Free communications

FC01-01
First stereotactic application of eladocagene exuparvovec into the putamen of a 3-year-old AADC-patient in Germany
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Introduction: Aromatic L-amino acid decarboxylase deficiency (AADC) is a very rare autosomal-recessive inherited neurotransmitter disorder. In most cases it results in severe neurologic and vegetative impairments due to the impaired synthesis of dopamine and serotonin. The clinical effect of standard drug therapy consisting of dopamine agonists, monoamine oxidase inhibitors and vitamin B₆ is still very limited. The intracerebral application of eladocagene exuparvovec, an adeno-associated virus-2 (AAV-2) based gene therapy, is the first causal therapeutic approach.

Patient(s) and methods: We present clinical and biochemical data of a 3-year-old girl with AADC deficiency, who was treated as the first patient in Germany with an intracerebral application of eladocagene exuparvovec within the framework of an individual healing attempt.

Results: The patient was diagnosed at the age of 6 months. She presented a severe phenotype with striking muscular hypotonia, lack of head control, very limited spontaneous movements, recurrent oculogyric crises, no active speech, feeding and sleeping disorders, excessive salvation and sweating as well as nasal congestion. In September 2021 eladocagene exuparvovec was administered bilaterally into the putamen by stereotactic surgery. Postoperative MRI scans showed correct placement of the vector and no indication for any perioperative complications. Two weeks after the intervention the patient developed dystonia and uncontrolled movements, requiring the administration of midazolam despite the reduction of the standard medication (apart from vitamin B₆). Regular monthly follow-ups revealed constant neurological progress. 4 months after application the child shows significant more and better coordinated spontaneous movements, has gained head control for 20 s, can lift the head in prone position and is more awake. Vegetative symptoms including sleeping disorders have improved while oculogyric crises and nasal congestion still appear nearly unchanged.

Conclusion/Discussion: The gene therapy vector was safely administered in our patient. Regular follow-ups document significant neurological improvements. Gene therapy might be a milestone in the treatment of AADC deficiency. Treatment response is age-dependent, emphasizing the need for early diagnosis. Approval of eladocagene exuparvovec in the European Union is expected during 2022.

FC01-02
Rosa canina L. extract can normalize the lipid profile and protein trafficking of various Niemann-Pick C1 gene mutations
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Introduction: Niemann NPC1 or NPC2 gene mutations cause Pick type C (NPC) illness, an autosomal recessive lysosomal storage disorder. The NPC1 protein, which is primarily in charge of intracellular cholesterol mobilization, is essential for maintaining lipid homeostasis. Defective cholesterol mobilization and a buildup of unesterified cholesterol are linked to NPC1 protein deficiency. N-butyldeoxynojirimycin or Miglustat is one of the few available treatments for NPC, and it is widely used in Europe. However, Miglustat’s effectiveness varies across NPC patients, and it also has a number of adverse effects. The current study’s objective is to identify an alternate therapy with fewer side effects. It has been demonstrated that Rosa canina L. methanol extract (RCME) improves protein trafficking and upholds cholesterol homeostasis.

Patient(s) and methods: To evaluate the effects of RCME on the trafficking of NPC1 and cellular cholesterol contents, skin-derived fibroblasts from healthy donors or patients carrying homozygote, heterozygote, or compound heterozygote mutations, wild type Chinese hamster ovary (CHO-WT) cells, and NPC1 knocked out CHO (CT43) cells were used. Either RCME (100 g/ml), Miglustat (100 M), or both treatments were applied to the cells. Endoglycosidase H treatment was used to evaluate the trafficking of NPC1 between the endoplasmic reticulum (ER) and the Golgi, and is more awake. Vegetative symptoms including sleeping disorders have improved while oculogyric crises and nasal congestion still appear nearly unchanged.

Conclusion/Discussion: The gene therapy vector was safely administered in our patient. Regular follow-ups document significant neurological improvements. Gene therapy might be a milestone in the treatment of AADC deficiency. Treatment response is age-dependent, emphasizing the need for early diagnosis. Approval of eladocagene exuparvovec in the European Union is expected during 2022.
**FC01-03**

Pathomechanism of CALFAN syndrome

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**Introduction:** Recurrent acute liver failure (RALF) is a rare, but life-threatening event. In recent years, various “new” causes for RALF have been identified due to the growing accessibility of whole exome sequencing. One of these is CALFAN syndrome (cholestatic acute liver failure and variable neurodegeneration), caused by variants in SCYL1 presenting with fever triggered episodes of liver failure with onset in infancy. SCYL1 is known to play a role in intracellular trafficking and Golgi homeostasis, but so far, little is known about the disease pathomechanism in patients with pathogenic SCYL1 variants.

**Patient(s) and methods:** Four patient fibroblast cell lines with different SCYL1 variants were available for comparison with two control fibroblast cell lines. We applied Western Blot to detect changes in protein level of SCYL1 and markers of the endoplasmic reticulum (ER) stress pathway. Immunofluorescence was used to examine protein (co-)localization of Collagen1-α1 with markers of various cellular compartments. Using Electron Microscopy, changes in the microstructure of cellular compartments were studied. To induce ER stress, temperature (40 °C) as well as a chemical stressor–Tunicamycin blocking the N-glycosylation of proteins–were applied, respectively.

**Results:** Collagen1-α1 was retained in the ER of the patient cells. When studying the colocalization of Collagen1-α1 with various compartments of the autophagic pathway, we could not detect a clearance by autophagy; however patient cells showed increased levels of lysosomal vesicles. The microstructure of the Golgi-apparatus was changed, whereas the macrostructure appeared to be intact. When challenged by ER stress, patient cells showed an early activation of the Ire1 pathway, and cell death occurred at an earlier timepoint.

**Conclusion/Discussion:** The observed Collagen1-α1 retention confirms a defect of cellular trafficking in CALFAN syndrome, potentially contributing to increased stress responses. Elevated temperature (mimicking fever) leads to a severe ER stress response and eventually apoptosis. We hypothesize that this may be the mechanism underlying hepatocytolysis (and thereby acute liver failure) during febrile infection in patients with CALFAN syndrome.

**FC01-04**

Subdural hematoma in glutaric aciduria type 1: High excreters are prone to incidental SDH despite newborn screening

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**Introduction:** Background: Glutaric aciduria type 1 (GA1), a neurometabolic disorder of L-lysine metabolism, is characterized by accumulation of neurotoxic metabolites and acute- or insidious-onset of striatal injury. Two biochemical subtypes (high and low excretors) have been arbitrarily defined. Incidence of subdural hematoma (SDH) was reported in up to 30% of symptomatic patients. A recent retrospective study found SDH in 4% of patients, but not in individuals identified by newborn screening (NBS).

**Patient(s) and methods:** In order to investigate localization, frequency, age distribution, additional MR and clinical findings and potential risk factors of SDH in GA1, we systematically analyzed MRI scans in a cohort of GA1 individuals, of which 38 were prospectively followed after identification by newborn screening (NBS), while n = 27 were diagnosed by targeted metabolic screening (TMS) and n = 4 by high-risk family screening.

