mitoNET Treffen und **Dreiländer-Kongress Mitochondriale Medizin 2019**

Deutschland – Österreich – Schweiz

Hörsaal
Universitäts-Kinderspital Zürich

Programm
Abstracts (freie Vorträge)

Mittwoch, 3. Juli 2019		**mitoNET Treffen**	
13.00 – 19.00		mitoNET Treffen	Thomas Klopstock – München

Donnerstag, 4. Juli 2019		**Dreiländer-Kongress Mitochondriale Medizin** Hauptthema: **Diagnostik**	
ab 08.00		**Registrierung** Kaffee und Gipfeli	
09.00 – 09.15	15	**Kongresseröffnung** **Grusswort**	Georg M. Stettner – Zürich Jean-Marc Nuoffer – Bern Robert Steinfeld – Zürich Matthias Baumgartner – Zürich
Diagnostik von Mitochondriopathien **Diagnostik-Guidelines**			Jean-Marc Nuoffer – Bern Holger Prokisch – München
09.15 – 09.40	25	Überblick: Biochemische Diagnostik	Jean-Marc Nuoffer – Bern
09.40 – 10.05	25	Überblick: Morphologische Diagnostik	Walter Schulz-Schaeffer – Homburg/Saar
10.05 – 10.30	25	Überblick: Genetische Diagnostik	Holger Prokisch – München
10.30 – 10.50	20	Diagnostik-Guidelines	Saskia Wortmann – München/Salzburg
10.50 – 11.00	10	Gemeinsame Diskussion	
	30	**Kaffeepause**	
Herausforderungen in der Diagnostik von Mitochondriopathien			Johannes Mayr – Salzburg Saskia Wortmann – München/Salzburg
11.30 – 11.50	20	Expected and unexpected results in investigations for mitochondrial cytopathies	Johannes A. Mayr – Salzburg
11.50 – 12.05	15	FB: Mitochondrial DNA mutation analysis from exome sequencing - a holistic approach in diagnostics of mitochondrial disease	Matias Wagner – München
12.05 – 12.20	15	FB: Quantitative Proteomics as a complementary diagnostic tool for Mendelian disorders	Robert Kopajtich – München
12.20 – 12.35	15	FB: Molecular mechanisms of mitochondrial disease: pathological and genetic studies in Mendelian disorders of mtDNA maintenance	Diana Lehmann – Ulm
12.35 – 12.50	15	FB: Mitochondriopathy in pediatric patients with unspecific neuropediatric disease	Amelie van der Ven - Hamburg
	70	**Lunch & Networking**	

Neue mitochondriale Erkrankungen, Patienten-Kohorten			Wolfgang Sperl – Salzburg Johannes A. Mayr – Salzburg
14.00 – 14.25	25	New mitochondrial diseases, an update	Johannes A. Mayr – Salzburg
14.25 – 14.40	15	FB: Delineating the phenotypic spectrum of MT-ATP6 associated disease from isolated neuropathy to infantile-onset neurodegeneration	Claudia Stendel – München
14.40 – 14.55	15	FB: Compassionate treatment of an adolescent with a m.9176TG MT-ATP6 mutation with sildenafil	Markus Schuelke – Berlin
14.55 – 15.10	15	FB: The genetic landscape of paediatric mitochondrial disease exploration of almost 2000 cases by whole exome sequencing	Sarah L. Stenton – München
15.10 – 15.25	15	FB: Reanalysis of WES-data of initially unsolved cases in patients with a suspected mitochondrial disorder	Tekla Wolstein – München
15.25 – 15.40	15	FB: Twin Sisters with same Phenotype of a Mitochondrial Disease without detectable Deletion or Mutation	Michael J. Scherrer – St. Gallen
	30	**Kaffeepause**	
Nicht-neurologische Manifestationen von Mitochondriopathien, LHON			Robert Steinfeld – Zürich Andrew Hall – Zürich
16.10 – 16.35	25	Mitochondrial dysfunction in the kidney	Andrew Hall – Zürich
16.35 – 16.50	15	FB: Expression studies of OTC in mouse liver mitochondria using naked-DNA vectors for gene therapy: internal Flag-epitope tagging and stable expression under control of an endogenous Otc promoter-enhancer	Sereina Deplazes – Zürich
16.50 – 17.10	20	Ophthalmologische Befunde bei Mitochondriopathien	Christina Gerth-Kahlert – Zürich
17.10 – 17.25	15	FB: Leber's hereditary optic neuropathy caused by rare mutations of the mitochondrial DNA clinical and genetic spectrum	Claudia B. Catarino - München
17.25 – 17.40	15	FB: Positive final readout from REVERSE Phase III clinical trial of GS010 for the treatment of Leber Hereditary Optic Neuropathy (LHON)	Magali Taiel – Paris
17.40 – 18.25	45	**Keynote Lecture** **Pharmakologie bei Mitochondriopathien**	Stefan Krähenbühl – Basel

19.00 – 23.00		**Schifffahrt auf dem Zürichsee mit festlichem Abendessen**	Abfahrt 19.30 Uhr Rückkehr 22.30 Uhr **Zürich Bürkliplatz**

Freitag, 5. Juli 2019		**Dreiländer-Kongress Mitochondriale Medizin** Hauptthemen: **Forschung, Therapie**	
ab 08.00		**Registrierung** Kaffee und Gipfeli	
09.00 – 09.45	45	**Keynote Lecture Genome editing in mitochondria – past, present and future**	Michal Minczuk – Cambridge
Grundlagenforschung, angewandte Forschung			Valerie Gailus-Durner – München Laura Kremer – Stockholm
09.45 – 10.15	30	Mouse models for mitochondrial diseases: Comprehensive phenotyping in the German Mouse Clinic	Valerie Gailus-Durner, München
10.15 – 10.40	25	Bottleneck und Selektion von mtDNA	Laura Kremer – Stockholm
10.40 – 10.55	15	FB: Mitochondrial translation requires folate-dependent tRNA methylation	Raphael J. Morscher – Zürich
10.55 – 11.10	15	FB: The Impact of Mitochondrial Bioenergetics on Metabolic Pathways by Real-time NMR of 3D Cell Cultures	Damian Hertig – Bern
	30	**Kaffeepause**	
Klinische Forschung			Michael Ristow – Zürich Hans Jung – Zürich
11.40 – 12.10	30	Mitohormesis	Michael Ristow – Zürich
12.10 – 12.30	20	Exercise effects in mitochondrial cytopathies	Hans Jung – Zürich
12.30 – 12.45	15	FB: New insights into mitochondrial complex I assembly defects	Fabian Baertling – Düsseldorf
12.45 – 13.00	15	FB: Perilipin 5 deficiency results in neurodevelopmental disorder and dysfunctional adaptive response to fasting: a case report and investigation of patient-derived iPSC-hepatocytes	Andrea Felser – Bern
	60	**Lunch & Networking**	

