss. Interestingly, he did not show total dilution of pigmentation but presented with several pigmented macules distributed all over the body. Evaluation of the pedigree over three generations and the family medical history revealed that hearing impairment, sectorial heterochromia irides and mild skin pigmentation anomalies, all clinical features pointing to a WS phenotype, segregated within the family. We also observed a variable expressivity of pigmentation anomalies and variable severity of deafness. Additional evaluation of the pedigree revealed that the index' parents were siblings. The index' phenotype in contrast did not resemble the phenotype of the other affected family members since it was much more pronounced and additionally presenting with mild facial dysmorphic features actually a property of WS1.

Molecular diagnostics by whole exome sequencing ─ index, mother, father and grandmother ─ revealed no exonic pathogenic variants in known WS associated genes like *MITF*, *PAX3*, *SNAI2*/*SLUG*, *SOX10*, *EDNRB*, *EDN3* and *KITLG*. Further analysis uncovered an intronic *MITF* sequence variant, the underlying gene for WS2A, influencing the splicing efficiency of *MITF* transcripts. The affected family members were

subsequently classified having a WS2A phenotype according to the diagnostic criteria proposed by the Waardenburg Consortium. Moreover, we identified ─ in regard of the consanguineous background ─ for the first time a homozygous *MITF* mutation in the index patient. The homozygosity in the autosomal dominant inheritance severely enhanced the index phenotype and brings into question if a WS2A classification is still indicated for the index, as we would expect in accordance with an underlying *MITF* mutation. Up to date homozygous WS mutations were known e.g. from *PAX3* in WS1 families leading to a more severe WS3 phenotype in the offspring. This report expands the WS phenotypical spectrum and gives new insights into the genotype-phenotype correlation of Waardenburg syndrome.

P-MonoG-191

Analysis for novel ELOVL5 mutations in German ataxia patients

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Hereditary spinocerebellar ataxias (SCA) are a genetically heterogeneous group of neurodegenerative disorders. Clinical features include gait and limb ataxia, dysarthria, and nystagmus in combination with other pyramidal and extrapyramidal signs and symptoms. To date, a minimum of 35 different SCA loci and 26 genes are known (1). Among those, mutations in the “elongation of very long chain fatty acids protein 5” (*ELOVL5*) gene were identified as the cause of SCA38 (2). The deduced protein is involved in the elongation of polyunsaturated very long fatty acids and belongs to a protein family comprised of ELOVL 1-7. *ELOVL5* is composed of 9 exons, and four protein-coding transcripts with a variable number of exons ranging from 3-9 are known. No data is available for tissue-specific expression of these transcripts. To date, only two missense mutations located in *ELOVL5* have been identified in single Caucasian families. One mutation affects codon 72 of exon 3 (p.Leu72Val) (2), the other is located in exon 7 (p.Gly230Val) (2). Interestingly, exon 7 is only part of two of the protein-coding transcripts.

48 independent patients with a family history of ataxia were screened for mutations in *ELOVL5*. Other known SCA-loci (SCA1-3, 6-8, 10, 12-14, 17, 27) had been excluded previously. We sequenced all 9 exons including exon-intron boundaries of *ELOVL5* in the patients and found a novel heterozygous C to T transition in exon 4 of the gene in one patient. The base change results in the substitution of glutamine for a stop codon which results in a premature polypeptide. Since exon 4 is only included in the longest transcript variant, relevance of the observed change in relation to disease-symptoms is unclear. Nevertheless, because the altered transcript and/ or the truncated polypeptide might be non-functional, we postulate a pathogenic effect for this alteration.

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P-MonoG-192

Congenital anonychia and uncombable hair syndrome: co-inheritance of homozygous mutations in RSPO4 and PADI3

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Ectodermal dysplasia comprises a heterogeneous group of genetic disorders defined by developmental defects in ectoderm-derived tissues. The great heterogeneity of the symptoms is often an obstacle for the identification of the causative gene, although the use of next generation sequencing has brought new insights.

Here, we investigated a 4-year-old Kuwaiti boy showing both congenital anonychia and uncombable hair syndrome. Through whole exome sequencing (WES) we identified mutations in two separate genes, demonstrating that the patient’s phenotype comes from the overlap of two autosomal recessive disorders.

With regards to anonychia, we identified a previously known homozygous splice-site mutation in RSPO4. The encoded protein, R-spondin, is expressed in nail mesenchyme and is an activator of the Wnt/β-catenin

pathway. For the hair abnormality, we found a novel homozygous missense mutation in PADI3. This gene encodes for peptidylarginine deiminases 3 (PADI3), which is involved in deiminating trichohyalin in the hair follicle, contributing to its structure.

All mutations were verified by Sanger sequencing. Furthermore, the PADI3 mutation was investigated through immunoblotting and immunofluorescence in a keratinocyte cell line, showing both lower expression and formation of aggregates for the mutant protein compared to wild-type.

In conclusion, our case highlights the value of WES in identifying co-inheritance of two distinct conditions in consanguineous pedigrees, giving rise to an ectodermal dysplasia phenotype.

P-MonoG-193

Unravelling the genetic causes of pontocerebellar hypoplasia type 1: clinical and genetic findings of 31 families

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Pontocerebellar hypoplasia (PCH) is a clinically and genetically heterogeneous group of rare developmental disorders of the cerebellum and hindbrain. Prior to the advent of genetic classification PCH was pathoanatomically subdivided into type 1 and type 2 by the presence or absence of spinal motor neuron degeneration (Barth. Brain Dev 1993;15:411-22). PCH1 follows autosomal recessive inheritance and is an important differential diagnosis of infantile spinal muscular atrophy (SMA variant).

Over a period of more than 20 years we recruited 41 patients out of 31 families with the clinical or pathoanatomical diagnosis of PCH1 in order to identify the genetic cause. At the beginning of our study in 1995, no gene locus was known. All we knew at that time was that mutations in SMN1 are not responsible for this complicated form of SMA. The first gene associated with PCH1 was VRK1 (Renbaum et al. Am J Hum Genet 2009;85:281-9), but only one family has been reported. The 2nd gene, EXOSC3, encoding a constituent of the exosome complex, was discovered in 2012 (Wan et al. Nat Genet 2012;44:704-8) and turned out to be the most important gene for PCH1, being responsible for 12/31 (39%) of our families. The clinical picture can be highly variable as regards survival, but global developmental delay is usually profound. The 3rd gene to be identified was EXOSC8, another constituent of the exosome complex. EXOSC8 mutations were reported in 3 families hitherto, affected children showed cerebellar and corpus callosum hypoplasia and abnormal myelination (Boczonadi et al. Nat Commun 2014;5:4287). EXOSC8 tested negative in our cohort, as well as other subunits of the human exosome complex. The 4th gene, SLC25A46, was initially reported as a gene responsible for complicated optic atrophy in 2016 (Abrams et al. Nat Genet 2015;47:1493-8). However, SLC25A46 mutations turned out to cause a variety of neurological conditions. Among our patients we detected 3 families with bi-allelic SLC25A46 mutations, with severe pontocerebellar hypoplasia or without optic atrophy and. One of these families was the original Dutch PCH1 family reported by Barth in 1993 (Van Dijk et al. Brain 2017;8:e46). Meanwhile, we and others discovered a 5th gene which plays a role in posttranslational modifications of tubulins and the microtubule-associated transport in neurons (Shashi et al., submitted; Magiera et al., submitted).

Altogether, the underlying gene defect has been discovered in more than half of our families (16/31) up to now. We will present major clinical features, brain imaging and pathoanatomical data of the mutation-positive patients.

P-MonoG-194

Phenotype diversity in Hutchinson-Gilford progeria syndrome

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Lamins A and C as alternatively spliced products of the A-type lamin gene (*LMNA*) are key components of the nuclear lamina and have a major influence on the integrity of the nuclear architecture in human cells. Hundreds of mutations in the *LMNA* have been identified which lead to a wide range of genetic disorders including cardiomyopathy, muscular dystrophy, neuropathy, restrictive dermopathy and Hutchinson-Gilford progeria syndrome.

Here we report a 4 years old patient who had been referred to the department of pediatric genetics with the initial diagnosis of stiff skin syndrome. The patient showed growth retardation and microcephaly as well as generalized contractures and a lipoatrophic skin. He additional featured sparse hair, a prominent forehead and veins visible over the scalp. No mental retardation was observed by now.

Trio whole-exome sequencing was performed and revealed the heterozygous mutation c.1824C>T [p.(G608G)] in exon 11 of *LMNA* which was not present in the parents. This mutation has already been reported several times as the most common cause for classic Hutchinson-Gilford progeria syndrome. It is known that this substitution activates a cryptic splice donor site resulting in the removal of 50 amino acids near the C-terminus of prelamin A. As a consequence, progerin accumulates at the nuclear periphery which disrupts the integrity of the nuclear lamina.

The symptoms of our patient overlap with the classical phenotype of Hutchinson-Gilford progeria syndrome. However, most cases described with Hutchinson-Gilford progeria syndrome did not show a microcephaly. Further investigations are necessary to identify mechanisms which might be causative for this phenotype diversity.