**Results:** A total of 168 MRIs of 69 patients (54 high and 15 low excretors; age at MRI 9 days–73.8 years, median 3.2 years), were systematically reviewed. SDH was observed in eight high-excreting patients (NBS: n = 6; TMS: n = 2) imaged between 5.8 and 24.4 months, namely space-occupying SDH in two patients after minor accidental trauma and SDH as an incidental finding in six patients without trauma. In patients without trauma imaged at 3 to 30 months (n = 36; 25 NBS, 27/9 high/low excreters), incidence of SDH was 16.7% (16% in NBS). SDH was more common after acute (33.3%) than insidious onset of dystonia (14.3%) or in asymptomatic patients (5.9%). It was only found in patients with a high excreting phenotype and associated with wide frontoparietal CSF spaces and frontotemporal hypoplasia. High excreters were over-represented among patients with SDH (6/27 vs 0/9 low excretors), acute onset (10/12), and wide frontotemporal CSF spaces (16/19).

**Conclusion/Discussion:** Incidental SDH occurs in approximately one in six patients with GA1 during a vulnerable period of late infancy and early childhood despite early identification by NBS and treatment. Greater risk of high excreters is morphologically associated with more frequent enlargement of external CSF spaces including frontotemporal hypoplasia, and may be further aggravated by more pronounced alterations of cerebrovascular blood volume and venous pressure.

**FC01-05**

Analysis of emotional and cognitive resources of patients with hyperphenylalaninemia (HPA)

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**Introduction:** Today the long-term outcome of early diagnosed and treated patients with hyperphenylalaninemia (HPA) is almost inconspicuous. Nevertheless, problems with implementation of therapy and accompanying increased blood phenylalanine (Phe) concentrations beyond the target range may become apparent, with negative consequences such as behavioral problems, nervousness and lack of concentration especially in adolescence. These in turn interact with each other demanding a conjoined consideration of all sub-areas. Patient-oriented approaches (analysis of executive functions, psychological distress/needs) are indispensable...
Impact of pregnancy planning and preconceptual dietary intervention on metabolic control and offspring's outcome in phenylketonuria

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Introduction: Phenylketonuria (PKU) is one of the most common inborn errors of metabolism and is caused by a monogenic mutation in the phenylhydroxylase gene. The lifelong therapy in most patients consists of a low-protein diet. For metabolic adjustment, regular laboratory determination of phenylalanine in serum (PHE) and outpatient presentation is necessary. The COVID19 pandemic has led to major changes in the lives of many people through various measures such as lockdown, contact restrictions etc. It has also significantly changed the care of patients with chronic diseases. How the COVID19 pandemic has changed the care and metabolic adjustment of adult PKU patients in Germany will be shown by this work for the first time.

Patient(s) and methods: Medical records of 190 PKU patients presenting to our outpatient clinic from 02/2018 to 02/2022 were retrospectively analyzed. Patients who became pregnant during the period (22), started a new drug therapy (6), lost to follow up after outbreak of the pandemic (26) or presented only after onset of the pandemic (33) were excluded. The records of 115 patients were included (range 19–66 years, W:M 66:49). The study was performed after obtaining approval from the ethics committee and informed consent from the patients. The data were collected and retrospectively analyzed for the number of outpatient presentations, metabolic adjustment (PHE concentrations) and patient adherence. The impact of the COVID pandemic on the number of outpatient presentations, metabolic adjustment and patient adherence was analyzed. The number of additional DBCs sent increased significantly after the outbreak of the COVID pandemic (6), lost to follow up after outbreak of the pandemic (26) or presented only after onset of the pandemic (33) were excluded. The records of 115 patients were included (range 19–66 years, W:M 66:49) and retrospectively analyzed for the number of outpatient presentations, dry blood chart (DBC) submissions and their metabolic adjustment before and after the outbreak of the COVID pandemic.

Results: Comparing the two years before the outbreak of the COVID pandemic (02/2018–02/2020) with the two years after, the number of outpatient presentations increased from 247 ± 467 (mean ± SD) by 35% to 325 ± 414 (p < 0.001) and lower plasma phenylalanine concentrations during pregnancy, particularly in the first trimester (0–7 weeks of gestation: 247 ± 467 µmol/L unplannd, p < 0.0001; 8–12 weeks of gestation: 235 ± 414 µmol/L planned, p < 0.0001). Furthermore, preconceptual dietary intervention increased the success rate of achieving the phenylalanine target before conception compared to women without training (19 weeks before conception with vs. 9 weeks of gestation without training, p < 0.001). Mode of nutrition before pregnancy showed no clear advantage, and maternal educational level had no apparent effect on target achievement. The majority (93%) of children had normal IQ (mean 103, range 69–132, median age 7.3 years); however, IQ decreased with increasing phenylalanine concentration during pregnancy.

Conclusion/Discussion: This study demonstrates that pregnancy planning has a major impact on the achievement of target phenylalanine levels during pregnancy and is a prerequisite for normal intrauterine development and cognitive functions of offspring. Preconceptional dietary training is also very helpful for reaching target phenylalanine levels during pregnancy. In contrast, lifelong diet and the educational level of women with PKU seem less important for reaching therapy targets. Key points remain the careful education about MPKUS and the harmful high phenylalanine concentrations with the necessity of consistent contraception.
A network medicine approach identifies key TCA cycle enzymes as potential therapeutic targets in organic acidemias

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Introduction: Organic acidemias (OAs) are an important class of inherited metabolic disorders (IMD) arising due to defect in intermediary metabolic pathways of carbohydrate, amino acids, and fatty acid oxidation. Current treatment is mainly based on special diet, but patients still develop chronic organ dysfunction, such as kidney disease in glutaric acidemia type 1 (GA1). In search for causal intervention, increased lysine acylation of proteins may be involved in the OAs pathomechanism. To define such mechanisms in an unbiased manner, i.e., not from canonical pathways, relevant proteins and genes including those from disease-disease relationships are networked based on the protein-protein interactome (PPI) and graph theories to novel so-called disease modules.

Patient(s) and methods: Here we incorporate six proteomics data sets, studying lysine acylation in GA1, hydroxy-methylglutaryl (HMG)-CoA-lyase deficiency and malonic acidemia, to generate the glutationylome, HM-Glyome and malonylome modules. Intersecting these acylomes resulted in a common network of acylated proteins connected via PPI, the combined-acylome (CCA). Network-based centrality measures were then applied to determine targets for in-vitro validation via enzymatic activity, functional in-gel activity assay and complexome profiling.

Results: The CA module consisted of 80 proteins that were acylated in all OA of these, 21% appeared to be highly modified and form the core-combined-acylome (CCA). The network analysis suggested central roles for dihydroxacyl dehydrogenase (DLD), malate dehydrogenase (MDH2) and succinate dehydrogenase flavoprotein subunit a (SDHA). Moreover, results revealed that three alpha-ketoadic dehydrogenase complexes, and OXPHOS complexes I, II and V were affected. In-vitro validation experiments in GA1 fibroblasts under acylating conditions showed indeed decreased activity of MDH2.