14.00 – 14.15	15	Auszeichnung des mitoNET mit dem **Kristall-Award der Diagnosegruppe Mitochondriale Erkrankungen (Deutsche Gesellschaft für Muskelkranke e.V.)** anlässlich des 10-jährigen Bestehens	Claus-Peter Eisenhardt – Lauffen (DGM)
14.15 – 14.50	35	**Keynote Lecture Mitochondrial optic neuropathies – state of the art**	Valerio Carelli - Bologna
Therapieansätze und klinische Studien			Felix Distelmaier - Düsseldorf Thomas Klopstock – München
14.50 – 15.10	20	Cofaktoren und Vitamine in der Behandlung von Mitochondriopathien	Felix Distelmaier – Düsseldorf
15.10 – 15.25	15	FB: Novel therapeutic strategies for inborn errors of coenzyme Q10 biosynthesis	Felix Distelmaier - Düsseldorf
15.25 – 15.45	20	Management von Schmerzen und Dystonie bei Mitochondriopathien	Sebastian Grunt - Bern
15.45 – 16.00	15	FB: MITOCHONDRIAL TRANSPORTERS – a focus on treatment	Bigna K. Bölsterli – Zürich
16.00 – 16.15	15	FB: Ketogenic diet as adjuvant therapy for melanoma	Sepideh Aminzadeh-Gohari – Salzburg
16.15 – 16.45	30	Übersicht über klinische Studien	Thomas Klopstock – München
16.45 – 17.00	15	Auszeichnung des besten freien Beitrags zum Dreiländer-Kongress mit dem **Wissenschaftspreis 2019 des Deutschen Netzwerks für mitochondriale Erkrankungen (mitoNET)**, gestiftet von Santhera Pharmaceuticals AG	Stettner + Gutachtergremium + Santhera Pharmaceuticals AG
17.00		**Abschied & Apéro**	Jean-Marc Nuoffer – Bern Robert Steinfeld – Zürich Wolfgang Sperl – Salzburg

FB: freier Beitrag

Ketogenic diet as an adjuvant therapy for melanoma

Sepideh Aminzadeh-Gohari[1], Daniela D Weber[1], René G. Feichtinger[1,2], Peter Kölblinger[3], Johann Bauer[3], Barbara Kofler[1,2], Roland Lang[3]

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Although, malignant tumors show substantial biological heterogeneity, the majority shares a remarkable and fundamental feature, which is the switch from oxidative phosphorylation (OXPHOS) to glycolysis (Warburg effect). Even in the presence of sufficient oxygen for cellular respiration, tumor cells prefer glycolysis for energy production and down-regulate OXPHOS. Based on these metabolic features, the glucose dependency combined with a decreased capacity to utilize alternative substrates by OXPHOS should render cancer cells susceptible to a high-fat, low-carbohydrate diet (ketogenic diet; KD). In addition, owing to the KD's low-carbohydrate content, the KD provokes reduction of circulating glucose, consequently lower insulin and IGF-1 levels. Insulin and IGF-1 receptor signaling pathways are significantly involved in tumorigenesis. It has been shown that a variety types of tumors with high glycolytic activity can be successfully targeted by a KD. The aim of the present study was also to determine the effect of KDs on highly glycolytic melanoma with different genetic alterations and varying OXPHOS competence.

Human BRAF/NRAS/NF1 wild-type (triple-WT) (WM3311) and BRAF V600E mutated (BRAF V600E) (WM47 and A375) melanoma xenografts were established in CD1 nu/nu mice. The melanoma xenografts-bearing mice were fed with a standard diet (SD) and different KDs (based on long-chain triglycerides only or supplemented with medium-chain triglycerides). The effects of the KDs on tumor growth, body weight, blood parameters such as β-hydroxybutyrate and glucose levels, and liver inflammatory parameters were evaluated.

As expected, KDs significantly increased the level of ketosis and decreased the blood glucose levels in melanoma bearing mice. KDs significantly decelerated the growth of triple-WT and both BRAF V600E melanoma xenografts. A375 xenograft showed lower levels of OXPHOS and mitochondria mass compared to Wm3311 and WM47 xenografts. Moreover, the long-chain triglyceride-based KD significantly increased the survival of triple-WT melanoma bearing mice compared to mice fed with SD. Previously, we have shown that KDs can be deleterious in the treatment of renal cell carcinoma by provoking a raise of interleukin-6 (IL-6) and C-reactive protein (CRP) expression in the liver. However, IL-6 and CRP were not altered in melanoma bearing xenografts by KDs compared to SD fed mice, indicating that a KD does not induce liver toxicity during the treatment of melanoma.

Taken together, our data show the KD could target melanomas, irrespective of the mutation status (BRAF V600E) or OXPHOS competence, suggesting the KD could be considered as an adjuvant therapy for a variety of melanoma subtypes.

New insights into mitochondrial complex I assembly defects

Fabian Baertling[1], Laura Sanchez-Caballero[2], Mariel AM van den Brand[2], Felix Distelmaier[1], Ertan Mayatepek[1], Jan AM Smeitink[2], Richard JT Rodenburg[2], Leo GJ Nijtmans[2]

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Inborn mitochondrial diseases affect approximately one in 5000 newborns and are most commonly associated with isolated mitochondrial respiratory chain complex I deficiency. Mutations in genes encoding components of complex I lead to complex I assembly defects. Therefore, the assembly pathway of complex I is central to the pathogenesis of inborn mitochondrial diseases. Complex I consists of 44 different subunits and can be grouped into three functional modules: the Q-, the P- and the N-module. Its assembly requires assembly factors that temporarily bind to assembly intermediates but are not part of the holo-complex.