P-MonoG-195

Identification of rare compound heterozygous PNKP variants in a German patient with ataxia-oculomotor apraxia 4 and pilocytic astrocytoma by whole exome sequencing

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Background

Ataxia-oculomotor apraxia type 4 (AOA4) are rare autosomal recessive neurologic disorders. The phenotype is characterized by ataxia, oculomotor apraxia, peripheral neuropathy and dystonia. AOA4 is caused by biallelic mutations in the *PNKP* gene encoding a polynucleotide kinase 3'-phosphatase with an important function in DNA-damage repair.

Material and Methods

We report on a female German patient, daughter of non-consanguineous parents, with first unclear polyneuropathy of the lower legs at 21 years of age. At 23 years of age, broken smooth pursuit was observed on eye movement testing. Additionally, a cerebellar pilocytic astrocytoma was diagnosed and surgically removed. Meanwhile, at 27 years of age, she is wheelchair bound and reports of progressive sensory and motor deficits of her arms. After extensive conventional molecular genetic diagnostics for Friedreich ataxia, AOA1, AOA2, spinocerebellar ataxia (SCA) type 1-3, type 6-8, type 17, Charcot-Marie-Tooth hereditary neuropathy (CMT) type 1A ,1B, CMT2A2, CMT2E/1F, CMT2K, CMT4A, CMTX1, and distal hereditary motor neuropathy type 5A yielding negative result, whole-exome sequencing (WES) on a peripheral blood sample was performed. Detected variants were filtered based on an autosomal recessive rare genetic disease model in non-consanguineous parents

Results

We identified two variants within the *PNKP* gene. One variant, a duplication in exon 14 resulting in the frameshift c.1253_1269dup p.(Thr424fs*49), has previously been described as pathogenic, e.g. in cases of AOA4. The second variant, representing a nonsense mutation in exon 17, c.1545C>G p.(Tyr515*), has not yet been described and is predicted to cause a loss of the seven C-terminal amino acids.

Conclusions

This is the first description of AOA4 in a patient with central European descent. Furthermore, the occurrence of a pilocytic astrocytoma has not been described before in an AOA4-patient. Our data demonstrate compound heterozygous *PNKP* germline mutations in a German patient with AOA4 and provide evidence for a possible link with tumor predisposition.

P-MonoG-196

Microcephaly, Short Stature, And Limb Abnormality Disorder Due to Novel Autosomal Recessive DONSON Gene Mutations in Two German Siblings

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Mutations in *DONSON* have only recently been identified to cause a syndrome of short stature, microcephaly and limb malformations. We identified novel compound heterozygous mutations in two affected siblings of German descent by Whole Exome Sequencing. Interestingly, one of the siblings had a previous clinical diagnosis of Fanconi anemia. This diagnosis was also suggested in three of the previously described *DONSON* mutation carriers. This diagnosis was suggested not only by primordial microcephalic dwarfism in the affected child but particularly due to a radial aplasia. Both the DONSON and Fanconi anemia proteins act in maintaining genome integrity. We hypothesize that the observed clinical overlap between Fanconi anemia and carriers of DONSON mutations indicates the proteins have similar functions during embryonic development. We conclude that *DONSON* mutations should be considered as part of the differential diagnosis when a clinical diagnosis of Fanconi anemia cannot be molecularly confirmed.

P-MonoG-197

Identification of SLC20A1 and SLC15A4 among other genes as potential risk factors for Combined Pituitary Hormone Deficiency

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Combined pituitary hormone deficiency (CPHD) is characterized by a malformed or underdeveloped pituitary gland resulting in an impaired pituitary hormone secretion. Several transcription factors have been described in its etiology, but defects in known genes only account for a small proportion of cases. To identify novel genetic causes for congenital hypopituitarism, we performed exome sequencing studies on 10 patients with CPHD and their unaffected parents. Two candidate genes were sequenced in further 200 patients. Genotype data of known hypopituitary genes are reviewed.

We discovered 51 likely damaging variants in 38 genes; 12 of the 51 variants represent *de novo* events (24%); 11 of the 38 genes (29%) were present in the E12.5/E14.5 pituitary transcriptome. Targeted sequencing of two candidate genes, *SLC20A1* and *SLC15A4*, of the solute carrier membrane transport protein family in 200 additional patients demonstrated two further variants predicted as damaging. We also found combinations of de novo (*SLC20A1/SLC15A4*) and transmitted variants (*GLI2/LHX3*) in the same individuals, leading to the full-blown CPHD phenotype. This data expands the pituitary target genes repertoire for diagnostics and further functional studies. Exome sequencing has identified a combination of rare variants in different genes that might explain incomplete penetrance in CPHD.

P-MonoG-198

Identification of the first genomic inversion in familial cerebral cavernous malformations by whole genome sequencing

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Cerebral cavernous malformations (CCMs, OMIM 116860, 603284, 603285) are autosomal dominantly inherited vascular lesions of the central nervous system that predispose to epileptic seizures and hemorrhagic stroke. Symptomatic hereditary CCMs have been estimated to have a prevalence of about 1/5400 to 1/6200 among the general population. Sequencing of the coding regions of *CCM1*, *CCM2*, and *CCM3* combined with quantitative analyses to detect copy number variations currently result in a mutation detection rate of up to 98% for familial CCM.

Standard diagnostic analyses, NGS-based screening of the entire genomic regions of *CCM1-3*, and whole exome sequencing did not reveal a causative mutation in a 12-year-old boy who presented with focal seizures, intermittent anomic aphasia, manual automatisms and two affected family members. Finally, whole genome sequencing (WGS) with an average coverage of 45x and data analyses using the Manta algorithm for detection of structural variants (Illumina®, Chen et al. Bioinformatics, 2016,32(8):1220-2) revealed a 24 kB inversion including the ATG containing exon 1 of *CCM2*. Its breakpoints were fine-mapped to the long terminal repeats MER34C_ and MER49 which may have triggered the rearrangement. Expression analyses on RNA derived from blood lymphocytes showed a significant reduction of the *CCM2* transcript to 59% when compared to healthy controls. The mother, maternal aunt and grandmother were also identified as carriers of the familial inversion. Notably, the 43-year-old mother is still asymptomatic highlighting the importance of considering incomplete CCM penetrance. PCR-based screening of 19 sporadic mutation-negative CCM cases with wildtype and allele-specific primers did not reveal further carriers of this specific inversion. Our data expand the spectrum of *CCM* mutations and indicate that the existence of a fourth *CCM* disease gene is rather unlikely.

P-MonoG-199

Functional characterization of homozygous frame shift variant c.19_20del in Exon 1 of ATP7B in a patient without Wilson's disease symptoms

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Case report: Wilson's disease (WD) is an autosomal recessive disease of copper excess due to pathogenic variants in the *ATP7B* gene coding for a copper-transporting ATPase of 157 kDa. We present a 5-year-old girl with homozygous frame shift variant c.19_20del in exon 1 of *ATP7B* leading to a premature stop codon (PTC) in exon 2. The variant was detected as secondary finding in whole exome sequencing (WES). Primary indication for WES was chronic asthma, visual impairment and progressive hearing loss. The girl presented without WD symptoms and without abnormalities in liver biopsy.

Background: Interestingly, Loudianos et al. described an asymptomatic 45-year-old man carrying the same frame shift variant compound heterozygous with a known pathogenic variant. This suggests no or only a mild pathogenic effect of c.19_20del. Nonsense or frame shift variants normally lead to nonsense-mediated mRNA decay (NMD), a surveillance function degrading PTC-containing mRNA to prevent production of truncated proteins. NMD is harnessing splice junctions to identify PTCs. Bypassing of NMD occurs if a PTC is less than 50 bp upstream of a junction or if translation reinitiation takes place before the next splice junction is reached. The latter is often observed when the PTC is localized in the first 200 bp downstream of the start codon. This holds true for c.19_20del.

Methods: To verify this hypothesis for c.19_20del, we investigated patient's *ATP7B* expression in liver compared to healthy controls and to patients with other nonsense variants by qPCR and northern blot. *ATP7B* 5'UTR as well as intron 2 were cloned into the *ATP7B* open reading frame in a pcDNA3 expression vector. We introduced c.19_20del by site directed mutagenesis as well as nonsense variant c.915T>A, also localized in exon 2, as control, and overexpressed the constructs in HEK293T cells. We analyzed ATP7B expression by qPCR, northern and western blot.

Results: *ATP7B* liver expression of c.19_20del patient was similar to healthy controls and significantly higher than those of patients with other nonsense variants. In contrast, northern blot and qPCR of transfected

HEK293T cells revealed reduced *ATP7B* expression for c.19_20del samples compared to wild-type (WT). However, control c.915T>A showed even more reduction. Western blot analysis of transfected HEK293T revealed that c.19_20del results in an ATP7B protein of about 150 kDa (albeit in less amount compared to WT), whereas for control c.915T>A no band was detectable.

Conclusions: Our results indicate that c.19_20del in *ATP7B* is able to bypass NMD by translation reinitiation which may explain the lacking WD phenotype. Several downstream in-frame start codons exist and due to six N-terminal copper binding motifs a truncated N-terminus may retain partial activity. To further complement our results, we plan to determine copper transport activity as well as the exact mass of the expressed proteins.