Conclusion/Discussion: Thus, unbiased network medicine is a novel approach to decipher previously unrecognized common pathomechanisms of OA. The identification of the CA and CCA adds mechanistic insights into affected protein complexes. Future research will focus on in vivo and clinical validation as well as expanding the CA by more OA and applying drug-repurposing or nutraceutical strategies on their target proteins.
FC02-03
Sudden neonatal death in individuals with medium-chain acyl-coenzyme A dehydrogenase deficiency, 2005–2021 in Germany: limitations of newborn screening

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Introduction: Medium-chain acyl-coenzyme A dehydrogenase (MCAD) deficiency leads to hypoketotic hypoglycemia, hepatopathy and often fatal outcome in undiagnosed individuals. Introduction of tandem mass spectrometry-based newborn screening (NBS) programs significantly reduced morbidity and mortality in MCAD deficiency; however, severe hypoglycemia and neonatal death in newborns with early disease manifestation may still occur.

Patient(s) and methods: Retrospective case collection (Long-term observational study NGS2025) of neonatally deceased infants with MCAD deficiency in Germany from 2005–2021.

Results: Eight individuals with MCAD deficiency died neonatally (median at age 3.5 days) in a metabolic decompensation with severe hypoglycemia before NBS result was available. All were born at term, with normal birth weight and postnatal adaption. For 80% poor feeding was reported. 50% were still at maternity hospital. Confirmatory genetic analysis revealed homozygosity for the classic pathogenic ACADM variant c.985A>G (Lys329Glu) for all tested patients (n = 7), revealing a neonatal mortality rate of 1.3% for this genotype despite NBS.

Conclusion/Discussion: Early fatal neonatal metabolic decompensations occurring in the first days of life cannot be prevented by NBS, however, we recommend sampling of NBS very early in the time frame recommended by national specifications. The common pathogenic ACADM variant c.985A>G is a major risk factor. Algorithms to prevent, diagnose and treat hypoglycemia in neonates in maternity and neonatal units should be optimized. Systematic post-mortem diagnostic protocols are needed for sudden neonatal deaths.

FC02-04
Feasibility of glycosylation analysis from newborn screening cards: a case series

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Introduction: Newborn screening programs have transformed metabolic medicine and allow early targeted treatment of previously untreatable and often fatal disorders. Among inborn errors of metabolism, Congenital Disorders of Glycosylation (CDG) represent an ever-growing group of severe diseases, some of which are amenable to either symptomatic or causative treatment. So far, early diagnosis has been hindered by the fact that defective glycosylation can be absent in the early postnatal period. Here, we present a series of retrospective glycosylation analyses performed from dried blood spots (DBS) derived from newborn screening programs allowing, in principle, early postnatal diagnosis of CDG.

Patient(s) and methods: Eight patients who were later diagnosed with various type I and II CDG were included in this study following informed consent. Dried blood spots obtained as part of the German newborn screening program were collected and analysed. After water-based elution with agitation overnight at 4 °C, samples were analysed using conventional glycosylation analyses using isoelectric focusing of serum transferrin.

Results: Dysglycosylation was readily detected in DBS obtained shortly after birth from all patients. Follow-up analyses showed marked deterioration of transferrin glycosylation over the following weeks. Generally, patients in whom dysglycosylation was found in DBS samples exhibited more severe phenotypes.

Conclusion/Discussion: In contrast to previous studies, we found that early postnatal diagnosis of CDG from DBS is possible. However, all cases presented here exhibited severe phenotypes and showed worsening glycosylation over time. This might indicate that dysglycosylation is only seen in more severe cases. In principle, early screening for CDG might be possible although more detailed analyses and controlled studies using alternative methods of analysis might be needed.

FC02-05
Metabolic profiling and mitochondrial function in hepatic glycogen storage diseases—a fibroblast study

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Introduction: Hepatic glycogen storage disease (GSD) subtypes I and III are autosomal-recessive disorders that alter cellular energy metabolism by impairing the break down of glycogen into glucose. Abnormal mitochondrial function has been described in murine GSD Ia hepatocytes, yet significantly less research is available in human cells and ketotic GSDs. We hypothesized that impaired glycogen metabolism results in distinct metabolic phenotypes in the extra- and intracellular compartments that may play a role in the pathogenesis of these disorders.

Patient(s) and methods: In this study, we investigated cultured human GSD fibroblasts (GSD Ia, GSD Ib, and GSD III) in comparison to healthy controls. We performed mitochondrial function tests by measuring the oxygen consumption rate and lactate production of the cultured fibroblasts. Mitochondrial organization and mitochondrial content were examined in live cells by spinning-disc confocal microscopy. Additionally, we profiled extra- and intracellular metabolites by targeted LC-MS/MS.

Results: Mitochondrial content and network morphology of cells of all 3 GSD subtypes were comparable to that of healthy controls. Likewise, we did not observe significant differences in the basal oxygen consumption rates between healthy controls and GSD cells. Targeted metabolomics followed by principal component analysis (PCA) and hierarchical clustering (HC) uncovered metabolically distinct poises of healthy controls and GSD subtypes. Metabolic profiling was compatible with dysfunctional energy production (glycolysis, Krebs cycle, succinate), and showed reduced creatinine export in GSD Ia and GSD III, as well as reduced antioxidant defense of the cytochrome and glutathione systems.

Conclusion/Discussion: Although we did not observe impairment of mitochondrial content and network morphology in GSD fibroblasts, we could show that extra- and intracellular metabolite profiles distinguished GSD subtypes from healthy controls. Research on human GSD hepatocytes is needed to further elucidate disease mechanisms and to help opti-
Heterozygosity for glycogen storage disease type IIIa might add to the risk for developing type 2 diabetes

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Introduction: Some genetic conditions are diagnosed frequently in the Faroe Islands, e.g. type IIIa glycogen storage disease (GSD IIIa) caused by mutations in the AGL gene coding for the debrancher, the enzyme which is necessary for a complete breakdown of glycogen. Type 2 diabetes (T2D) may occur in affected persons. We hypothesized that being heterozygous for GSD IIIa might impair glucose-homeostasis and thus increase the risk for developing prediabetes or manifest T2D.

Patient(s) and methods: DNA of 850 participants of the Faroese Diabetes Project [Veyhe AS et al. Diabetes Res Clin Pract. 2018] were analyzed for the presence of the Faroese mutation in the AGL gene (c.1222C>T; p.R408X): 382 healthy controls, 232 pre-diabetic individuals and 236 persons with confirmed T2D.

Results: A total of 29 (3.4%) participants were identified being heterozygous for R408X. Gender and age as well as BMI and waist-hip-ratio did not differ significantly between GSD- wild type individuals and GSD-heterozygotes. However, risk ratio for abnormal glucose metabolism defined as being pre-diabetic or suffering from T2D was slightly higher in R408X-heterozygotes (RR = 1.03 with a 95% CI 0.45–2.35).

Conclusion/Discussion: Our results show no evidence of vascular dysfunction in patients with GSD I up to the age of at least 20 years. This suggests that protective mechanisms exist to protect patients from early atherosclerosis and its subsequent complications. Further studies are needed to uncover these protective mechanisms and to investigate the influence of other factors such as dietary measures, medication, disease complications, and genetic predisposition.

Evaluation of vascular dysfunction and risk of atherosclerosis in patients with glycogen storage disease type I

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Introduction: GSD I is an inborn error of metabolism with impaired glycogenolysis and gluconeogenesis that is clinically characterized by hepatomegaly and recurrent hypoglycemia. Hyperlipidemia with elevated concentration of both triglycerides and cholesterol is a common laboratory finding in patients with GSD I. Hyperlipidemia is a known risk factor for atherosclerosis. However, the cardiovascular risk in GSD I is still not known, and previous studies have shown contradictory results.