We developed a detailed model of the entire complex I assembly pathway by combining a conditional complex I assembly model with complexome profiling. After deciphering the complex I assembly process, we performed assembly analyses via 2D-BN-PAGE/SDS-PAGE in patient fibroblasts carrying novel mutations in complex I assembly factors NDUFAF3 and NDUFAF4 and in complex I subunit NDUFA9. Blots were stained with antibodies directed against subunits representative of every functional module. The groundwork laid by our complex I assembly model was used to interpret the patients' assembly defects.

Even though NDUFAF3 and NDUFAF4 are assembly factors that physically associate with the Q-module, mutations in their genes lead to accumulation of assembly intermediates of all other functional modules except the Q-module. This demonstrates that they are required for Q-module assembly and/or intermodular connection in the final complex I assembly steps. NDUFA9 mutations cause an accumulation of assembly intermediates either of the Q-module alone or the Q- and P-module. This shows that different mutations in the same subunit gene can variably affect different complex I assembly pathway branches.

Our comprehensive complex I assembly model facilitates interpretation of assembly defects in patient fibroblasts allowing valuable insight into disease mechanisms.

MITOCHONDRIAL TRANSPORTERS – a focus on treatment

Bigna K. Bölsterli[1], Felix Distelmaier[2], Wolfgang Sperl[3], Holger Prokisch[4,5], Johannes A. Mayr[3], Saskia B. Wortmann[3,4,5]

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BACKGROUND More than 300 human disease genes associate with mitochondrial disease. Knowing the physiological base of disease guides successful personalized treatment, e.g. riboflavin supplementation for SLC52A3-deficiency or bypassing pyruvate metabolism with ketogenic diet in PDHc deficiency. We aimed to evaluate the subgroup of mitochondrial transporter deficiencies (excluding the mitochondrial protein import machinery) for treatment options.

PATIENTS AND METHODS A PubMed search yielded 19 human disorders: beside 16 members of the solute carrier 25 (SLC25) - family of transporters, these were the mitochondrial pyruvate carrier (encoded by *MPC1*), and the mitochondrial calcium uniporters 1 and 2 (*MCU1, MCU2*). Next, we queried our databases for affected individuals and details on treatment.

RESULTS The most frequent disorder was citrin deficiency/citrullinemia type II (*SLC25A13*) which is common in Asia (> 400 patients reported), but has only been reported in about 10 non-Asian families. While treatable with a low carb diet, misdiagnosing it as citrullinemia type I and consequent treatment with high i.v. glucose can lead to fatal deterioration. We here present six new non-Asian patients. In literature two patients with aspartate-glutamate carrier 1-deficiency (*SLC25A12*) have been described with severe developmental delay, muscular hypotonia, seizures and myelination deficits; in one ketogenic diet improved epilepsy and myelination. We here present four additional patients of whom three clearly improved under ketogenic diet (videos will be presented). Three families with mitochondrial pyruvate carrier deficiency (*MPC1*) have been published with severe global delay, hepatomegaly, and encephalopathy. Ketogenic diet was shown successful in a fetal mouse model. We here present two additional families and for the first time successful treatment (seizure control) in human. Beside one patient in literature no additional patients with riboflavin responsive exercise intolerance (*SLC25A32)* could be identified.

DISCUSSION AND CONCLUSION Knowing the genetic and consequently the pathophysiologic base of disease can guide successful personalized treatment. AGC1-deficiency and MPC1-deficiency should be added to the short list of biochemically based indications for ketogenic diet (PDHC-deficiency, GLUT1-deficiency).

Leber's hereditary optic neuropathy caused by rare mutations of the mitochondrial DNA: clinical and genetic spectrum

Claudia B. Catarino[1], Felice Lob2, Susanna König[2], Bettina von Livonius[2], Claudia Priglinger[2], Günther Rudolph[2], Thomas Klopstock[1]

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Background and aims: Leber's hereditary optic neuropathy (LHON) is the most common primary mitochondrial DNA disorder and frequently results in bilateral, severe central vision loss. Only 90% of LHON is explained by three primary causal mutations of the mitochondrial DNA (mtDNA).

Methods: Single-centre cross-sectional analysis of prospective cohort study. Patients seen in our outpatient clinic with clinical diagnosis of LHON and one rare causal LHON mutation were identified. All underwent complete mtDNA sequencing. Patients with rare mtDNA variants of uncertain significance (VUS) or having one other known LHON causal mutation were excluded from this analysis.

Results: In our cohort of 231 LHON patients seen in our outpatient clinic, we identified 17 LHON patients (7.4%) from 12 families with rare mtDNA mutations interpreted as causal for LHON. We found ten different rare causal mtDNA mutations, located in the genes MT-ND1, MT-ND6 and MT-ND4L. Five patients were female (29.4%). Age at onset was 6.0 to 59.0 years (mean 21.1± 15.3). Seven had clinical onset at or before 16 years old. Two female patients with childhood onset of mild to moderate visual loss could be retrospectively diagnosed in adulthood, after LHON had been diagnosed to their sons. No patient presented with additional neurological features. Clinically significant improvement was documented in six patients. Four of eight patients treated with idebenone had follow-up longer than six months; three of them showed significant clinical improvement.

Conclusion: There was phenotypical variability in age at onset, severity and prognosis. Awareness of causal rare LHON mutations is essential. By high clinical suspicion of LHON and no frequent causal mutation, complete mtDNA sequencing is warranted. Prompt confirmation of a LHON diagnosis is essential, given availability of potential disease-modifying treatment.