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P-MonoG-200

Mutations in SEMA3A are not a common disease modifying factor in CHARGE syndrome.

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CHARGE syndrome is a complex congenital disorder caused by heterozygous loss of function mutations in the chromatin remodeler CHD7. The clinical phenotype is highly variable suggesting additional modifying factors. Recently, we could demonstrate that CHD7 regulates SEMA3A, a gene involved in the pathogenesis of Kallmann syndrome. Kallmann syndrome is a heterogeneous disorder characterized by the combination of hypogonadotropic hypogonadism and anosmia, two features which are also frequently observed in CHARGE syndrome patients. To analyze if in addition to pathogenic CHD7 mutations SEMA3A variants can modify the CHARGE phenotype, we screened 32 CHD7-positive patients for SEMA3A mutations. In one patient we could identify the non-synonymous variant c.2002A>G (p.I668V) in SEMA3A in a heterozygous state. Further functional analysis using HEK293 cells and the Xenopus laevis system were carried out for this variant together with the recently in a Kallmann syndrome patient described SEMA3A mutation, c.196C>T (p.R66W). In the HEK293 cell system the variant c.2002A>G (p.I668V) shows no secretion or processing defect, while the variant c.196C>T (p.R66W) caused a secretion defect. CHD7 loss of function in the Xenopus system can be partially rescued by overexpression of the human SEMA3A wildtype or p.I668V variant but not the p.R66W variant, demonstrating a pathogenic effect of the p.R66W variant, while the p.I668V variant seems to be benign. Our data suggest that variants in SEMA3A are not a common disease modifying factor in CHARGE syndrome.

P-MonoG-201

Detection of mosaic NRAS mutation c.181C>A; p.Gln61Lys in Neurocutaneous melanosis with Encephalocraniocutaneous lipomatosis

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Neurocutaneous melanosis (NCMS, MIM #249400) and Encephalocraniocutaneus lipomatosis (ECCL, MIM #613001) including oculoectodermal syndrome (OES MIM %600268) which has been discussed as a mild variant of ECCL are rare non-inherited congenital disorders that have been shown recently to result from postzygotic mutation of the genes NRAS (CMNS) and KRAS or FGFR1 (ECCL,OES), respectively. Previously, overlapping phenotypes between NCMS and ECCL were observed in only two patients reported. However, mutation analysis was not performed. We describe for the first time molecular analysis of a patient with an overlapping NCMS-ECCL phenotype. The female patient presented with a giant congenital melanocytic nevus (CMN) extending over the neck to the lower back and multiple smaller satellite nevi. On the left temporal side of the scalp a tumour suspicious of a lipoma was detected that was covered by a mixed nevus of CMN and a histologically confirmed nevus sebaceous. MRI showed lipomas on both cerebellopontine angles. The patient suffered from seizures. Opthamological examination demonstrated a lateral epibular dermoid at the left eye.

Mutation analyses of the genes NRAS, KRAS and FRGR1 for hotspot mutations was performed on multiple tissue samples obtained from the patient by conventional Sanger sequencing. This revealed the known NRAS mutation c.181C>A; p.Gln61Lys at varying degrees ranging from 9-33%. To increase the detection limit of Sanger sequencing for mutations in target genes a TruSeq Custom Amplicon Low Input Library Prep Kit (Illumina) was applied and sequencing was performed on a Next-generation sequencing platform (MiSeq, Illumina). Target regions showed a read depth of 1000 - >5000 reads. Sequence data were further analysed by Variant Studio Data Analysis Software v3.0 (Illumina). This confirmed mosaicism for NRAS mutation c.181C>A; p.Gln61Lys in selected samples. However, hotspot mutations in KRAS and FGFR1 previously demonstrated for ECCL phenotype were not observed at a detection limit as low as 1% and no other somatic mutation in the panel genes was consistently detected. These results suggest that the NCMS-ECCL phenotype in the patient is most likely caused by a somatic mutation c.181C>A; p.Gln61Lys in the NRAS gene. This observation documents the clinical overlap between mosaic RASopathies and demonstrates that the ECCL phenotype may also be caused by mutations in genes other than KRAS and FGFR1.

P-MonoG-202

Mutation analysis of a candidate gene for male infertility due to meiotic arrest

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Introduction Infertility is a wide spread problem for couples wishing to have children. In 20-70% of these cases, male infertility is the cause for not being able to get pregnant. Azoospermia is a form of male infertility that affects approximately 1% of all males and 10-15% of infertile. Already established genetic causes of azoospermia are chromosomal aberrations and Y-chromosomal AZF deletions. However, only very few monogenetic causes have been elucidated so far. Recently, mutations in *STAG3* have been identified as autosomal-recessive cause for primary ovarian failure (POF). *STAG3* encodes a protein crucial for the stability of meiotic cohesin complexes. Because *STAG3* knockout mice develop azoospermia, this gene is also a potential candidate for male infertility due to meiotic arrest.

Patients and methods Twenty-seven male patients from the Centre of Reproductive Medicine and Andrology (CeRA) of the University Clinic Münster with non-obstructive azoospermia were included. Other aetiologies of the azoospermia were excluded in advance. All patients underwent testicular biopsy, which demonstrated complete bilateral meiotic arrest. The full coding region of *STAG3* (NM_012447.3) was in part evaluated from whole-exome-sequencing data or analysed by Sanger sequencing. Variants were assessed by several *in silico* algorithms.

Results In our cohort of 27 patients we found one patient with two likely pathogenic variants in exon 13 of the *STAG3* gene. The first variant is a nonsense mutation c.1312C>T that introduces a premature stop codon (p.Arg438Ter). The second variant is a missense mutation c.1262T>G (p.Leu421Arg), that has not been described in public databases. This substitution changes a highly conserved aminoacid and is predicted to be disease causing by several prediction programmes (PolyPhen, SIFT, MutationTaster). From the evaluation of the NGS raw data, it becomes clear that both variants are present in compound heterozygosity. Genetic testing of the parents and other family members is on the way to confirm this.

Conclusions Based on our results we propose that homozygous or compound-heterozygous mutations in *STAG3* are causing autosomal-recessive male infertility due to meiotic arrest.

This work was carried out within the frame of the DFG Clinical Research Unit ‚Male Germ Cells: from Genes to Function' (CRU 326).

P-MonoG-203

Mutational Spectrum and Functional Relevance of the Adhesion Receptor ADGRG2 in X-Linked CBAVD

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In most men with obstructive azoospermia caused by a congenital bilateral absence of the vas deferens (CBAVD), mutations are identified in the cystic fibrosis transmembrane conductance regulator gene (CFTR). Comprehensive CFTR analysis by sequencing and screening for rearrangements allows a mutation detection rate of about 80% in CBAVD patients. Thus, despite extensive testing, the origin of the CBAVD remains unknown for a considerable portion of cases. This could be attributable to the presence of additional genetic

causes. Recently, loss-of-function mutations were described in the adhesion G protein-coupled receptor ADGRG2 (Adhesion G Protein-Coupled Receptor G2). We performed next generation sequencing of all 29 exons of ADGRG2 in our well-phenotyped cohort of CBAVD patients (25 subjects) and identified previously unknown deleterious ADGRG2 variants (protein-truncating mutation: c.2033_2035delins17 p.(Val678Aspfs*42) and missense mutation: c.2441G>A p.(Arg814Gln)). This widened the spectrum of pathogenic mutations in ADGRG2, as the original publication focused on loss-of-function mutations exclusively. We estimate the prevalence of X-Linked CBAVD and the functional annotation of identified variants allows further risk assessment in patients. This established ADGRG2 as a novel key-player in the male reproductive system. Sequencing and functional analysis are ongoing to identify and characterize additional ADGRG2 variants. Further mechanistic studies could focus on ADGRG2 ligand discovery and elucidation of G protein-coupled receptor signaling to benefit the understanding of the molecular mechanism of X-linked CBAVD.

This work was carried out within the frame of the DFG Clinical Research Unit ‚Male Germ Cells: from Genes to Function' (CRU 326).

P-MonoG-204

Using CRISPR/Cas9 mediated genome editing to obtain DYNC2LI1 deficient cell lines

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The primary cilium is a single, immobile organelle present on almost all vertebrate cells. It is composed of the axoneme and the basal body. Mutations in ciliary proteins are involved in formation, maintenance and function of the primary cilium and associated with developmental defects, like brain malformations, polydactyly, kidney cysts and skeletal abnormalities. This phenotypic spectrum is present among patients with short rib-polydactyly syndromes (SRPS). This group constitutes the most frequent lethal autosomal recessive osteochondrodysplasias.

In previous studies, we demonstrated that diallelic mutations in *NEK1*, encoding for a basal body protein, are the underlying cause of SRPS II. In a yeast two-hybrid screen we identified DYNC2LI1 as interaction partner and reported the first mutations in *DYNC2LI1* in a family with short-rib thoracic dysplasia. *DYNC2LI1* is ubiquitously expressed in adult and fetal tissues, with the highest expression levels in chondrocytes. The protein is a part of the dynein-2 complex, involved in the retrograde intraflagellar transport (IFT). We were able to demonstrate that defects of *DYNC2LI1* lead to significantly reduced cilia length and altered ciliary morphology with broadened ciliary tips, confirming the retrograde IFT defect. As defects in other members of the retrograde IFT (DYNC2H1, WDR34, WDR60) have been identified in patients with overlapping clinical features, we aimed to identify potential overlapping ciliary transduction pathways.