Patient(s) and methods: We investigated vascular dysfunction in a cohort of 32 GSD I patients [26 la/6 lb; mean age 20.7 (4.8–47.5) years] compared to 32 healthy controls matched for sex, age, and BMI. In addition to the lipid profile, we measured sulfur-containing metabolites, so-called aminothiols to evaluate oxidative stress as well as hsCRP and Lp PL2 as parameters of vascular inflammation. Determination of intima media thickness, retinal vessel analysis, and 24-h blood pressure measurement were performed to evaluate endothelial function.

Results: In addition to the expected atherogenic lipid profile, GSD I patients showed an increased burden of oxidative stress. This was reflected by increased concentrations of oxidized cysteine, oxidized glutathione, and increased ratios of oxidized/reduced aminothiols. Moreover, both inflammatory parameters, hsCRP and Lp PL2, showed elevated levels in GSD I patients, confirming vascular inflammation. Despite this risk constellation, no evidence of vascular end-organ damage could be detected in the functional-structural examinations: Retinal vessel analysis showed no differences between the two cohorts, and intima-media thickness measurements even yielded a significantly thinner intima media in the GSD I cohort. The 24-h blood pressure analysis also showed no clear differences except for single blood pressure values; above all, pulse wave velocity and pulse pressure as markers of arterial stiffness did not differ.

Conclusion/Discussion: Our results show no evidence of vascular dysfunction in patients with GSD I up to the age of at least 20 years. This suggests that protective mechanisms exist to protect patients from early atherosclerosis and its subsequent complications. Further studies are needed to uncover these protective mechanisms and to investigate the influence of other factors such as dietary measures, medication, disease complications, and genetic predisposition.
Abstracts

Posters

P01 „Acht auf einen Streich – Achtung Gangliosiden!” – erste Daten einer patientenorientierten, industrienunabhängigen Registerstudie

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Einleitung: Gangliosidosen gehören zu den angeborenen neurodegenerativen lysosomalen Speichererkrankungen. Die Patienten zeigen progressive Makrozeaphalie, Entwicklungsverzögerung und eine Regression, die entsprechend der Verlaufsform mit einer frühen Morbidität und Mortalität einhergehen. Eine objektive Charakterisierung des natürlichen Verlaufs der Erkrankungen kann die klinische Forschung insbesondere im Hinblick auf die Entwicklung von neuen Therapien voranbringen.

Patienten und Methoden: Bei diesem gemeinsam mit der Patientenorganisation Hand in Hand gegen Tay-Sachs und Sandhoff e.V. entwickelten Projekt handelt es sich um eine Register-Studie zur Erfassung der klinischen Manifestationen von 8 Gangliosidosen (GM-2-Gangliosidosen M.Tay-Sachs, M.Tay-Sachs-Variante B1, M. Sandhoff, GM2-Activator Mannosegalaktosidose, GM-1-Gangliosidosen, M. Morquio B [Mukopolysaccharidose Typ IV B, Sialidosen und Galaktosialidosen NCT04624789]). Es wurde eine Querschnittanalyse der Baseline-Daten bei 26 Patienten durchgeführt. Der primäre Endpunkt ist der Schwergrad der Erkrankung anhand des neu konspierten 8 in 1-Scores. Sekundäre Endpunkte waren das erstmalige Auftreten neurologischer Zeichen und Symptome, die von Eltern und Ärzten beobachtet wurden sowie die zeitliche Verzögerung in der Diagnosestellung und die phänotypische Charakterisierung der Patienten. Tertiärer Endpunkt waren die Ergebnisse der neurologischen Untersuchungen (Entwicklung, Ataxie, Geschicklichkeit) und die körperlichen und kognitiven Einschränkungen.

Ergebnisse: Der 8 in 1-Score erfasst quantitativ den Schwergrad der Erkrankung. Die Eltern erkennen das Erstsymptom, akustische Startles-reaktionen, deutlich früher als die behandelnden Ärzte, die dann die Entwicklungsstörung und Hypotonie sehen. Im Median betrug die zeitliche Verzögerung bis zur Diagnosestellung 3,16 [IQR 0,69 … 6,25] Jahre. 8 Patienten zeigten eine spät-infantile Verlaufsform.

Diskussion: Die Daten dieses Registers sensibilisieren behandlende Ärzte, Therapeuten, Betreuer über diese seltenen und schwer verlaufenden Erkrankungen, um eine früh Diagnose und eine fachliche Beratung der Eltern frühzeitig zu ermöglichen. Des Weiteren dienen die Daten dazu bessere, quantitativ zu erfassende Endpunkte für zukünftige klinische Studien zu bestimmen. Die bisherigen Daten zeigen eine genauere Charakterisierung der Phänotypen insbesondere wird der bisher kaum beschriebene spät-infantile Phänotyp charakterisiert. Die Erhebung longitudinaler Verlaufsdaten ist geplant.

P02 Multisystemic manifestations of a leukodystrophy: Aicardi-Goutières syndrome caused by IFIH1 variant

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Introduction: Pathogenic autosomal dominant gain of function IFIH1 variants are known to cause the leukodystrophy Aicardi-Goutières syndrome (AGS), an autoimmune inflammatory Type I interferonopathy with variability in disease expression. Type I interferonopathies are characterized by an aberrant and uncontrolled activation of the IFN-alpha pathway often leading to exuberant and uncontrollable inflammatory processes with multisystemic involvement within the first years of life. Leukodystrophy with basal ganglia calcifications, cerebral atrophy, and cerebrospinal fluid findings of chronic lymphocytosis and highly elevated interferon-alpha (INF-alpha) are frequently found. Neurologic and extra neurologic manifestations lead to severe progressive disabilities and premature death. To date treatment of AGS can only rely on supportive care to limit subsequent sequelae. However, recent studies have indicated that the use of Janus Kinase (JAK) inhibitors may be effective in controlling the course of interferonopathies.

Patient(s) and methods: We report on a 5-year-old girl born with AGS in which an individualized therapeutic attempt with Ruxolitinib, a JAK inhibitor, was initiated at 2.5 years of age. The patient initially presented with an intrauterine growth retardation, primary microcephaly, neonatal anaemia and thrombocytopenia. Increased crying occurred after a CMV infection at the age of 3 months and loss of gained abilities after a vaccination at the age of 7 months followed by delayed dentition, failure to thrive, severe psychomotor retardation with tetraspastic tetraparesis, epileptic seizures, hearing loss, reduced visual abilities, autoimmune hepatitis, arterial hyper- tension with cardiac septal hypertrophy and daily episodes of shivering and discomfort. A de novo missense alteration in IFIH1 (p. Ala719Val) was identified.

Results: Ruxolitinib was started at 0.2 mg/kg/d and slowly increased to a target dose of 0.5 mg/kg/d. A significant decrease of the initially measured hyper-expression of IFN-stimulated genes (so called “IFN-signature”) was evident after three months of treatment. A remarkable improvement of the neurological picture was evident with a reduction of asthenia and irritability, better fine motor skills and balance competencies and a considerable decrease in the frequency of seizures. Furthermore, the autoimmune hepatitis alleviated.