Expression studies of OTC in mouse liver mitochondria using naked-DNA vectors for gene therapy: internal Flag-epitope tagging and stable expression under control of an endogenous *Otc* promoter-enhancer

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Ornithine transcarbamylase deficiency (OTCD) is the most common inherited defect of the urea cycle resulting in severe hyperammonemia and death if left untreated. We are aiming at correcting OTCD using naked-DNA minicircle (MC) vector mediated gene therapy in the *spf-ash* mouse with limiting low OTC activity and a model for human OTCD. A critical parameter is delivery to periportal hepatocytes, also called metabolic zone 1, where the urea cycle is located. Functional OTC is assembled as a homotrimer in the mitochondria. To distinguish between MC-born and endogenous OTC enzyme, we generated an expression cassette with an internally Flag-tagged OTC enzyme. Internal epitope tagging is required because of the N-terminal mitochondrial import sequence. A corresponding Flag-tag sequence, flanked on both sides by two glycine residues as flexible spacers, was introduced C-terminally of the mitochondrial import signal. The tagged protein performed similar to its non-tagged OTC enzyme upon hydrodynamic tail vein injection for liver targeting in *spf-ash* mice indicating that the tag neither interferes with the mitochondrial import nor the formation of a functional OTC trimer. Furthermore, we generated an endogenous *Otc* promoter-enhancer construct, termed PmO1, for potential specific or "natural" OTC transgene expression. According to others, the promoter (672 bp) sequence derived from mouse *Otc* was not sufficient for liver specificity (Veres et al, J Biol Chem 261: 7588-7591, 1986) and requires a corresponding enhancer sequence (Nishiyori et al, J Biol Chem 269: 1323-1331, 1994). An enhancer sequence (232 bp) originating from rat *Otc* was used that contains several binding sites for liver-selective transcription factors (Murakami et al, Mol Cell Biol 10: 1180-1191, 1990). The resulting MC-vector expressing OTC from this PmO1 promoter-enhancer construct was delivered to *spf-ash* mice via hydrodynamic injection, resulting in similar enzymatic activity in whole liver extracts compared to wild-type mice. Vectors designed to express Flag-tagged OTC driven from a natural *Otc* promoter might be useful to study biodistribution and stable expression in periportal (zone 1) hepatocytes following MC-based gene therapy for OTCD.

Novel therapeutic strategies for inborn errors of coenzyme Q_{10} biosynthesis

Felix Distelmaier

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Coenzyme Q_{10} (CoQ_{10}) is a lipid-soluble compound that plays an important role during electron transport within the mitochondrial respiratory chain. The cellular demand of CoQ_{10} is mainly derived from endogenous biosynthesis. Inherited genetic defects affecting CoQ_{10} biosynthesis may cause a broad spectrum of clinical phenotypes ranging from fatal neonatal presentations to adult-onset isolated myopathy or ataxia.

CoQ_{10} biosynthesis defects are generally regarded as mitochondrial disorders with a specific treatment option (e.g. oral CoQ_{10} supplementation). However, clinical response in affected individuals is mostly unfavourable. The reasons for this unsatisfactory treatment effect are manifold and include poor bioavailability as well as ineffective penetration into the CNS compartment. An additional specific problem might be that targeting of CoQ_{10} to lipid membranes requires shuttling via the proteins COQ10A/B and it remains unclear in how far exogenous CoQ_{10} effectively enters this pathway.

In view of these problems, an alternative strategy could be the re-activation of endogenous CoQ_{10} biosynthesis in affected individuals. This might be achieved by providing metabolic intermediates that are able to circumvent the enzymatic block (e.g. CoQ precursor compounds without polyisoprenoid tail).

Perilipin 5 deficiency results in neurodevelopmental disorder and dysfunctional adaptive response to fasting: a case report and investigation of patient-derived iPSC-hepatocytes

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Background: Perilipin 5 (Plin5) is a lipid droplet coating protein that is highly expressed in oxidative tissues and plays a critical role in the regulation of intracellular lipid storage according to metabolic demand. Under fed conditions, Plin5 reduces lipolysis and sequesters fatty acids (FAs) into lipid droplets. Upon fasting, it stimulates lipases, directs FAs to mitochondrial beta-oxidation and increases transcription of important players in FA oxidation. Plin5 deficiency has so far not been associated with a disease phenotype in humans, but it is associated with cardiac, muscular and hepatic dysfunction in knockout mice.

Case study: We report a case of an adolescent who presented with congenital cerebellar ataxia and psychomotorical retardation. During the first years of life, he presented with recurrent hyperketotic acidosis (pH 7.18, total ketone bodies of 8.3 mmol/L) triggered by infections and repeated vomiting. We identified a compound heterozygous mutation in the *PLIN5* gene (p.R145C; p.P370L) predicted to be deleterious. To this day, the patient suffers from a dyskinetic-ataxic movement disorder and mental disability. However, he does not display any clinical signs of cardiac dysfunction or liver injury.

Results: To study the hepatic phenotype, we reprogrammed patient-derived skin fibroblasts to generate induced pluripotent stem cells which we differentiated into iPSC-derived hepatocytes (iPSC-heps). While Plin5 was expressed in control and patient iPSC-heps, we found an increased compensatory expression of Plin2 in the latter. After 24 hours fasting, both iPSC-hep lines were able to stimulate palmitate oxidation rate, but patient iPSC-heps failed to adequately increase PPARα signalling pathway.

Discussion: Plin5 deficiency is associated with a deficient adaptive response to fasting, which could explain the disinhibited FA oxidation and hyperketosis observed in this patient. Recent studies have identified the importance of lipid droplets and FA oxidation in neurogenesis. Further investigations are ongoing in an iPSC-derived neural cell model to study Plin5 deficiency-associated pathophysiology in the brain.

The Impact of Mitochondrial Bioenergetics on Metabolic Pathways by Real-time NMR of 3D Cell Cultures

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Background: Mitochondria are the cell's powerhouse and important signalling organelles. Dysfunctions either originates from primary genetic defects or are induced by signaling pathways, toxins or drugs. Thus, different mitopathogenic mechanisms are being revealed across all medical disciplines, highlighting the interdependence of bioenergetic function with a multitude of metabolic and signaling pathways. To our knowledge, there is no method enabling simultaneous analysis of both: mitochondrial function and metabolomic changes in living cells. Therefore we aim simultaneous real-time metabolomic and bioenergetic OXPHOS analysis of living cell culture system inside NMR. This bioreactor system may than be used for characterisation of pathomechanism and evaluation of potential treatment strategies.