We now used CRISPR/Cas9 mediated genomic editing to introduce disrupting mutations in exon 3, 5 and 12 of *DYNC2LI1* in HEK293T cells. Exons 3 and 5 code for the dynein light intermediate chain, which includes a predicted P-loop containing nucleoside triphosphate hydrolase domain, whereas exon 12 encodes for a part of the coiled-coil domain. The latter is involved in protein interaction and dimerization. After successful transfection of the CRISPR/Cas9-Vector, cells were separated via FACS and sequenced to detect NHEJ-based mutations. 84 % of the retrieved cell lines presented with a mutation. 54 % of these are predicted to disrupt protein function (frameshift mutations) on at least one allele. For each exon two cell lines with frameshift mutations were selected for further analysis.

Based on the previous observation, mutations in *DYNC2LI1* are proposed to lead to a loss of function of the protein. With these CRISPR/Cas9 edited cell lines we aim to mimic the functional loss of DYNC2LI1. After confirmation of the expression defect and consecutive protein loss in real-time qPCR and Western Blot experiments, we intent to perform genome wide expression analysis (RNASeq) and compare these results with the expression profile of other SRPS genes. In order to perform cluster analysis of ciliated and non-ciliated cells, transcriptome analysis will now be performed by single cell sequencing.

P-MonoG-205

PDE6C is a minor gene for achromatopsia as determined by an amplicon based targeted resequencing approach

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Achromatopsia (ACHM) is a rare autosomal recessive cone disorder characterized by color vision defects, photophobia, nystagmus, and severely reduced visual acuity. To date, six genes have been linked to ACHM. Approximately 80% of the patients carry mutations in the genes CNGA3 and CNGB3 encoding the two subunits of the cone photoreceptor cyclic nucleotide-gated (CNG) channel. Much less frequently, causative mutations have been found in other genes encoding crucial components of the cone phototransduction cascade, namely GNAT2, PDE6C, and PDE6H, and in ATF6, which is not involved in the phototransduction cascade, but in the unfolded protein response pathway.

Here, we present the results of a study comprising 174 genetically pre-selected patients who remained unsolved after Sanger sequencing of the most frequent genes accounting for ACHM, namely CNGA3, CNGB3 and GNAT2, in a large cohort of families clinically diagnosed with achromatopsia. These 174 patients were screened for variants in the PDE6C gene using an amplicon-based next generation sequencing (NGS) approach. Target enrichment was performed using the Fluidigm 48.48 Access Array™. NGS was performed using the Nextera XT DNA Library Prep Kit from Illumina. The purified combined libraries were sequenced using the MiSeq instrument from Illumina. All putative disease-associated variants identified by our NGS approach were validated and tested for segregation with the phenotype in available family members by Sanger sequencing. We were able to identify potentially pathogenic biallelic variants in 13 cases, thereby achieving a detection rate of 7.5% .The mutation spectrum comprises 18 different alleles, 15 of which are novel. The Human Gene Mutation Database currently lists 38 variants in PDE6C that explain the disease phenotype in the respective patients. Our study significantly contributes to the mutation spectrum of PDE6C and allows for a realistic estimate of the prevalence of PDE6C mutations in ACHM since our entire ACHM cohort comprises 1074 independent families. Considering a prevalence of 1:30.00 to 1:50.000 for ACHM, this number is certainly high enough to give a comprehensive view on the spectrum and prevalence not only of the more common CNGB3 and CNGA3 mutations, but also on the four non-CNG channel encoding ACHM genes. Taking together the results of the present study and previous screening results, we calculate a prevalence of 2% for PDE6C mutations in our cohort which is most probably representative for ACHM in general.

P-MonoG-206

Rare heterozygous SPG7 variants in patients with amyotrophic lateral sclerosis: a genetic link with hereditary spastic paraplegia

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Amyotrophic lateral sclerosis (ALS) is the most common adult-onset motor neuron disease (MND). It is characterized by rapidly progressive degeneration of upper and lower motor neurons in the brain and spinal cord, resulting in paralysis and ultimately death due to respiratory failure within 3-5 years. MNDs also comprise the large heterogeneous group of hereditary spastic paraplegias (HSP). In HSP, selective degeneration of axons of the corticospinal tract occurs, thus the upper motor neurons are affected, leading to slowly progressive lower limb spasticity and weakness. Apart from the significant overlap in clinical characteristics between MNDs, shared molecular pathomechanisms can be assumed because several genes have been found mutated in both ALS and HSP patients, such as *ALS2* and *SPG11*. In 2016, heterozygous variants in

the *SPG7*-gene, biallelically mutated in around 5% of HSPs, were identified in four of 80 German ALS patients (Krüger et al. 2016, Front Mol Neurosci). Paraplegin, encoded by *SPG7*, belongs to a family of ATPases and localizes to mitochondria. Paraplegin-deficient mice develop mitochondrial morphological abnormalities and swelling of spinal and peripheral axons due to an accumulation of organelles and neurofilaments. Here, by whole exome sequencing of 20 sporadic ALS patients, we identified rare heterozygous *SPG7* variants in two cases, both located within the AAA+ domain encoded by exons 7-13. The AAA+ domain of paraplegin links ATP hydrolysis to protein remodeling and was shown to be crucial for proper protein function. Therefore, we performed targeted sequencing of exons 7-13 of *SPG7* in a cohort of over 200 ALS patients, mainly of central European origin. All patients had been analyzed by detailed clinical, electrophysiological, and neuroradiological characterization and had undergone at least one clinical follow-up examination. Using all sequencing approaches, we identified a total of five rare heterozygous variants, i.e. four missense and one essential splice site variant, predicted to be deleterious in the AAA+ domain of 9 ALS patients, one of which had already been associated with ALS by Krüger et al. None of these patients carried an additional *SPG7* mutation, providing evidence for a potential dominant effect of some variants in ALS. Genetic analyses will be followed by reverse phenotyping efforts in *SPG7* mutation carriers to explore genotype-phenotype relationships with respect to common HSP features. In conclusion, our preliminary data demonstrate the presence of rare heterozygous *SPG7* variants in ALS patients of mainly central European origin. Comparing the phenotypic variability of *SPG7*-mutation to non-mutation carrying ALS patients may help to confirm the genetic link between ALS and HSP and to identify distinct disease subgroups, prospectively with relevance for more specific diagnostic and therapeutic approaches.

P-MonoG-207

MID1 overexpression increases migration velocity

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Mutations in the *MID1* gene cause the rare X-linked monogenic disorder Opitz BBB/G syndrome (OS) (Quaderi et al., 1997). The disease is characterized by malformations of the ventral midline, including hypertelorism, hypospadias, heart defects, structural brain abnormalities and/or cleft lip/palate. Additionally, some patients suffer from intellectual disability and/or developmental delay. OS belongs to the neurocristopathies, but the exact role of the gene product, the MID1 protein, in neural crest cells is still unknown. Ubiquitously expressed, the microtubule-associated MID1 protein is a ubiquitin ligase, that activates mTOR signaling by degrading its antagonist PP2A (Trockenbacher et al., 2001, Liu et al., 2011). The mTOR pathway plays an important role in cell growth and proliferation.

So far, numerous mutations in the *MID1* gene have been identified and there is evidence for both, loss- and gain-of-function as a result of different mutation types. Since MID1 is believed to be involved in neural crest cell migration and epithelial-mesenchymal transition and regulates mTOR signalling, it can be speculated that migration and proliferation behavior of cells changes after influencing MID1 expression.

We show here, that surprisingly neither overexpression (OE) nor knockdown (KD) of *MID1* in HeLa cells did affect proliferation rates. By contrast, velocity of migration was clearly increased after OE of *MID1*. However surprisingly KD did not show any significant differences and no significant effect on neuronal migration was seen in vivo after in utero electroporating MID1 specific shRNAs into E13 mouse embryos to decrease *MID1* expression during embryogenesis. These results are supported by the analysis of a male OS patient fibroblast cell line that carries a four base pair deletion in exon 9 of the *MID1* gene. No change of migration properties was detected.

Taken together, other than predicted by known functions of the MID1 protein, KD or loss of function of MID1 does not cause clear defects in cell proliferation and migration. Solely overexpression of MID1 influences cell migration. In a subsequent series of experiments we will (i) look for effects of MID1 overexpression on neuronal development and migration *in vivo* after *in utero* electroporation and (ii) will use cell lines with *MID2* deletions to look for effects of *MID1* KD. MID2 is highly homologues to MID1 and is likely to compensate for MID1 function in a variety of cell lines and tissues. We hypothesize that only in a small number of cells MID2 cannot fully compensate MID1, which might explain the very specific phenotype of OS.