Conclusion/Discussion: Overall, the treatment of patients Aicardi-Goutières syndrome with JAK Inhibitor Ruxolitinib can result in a substantial improvement in clinical multisystemic symptoms and quality of life.

P03 Pulmonary fibrosis and hyperinflammation in heme oxygenase 1 deficiency

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Introduction: Homozygosity for pathogenic variants in HMOX1 leads to heme oxygenase 1 deficiency, disrupting the rate-limiting step of heme degradation. Subsequent oxidative damage causes chronic inflammation with a severe and ultimately fatal disease course. Allogeneic stem cell transplant has been proposed as a curative treatment with no other causative treatment options available. We present a case of HMOX1 deficiency and thrombocytopenia which an individualized therapeutic attempt with Ruxolitinib, a JAK inhibitor, resulted in a substantial decrease in the frequency of seizures. Furthermore, the autoimmune hepatitis alleviated.

Results: Ruxolitinib was started at 0.2 mg/kg/d and slowly increased to a target dose of 0.5 mg/kg/d. A significant decrease of the initially measured hyper-expression of IFN-stimulated genes (so called “IFN-signature”) was evident after three months of treatment. A remarkable improvement of the neurological picture was evident with a reduction of asthenia and irritability, better fine motor skills and balance competencies and a considerable decrease in the frequency of seizures. Furthermore, the autoimmune hepatitis alleviated.

Conclusion/Discussion: Overall, the treatment of patients Aicardi-Goutières syndrome with JAK Inhibitor Ruxolitinib can result in a substantial improvement in clinical multisystemic symptoms and quality of life.
ro. Cell viability was analyzed in addition to protein carbonylation, which served as a correlate of oxidative damage to protein. HMOX1 expression in patient and control cells was studied by immunoblotting following CdCl₂ stimulation.

**Results:** Exome sequencing identified the pathogenic variants c.55dupG (p.Glu19Glyfs*14); c.262_268delinsCC (p.Ala88Profs*51) in HMOX1. HMOX1 was absent from patient cells, thus establishing the diagnosis. Hemin challenge resulted in severely reduced cell viability in patient-derived LCL, while protein carbonylation was significantly increased compared to controls.

**Conclusion/Discussion:** Deficiency in HMOX1 results in a severe, ultimately fatal phenotype characterized by impaired heme metabolism and subsequent oxidative damage. Our findings support the presence of pulmo-

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**P04 Characterization of oxidative and mitochondrial stress in X-linked adrenoleukodystrophy fibroblasts**

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**Introduction:** X-linked adrenoleukodystrophy (X-ALD) is an inherited metabolic disorder caused by ABCD1 gene mutations, leading to a dysfunctional ABCD1 protein. ABCD1 is a peroxisomal transporter crucial for fatty acid transport and β-oxidation of very-long-chain fatty acids (VLCFA). ABCD1 mutations lead to VLCFA accumulation in body fluids and tissues. Clinical presentation is heterogeneous and ranges from asymptomatic carriers to rapidly progressing childhood cerebral ALD, distinct pathophysiological mechanisms are still unclear. Several studies showed elevated oxidative stress parameters and mitochondrial dysfunction in X-ALD cells and tissues, while detailed processes and correlation to clinical presentation need further investigation.

**Patient(s) and methods:** This study aimed to analyze oxidative stress and mitochondrial dysfunction in 10 X-ALD fibroblast cell lines from 5 families and two healthy control cell lines. Quantification of cellular reactive oxygen species (ROS) was performed using the 2′,7′-dichlorofluorescein diace-

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**P05 Correlation of therapy regimen, biochemical monitoring and in vivo brain proton MR-spectroscopy in a patient with GAMT deficiency and normal neurocognitive development**

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**Introduction:** GAMT codes for guanidinoacetate methyltransferase (GAMT) which catalyzes the final step of creatine synthesis. Creatine serves as an essential energy shuttle facilitating manifold functions in neurometabolism. GAMT deficiency is a rare autosomal recessive disease and patients suffer from intellectual disability, seizures, speech and movement disturbances. Treatment consists of daily creatine supplementation to increase cerebral creatine and strategies to reduce toxic guanidinoacetate (GAA) levels.

**Patient(s) and methods:** Case study: Our patient was followed over 14 yrs starting at the age of 15 mo. Developmental milestones, brain MRI, quantitative single voxel ¹H MRS and biochemical analyses were assessed. Molecular genetic studies confirmed the diagnosis.

**Results:** GAMT deficiency was diagnosed by characteristic brain MRI, ¹H MRS and biochemical findings at age 15 mo, prompting initiation of treatment with creatine/ornithine supplementation. Genetic testing revealed compound heterozygosity for a GAMT frameshift mutation (c.442dupC, p.Gln148ProfsX43) and a null allele. Doses of creatine/ornithine were adapted to body weight (100–400 mg/kg per day). Follow-up examinations documented close to normal neurocognitive development, resolution of brain MRI alterations, significant increase of cerebral creatine concentration and improvement of metabolite changes.

**Conclusion/Discussion:** Our study presents the first long term follow-up over 14 yrs that provides clinical data, biochemical and multimodal neuroimaging results. It allows for correlations of therapeutic regimen with clinical course, biochemical data and brain ¹H MRS creatine levels. The results reveal insights into the dose dependent effects of creatine/ornithine supplementation and expand the phenotypic spectrum of GAMT deficiency. This is the second report of a patient with normal neurocognitive development after initial symptomatic clinical presentation including developmental delay. Early start of supplementation and strict dosing regi-

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**P06 Metabolic neuropathies in children and adolescents with LCHAD/MTP deficiency: Insights from in-vivo magnetic resonance neurography**

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**Summary:**

- Cell viability was analyzed in addition to protein carbonylation.
- Exome sequencing identified pathogenic variants in HMOX1.
- Hemin challenge reduced cell viability and increased carbonylation.
- Deficiency in HMOX1 leads to severe oxidative damage.
- Our findings support the presence of pulmoperi-
- X-linked adrenoleukodystrophy fibroblasts were studied.
- major progress in understanding the disease.

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**Conclusion:**

- New treatment strategies are needed.
- Further research is required to establish specific therapies.
- Oxidative stress and mitochondrial dysfunction in X-ALD cells were characterized.
- Initial symptomatic clinical presentation was improved.
- Insights into dose-dependent effects were gained.

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**P07** Intracellular localization of Glycine N-acyltransferase-like protein 1 (GLYATL1)

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**Introduction:** The phase 2 enzyme Glycine N-acyltransferase-like Protein 1 (GLYATL1; EC 2.3.1.68) is an acyltransferase which transfers an acyl group to the N-terminus of glutamine and can use phenylacetyl-CoA as an acyl donor. GLYATL1 contributes to the elimination of compounds such as N-acetylglutamine, lactacidosis, and very high concentrations of plasma citrulline. However, the severe form of pyruvate carboxylase (PC) deficiency also presents in the first days of life with a high-biochemical trait including elevated plasma citrulline.

**Patient(s) and methods:** The index patient was a newborn male, who was transferred on the third day of life from the postnatal ward to the NICU because of respiratory distress and lethargy. A dried blood sample (DBS), plasma, and cerebrospinal fluid (CSF) were taken on day 4. The baby had hyperammonemia (2004 µmol/L), lactacidosis (94 mg/dL), mild cardiac dysfunction, and convulsions. He died on day 6 of multi-organ failure.