Methods: We established a perfused bioreactor inside the NMR spectrometer. An HPLC pumping system is used for constant perfusion of substrates via the perfusion apparatus InsightCell™. Online metabolic profiling of 30 metabolites is performed using proton NMR of 7min acquisition per metabolomic profile. Oxygen is measured using T_1 relaxation times and cell viability was controlled by LDH release and trypan blue staining. Reproducibility was evaluated in "stop-and-go" experiments (6 cycles) over 12h. First mitochondrial stress tests were performed investigating the degradation rate of Glycolytic-, Glutamine-, Fatty acids and other pathways.

Results: We implemented a perfused bioreactor system within the NMR spectrometer, evaluated embedding methods for high cell density (20 million cells), viability (>90%), stability (up to 12h) and reproducibility of metabolic and oxymetric responses.
1. Degradation of Glycolytic metabolites, Glutamine, and Fatty acids as well as O_2-consumption was clearly dependent on perfusion rate. Switch from high to low flow rate resulted increased degradation of Glucose and Glutamine. Increase of Fatty acid content was observed simultaneously.
2. Changes of Glucose, Pyruvate and Lactate turnover remained constant over 12 hours in its direction and amplitude during stop and go experiments. This shows stability and reproducibility over 12h measurement time.
3. Sequential injection of Rotenone + Oligomycine and 2-Deoxy-Glucose resulted immediate activation and deactivation of glycolytic rate.

Discussion: Preliminary results confirm the feasibility of simultaneous assessment of bioenergetic and metabolomic data. We show sensitivity to detect substrate degradation rates of major mitochondrial fuel pathways and were able to measure cellular oxygen consumption. In a next step, we aim to investigate different types of OXPHOS deficient cell lines and the use of different mitochondrial stress tests.

Quantitative Proteomics as a complementary diagnostic tool for Mendelian disorders

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The care of rare Mendelian diseases has been revolutionized by genome sequencing. While in the past it could be a long, frustrating and often losing battle for parents with an affected child to find the cause of their child's suffering, the availability of genome sequencing has made this - at least conceptually - possible for every patient. However, across a large variety of Mendelian diseases, analysis of the coding sequence does not lead to diagnosis for 50-75% of patients. This indicates that in many cases the pathogenic variant evaded detection, was detected but remained of uncertain significance (VUS), or is involved a more complicated interaction.

We recently demonstrated the power of combining DNA and RNA sequencing to tackle unsolved WES cases. We identified 3 situations in which to prioritize candidate disease-causing genes for a rare disease. *Firstly*, genes with expression outside physical range can be identified as expression outliers. *Secondly*, RNAseq can reveal cases of allele-specific expression. Detection of mono allelic expression can help in re-prioritizing heterozygous rare variants. *Thirdly*, splicing of a gene can be affected.

Still, many pathogenic alterations cannot be seen at the RNA level, but they may affect protein folding and stability. We therefore re-investigated protein levels in fibroblast cell lines using antibodies specific for the proteins affected by pathogenic mutations and found significantly reduced levels in 93 out of 101. The large dynamic range and highly sensitive analytical capabilities of recent MS-based proteomics platforms now allows systematic, near comprehensive, quantification of the proteins expressed in cells. Hence, proteomics has the potential to be an important tool to screen for

pathogenic variants. Moreover, most proteins function in multiprotein complexes, the stability of which depends on the availability of all components. We established a protocol for quantitative deep proteome analysis using TMT-10plex labeling and Trinity fractionation combined with the MultiNotch MS3 method for peptide quantification that allowed us to quantify about 8,000 proteins. Proteomics of 120 fibroblast cell lines from patients with suspected mitochondrial disorder quantified more than 700 mitochondrial proteins including two thirds of the known mitochondrial disease with missing values of less than 1%. Proteomic data helped us to interpret the destabilizing effect of several missense VUS and small in frame deletions and insertions that did not alter transcript level. In one case we identified almost all subunits of the mitochondrial ribosome to be strongly reduced indicating a defect in mitochondrial translation. In summary, quantitative proteomics has proven to be a powerful complementary tool to genome and transcriptome sequencing. It delivers functional data supporting interpretation of VUS not only on the level of the affected protein but also on protein complexes sometimes providing insights in disease mechanisms.

Molecular mechanisms of mitochondrial disease: pathological and genetic studies in Mendelian disorders of mtDNA maintenance

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Background and aims: Mitochondrial DNA (mtDNA) deletions are an important pathological mechanism in adults with mtDNA maintenance disorders. We attempt to correlate mitochondrial genetics and respiratory chain deficiency at a single cell level in patients with mitochondrial disorders.

Methods: We used immunofluorescence to quantify mitochondrial respiratory chain deficiency in muscle biopsies from patients with mtDNA maintenance disorders (n=16). Further studies on 6/16 patients (2 *POLG*, 2 *RRM2B,* 1 *TWNK*, 1 *SLC25A4* (ANTI)) included the correlation of the biochemical deficiency with the mtDNA abnormality in individual cells, following laser microcapture and determination of the size and level of clonally-expanded mtDNA deletion within fibres by real-time PCR, long-range PCR and sequencing of breakpoints.

Results: Quadruple immunocytochemical studies show that the muscle biochemical phenotype is different in patients with multiple mtDNA deletions compared to other mtDNA mutations, but showed no difference between genotypes. Genetic analysis demonstrated major arc deletions to be more common and showed a dear correlation between deletion level and respiratory chain defiriency. We find that 62.8% of respiratory chain deficient muscle fibres contained a single deletion, 34.6% two deletions and 2.6% three deletions.

Conclusions: Patients harbouring multiple deletions have a distinct muscle respiratory chain profile, however, no genotype based pattem of respiratory chain defidency has been found. A clear correlation between the level of mtDNA deletion and extent of respiratory chain defidency within a single cell has been showed.

Mitochondrial translation requires folate-dependent tRNA methylation

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Mitochondrial co-factor utilization and protein expression have recently developed into highly dynamic fields within the study of inborn errors of mitochondrial metabolism. Here we present an unexpected link between the two fields, identifying mitochondrial folate one-carbon (1C) activation as being essential for mitochondrial translation.