P-TECHNOLOGY AND BIOINFORMATICS

P-Techno-208

Evaluation of small copy number variant analysis based on NGS data of panel diagnostic

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Introduction and objectives: Next generation sequencing (NGS) is widely accepted in routine diagnostics for detecting changes in DNA sequence. However, copy number variant (CNV) detection based on NGS data is still considered as an exploratory add-on, but not as a routine method. To address this point, we evaluated sensitivity, specificity and added value of CNV analysis of NGS data in a routine gene panel diagnostic setting.

Materials and Methods: We use TruSight One (TSO) from Illumina as an enriching kit (4813 genes, ca. 11 Mb). Sequencing is performed on the NextSeq550. Raw data is processed by the Varfeed software and annotation and evaluation of the identified variants are performed using Varvis software (Limbus Medical Technologies, Rostock). Analyzed samples include all sent samples to our molecular genetic lab. Here, we present the data of the first 60 patients.

Results: In 10 patients, we identified deletions in genes with relevance to the phenotype. Validation using MLPA or qPCR is ongoing. For 12 patients we had additional trio exome data that we have used to identify 62 segregating deleted target regions. To evaluate sensitivity, we compared these 62 deletions with the corresponding TSO target regions of the patients. Fifty deletions were equally recognized, 2 were false negative and 10 could not be interpreted due to fluctuation of coverage in the reference cohort. Using the current quality thresholds we defined the sensitivity as 81%. To characterize specificity of the CNV analysis, we compared the targeted regions in TSO with high quality exome data. This revealed a specificity of 99.96% and a positive predictive value (PPV) of 20%. Subsequent adjusting of the actual thresholds enabled a PPV of 51%.

Discussion: The evaluation of CNVs is essential in genetic diagnosis. Sequencing cannot detect variants that are larger than 20bp. Array is blind for variants smaller than 10kb. CNV based on NGS is the only chance to detect these variants in a large number of genes. We thus conclude that deletion analysis of NGS data should be a part of the routine diagnostics. This study includes a major weakness since the positive controls have not been validated in the wetlab, but using an only slightly different approach. Weakness of CNV analysis based on NGS data includes missing sensitivity and low PPV. The PPV of 51% is tolerable in the daily clinical life, but not optimal. Ultimate solutions are high covered and long read genome sequencing. Until this is possible, information of the NGS techniques should be exhaustively obtained.

Outlook: Encouraged by this pilot study we started analyzing the data of all our TSO samples (ca. 400) as well as comparing them with all available trio exome data (ca. 60) to get stable calculation basis. This would lead to specific thresholds for single exon deletions and enable adding CNV analysis to our routine diagnostic pipeline. Final results will be presented in the poster.

P-Techno-209

Site-specific gene expression analysis from archived human in-testinal samples by laser capture microdissection combined with nCounter analysis

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Site-specific alterations of gene expression of relevant networks within specific subregions of the gut may lead to gastrointestinal disorders, like inflammatory bowel disease (IBD) or irritable bowel syndrome (IBS). This may lead to symptoms seen in both conditions like diarrhea, constipation, impaired motility, abdominal pain and visceral hypersensitivity. In order to determine site-specific changes in gene expression within histological heterogeneous tissues, laser capture microdissection represents a feasible tool to detect gut layer-specific expression patterns. nCounter nanoString analysis (www.ncounter.uni-heidelberg.de) for detection and quantification of gene transcripts provides high sensitivity. The major advantages of the technology represent very low input material, no need of further RNA modification, no reverse transcription and amplification and reliable quantification even from compromised input material.

Full-thickness specimens of the colon were acquired from patients who underwent surgery due to pathological conditions such as colon cancer or diverticulosis and were subsequently cryo-conserved. 12µm cryo-sections were mounted on membrane-coated slides and stained with cresyl violet. Epithelium, lamina propria, submucosal and muscular layer and myenteric ganglia were isolated by optimized laser capture microdissection. Total RNA was isolated using a modified Trizol protocol and RNA quality assessed by Bioanalyzer measurement. In nCounter analysis molecular probes labeled with a specific color „barcode“ so called CodeSets are hybridized to target genes of interest and color codes for each target are counted. Subsequent nCounter analysis for site-specific markers and key molecules of pathways of interest was performed using total RNA isolated from particular tissue subregions.

Collecting ~10mm² (equals ~100,000-250,000 cells) of tissue from the epithelial layer, lamina pro-pria and myenteric ganglia provided sufficient amounts of RNA (509 µg; range 123-1814 µg) of appropriate quality (5.3; range RIN 2.5-7.2) for subsequent analysis. In contrast, ~40mm² (equals ~250,000-650,000 cells) of tissue were dissected from the less cell-rich submucosal and muscular layer. nCounter analysis revealed site-specific expression patterns of cell type specific marker genes in specific subregions of the colon.

Laser capture microdissection in combination with nCounter gene expression analysis enables site-specific, sensitive detection and quantification of mRNA from histological heterogeneous tissues.

P-Techno-210

Importance of intronic variants in unsolved whole exome sequencing genetic diagnosis

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Introduction/Background: Recognition of well-known intronic consensus sequences by the splicing machinery is the basis for the correct removal of introns from the pre-mRNA (Cooper and Mattox 1997; Hastings et al. 2001). Impairments in this process can lead to the production of aberrant mRNAs. It has been reported that a significant number of disease causing mutations either disrupts consensus splicing sequences or creates cryptic splice sites and consequently affects pre-mRNA splicing (Krawczak et al. 1992). Identifying these mutations and assessing their potential contribution to disease development is important for routine diagnostics. To assess the potential pathogenicity of splicing mutations in silico prediction tools can be used that analyze their potential effects on splicing (Houdayer et al. 2012). Here, we re-analyzed whole exome sequencing data of unsolved cases for the presence of intronic mutations potentially affecting splicing.

Patients and Methods: Fifty-one cases of unsolved whole exome sequencing data that had been sequenced at the Institute of Human Genetics at the University Medical Center Mainz were re-annotated by introducing a 250 bp padding size around each exon using GATK (McKenna et al. 2010) and analyzed using Alamut Software (Interactive Biosoftware). Only rare variants (ExAC allele frequencies < 5%) were further analyzed with the combination of splice site prediction tools MaxEntScan (Yeo and Burge 2004) and SpliceSiteFinder (Shapiro et al. 1987; Zhang et al. 1998) with a mutant score reduction of 15% and 5% (as recommended by Houdayer et al.) to reach a specifity of 83% and sensitivity of 96% (Houdayer et al. 2012). After these filtering steps a downstream analysis of the remaining intronic variants was performed.

Results: A total of 1342 splice site variants were found with a median of 26 per patient (range: 12 to 46 variants per patient). Of these a total of 698 variants were found each in at least two unrelated patients and were therefore considered as polymorphisms and discarded from further analysis. Another 637 variants were eliminated from further analysis due to unmatched inheritance pattern to the disease and/or patient’s phenotypes. After this removal seven different intronic variants (found in 6 patients) remained, including five variants predicting an exon skipping. Three out of these seven mutations had already been reported before this study to be pathogenic. Currently, we are validating the identified mutations by Sanger sequencing and carrying out functional studies to verify their effects on splicing.

Conclusions: Our findings not only show the underestimated importance of intronic variants in clinical diagnostics, but also the possibility to increase the diagnostic rate of whole exome sequencing by 10%. Furthermore, this finding highlights the importance of integrating intronic splice site prediction in the routine clinical diagnostics pipeline.

P-Techno-211

Establishing of the Agilent SureSelectQXt target enrichment protocol on a TECAN Freedom EVO NGS

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Background Targeted enrichment gene panels reduces the costs and enhances the efficiency of next generation sequencing (NGS) for identification and analysis of variations in specific regions of the genome as thus presents a helpful tool especially in clinical genomic research. SureSelect^{QXT} Target Enrichment is using a shearing-free, transposase-based library preparation protocol in combination with a quick hybridization technology. The workflow enables single molecule resolution of variants with a streamlined one day workflow starting with only 50 ng sample input, allowing analysis of a wide range of sample types including those with limited availability. This poster describes the automated protocol for preparation of 1-48 sequencing-ready DNA libraries using the SureSelect^{QXT} Target Enrichment for Illumina Multiplexed Sequencing System on the Freedom EVO NGS workstation.

Methods Library preparation for Illumina sequencing was performed using 50 ng of nine random anonymized DNA samples with the Agilent SureSelect^{QXT} Target Enrichment Kit and a custom designed Agilent SureSelect panel of 34 breast and ovary cancer associated genes including BRCA1, BRCA2, CHEK2, RAD51C, RAD51D, PALB2, TP53, CDH1, NBN, ATM, following the manufacturer's recommended protocol. To determine the quality and reproducibility of the automated procedure, libraries were prepared from eight replicates of sample A as well as from eight individual samples using the Freedom EVO NGS workstation. Libraries were analyzed using an Illumina NextSeq 500. Alignment was calculated with BWA v0.7.16a (mem -M) using GRCh37.75 as reference genome. Calibration was performed using GATK v3.8 according to best practice recommendations. Library validation and quality control was performed using a 2200 Tape Station.