**Results:** Amino acids in DBS, plasma, and CSF were highly suggestive of PC deficiency as in addition highly elevated to citrulline (1406 µmol/L), the patient had elevated plasma levels of alanine (2813 µmol/L), proline (756 µmol/L), glycine (804 µmol/L), and lysine (854 µmol/L). Genetic analysis revealed homozygosity for the c.470G>A [p. (Arg157His)] mutation in the ASS1 gene. No variants in the PC gene were detected.

**Conclusion/Discussion:** According to literature, the amino acid profile was highly suggestive of PC deficiency. We also compared it to the amino acid profiles in DBS from 16 previously diagnosed cases of citrullinaemia type I which showed a different amino acids pattern. We conclude that the amino acid profile from DBS samples taken in the first days of life cannot reliably distinguish between citrullinaemia type I and PC deficiency.

**P08** Severe citrullinaemia type I with atypical amino acid pattern

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**Introduction:** Cystinurinaemia type I is a severe autosomal recessive urea cycle defect, with a defect in argininosuccinate synthetase (ASS1). Patients usually present during the first days of life with hyperammonemia, lactacidosis, and very high concentrations of plasma citrulline. However, the severe form of pyruvate carboxylase (PC) deficiency also presents in the first days of life with a high-biochemical trait including elevated plasma citrulline.

**Patient(s) and methods:** The index patient was a newborn male, who was transferred on the third day of life from the postnatal ward to the NICU because of respiratory distress and lethargy. A dried blood sample (DBS), plasma, and cerebrospinal fluid (CSF) were taken on day 4. The baby had hyperammonemia (2004 µmol/L), lactacidosis (94 mg/dL), mild cardiac dysfunction, and convulsions. He died on day 6 of multi-organ failure.

**Results:** Amino acids in DBS, plasma, and CSF were highly suggestive of PC deficiency as in addition highly elevated to citrulline (1406 µmol/L), the patient had elevated plasma levels of alanine (2813 µmol/L), proline (756 µmol/L), glycine (804 µmol/L), and lysine (854 µmol/L). Genetic analysis revealed homozygosity for the c.470G>A [p. (Arg157His)] mutation in the ASS1 gene. No variants in the PC gene were detected.

**Conclusion/Discussion:** According to literature, the amino acid profile was highly suggestive of PC deficiency. We also compared it to the amino acid profiles in DBS from 16 previously diagnosed cases of citrullinaemia type I which showed a different amino acids pattern. We conclude that the amino acid profile from DBS samples taken in the first days of life cannot reliably distinguish between citrullinaemia type I and PC deficiency.
Glutaric acidemia type-1: Therapeutic strategies in a mouse model

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Introduction: Glutaric aciduria type-1 (GA1) is a rare inherited disease affecting newborns caused by a deficiency of glutaryl-CoA dehydrogenase (GCDH). Despite dietary treatments, one-third of patients still develop chronic kidney disease and white matter changes. To address this challenge, we aim to target enzymes upstream of GCDH in a mouse model. GCDH is a mitochondrial enzyme of the L-lysine, -hydroxylysine, and -tryptophan catabolism. This depletion leads to a neurotoxic accumulation of glutaric acid and related metabolites. Current treatment consists of a low lysine diet, arginine, and carnitine supplementation. Thus, we have chosen AASS and AADAT upstream of GCDH in the L-lysine degradation pathway as potential therapeutic targets since their deletion causes benign or no phenotype.

Patient(s) and methods: Furthermore, to evaluate this aim, the GA1 mouse model (GCDH−/−), the GA1 rescued mouse models (GCDH+/−AASS−/−, GCDH+/−AADAT−/−), and control wild-type mice will be analyzed. Mass spectrometry will be used to screen for metabolite changes in body fluids. Western blots will be performed on extracted organs, and immunohistochemistry to check for morphological changes.

Results: Our preliminary data confirm the efficacy of the GA1 mouse model, where the concentrations of the analyzed metabolites increased in GCDH−/− mice compared to control mice. Moreover, through the western blot technique, we show a lack of GCDH protein concentration in diseased mice compared to the control mice in the brain.

Conclusion/Discussion: Ultimately, we envisage rescuing the phenotype in GA1 mouse models. We hope to translate these insights into a potential therapeutic approach for GA1 patients.
Results: The reciprocal immunoprecipitations with either mAb revealed by Western blotting interactions between MGAM and SI concomitant with a hetero-complex assembly of these proteins in the BBM. The inclusion of DOC into the solubilization buffer resulted in a reduction of the enzymatic activities likely due to alteration in the quaternary structure of either enzyme.

Conclusion/Discussion: In view of their interaction, SI and MGAM regulate the final steps in starch digestion in the intestine, whereby SI assumes the major role by virtue of its predominant expression in the intestinal BBM, while MGAM acts in auxiliary supportive fashion. These findings will help understand the pathophysiology of carbohydrate malabsorption in functional gastrointestinal disorders (FGIDs), particularly in irritable bowel syndrome, in which gene variants of SI are implicated.

P13 Idiopathic pathological ketotic hypoglycemia and celiac disease

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Introduction: A diagnosis of idiopathic pathological ketotic hypoglycemia (IPKH) is made if recurrent symptomatic events of (mild) hypoglycemia and ketosis occur in the absence of acute infections and after exclusion of endocrine disturbances (such as growth hormone or cortisol deficiency) and metabolic disorders (such as glycogen storage disease type 0, III, VI and IX). Recently we identified several children fulfilling the criteria for IPKH who have an additional diagnosis of celiac disease.

Patient(s) and methods: Three patients diagnosed with celiac disease continued to have poor growth and symptoms consistent with IPKH despite full compliance with gluten-free diet and negative transaminase antibodies and gliadin peptide IgG. Because of the high frequency and awareness of IPKH in the community, glucose and beta-hydroxybutyrate were evaluated with a point-of-care device and found to be abnormal with early ketosis. Growth hormone and thyroid hormone deficiency were ruled out. Because of the finding of hepaticomegaly, a clinical diagnosis of mild GSD-like condition was made and treatment with uncooked cornstarch (UCCS) and high-protein diet was implemented. UCCS was administered at night at a dose of 0.7 to 2 g/kg sufficient to prevent morning ketosis. High-protein diet was commenced with a goal of achieving total protein levels in the blood of 70 g/L and pre-albumin levels of 0.20 g/L.

Results: After starting a dietary treatment, significant subjective and objective improvement was noted in all patients. Improved growth and body mass index, normalization of liver size and decreased echogenicity of liver were demonstrated. Patients reported feeling more energetic with higher activity level and better endurance.

Conclusion/Discussion: The co-incidence of IPKH and trisomy 21 has been described (Drachmann et al., JIMD reports 2021). We describe a series of patients with IPKH and celiac disease. The key clinical feature was onset of diabetes mellitus in one patient, which is rather surprising because the corneal epithelium contains remarkable glycogen stores, both the enzymatic system for synthesis and degradation of glycogen, and GLUT1 as the major glucose transporter. Since glucose supply to the cornea is a diffusive process, as the cornea is and must remain an avascular structure to prevent vascular formation from interfering with light transmission and thus vision, glycogen metabolism is tightly regulated by the c-kit/FIH-1/Akt/GSK3-β pathway.