The essential vitamin folate is well known for its role in the activation and transfer of 1C units for the biosynthesis of purines, thymidine and methionine. Studies on mitochondrial folate enzymes have thus focused on their support of anabolic metabolism in the cytosol of proliferating lymphocytes and human cancers. The full range of uses of folate-bound one-carbon units in the mitochondrial compartment itself, however, has not been explored. When characterizing a set of human CRISPR-deletion cell lines lacking folate 1C enzymes, we serendipitously discovered that loss of catalytic activity of the mitochondrial folate enzyme serine hydroxymethyltransferase 2 (SHMT2), but not of other folate enzymes, leads to a combined respiratory chain deficiency phenotype. We find that SHMT2, by generating mitochondrial 5,10-methylenetetrahydrofolate, provides methyl donors to produce the taurinomethyluridine base at the wobble position of select mitochondrial tRNAs. Mitochondrial ribosome profiling in SHMT2-knockout cells reveals that the lack of this modified base causes defective translation, with preferential mitochondrial ribosome stalling at certain lysine (AAG) and leucine (UUG) codons. This results in the impaired expression of respiratory chain subunits. Stalling at these specific codons also occurs in certain inborn errors of mitochondrial metabolism, either directly affecting enzymes catalyzing the modification reaction (MTO1) or mt-DNA mutations precluding tRNA modification (MT-TL1/MELAS). Disruption of whole-cell folate metabolism, by either folate deficiency or antifolate treatment, also impairs the respiratory chain.

In summary, mammalian mitochondria use folate-bound one-carbon units to methylate tRNA, and this modification is required for mitochondrial translation and thus oxidative phosphorylation.

Twin Sisters with same Phenotype of a Mitochondrial Disease without detectable Deletion or Mutation

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Medical History: Since birth, currently 25-year-old monozygotic female twins (otherwise negative family history) suffer from proximal accentuated, symmetrical muscle weakness, fatigue, exercise intolerance, mental retardation in early childhood, ptosis of both eyes and progressive external ophthalmoplegia. There are also vegetative symptoms like chronic obstipation with recurrent stomach pain and urinary retention with spontaneous recovery at the age of 20 as well as a chronic hypohidrosis. Due to frequent periods of apnea during sleep, a congenital central hypoventilation syndrome was diagnosed at the age of four, treated with a Variable Positive Airway Pressure (VPAP) device.

Diagnostics: Ophthalmological examination showed retinitis pigmentosa sine pigmento in both twins. In muscle biopsy (2014), an increased variation of fiber thickness and some lobulated fiber profiles were found. However, detailed molecular genetic analysis of the mitochondrial and nuclear genome provided no evidence of deletion or point mutation. Also, a mutation in PHOX2B gene – defining for the congenital central hypoventilation syndrome (CCHS) - was not detectable. Up to now, periodic echocardiography and electrocardiogram were normal.

Background: Monozygotic (identical) twins share the same mitochondrial DNA (mtDNA) from early life (1), but even in ‘‘identical’’ twins there can be remarkable differences in the levels of mutated mtDNA in different tissues (2). Therefore, it is possible – like in our case – that both twins are affected by a mitochondrial disease in a similar degree, but also that one of the twins develops a symptomatic mitochondrial disease whereas the other is completely asymptomatic.

Conclusion: The clinical evaluations, diagnostic findings and course of disease are highly suspicious for the same mitochondrial disease (such as CPEO plus or Kearns-Sayre syndrome) in both twins, even if we couldn’t find a causal deletion or mutation yet. Due to negative family history and clinical findings, a sporadic respectively new deletion (or mutation) of mtDNA is most likely, shared as twins in the same oocyte before development of the embryos. No relation between the congenital central hypoventilation syndrome and the mitochondrial disease was described until now.

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Compassionate treatment of an adolescent with a m.9176T>G *MT-ATP6* mutation with sildenafil

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The lack of animal models for Leigh syndrome due to *MT-ATP6* mutations led us to develop an experimental system of patient iPSC derived neuronal precursor cells (NPCs), in which we were able to demonstrate a biochemical effect. Affected NPCs exhibited defective ATP production, an abnormally high mitochondrial membrane potential (MMP), as well as altered calcium homeostasis. We used these cells to screen for FDA-approved compounds and discovered the PDE5 inhibitor avanafil to partially rescue the calcium defect.

A 15-year-old boy was known to suffer from ataxia and a combined motor-sensory neuropathy. During a febrile episode he drifted into catabolic state with lactic acidosis, severe cardiomyopathy, encephalopathy, epileptic seizures, and profound muscle weakness. He was not able to lift his arms against gravity. Respiratory failure secondary to diaphragmatic weakness required continuous mechanical ventilation. Molecular diagnostics revealed a homoplasmic *MTATP6* m.9176T>G mutation.

At this stage, having no causative treatment available, we suggested to the parents compassionate treatment with a PDE5 inhibitor. As no dosage scheme was known for avanafil, we settled for another PDE5 inhibitor, sildenafil, for which data and dosage schemes for long term treatment were available. Sildenafil is an established drug to treat pulmonary hypertension in infants and adults. During treatment with sildenafil (2 mg/kg BW/day) he was able to be weaned completely from the ventilator. Cardiomyopathy improved from a left ventricular ejection fraction (LVEF) of 45% to 65% and his muscle strength improved. Presently he is about to attend school again in a wheel chair. Based on these clinical data we plan to perform a controlled clinical study using a PDE5 inhibitor.

Delineating the phenotypic spectrum of MT-ATP6 associated disease: from isolated neuropathy to infantile-onset neurodegeneration

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Background and Methods: Mutations in the *MT-ATP6* gene are an established cause of mitochondrial disorders and show a broad spectrum of symptoms. Previous studies presented single case reports or small case series. The objective of this study was the characterization of the genetic and phenotypic spectrum on an extended cohort of patients with MT-ATP6 associated disorders. We analysed all available medical data of probands with confirmed *MT-ATP6* mutations from national registries and local databases from Europe, USA, Japan and China.