Results The highly homogeneous sequencing data for eight repetitions of sample A demonstrates the robustness of the automated library preparation on the Freedom EVO NGS workstation. Additionaly, all nine samples show a similar sequencing depth and coverage, demonstrating the robustness of the successfully established Agilent SureSelect^{QXT} protocol on a TECAN Freedom EVO NGS for the first time.

P-Techno-212

MutationDistiller - User-driven identification of disease mutations

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MutationDistiller is a web-based, user-driven tool aimed to assist human geneticists in finding disease mutations implicated in the onset of inherited diseases. It offers a wealth of options in a user-friendly interface and makes the analysis of Next Generation Sequencing data simple. Its analyses are based on biological data on disease processes and enable a combined view on a patient's genotype and their phenotype. Thus, it enables users to take every relevant piece of information into account when assessing a clinical case.

Its input options go far beyond the Human Phenotype Ontology (HPO) and include complete diagnoses, biological pathways, tissue specific expression, and gene function. We also allow to specifically exclude genes associated with selected symptoms. Additionally, users can run a virtual panel gene analysis based on Genomic England's PanelApp.

Potentially harmful variants are identified by MutationTaster, providing a deleteriousness estimate together with data on a variant's effect. MutationDistiller can handle non-synonymous as well as intragenic non-coding or synonymous variants. Moreover, it incorporates the known modes of inheritance of disease genes and the genotype of the queried variants (including compound heterozygosity).

The output assembles all relevant information in one page: A concise overview table of the most likely disease genes and variants lists crucial data, such as the variant's effect on the protein, known diseases and frequencies in ExAC and 1000G. Detailed gene and disease information as well as hyperlinks to external data are provided below. This comprehensive presentation of results allows users to draw their own conclusions on a variant's pathogenicity without any tedious collection of relevant information from scattered sources.

Visit MutationDistiller at http://mutationdistiller.org.

P-Techno-213

Including „opposite-of"-relations improves similarity calculations across phenotype ontologies

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One of the most valuable uses of phenotype ontologies is the calculation of similarity scores between the phenotype profile of patients or diseases annotated with a set of classes from an ontology. However, the hierarchical subclass structure of an ontology does not necessarily reflect all relevant aspects of the field it is modelling. This can reduce the performance of ontology-based similarity algorithms for search and retrieval.

For instance, the classes may be arranged according to anatomical criteria, but individual phenotypic features may affect anatomic entities in opposing ways. Thus, classes that represent opposing concepts may be located in close proximity in an ontology. For example, enlarged liver and small liver are grouped as subclasses under abnormal liver size. Using standard similarity measures, these would be scored as being highly similar, despite describing converse phenotypes.

Here, we describe our approach to add "opposite-of"-relations to two large phenotype ontologies, the Human Phenotype Ontology (HPO) and the Mammalian Phenotype Ontology (MPO). We show that those relations can be used to improve rankings by incorporating it in three different similarity measures. Our results support the hypothesis that structured descriptions of concepts in an ontology beyond simple subclass-of or part-of relations improve the quality of computational results based on that ontology.

P-Techno-214

Analyzing human genomes by nanopore sequencing

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Short-read sequencing technologies have several limitations. They require amplification steps and, in case of RNA, reverse transcription, which prevents detecting base modifications at their native state. Moreover, de novo genome assembly or mapping of reads to reference genomes can be complicated or even impossible using short-reads due to repeats and homologies. Of the very recently developed 3rd generation sequencing technologies, nanopore sequencing is the only technique that can generate both ultra-long reads (up to 1 million bases) and sequence nucleic acids in their native state. Although the idea of sequencing DNA via nanopores had its origins in the 1980s, it has only become feasible recently after a sequencing device from Oxford Nanopore Technologies® (ONT) reached market maturity. In nanopore sequencing, a tiny protein pore is embedded in an electrically resistant polymer membrane, and an ionic current is passed through the nanopore by setting a voltage across the membrane. When the DNA or RNA passes via a motor protein through the pore, this creates a characteristic change in the current, which provides information on the respective nucleotides in the pore. In contrast to single molecule real time sequencing (SMRT, e.g. Pacific Biosciences®), nanopore sequencing does not depend on a polymerase and allows the sequencing of native DNA and RNA. The ONT MinION sequencer was the first portable sequencing device which allowed real-time sequencing of smaller-sized genomes, however, like the first Illumina® GA systems, sequencing larger genomes such as the human genome was largely inefficient and time consuming. We will here present initial results on library preparation, sequencing and data analysis of human samples on the recently released larger ONT GridION X5 benchtop sequencer. Applying this technique to human genetics will provide insight into the yet unsequenced parts of the human genome, into structural variation, as well as base modifications of both native DNA and RNA.

P-Techno-215

Sciobase: A platform for the evaluation of variants from next-generation-sequencing experiments

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We developed Sciobase a platform to annotate, evaluate and store variants from next-generation-sequencing experiments. Variants are called using a standard GATK workflow complemented by diverse

preprocessing, quality control and visualization programs. Afterwards perl and shell scripts calculate and fetch annotations from multiple public databases and store these together with data from the run output files (e.g. vcf-files, quality reports, links to bam files) into the database. A web front-end allows the visualization and filtering of variants, the analysis of coverage profiles, the creation of reports and the design of primer oligos to validate variants by Sanger sequencing or to resequence poorly covered regions.

At the moment Sciobase contains about 5200 samples in total. These were analyzed in 400 sequencing runs on PGM, Proton, MiSeq, NextSeq and HiSeq sequencers. The used capture kits range from smaller gene panels with only a few genes over whole exome up to whole genome data.

Variants can be analyzed on a single sample basis or compared between different samples. Another module allows the analysis of pedigree data for compound heterozygous variants. A couple of search functions allows to search the database for known variants or samples by chromosomal position, gene symbol, HGVS notation or variant class and also for panels containing genes to analyze.

The database is tightly coupled with our laboratory information management system (LIMS), so information needed to analyze the raw data e.g. used barcodes or gene panels is exported from the LIMS and after the analysis completes reports including classified variants are written back.

Based on the variants stored in the database so far we identified a small set of variants able to uniquely identify samples. With this set of variants we implemented a SNPshot approach to detect sample swaps.

P-Techno-216

Nanopore sequencing: ready for the clinic?

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Background: In medical genetics, short read next generation sequencing has proven to be very useful for detecting single nucleotide variants and smaller indels. However, in a large fraction of cases no molecular diagnosis can be made despite the exhaustive usage of short read technologies for sequencing gene panels, exomes or genomes. Except for mosaicism, three types of genetic variants appear to be mainly responsible for this gap: larger indels and structural DNA variants (SVs), variants affecting RNA splicing, and epigenetic DNA modifications. Using nanopores to sequence nucleotide strands can potentially address these three types of variations. With nanopore sequencing, it is possible to sequence an individual DNA strand in the kilobase range and to simultaneously query its methylation status, or to sequence highly expressed full length mRNA transcripts (cDNA or native RNA). However several aspects, like low accuracy, the need for high quality DNA and especially the difficult handling of the large amount of raw sequencing data have prevented nanopore sequencing from becoming established in human genetics diagnostics, but the technique is rapidly evolving. Here, we describe our analysis of patient samples using the MinION sequencing device (Oxford Nanopore Technologies, ONT), addressing several types of variants relevant in human genetics.

Materials and Methods: High molecular weight DNA was used as input (1-5µg). 1D sequencing kits (ONT) were used for library preparation for whole genome sequencing SV detection or sequencing GC rich regions using long range *APOE* gene PCR fragments. Purified poly A+ mRNA from a peripheral blood sample from a patient with a known *ACVRL1* splice variant c.772+5G>A was used as input for library preparation with the Direct cDNA sequencing kit (ONT). Sequencing was performed using R9.5 flow cells on a single MinION device. Alignment was done using BWA mem, Albacore 2.0.2 was used for basecalling, and Sniffels for SV calling.

Results: Average output per MinION flow cell was 5-6 gigabases, and for DNA sequencing, the average read length was above 10 kilobases. Read length is highly dependent on the type and quality of the input nucleotide material. The average base calling accuracy was 90%, even in the highly GC rich intronic regions of the *APOE* gene. In the cDNA sequencing experiment, an aberrant *ACVRL1* transcript lacking 105 nucleotides could be further characterized.

Conclusion: Nanopore sequencing is a promising tool to address variants that escape detection with conventional short read sequencing techniques, but using high molecular DNA is mandatory, single nucleotide variant detection accuracy is still low, and data handling of this new type of data is challenging.

P-Techno-217

*** RegulationSpotter: Interpretation and annotation of extratranscriptic DNA variants

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In the quest to elucidate the causes of rare genetic disorders, high-throughput DNA sequencing techniques such as Whole Genome Sequencing (WGS) have found their way into the realm of clinical medicine and routine diagnostics, with the UK100K project as the best-known example.

Unfortunately, many tools for the evaluation of non-coding sequence alterations output scores instead of human-readable annotations. However, the comprehensible and understandable presentation of analysis results is a crucial aspect in successfully translating WGS from basic science to patient-driven applications. We think that software should allow clinicians and life scientists to actively participate in the data analysis, as their knowledge about their patients' phenotypes and biological concepts is indispensable for a meaningful, in-depth interpretation of variants.