P14 Cytosolic phosphoenolpyruvate carboxykinase deficiency: genotypic and phenotypic spectrum

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Introduction: Cytosolic phosphoenolpyruvate carboxykinase (PEPCK-C) deficiency is a rare disorder of gluconeogenesis caused by pathogenic biallelic variants in the gene PCK1. Patients present with hypoglycemia, lactacidosis, and hepatopathy. To date, there has been no systematic analysis of its phenotypic, biochemical, and genetic spectrum.

Patient(s) and methods: All currently published cases and a novel patient with genetically confirmed PEPCK-C deficiency were included. Clinical, biochemical, and genetic characteristics were analyzed. Protein and in-silico prediction score modeling was applied to analyze potential variant effects.

Results: 32 individuals from 25 families were found, including one previously unreported patient. Symptom onset usually occurred in infancy with a broad range from neonatal age to adulthood. The typical biochemical pattern was hypoglycemia triggered by fasting or illness, increased urinary concentrations of tricarboxylic acid cycle metabolites, mildly elevated hepatic transaminases and lactate concentrations in serum. Plasma glutamine concentrations were elevated in some patients and may be a suitable marker for newborn screening. With adequate treatment, biochemical abnormalities usually normalized after a hypoglycemic episode. Regardless of the genotype, a broad clinical spectrum was found for different phenotypes. To date, eight genotypes with nine different PCK1 variants were identified, of which alleles with the recurrent variant c.925G>A; p.(Gly309Arg) are predominant and appear to be endemic in the Finnish population. Protein modeling suggests altered manganese- and substrate-binding as superordinate pathomechanisms.

Conclusion/Discussion: Based on the biochemical pattern, PEPCK-C deficiency is a recognizable cause of childhood hypoglycemia. Newborn screening for PEPCK-C deficiency may identify at least a sub-cohort of affected individuals through elevated glutamine concentrations in dry blood and should be pursued. Environmental factors appear to be the main determinant for phenotypic differences in patients with biallelic variants in PCK1. Since it is a treatable disorder of gluconeogenesis, early diagnosis is crucial to prevent metabolic derailment and morbidity.

P15 Corneal involvement in glycogen storage disease type IV

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Introduction: Apart from extremely rare cases of retinopathy in myophosphorylase deficiency (glycogen storage disease type V, GSD V) and generalized lysosomal storage in (treated) Pompe disease (GSD II) patients, ocular involvement has not been described in GSDs. This includes corneal disease, which is rather surprising because the corneal epithelium contains remarkable glycogen stores, both the enzymatic system for synthesis and degradation of glycogen, and GLUT1 as the major glucose transporter. Since glucose supply to the cornea is a diffusive process, as the cornea is and must remain an avascular structure to prevent vascular formation from interfering with light transmission and thus vision, glycogen metabolism is tightly regulated by the c-kit/FIH-1/Akt/GSK3-β pathway.
Patient(s) and methods: Here, we present for the first time the case study of a now 14-year-old boy with congenital neuromuscular form of GSD IV and bicuspid perforating corneal dystrophy.

Results: Patient presented prenatally with polyhydramnios, shown muscular hypotonia and contractures at birth, developed progressive neuro-myopathic scoliosis treated with growing rods (age 13) and before surgical correction of multiple contractures. Minor liver involvement (increased echogenicity), minimal mitral regurgitation, normal mental development. At age 10, diagnosis of GSD IV based on detection of compound heterozygosity for GBE c.691+2>T>C (splice site) und c.706G>C (p.Gln236His) by multiple parallel sequencing. A corneal infiltrate was first observed at age 6 which developed into thinning and ulceration that eventually required penetrating keratoplasty of both eyes at ages 11 and 12 respectively.

Conclusion/Discussion: This is the first report of corneal involvement in a patient with GSD IV. The latter diagnosis is unequivocal because two variants were detected that have been repeatedly found in patients with a similar myoneuropathic course of branching enzyme deficiency. Despite highly suggestive, the causality for corneal involvement has not been established with absolute certainty, albeit no other causes, particularly infectious, have been found. Massive glycogen deposition in the epithelium was not detected, however in cases with liver involvement, it is the atypical amylopectin-like glycogen rather than the absolute amount that could trigger an inflammatory response. Limbal stem cells could also be involved, but this would likely result in a different clinical presentation. Finally, neovascular factors could also be involved in the pathomechanism.

P16
Unexpected high stature in young adults with GSD type IIIa

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Introduction: Glycogen storage disease type IIIa (GSD IIIa) is caused by biallelic pathogenic changes in the AGL gene coding for the glycogen debranching enzyme (OMIM 232400). Typical manifestations include hepatomegaly, myopathy, fastiging ketotic hypoglycemia, short stature and more. We reviewed records of five young Faroese GSD IIIa adults and found an unexpected good growth.

Patient(s) and methods: Out of seven young Faroese GSD IIIa patients, records of five of them were included in this report (one moved abroad, one just entered puberty). All are homozygous for c.1222C>T (p.R408X) leading to a truncation in exon 11 and thus affecting both enzymatic activities of the debranching enzyme.

Results: Height with last control (age 22 to 28 years) was 0.8 to 16.5 cm above parental target height (PTH) (ratio height/PTH 1.0 to 1.1). No severe overweight (BMI 20.0 to 27.9) or hepatomegaly (1.0 to 4.2 cm below costal margin) were observed. Creatinine kinase was normal in all of them, liver enzymes slightly elevated in most patients (ALT max. 203 U/L). Total protein and pre-albumin surpassed target range (>69 g/L resp. >0.19 g/L) in four patients. Uncooked cornstarch at bedtime was used by two patients, before school by one; protein supplements before/around sports by two. All of them are exercising regularly, up to 10 h per week.

Conclusion/Discussion: There are several possible explanations for the unexpected good growth in these patients: 1) Our observation describes the normal trend of a generational effect on height. 2) Suboptimal growth of obligate carrier parents caused a lowered final height (also in heterozygous sibs of patients ratio of final height to PTH was lower than in patients themselves). 3) Traditional Faroese diet leads to a high-protein intake (1.5–2.0 g/kg/day protein), which became even higher after re-ensuring high protein intake since patients were about 14 years old. However, compliance with recommended diet was Limited. Thus, recurrent mild (nocturnal) hypoglycemia might have occurred and stimulated excessive release of growth hormone. With availability of sufficient protein, this might have caused extra growth.

P17
SGLT2 inhibition in pediatric and adult GSD Ib patients—a monocentric experience

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Introduction: GSD Ib is caused by variants in the SLC37A4 gene coding for glucose-6-phosphate translocase. The genetic defect results in impaired glycogenolysis and gluconeogenesis plus neutropenia and neutrophil dysfunction. In addition to hypoglycemias, patients suffer from recurrent infections, oral ulcers and inflammatory bowel disease, which are not sufficiently controlled by GCSF. Recently, the SGLT2 inhibitor empagliflozin has been shown to reduce accumulating 1.5-anhydroglucitol-6-phosphate in GSD Ib patients resulting in an increased neutrophil count with improved function.