Results: We identified 113 clinically affected and 19 asymptomatic individuals with a known pathogenic mt-ATP6/8 mutation. The most frequent mutations were m.8993T>G (53/132, 40%), m.8993T>C (23%), m.9176T>C (23%), and m.9185T>C (9%). The degree of heteroplasmy was high both in affected (mean 95%, range 15-100%) and unaffected individuals (mean 73%, range 20-100%). Age of onset ranged from the prenatal period to the age of 75 years but almost half of the patients (49/103) became symptomatic before their first birthday. In 28 deceased patients, the median age of death was 14 months. If age at onset was in infancy, the mean delay in diagnosis was 1.87 years (range 0-19, median 1), while it was 5.85 years (range: 0-31, median 1.5) if symptoms started between one and six years, and 10.61 years (range 0-60, median 5) if disease onset was later than six years. The most frequent symptoms were neuropathy (90%), ataxia (80%), cognitive dysfunction (49%), seizures (37%), and retinopathy (14%). A diagnosis of Leigh syndrome was made in 55% of patients while the classical syndrome of NARP was rare (8%).

Conclusion: Our study presents the currently largest series of patients with mitochondrial disease. The phenotypic spectrum ranged from asymptomatic to early onset, multisystemic neurodegenerative disease with severe disability. Oligo- and monosymptomatic presentations appeared recurrent in our cohort, especially in adult onset patients. Early onset was associated with a more severe course of disease, while later onset showed a significant correlation with a larger delay until molecular diagnosis. As degree of heteroplasmy was high both in affected and unaffected individuals it strongly supports the notion that the degree of heteroplasmy alone cannot reliably predict disease severity.

The genetic landscape of paediatric mitochondrial disease: exploration of almost 2000 cases by whole exome sequencing

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Mitochondrial diseases (MD) pose a diagnostic challenge due to clinical and genetic heterogeneity, propelling unbiased WES into early diagnostics. To date, over 300 disease-genes implicated in mitochondrial energy metabolism are recognised, and this number continues to grow. Through global collaboration, initiated by the German and European Networks for Mitochondrial Diseases, mitoNET and GENOMIT, we assimilate data from almost 2000 paediatric patients investigated by WES under the clinical suspicion of MD.

Systematic analysis establishes a genetic diagnosis in 50% of cases. Genetic diagnoses span over 350 genes, the majority of which are seen in single cases, highlighting the vast genetic underpinning of MD. Variants in 70% of solved cases harbour within one of more than 150 MD disease-genes, with defects in *ECHS1*, *ACAD9, PDHA1*, and *MT-ATP6* arising most frequently. Variants in 30% of solved cases harbour within one of almost 200 non-MD OMIM disease-genes, underlining phenotypic mimicry in other genetic diseases. As is typical for MD, all modes of inheritance are observed: 70% autosomal recessive, 10% maternal, and less than 10% autosomal dominant, x-linked, and *de novo* inheritance, respectively.

Reaching a definitive diagnosis paves the way for development of efficacious treatment. Amongst the molecularly confirmed MD cases, 20% have a potential treatment strategy, such as cofactor metabolism defects amendable to specific supplementation. However, defect-targeted treatments for the majority are missing and, with genotyping fast becoming the prerequisite for clinical trial inclusion, the search for a molecular diagnosis is ever more important.

The study unites genetic- with clinical-, and biochemical-data, available for more than 95%, and 50% of cases, respectively. Age of symptom onset ranges from 0-17 years (mean 1.5 years), with a median of 5 clinical HPO terms available per case. The clinical data represents a "snapshot in time", allowing analysis of genotype-phenotype correlation, and retrospective calculation of the Mitochondrial Disease Criteria score, an objective measure of clinical likelihood of MD. This reveals a continuum of clinical suspicion from, unlikely, to possible, probable, and definite MD, with the majority falling within the possible (50%), and probable (30%) subgroups. Increase in clinical likelihood is reflected by increase in both diagnostic rate and the proportion of MD molecular-diagnoses.

In summary, this study brings together a substantial cohort of suspected paediatric MD cases, integrating molecular and health (HPO) data to aid diagnosis, further elucidate the heterogeneous genetic landscape of MD, and to strengthen on genotype-phenotype associations. Moreover, it exemplifies the value of international collaboration in the rare disease setting, by establishing a valuable global registry for both clinical and genomic data.

Positive final readout from REVERSE Phase III clinical trial of GS010 for the treatment of Leber Hereditary Optic Neuropathy (LHON)

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Objective: To assess the efficacy of a single intravitreal (IVT) injection of rAAV2/2-*ND4* (GS010), an investigational gene therapy for vision loss due to *ND4*-LHON.

Background: LHON is a mitochondrial inherited disease that causes bilateral central vision loss. A point mutation in the mitochondrial *ND4* gene accounts for 75% of all LHON cases. rAAV2/2-*ND4* is a gene therapy enabling allotopic expression and delivery of the wildtype ND4 protein to mitochondria of retinal ganglion cells.

Design/Methods: REVERSE (NCT02652780) is a Phase III, randomized, multicenter, double-masked, sham-controlled trial of 37 LHON subjects with the G11778A-*ND4* mutation and a vision loss duration between 6 months and one year. All received a single unilateral intravitreal injection of rAAV2/2-*ND4*. Visual functions and measurements of relevant retinal anatomy were monitored throughout the study with a final evaluation at Week 96 post-treatment.

Results: There was no withdrawals and all 37 subjects performed their End of Study visit at Week 96. A Best Corrected Visual Acuity (BCVA) improvement of more than +15 ETDRS letters was seen in GS010 treated eyes at Week 96. Sham-treated eyes showed improvement in BCVA (+13 ETDRS letters) as well. The BCVA improvement from Nadir (the worst BCVA post-baseline BCVA value up to Week 96 determined for each subject) was remarkable for GS010 treated eyes with an improvement of 28 letters (almost 6 lines), and 23 letters for Sham eyes. Contrast sensitivity (CS) also improved: GS010-treated and sham-treated eyes gained respectively on average +0.22 and +0.12 LogCS, compared to baseline. When looking at individual data after unmasking at Week 96, and response to GS010 therapy at the subject level, 68% of REVERSE ND4 LHON subjects attained a Clinically Relevant Recovery (CRR) in at least one eye; the CRR was defined as a subject showing, in at least one eye, an improvement of at least 10 letters for on-chart eyes at baseline, or moving from off-chart at baseline to reading at least 5 letters on the ETDRS scale (Magda et al, March 2019, “Natural History of Leber’s Hereditary Optic Neuropathy (LHON): Findings from a Large Patient Cohort”, NANOS Poster). The structural endpoints, including ganglion cell layer volume and temporal quadrant of the retinal nerve fiber layer, showed volume and thickness stabilization.