We introduce RegulationSpotter, a web-based tool for the user-friendly annotation and interpretation of non-coding variants located outside of protein-coding transcript regions (extratranscriptic variants). Users can upload single variants or complete VCF files and limit their analysis to variants located within candidate genes, including interacting genomic elements. Additionally, coding and non-coding variants within protein-coding transcripts will automatically be analysed by MutationTaster.

RegulationSpotter handles and displays 125 different genomic features, of which we found 54 to show discriminative power. These are included in the X-score, which indicates the functional relevance of a variant's genomic region. In contrast to scores generated by related software, the X-score is not based on machine-learning to reduce the common problem of overfitting which stems from the low number of known extratranscriptic disease mutations. Instead, we selected the features and estimated their contribution based on state-of-the-art biological knowledge. We then optimised the X-score on the basis of relative risks of features to appear in either a set of 295 extratranscriptic disease mutations from HGMD and the Genomiser publication or in 8,000 common polymorphisms from the 1000 Genomes Project.

For a meaningful interpretation, we give Boolean predictions of the functional impact of each variant, which can be either 'functional' or 'non-functional'. The predictive performance of RegulationSpotter is on par with state-of-the-art software. However, our main focus lies on RegulationSpotter's convenient usability for clinicians and life scientists without IT skills. Our website provides a thorough documentation as well as a tutorial.

Try RegulationSpotter at http://www.regulationspotter.org.

P-Techno-218

The Reliable HPO

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The **Human Phenotype Ontology** has become a widely used tool for the precise 'deep' phenotyping of patients. However, it remains unclear how accurate and reproducible HPO-based phenotyping is. Different physicians put emphasis on different features, provide different detail levels, and have different opinions on the inclusion of non-cardinal symptoms. The complex structure of the ontology and the sheer amount of terms may also add some uncertainty, especially for novel users.

With **The Reliable HPO**, we strive to address these issues. We encourage physicians and biomedical researchers to 'phenotype' simulated patients according to their health records. By comparing different HPO phenotype profiles of the same patient, we aim to determine the normal divergence in the HPO-based description of a patient's symptoms and to identify missing or misleading terms.

This knowledge will help us and other groups to better estimate a realistic 'fuzziness' of symptoms when developing software that utilises the HPO, for instance for the determination of mutations in clearly described diseases. In addition, the results can and will be used for an optimisation of the names, synonyms, and descriptions of HPO terms. We include functions to suggest more appropriate names of the symptoms and new synonyms and to point out missing symptoms.

We will of course share all our findings with the community.

Please contribute to The Reliable HPO at **http://www.human-phenotype-ontology.org/reliableHPO/**

P-Techno-219

*** A mega-analysis of expression quantitative trait loci (eQTL) reveals the regulatory architecture of gene expression variation in the liver

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Purpose: Genome-wide association studies (GWAS) in late stage age-related macular degeneration (AMD) have identified at least 52 independent gene variant signals in 34 loci with genome-wide significance. Although these associated signals explain over half of the genetic variability of AMD, in most loci, the causative genetic variant is unknown. Expression quantitative trait loci (eQTL) in disease relevant tissues provide an excellent approach to shed light on association signals and hitherto on the true underlying disease mechanisms. As a proof-of-principle, we explored the contribution of AMD associated variants to liver gene expression modulation.

Methods: To this end, we extracted publicly available datasets to compute the largest eQTL analysis for liver tissue to date. Genotypes from all studies underwent standard quality control and were imputed using the 1000 Genomes reference panel. The expression values were normalized across all samples using quantile normalization and ComBat, an empirical batch correction algorithm. MatrixeQTL algorithms helped to assess the influence of genetic markers on local (cis) gene expression.

Results: In total, liver tissues from 588 individuals (with unknown AMD status) were analysed and 202,489 significant eQTL variants affecting 1,959 genes (Q-Value < 0.001) were identified. In addition, a further 101 independent (secondary) eQTL signals were identified in 93 of the 1,959 eQTL genes. Overall, the eQTL variants were highly enriched in introns and other intergenic regions as well as in likely functional RegulomeDB classes compared to non-eQTL variants. Importantly, two independent AMD associated variants are significant eQTL for two genes involved in HDL metabolism (P-Values < 8.48 x10-05). Therefore, our results reinforce the notion that high density lipoprotein (HDL) metabolism plays a role in AMD pathogenesis.

Conclusions: Taken together, by combining four different studies we propose a first regulatory landscape of gene expression in liver and provide further evidence that lipid metabolism is one of the causative pathways involved in AMD disease pathology.

P-Techno-220

Disagreement of variant allele frequencies between methods utilized for cancer mutation screening and for targeted follow-up

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Next Generation Sequencing (NGS)-based amplicon panels have entered routine genetic diagnostic procedures as a method to screen for tumor-specific variations in defined genomic regions with high coverage and adequate reliability to infer the relative proportions of cells carrying tumor-sub-clones. In the context of haematological malignancies, the frequency of pathogenic mutations identified by screening methods is re-evaluated during patient treatment to estimate the levels of remaining cancer cells as the minimal residual disease (MRD) burden. However, the concordance of allelic frequencies reported by the different methods utilized for screening and MRD follow-up is poorly characterized.

We report our observations on two recurrent mutations in exon 12 of the *ASXL1* (additional sex combs like 1, transcriptional regulator) gene, c.1900_1922del and c.1934dupG (NM_015338.5), that were identified by screening of 132 samples with the Illumina TruSight Myeloid amplicon panel. The Illumina Nextera XT DNA single-amplicon approach was used for further analyses of the mutation sites. NGS was performed on a MiSeq instrument with v3 chemistry (Illumina).

The *ASXL1* c.1900_1922del (p.Glu635ArgfsTer15) mutation was identified in four samples with variant allele frequencies (VAF) ranging from 14.0-29.6% in the Myeloid panel approach, and the deletions were clearly visible in Sanger sequencing. One sample initially screened with 22.6% VAF was re-analyzed with the Nextera XT approach on two different amplicons. Here the deletion VAFs were only 4.9% and 5.8%. The difference was also evident in visual inspection of sequence read alignments (IGV). The deletion was not detected by Nextera XT in a follow-up sample from the same individual.

The *ASXL1* c.1934dupG (p.Gly646TrpfsTer12) mutation is located in a guanine 8-mer, a sequence context that is known to be both mutation prone and difficult for NGS-based approaches. Using the Myeloid amplicon panel, we generally observed elevated unspecific levels for both c.1934delG (2.1-5.6%) and c.1934dupG (2.0-5.5%), while the positions before and after the homopolymer exhibit only low overall levels of non-reference

bases (0.2-1.2% and 0.3-1.1%). In contrast, markedly increased VAFs for the c.1934dupG mutation (19.5-39.6%) were detected in eight samples, each confirmed by Sanger sequencing. Re-analysis of one sample with Nextera XT revealed a VAF of only 14.9% instead of 36.4%, with the difference also evident in visual inspection.

In summary, single amplicon analysis with the Nextera XT approach cannot reliably detect all classes of mutations and should be used cautiously in the context of MRD. We recommend the re-analysis of the originally screened sample as mandatory practice to establish the sensitivity for the specific target mutation. Although *ASXL1* c.1934G is in a homopolymer site with significant levels of delG or dupG artifacts, it should not be excluded from mutation analysis without regard to actual allele frequencies.

P-Techno-221

*** Targeted resequencing of non-coding risk loci for complex traits using single molecule molecular inversion probes (smMIPs)

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The genetic etiology of complex traits is largely associated with common risk variants that are located in non-coding regions. Resequencing of those regions might help to identify putative causative, rare variants in individual families. However, this is challenging for two main reasons: (i) the associated regions are often very large; and (ii) large cohorts of cases and controls are required. This results in high sequencing costs. In this project, we suggest to address these issues by applying the single molecule molecular inversion probes (smMIPs) method.

SmMIPs are 75 bp-long molecules composed of two region-specific oligonucleotide primers that are linked by a common backbone sequence. Upon contact with genomic DNA, smMIPs form a circular DNA fragment around the target region. Barcoded primers enable multiplexing of up to 384 samples, and unique molecular tags in the smMIPs backbone can be used to reduce PCR artifacts. Our general pipeline includes smMIP-design by MIPGEN, read alignment with BWA and variant calling with UnifiedGenotyper.

Our first smMIPs-approach for non-coding regions aims at characterizing non-coding susceptibility regions for non-syndromic cleft lip with/without cleft palate (nsCL/P). So far, genetic studies (mainly GWAS) have identified 40 risk loci associated with nsCL/P, and a substantial number of additional ones with suggestive significance. We selected 19 regions at four of those non-coding loci for resequencing in 1,061 cases and 1,591 matched controls (80% of the samples completed). Our downstream analysis will be based on MAF and annotation scores such as CADD, FATHMM-MKL, and LINSIGHT.