Patient(s) and methods: We describe the clinical course of 6 patients (2 children and 4 adults, age 9 months to 37 years) with GSD Ib receiving empagliflozin (0.27 to 0.58 mg/kg bw/d) for 1 to 20 months.

Results: All patients showed significant improvement of neutropenia. Those treated with GCSF (5 patients) were able to stop (3/5) or reduce (2/5) this medication. Frequency of infections, oral ulcers and bowel movements decreased in all patients. An infant had abdominal wall phlegmona after infection of gastrostomy despite GCSF treatment requiring surgical correction of multiple contractures. A child had binocular perforating corneal dystrophy.

Conclusion/Discussion: Neutropenia and neutrophil dysfunction in GSD Ib patients can be effectively treated with the SGLT2 inhibitor empagliflozin. The most frequent adverse effects are symptomatic hypoglycemias, requiring an effective monitoring and, in some patients, dosage reduction.

P18
Glycogen storage disease type Ib, SGLT2 inhibitors and liver transplantation: lessons to learn

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Introduction: In the past, the indication for liver transplantation (LTX) in patients with glycogen storage disease type Ib (GSD1b) has been markedly reserved. This is due to neutropenia and granulocyte (PMN) dysfunction, which result in higher propensity for infection and fear of wound healing problems or fistula formation. The widespread use of SGLT2 inhibitors in GSD1b and their impressive effect on PMN counts is now well documented [1]. We report the complicated clinical course of two GSD1b patients who received LTX that eventually led to normal glucose homostasis. Here, we focus on PMN counts before and after use of an SGLT2 inhibitor.

Patient(s) and methods: Patient 1 is a 3-year-old girl with typical signs of GSD1b (SLC37A4 c.1042_43delCT homozygous) including oral aphthous, frequent infections, and otoneura at first referral. Parents rejected tube feeding and reasonably satisfactory glucose homostasis was only achieved with extreme dietary measures unacceptable for parents. Therefore, LTX was considered and off-label treatment with the SGLT2 inhibitor
**Abstracts**

**P02**
Early diagnosis of neonatal vitamin B12 und cbl G deficiency: arguments for newborn screening in Germany

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**Introduction:** Newborn screening (NBS) including second-tier strategies measuring homocysteine (HC), methylenylacetic acid (MMA), and methylcitrate (MC) based on first-tier markers like methionine (Met) and propionylcarnitine (C3) is feasible to identify vit. B12 deficiency, organic acidurias, remethylation and cobalamin disorders, but a national NBS program for these disorders does still not exist in Germany.

**Patient(s) and methods:** Here we report 3 of those cases, two of them with early diagnosis following NBS in a pilot study and one later in the symptomatic phase.

**Results:** First case is a now 19-months-old female. NBS showed C3 of 5 µmol/l (<5), in recall C3 2.1 µmol/l and in the context of a pilot study MMA 17.8 µmol/l (<2.5), MC 1.4 µmol/l (<1) and HC 13 µmol/l (<13) were measured. Subsequently low vit. B12 (176 ng/l, >211) and holotranscobalamin (HTC) (24.7 µmol/l, 37.5–188) was found. Vit. B12 of the mother was 222 ng/l (>211), HTC 30 µmol/l (37.5–188), HC 18.76 (<13.9) and anti-parietal cell antibodies 1:1280 (<1:80) were shown. Initial musclar hypotonia and breastfeading difficulties of the newborn responded well to oral vita- min B12 substitution. Normal development and weight gain so far. Second case is a 6 months old breastfed female with normal national NBS (but retrospectively slightly elevated C3/C2) presenting with seizures and developmental delay. Metabolic workup showed C3 of 10 µmol/l, HC 203 µmol/l, MMA in urine 290 mmol/mol Krea, vit. B12 165 ng/l, HTC 4.2 µmol/l and low maternal vit. B12 (94 ng/l) as cause of vit. B12 deficiency. Third case is a 5 months old female with IUGR and microcephaly and consanguineous parents. NBS in the context of a pilot study showed Met 4.2 µmol/l, HC 58.7 µmol/l, MMA 0.4 µmol/l, MC 0.2 µmol/l. Vit. B12 was 216 ng/l. WES showed a homzygous duplication in MR1Tand confirmed cbl G deficiency. Hyperexercitability and opisthotonos improved rapidly under treatment with betaine, folic acid and hydroxocobalamin. Met reached a constantly normal level but HC remained moderately increased (<50 µmol/l). Low but growing head circumference (~2.2 z), no significant loss of developmental milestones so far.

**Conclusion/Discussion:** These cases indicate that vitamin B12 and cbl G deficiency, both conditions with a (more) favorable outcome if the treatment starts early, should be included in the national NBS in order to prevent the manifestation of irreversible neurologic symptoms of affected newborns und to avoid recurrence of vitamin B12 deficiency in future pregnancies.
Lipid Nanoparticle-mediated delivery of plasmid DNA for re-applicable, long-term gene therapy independent of pre-existing neutralizing antibodies

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Introduction: Adeno-associated viral vectors (AAV) have achieved tremendous success in clinical trials demonstrating safety and efficacy in delivering a functional copy of a defective gene. However, application is limited by exclusion of patients with pre-existing neutralizing antibodies (NAbs), wide patient-to-patient variation of expression levels and restriction to a single application.

Lipid Nanoparticles (LNP) enable non-viral delivery of nucleic acids independent of NAbs and thereby application and re-administration in all patients. In contrast to AAV, LNP do not deliver their payload to the nucleus but release it in the cytoplasm. This limits their application to RNA-based approaches, such as the clinical trial for GSD1a mRNA-LNP therapy. In addition, duration of gene expression from mRNA is restricted and frequent administrations are necessary to achieve long-term therapeutic effects. Together with Acuitas Therapeutics, we developed novel DNA vectors delivered with LNP, capable of translocating DNA to the nucleus to enable long-term gene therapy for all patients with the option of re-administration.

Patient(s) and methods: To enable nuclear localization of LNP-delivered DNA, we equipped a luciferase (luc)-expressing plasmid DNA (pDNA) vector with DNA-nuclear targeting sites, which represent binding-sites for transcription factors that shuttle the DNA through the nuclear pores. To reduce the innate immune response to the pDNA payload, we take advantage of a CpG-free pDNA backbone and an LNP-incorporated Dexamethasone-prodrug (Integrated Nanotherapeutics, Vancouver, BC). Acuitas Therapeutics formulated the pDNA in liver-specific LNP.

Results: LNP-pDNA injected BALB/c wt mice showed efficient expression 7 days post-injection (p.i.), which declined by the 1-month time-point. Restimulation of isolated splenocytes from these mice with a luc-peptide in an anti-Interferon-γ ELISPOT revealed a transgene-specific T-cell response as limitation for long-term expression. In contrast, immuno-deficient NSG mice showed stable expression until the endpoint 6-months p.i.

Conclusion/Discussion: In these initial proof-of-concept experiments, we could demonstrate efficient non-viral delivery of pDNA and expression of a reporter gene in vivo. Long-term expression was limited by an adaptive immune response against cells expressing the xeno-transgene luc. We aim to investigate whether a similar immune response will develop against more physiological proteins and explore approaches to induce immune tolerance against expressed transgenes.