The ocular adverse events were mostly mild and mainly related to the IVT injection itself. Episodes of intraocular inflammation, likely related to rAAV2/2-*ND4,* were responsive to conventional treatment, frequently with corticosteroid drops only.

Conclusions: The final efficacy readout of the REVERSE pivotal study, with a follow-up of 96 weeks post IVT injection, show a clinically meaningful improvement in visual functions and sustained preservation of LHON-relevant retinal anatomy in GS010-treated eyes, suggesting that the biological targets of this gene therapy were successfully engaged. The positive effect of rAAV2/2-*ND4* on the contralateral eyes is currently under exploration.

Mitochondriopathy in pediatric patients with unspecific neuropediatric disease

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Mitochondriopathies account for an important subgroup of neuropediatric conditions but often the clinical presentation may be too unspecific to hint to an a priori disease hypothesis.

We report on a deep phenotyping and trio-WES study of 391 unrelated children affected by previously undiagnosed and diverse complex neuropediatric disorders. All children underwent a standardized and comprehensive clinical work-up and trio-WES. In a subset of 32 children we had an a priori clinical suspicion of a mitochondriopathy.

Within this group we confirmed the diagnosis by identifying a pathogenic variant in a known mitochondriopathy-associated gene in 11 children (34%). In 11/32 children (34%) we discovered a pathogenic variant in a disease gene not associated with a mitochondriopathy. For 3/32 children we detected a pathogenic mutation in a candidate gene for a mitochondriopathy. In the group of 359 children with no suspicion of the underlying cause we identified pathogenic mutations in known mitochondriopathy-associated genes in 13 children (3,6%).In 177/359 children (49%) we detected pathogenic mutations in other known disease genes.

Overall, we molecularly established the diagnosis of a mitochondriopathy in 24 of 391 (6%) unselected neuropediatric patients. This study highlights the importance of WES for the identification of children with a clinically less characteristic presentation of their underlying mitochondriopathy.

Mitochondrial DNA mutation analysis from exome sequencing – a holistic approach in diagnostics of mitochondrial disease

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Background: Diagnostics for suspected mitochondrial disease (MD) can be challenging and necessitate invasive procedures like muscle biopsy. This is due to the extremely broad genetic and phenotypic spectrum, disease genes on both nuclear and mitochondrial DNA (mtDNA), and the tissue specificity of mtDNA variants. Exome sequencing (ES) has revolutionised the diagnostics for MD. However, the nuclear and mtDNA are currently investigated with separate tests, increasing costs and duration of diagnostics. The full potential of ES is often not exploited as the additional analysis of "off-target reads" deriving from the mtDNA can be used to analyse both genomes.

Methods: We performed mtDNA analysis by ES of 2,111 cases in a clinical setting. We further assessed the recall rate and precision as well as the estimation of heteroplasmy by ES data by comparison with targeted mtDNA next generation sequencing in 49 cases.

Results: ES identified known pathogenic mtDNA point mutations in 38 individuals, increasing the diagnostic yield by nearly 2%. Analysis of mtDNA variants by ES had a high recall rate (96.2±5.6%) and an excellent precision (99.5±2.2%) when compared to the gold standard of targeted mtDNA next generation sequencing. ES estimated heteroplasmy levels with an average difference of 6.6±3.8%, sufficient for clinical decision making.

Discussion: Taken together, the mtDNA analysis from ES is of sufficient quality for clinical diagnostics. We therefore propose ES, investigating both nuclear and mtDNA, as first line test in individuals with suspected MD.

Reanalysis of WES-data of initially unsolved cases in patients with a suspected mitochondrial disorder

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Diagnostics in Mendelian disease has been transformed by genomic sequencing. Despite the revolutionary impact of coding-sequence interrogation by whole-exome sequencing (WES) on average 35% (24-68%) of patients receive a genetic diagnosis (Clark MM et al 2018). The diagnostic success can be increased by reanalysis of the WES data. Ewans et al. (2018) report an improvement from 30 to 41% when applying reanalysis after 12 months, leading 15% of the unsolved cases to a new diagnosis.

With insight into newly published disease genes, we have already proven the efficacy of reanalysis in a cohort of 1123 unsolved cases in the Munich sequencing database. Via reanalysis of known mitochondrial disease genes (n=300) we were able to provide a diagnosis to a further 36 suspected mitochondrial disease cases. Now we will systematically re-analyse unsolved WES cases considering mitochondrial and non-mitochondrial disease genes, known pathogenic variants and rare variants, focusing on the clinical interpretation.

We selected 339 patients with suspected mitochondrial disorders for the reanalysis. 176 have been recruited in the “German Network for mitochondrial disorders (mitoNET)” and 163 patients have been analysed within the diagnostic setting at the Institute of Human Genetics, TU Munich. All samples were sequenced between 2010 and 2018.

Currently we are contacting the referring clinicians for a clinical update of the patient’s phenotype and disease progression and are in contact with 51 out of 86 hospitals.

For the reanalysis we defined a standardized procedure. For variant calling we use SAMtools as well as GATK variant caller. Within the tools we are following a 6 step search.
1. search for potential biallelic variants in known disease genes (autosomal recessive)
2. search for de novo variants, if a trio dataset is available
3. search for known pathogenic variants, both dominant and recessive, listed in ClinVar or HGMD
4. search for mutations in genes known to be associated with the signs and symptoms of the patient based on an OMIM full text search
5. search for known disease genes (listed in OMIM) in the patient`s CNVs
6. search for mutated mitochondrial DNA (only in GATK)
For some cases the interpretation is supported by multi-omics functional data.
This approach was already successful for a number of cases.