To investigate the general applicability of smMIPs in complex trait analysis and further reductions in costs, we also tested different smMIPs-options in our study. The use of different Illumina sequencing platforms revealed high-quality results on both MiSeq and HiSeq2500 RapidMode. By contrast, the HiSeq2500 HighOutput mode v4 consistently yielded reduced data output. We also performed coverage simulations to further improve cost-efficiency, demonstrating that a decrease from ca. 700x to 350x does not lead to a significant loss of detectable variants. Also, the insert size of our smMIPs (currently about 150 bp) was increased by computational approaches, suggesting that larger insert sizes should be possible, but this will have to be proven experimentally. Finally, we also assessed whether genotyping and resequencing can be confidentially combined in one smMIPs assay, and tested the concordance between genotype information obtained by array genotyping and smMIPs genotyping. For three analyzed common SNPs in 1,400 samples, concordance rate was 100% after filtering out low-quality smMIPs variant calls. Our results indicate that smMIPs resequencing is a powerful tool for targeted analysis of candidate sequences beyond the protein-coding regions, in large samples, in a cost-efficient way.

P-Techno-222

*** Integration of large-scale genetic and functional data sets in craniofacial development

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In complex trait genetics, numerous risk loci that are associated with specific phenotypes have been identified in the last years thanks to genome-wide association studies (GWASs). One major finding of these GWAS is that up to 95% of the identified risk variants are located in non-coding regions, and it has been hypothesized that they confer risks via modification of the “regulatory landscape” of the genome. Consistent with this finding, at least 38 of the 40 risk loci associated with nonsyndromic cleft lip with or without cleft palate (nsCL/P) are located in non-coding regions. As next step it is now important to understand how this variation in non-coding regions contributes to phenotypes, and numerous efforts that aim at integrating functional, cell-type specific data are currently underway. In particular, these studies focus on functionally-relevant *cis*-regulatory modules (CRMs) that are characterized by 1) open chromatin structures that enable the access of transcription factors (TFs) and 2) epigenetic modifications - such as histone acetylation and methylation - which may activate or silence specific CRMs. These datasets are generated via chromatin immunoprecipitation followed by sequencing (ChIP-seq) in relevant cell-types. For nsCL/P, those analyses are particularly challenging since the relevant biological processes occur during early embryonic development, and access to relevant human tissue for follow-up analyses is therefore limited.

In the present study we aim at elucidating the functional architecture of risk loci associated with nsCL/P using a comprehensive data integration-approach that includes ChIP-seq data from different sources as well as GWAS findings. We re-analysed published ChIP-seq data sets from human embryonic craniofacial tissue (Carnegie stages (CS) 13-17) and human neural crest cells, in a joint pipeline of peak calling, chromatin segmentation and epigenetic imputation based on public available data from ENCODE project and Roadmap Epigenomics Consortium. Moreover, in-house ChIP-seq data will be generated for selected craniofacial TFs in human embryonic palatal mesenchyme cells, one of the rare in vitro cell models for nsCL/P. Finally, we will use these ChIP-seq datasets to integrate them with genetic results from GWAS, to 1) identify potential regulatory effects of risk variants at the known risk loci, and 2) identify potential novel risk variants. Our “functional genomics” approach will reveal new insights in regulatory processes affecting craniofacial development and will close the gap in the functional understanding and validation of genetic risk loci. As one preliminary example, we identified some risk variants in peak regions of chromatin modifications associated with active enhancer states (H3K27ac) in craniofacial tissue of CS 13. Notably, the systematic data integration strategies that are currently developed for nsCL/P can also be applied to other complex traits.

P-THERAPY FOR GENETIC DISEASES

P-Therap-223

Chemical compound screening in Best vitelliforme macular dystrophy (BVMD)

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Purpose: Human Bestrophin-1 (BEST1) is a Ca^{2+}-activated volume-sensitive chloride channel and is localized in the back of the eye 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 dystrophy (BVMD). In this study, we developed an assay targeting mutated BEST1 localization and function with an application range for high and small-scale compound screening.

Methods: To assess BEST1 channel function we developed the halide assay. Briefly, MDCKII cell lines were established, stably expressing wildtype BEST1 or BVMD-associated BEST1 mutations together with a yellow fluorescent protein (YFP)-based halide sensor. Cells were stimulated with extracellular addition of iodide known to pass the plasma membrane through anion cannels, consequently quenching YFP fluorescence intracellularly. 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. Positive test compounds were reanalyzed by immunocytochemistry and Western blot analysis and transferred to a cellular model of BVMD-patient derived human induced pluripotent stem cell RPE. In whole-cell patch clamp

recordings, the effect of candidate compounds on endogenous BVMD-BEST 1 chloride conductance was analysed.

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 stimulation. Cells expressing mutant BEST1 showed 85% of default YFP fluorescence following the identical protocol setup. Two compounds were detected enhancing halide permeability associated with an enhanced membrane expression of mutant BEST1.

Conclusion: The current study established an assay suited for high-scale compound screening to address mutant BEST1 localization and function. So far, two compounds were identified with a significant effect on relocating mutant BEST1 to the plasma membrane and stabilizing channel function. These two substances are promising candidates for future therapies.

P-Therap-224

Characterization of retinoblastoma cell lines with different genetic backgrounds

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Retinoblastoma is the most common eye tumor in childhood and originates from retinal progenitor cells. Retinoblastoma is considered to be a paradigm for biallelic loss of tumorsupressor gene RB1 but a small subgroup (<2 %) of unilateral tumors harboring MYCN amplification but no loss of RB1 (RB1+/+MYCNA) were detected. These tumors are characterized by distinct histological features and are associated with early age at diagnosis. Up to now the role of MYCN in retinoblastoma is unknown.

In this study a panel of ten retinoblastoma cell lines was investigated regarding to their MYCN amplification status (FISH analysis) and their RB1 mutational (MLPA, Sanger sequencing) as well as promoter methylation status (next-generation bisulfite amplicon sequencing). In addition, expression levels of RB1 and MYCN (qRT-PCR, Western Blot) were determined. Targeted sequencing of cancer associated genes was performed to detect further mutations. Cell lines were treated with small molecule inhibitors targeting the MYCN protein stabilizer, AURKA, or the epigenetic regulator, BRD4, to investigate response of each cell line. Antitumoral effects were analyzed using MTT-assay, BrdU ELISA, Cell Death ELISA and cell cycle analysis.

Among the tested cell lines all variations of RB1 aberrations (mutation, deletion, promoter methylation) were detected and MYCN amplification was verified in 50 % of cell lines. Based on these results, cell lines were categorized into three groups, RB1-/- MYCNnon-A, RB1-/- MYCNA, RB1+/+ MYCNA. Our data revealed that MYCN is highly expressed in all retinoblastoma cell lines and that MYCN amplification status correlated with MYCN expression level. Targeting MYCN via AURKA (MLN8237, CD532) and BRD4 (OTX015) inhibitors demonstrated that inhibition caused reduction of MYCN protein levels and decreased cell viability, proliferation and induced apoptosis. Susceptibility of cells to inhibitor treatment, however, was independent of RB1 and MYCN status.

The thorough characterization of retinoblastoma cell lines establishes them as appropriate model to investigate cellular mechanisms and to develop new therapies in all genetic retinoblastoma subtypes. Moreover, our data provide first results for MYCN as potential therapeutic target in retinoblastoma. Nevertheless, more detailed analyses are necessary to investigate the role of MYCN in retinoblastoma.

P-Therap-225

Decision-making competence regarding the possibilities of genome editing by CRISPR/Cas9

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Theoretical background

The CRISPR/Cas9 technology is proclaimed as one of the greatest innovations of the last decade. In addition to the euphoria of the versatile possibilities of this new genome engineering technique, genome editing has triggered a lively public debate as to its ethical consequences. Young people may use this technology in the future and face these ethical issues.

To facilitate participation of adolescents in public debates, the competence field of decision-making is fixed as a part of the educational standard for the natural science curriculum in Germany. In the present study we intend to investigate decision-making competences of medical students and adolescents in the context of genome editing.

Research design and sample

The Oldenburg model of decision-making competences is a structural model displaying eight sub-competences, each describing three levels. The sub-competences include perception and awareness of moral relevance as well as of sources of the own attitude, assessment, judgement, argumentation, consequence reflection, change of perspective and basic ethical knowledge with regard to a bioethical context.

Based on a paper-pencil-test with open questions on authentic ethical dilemmas, regarding genome editing for somatic or germline gene therapy for RUNX1-associated familial leukemia, the above-mentioned sub-competences are recorded in sample of 50 medical students of Hannover Medical School and 50 adolescents from advanced level of a secondary school in Oldenburg.

The responses of participants are analyzed using the structured qualitative content analysis according to Mayring (2015). When analysing the test responses, points are awarded for the dimensions of decision-making competence. The test scores allow quantitative analysis of the study results.

Results and conclusion

First results of the study show, that medical students as well as adolescents are open-minded towards genome editing in case of healing heavy diseases. Nevertheless, they have great fears about a possible misuse of this technology for creating “designer babies” or “enhanced humans”. Some students reject genome editing. They bring forward the argument that human dignity might be violated while tinkering in the DNA of embryos. Missing guarantees and experiences fortify the contra position. Most of the students advocate the genome editing, but they see clear limits for its usage. None of them agree with the usage in general.

The results of this study will give a hint if there should be further education on decision-making competence and/or on basics about the CRISPR/Cas based genome editing in school and university in future.

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